**Federal State Budget Educational Institution of Higher Education**
**"North Ossetian State Medical Academy"**
**of the Ministry of Health of the Russian Federation**

**Department of Biology and Histology**

# CELL BIOLOGY

UDC 576

**Compilers:**

**Tseboeva A.A.**, PhD in Medical sciences, senior Lecturer of department of Biology and Histology of North Ossetian State Medical Academy

**Bibaeva L.V.** Professor, Grand PhD in PhD in Medical sciences, Head of department of Biology and Histology of North Ossetian State Medical Academy.

**Reviewers:**

**Skupnevskiy S.V.** - Senior Researcher of Institute of Biomedical Investigations - the Affiliate of Vladikavkaz Sciientific Centre of Russian Academy of Sciences: Vladikavkaz, North Ossetia Alania Republic

**Nikolaev N.I.** - PhD in Biology, Assoc. Prof of department of anatomy, physiology and botanic of North Ossetian State University

This book is especially designed to serve the needs of the 1st grade students of specialties 31.05.01 General Medicine, 31.05.02 Pediatrics, 31.05.03 Dentistry, 33.06.01 Pharmacy, 32.05.01 Preventive Medicine of medical universities to study one of the sections within the biology course in English.

It was prepared on the basis of educational training program of discipline “Biology” of FSBEI HE NOSMA MOH Russia in accordance with the requirements of federal state educational standard of higher education

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## ОглавЛЕНИЕ

# *Preface*

This book is especially designed to serve the needs of the 1st grade students of specialties 31.05.01 General Medicine, 31.05.02 Pediatrics, 31.05.03 Dentistry, 33.06.01 Pharmacy, 32.05.01 Preventive Medicine of medical universities to study one of the sections within the biology course in English. It was prepared on the basis of educational training program of discipline “Biology” of FSBEI HE NOSMA MOH Russia in accordance with the requirements of federal state educational standard of higher education

The book includes fundamental concepts of the topics in cell and molecular biology. The language of book is quite easy and understandable based on scientific approach. The book helps students to prepare classes in the “Teach Yourself” style. Many illustrations helps to visualized and understand difficult topics.

# *BIOLOGY AS A SCIENCE. INTRODUCTION TO CELL BIOLOGY*

Biology is a science of living matter.

**Biology as a science of life.**

***Biology*** is a branch of the natural sciences, which studies living organisms and how they interact with each other and their environment. It examines the structure, function, growth, origin, evolution, and distribution of living things.

The word "Biology" has been derived from two Greek words, «*bios-*» and «*-logos*». "*Bios*" means life and "*logos*" means "ground", "opinion", "expectation", "account", "reason", and "discourse. The term "Biology" was coined by ***Lamarck*** and ***Treviranus*** in ***1802***.

**The main disciplines of Biology.**

Biology is subdivided into separate branches for convenience of study, though all the subdivisions are interrelated by basic principles. Biology includes some disciplines:

**1. Morphology**, it deals with the form and structure of organisms. It comprises several branches:

**a) Anatomy**, it deals with the structures visible to the naked eyes such as in the dissection of organisms.

**b) Histology**, it is the study of finer details of structure of organs and tissues with the help of a microscope.

**c) Cytology (cell biology)**, it is the detailed study of structures and functions of cells.

**d) Embryology**, it is study of formation, growth and development of a new individual generally from an egg.

**2**. **Physiology**, it is the study of working and functions of organs within an organism.

**3. Ecology**, it is the study of relations of living things and their environment.

**4. Taxonomy**, it is the study of laws and principles of a natural classification of organisms.

**5. Evolution**, it is the study of origin, differentiation, and interrelationships of organisms of the present day and past ages.

**6. Genetics** is the study of heredity and variations.

**7. Molecular Biology**, the study of living things in terms of Physics and Chemistry of the molecules.

**8. Protozoology** - the study of Protozoa

**9. Parasitology** - the study of parasites

**10**. **Botany** the study of plants

**11**. **Zoology** - the study of animals

**12**. **Microbiology** - the study of microorganisms in.

## The main characteristics of living and non-living substances.

Living organisms can usually be distinguished from the non-living though this is not easy with lower forms of life. All living beings display the following properties:

**1) Cellular Structure.** It is defining property of living beings. Each living being is a complex entity which is formed of one or more cells.

**2) Chemical composition**. Living organisms are composed of chemical substances in definite proportions; these chemicals form complex organic molecules (lipids, sugar, proteins and nucleic acids) of great molecular weight which form a living substance.

**3) Nutrition and growth**. Living beings require nutrition which is used for building the body and repairing worn-out parts and also for supplying energy for their vital activities.

**4) Metabolism**. Various vital chemical changes take place constantly in living organisms, these changes are called *metabolism*. In metabolism organic substances are changed into new organic substances which replace old parts and build the body, this constructive process is called *anabolism*. The metabolic process of decay, decomposition into simpler substances is called *katabolism* (or catabolism).

**5) Irritability.** Any change in the environment to which an organism responds is called a *stimulus*, and the capacity of an organism react to stimuli constitutes its irritability.

**8) Reproduction**. It is the ability to duplicate itselves or produce new individuals resembling it in all essential features.

**9) Adaptation to environment**. Every living organism fits to its surrounding or it is adapted to the conditions of life called the *environment*. The ability to adapt themselves to their environment is the characteristic property of living organism.

**10) Heredity**. It's the passing on of traits from parents to their offspring

**11) Variations**. Living beings possess variations and have the ability to evolve with time.

**12) Homeostasis**. Homeostasis is the maintenance of a constant (yet also dynamic) internal environment in terms of temperature, pH, water concentrations, etc. Much of our own metabolic energy goes toward keeping within our own homeostatic limits.

## Levels of biological organization.

The ***molecular leve***l is the simplest level of biological organization. This level is about organic molecules and hereditary material (DNA and RNA).

At the ***cellular level*** we find a cell that is the basic structural unit of life, the simplest part of living matter that can carry on all of the activities necessary for life.

In most multicellular organisms, cells associate to form *tissues* - group of cell, having the same origin, structure, and fulfilling the same functions (***tissue level***), such as muscle tissue in animals or epidermis in plants.

Tissues arranged into functional structures called *organs* (***organ level***), such as the heart or stomach in animals or root and leaves in plants.

Functioning together with great precision, the organ systems make up the complex multicellular organism (***organism level***).

Organism interacts to form still complex levels of biological organization. All members of one species that live in the same area, mate and give the progeny called a *population* (***population level***).

Populations which are phenotypically similar and reproductively isolated from the other, but actually or potentially capable of interbreeding among themselves form the *species* (***species level***).

A community together with its non-living environmental factors is referred to as an *ecosystem (**ecosystem level)***.

All communities of living things on Earth are collectively referred to as the *biosphere* (***biosphere level***).

# *Cellular organization of living matter.*

## BRIEF HISTORY OF CELL DISCOVERY

The discovery of cells followed from the invention of the microscope by Robert Hooke. In 1665 *Robert Hooke* reported honeycomb like structures in a very thin slice of cork and coined the term "cell" to describe them. He published his findings in the form of a book titled "Micrographia". The next year he presented his findings to the Royal Society of London.

In 1674 *Anton Von Leeuwenhoek* improved microscope and was first who discovered protozoans, sperm cell and red blood corpuscles.

At the beginning of 19-th century (1802-1826), several scientists including a Frenchman, *H.J. Dutrochet* gave the idea of the cell theory. In 1830 *Purkinje* coined the term protoplast to describe the cellular substance. *Robert Brown* discovered a thick, rounded structure in a cell and gave it the name of nucleus in 1831.

The credit for proposing "cell theory" goes to two German scientists, *Mathias Schleiden* and *Theodore Schwann*. In 1838 *Schleiden* reported that all the plants are made of cells only. The following year, i.e., 1839, *Schwann* reported that all animals were also made of cells. *Schwann* also proposed that tissues were composed of cells and the cells were the functional units of all living organisms. Unfortunately both of them wrongly believed that cells originated from non-living substances.

*Robert Remak* and *Rudolf Virchow* reported that cells always originated from pre-existing cells only.

Our knowledge of a cell has reached molecular level with the discovery of the electron microscope by *Knoll* and *Ruska* in 1932.

## Modern Cell Theory

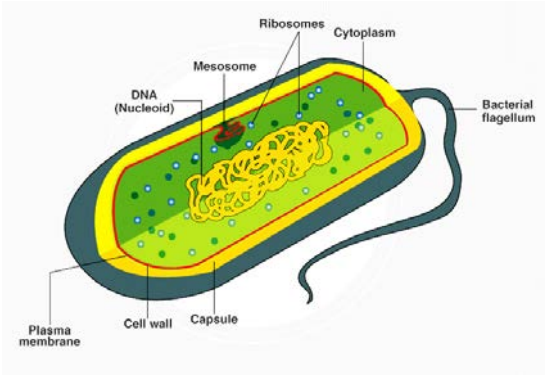
- The cell represents the elementary unit of construction and function in living organisms.
- All cells are basically alike in chemical composition and metabolic activities.
- All cells come from the division of pre-existing cells.
- All living organisms consist of one or more cells. Multicellular organisms compose of many cells which are connecting together and form unique system

## The types of cells.

There is a great variation in the form, size and number of cells present in the body of living beings. But all the cells found in nature are divided into two independent and radically different groups which are as follows:

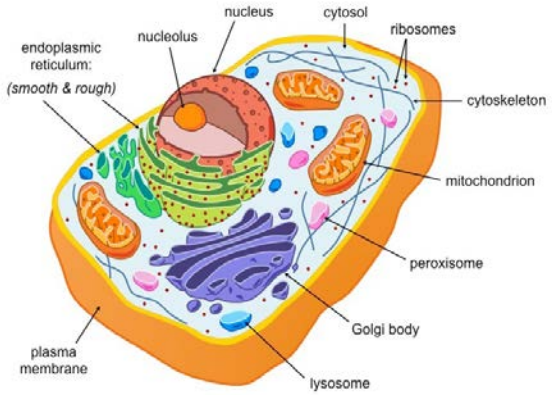
### Prokaryotic (pronuclear) cells.

All prokaryotes are single-celled (unicellular) organisms (Bacteria and Archaea). These cells are characterized by the absence of nucleus and most of cytoplasmic organelles. The cells have a plasma membrane, minute ribosomes, cytoplasm, and DNA. The genetic material (single circular DNA without histone proteins) in prokaryotes is not enclosed by nuclear membranes, and lies free in the cytoplasm.




### Eukaryotic cells.

These cells are found in all animals and higher plants, fungi and most of the algae. They have a distinct nucleus with a nuclear membrane.




### *SIMILARITIES AND DIFFERENCES BETWEEN PROKARYOTIC AND EUKARYOTIC CELLS*

| **characteristic** | **Type of the cells** | | | |
|---|---|---|---|---|
| | **Prokaryotic** | **Eucaryotic cells** | | |
| | | **Animal** | **Plant** | **Fungi** |
| | **(Bacteria and cyanobacteria)** | | | |
| Cell size | Avarage diameter 0.5 – 5 nm | Up to 40 nm diameter common, commonly 1000-10000 times volume of prokaryotic cells. | | |
| Form | Unicellular | Unicellular or truly multicellular | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Genetic material | | | Circular DNA without histone proteins (nucleoid and plasmids) spread out in the cytoplasm. Nucleus is absent. | Linear DNA, associated with proteins and RNA to form chromosomes, which are concentrated within the nucleus. | | |
| Cell division | | | Binary fission | Mitosis and meiosis | | |
| **Surface apparatus** | Plasma membrane | | Present. It forms invaginations, like mesosomes et al. | Present | | |
| | Cell wall | | Cell wall (murein). Sometimes capsule (mucopolysacharides) | Cell wall is absent. Glycocalyx (glycolipids, glycoproteins) is located over membrane | Cell wall (cellulose) | Cell wall (chitin) |
| **cytoplasm** | **<u>Hyaloplasm</u>** | | Present | Present | | |
| | **Organelles** | Endo-plasmatic reticulum (ER) | – | Mainly granular | Mainly smooth | present |
| | | Mitochondria | – | Present | | |
| | | Golgi apparatus (GA) | Absent | Forms piles of cisternae and vesicles | Flattened piles of cisternae (dictyosomes) | |
| | | Peroxy-somes | – | Present | Present only in higher plants | present |
| | | Lysosomes | – | Phagosomes | Autophagosomes | |
| | | Ribosomes | 70 S | 70 S – mitochondria<br>80 S – hyaloplasm and ER | | |
| | | Cell center | Absent | Present | Present only in lower plants | Present |
| | | Microtubules | Absent | Present | | |
| | | Microfilaments | Rare | Present | | |
| | | Plastids | Absent | Absent | Present | Absent |
| | | Vacuoles | Absent | Absent | Present | Present |
| | | Cilia | Absent | Present | Absent | Absent |
| | | Flagellae | Present in some species | Present | Absent. Present in some species (algae) | Absent |

| | | | | | |
|---|---|---|---|---|---|
| | **Inclusions** | Proteins, lipids, carbohydrates (glycogen), polyphosphates (volutin's granules) | Proteins, lipids, carbohydrates (glycogen), secretory granules, pigment et al. | Lipids, carbohydrates (starch), protein (gluten), calcium oxalate crystals | Proteins, lipids, carbohydrates (glycogen), secretory granules, pigment et al. |
| | **Cytoskeleton** | Absent | Microtubules, microfilaments and microtrabecular fibers | Occasional microtubules | present |

## General plan structure of eukaryotic cell.

### Origin of eukaryotic cell.

The eukaryotic cell seems to have evolved from a symbiotic community of prokaryotic cells. It is almost certain that DNA-bearing organelles like the mitochondria and the chloroplasts are what remains of ancient symbiotic oxygen-breathing proteobacteria and cyanobacteria, respectively, where the rest of the cell seems to be derived from an ancestral archaean prokaryote cell – a theory termed the **endosymbiotic theory**:

***The ingested cell under some circumstances could survive and reproduce within cytoplasm of the host cell. The relationship is stabilized by their mutual benefits of metabolic symbiosis and becomes obligatory.***

***Horizontal gene transfer from symbiont to host genome causes the loss of corresponding protein synthesizing ability of the symbiont and is likely to be selectively favoured. The development from symbiont to organelle is completed by the loss of its independent survival ability.***

## Subcellular components.

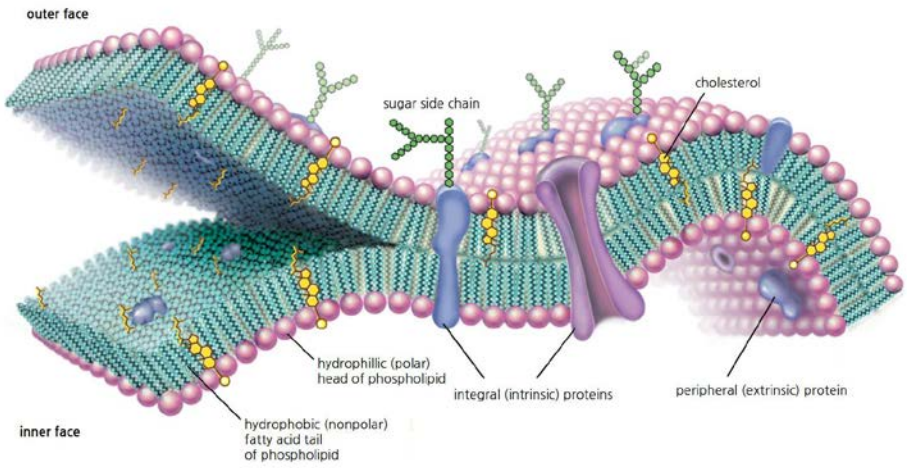
Each of these three main components or compartments of the cell (***nucleus, plasma membrane and cytoplasm***) contain several subcomponents or subcompartments.

## PLASMA MEMBRANE.

Structure. The structure that separates the cell contents from the external environment is the plasma membrane. This is two-layered film (6 to 10 nm thick) made of molecules of fats pressed between two sets of protein molecules, and is perforated by small holes. Most cell membranes also contain variable amounts of glycoproteins and glycolipids. This is three-dimension view of the membrane at the molecular level, in which the "***fluid mosaic***" ***model*** is reproduced by ***Singer*** and ***Nicolson*** in ***1972***. They observe that all plasma membranes are composed chiefly of proteins and lipids, not so much carbocchadrates (in human red cell – 52% of proteins, 40% - lipids, 8% - carbohydrates.

Lipids are cholesterol, but most of lipids are phosphoglycerides. The "head" of such molecules become ionized under pH conditions commonly found in cell, but "tails" end usually remain unchanged. The polar and non-polar regions of these molecules react quit differently where placed in water. The polar "head" tend to form hydrogen bonds with water molecules. Consequently, the charged regions of phosphoglycerides are said to be **hydrophilic** ("water-loving"), the uncharged "tails" are said to be **hydrophobic** ("water-hating"). The polar "heads" are in the contact with intra- and extracellular aqueous fluids, and the non-polar "tails" are directed toward the center of the bilayer, isolated from water molecules.

There are proteins ***peripheral*** and ***integral***, depending on how deeply they penetrate into the lipid bilayers. Hence, the membrane is highly asymmetrical. The molecular asymmetry of plasma membrane is further emphasized be the oligosaccharide chain of glycoproteins and glycolipids that protrude only at the surface of the membrane.




Properties and functions of plasma membrane.

*1.Plasma membrane is selectively permeable.*

Some substances pass across plasma membranes readily, others move across slowly only under certain conditions; and still others normally cannot enter cells at all.

2. *Absorption of materials (endocytosis).* Endocytosis and exocytosis are active processes involving the bulk transport of materials through membranes, either into cells (endocytosis) or out

of cells (exocytosis). ***Endocytosis*** occurs by an extension of the cell surface membrane to form a vesicle vacuole. It is two types:

***a) Phagocytosis*** ("cell eating") – material taken up is in solid form. Cells specializing in the process are called *phagocytes* and are said to be *phagocytic*; for example, some blood cells. The sac formed during uptake is called a *phagocytic vacuole*.

***b) Pinocytosis*** ("cell drinking") – material taken up is in the liquid form (a solution, colloid or fine suspension). Vesicles formed are often extremely small, in which case the process is known as *micropinocytosis* and the vesicles as *micropinocytotic*. Pinocytosis is particularly associated with amoeboid protozoans and many other, often amoeboid cells, such as leucocytes, embryo cells, liver cells and certain kidney cells involved in fluid exchange. It can also occur in plant cells.

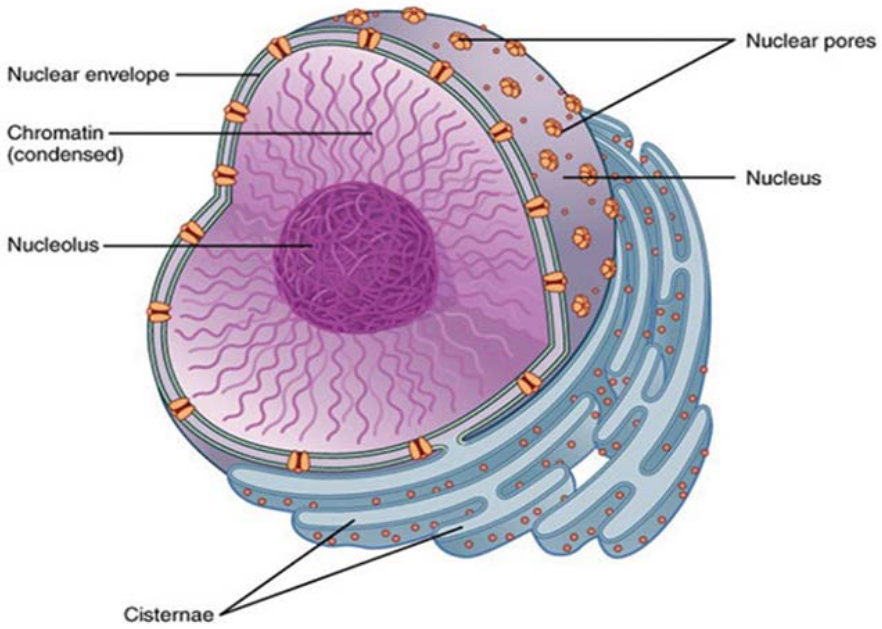
*3. Excretion of materials (exocytosis).* ***Exocytosis*** is the reverse process of endocytosis by which materials are removed from cells, such as solid, undigested remains from food vacuoles or reverse pinocytosis in secretion.

*4 . Transport of materials (diffusion, active transport, facilitated transport)*

5.*Locomotory function.* Many cell types are capable of locomotion, forming the locomotion structure- flagella (protozoa) and cilia (protozoa).

*6. Cell form.*

**NUCLEUS.**




Structure. The nucleus is the most important component of an eukaryotic cell. It contains four main structural elements: double-membranous nuclear envelope, nucleolus, nucleoplasm (karyoplasms) and chromatin.

The nucleus is bounded by the "double membrane" the so called "**nuclear envelope**". These two tightly attached membranes are of the same basic structure as the familiar lipid-protein bilayer. Scattered throughout the double membrane nuclear envelop are "**Nuclear Pores**", these are holes or passages through which large molecules can pass.

There are two major types of material within the nucleus.

1) The "**nucleoplasm**": the jelly-like matrix within which all other materials within the nucleus "float".

2) '**Chromatin**": this material is easily stained (Hence the name.)

It is composed of **DNA** and its associated protein **histone** which form the long strands called "**chromosomes**".

Also found within the nucleus are dark staining parts called "**nuceoli**" (little nucleus) which are rich in the other type of nucleic acid **RNA** (Ribonuelic acid). The nucteoli have the task of assembling (synthesizing) a special type of RNA used to create the **ribosomes** rRNA (Ribosomal RNA).

Functions:

1. To keep the hereditary material and regulates the transmission of it in generations.

2.To regulate the all metabolic processes in the cell.
3.To synthesize the ribosomes.

## CYTOPLASM.

Structure. The protoplasm of a cell laying the plasma membrane but outside the nucleus is known as ***cytoplasm***. The cytoplasm appears as a structureless fluid mass. It includes the ***cell organelles, cytosol and cell inclusions***.

Functions. It takes in raw materials and energy through its membranes which serve as conducting channels, and manufactures proteins and enzymes of various kinds. It is concerned with taking in food and changing it into living parts of a cell. It forms substances needed by the cell or for export outside the cell into other cells. The cytoplasm also extracts chemical energy from sugar and fats and transfers it to the special energy-rich molecules (ATP) which circulate in the cell.

## Eukaryotic organelles.

There are two groups of eukaryotic organelles, such as membranous and non-membranous.

***Membranous:***

1.Single-membranous organelle:

- endoplasmic reticulum;

- Golgy body;
- lysosomes;
- microbodies (peroxisomes);
- vacuoles (in plant cells).

2.Double-membranous organelles:

- mitochondria;
- plastids.

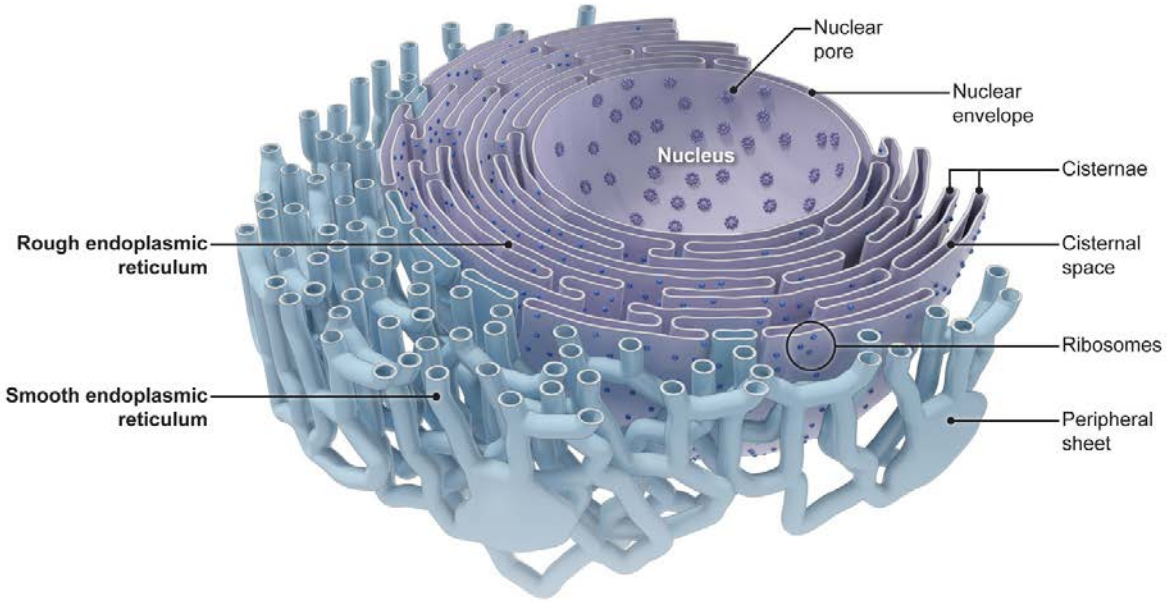
***Non-membranous organelles:***

- ribosomes;
- centrosome;
- microtubules;
- microfilaments.

## Membranous eukaryotic organelles.

**Single-membranous organelles:**

## Endoplasmic reticulum.



Structure. The complex of membranes which can form a significant part of the total volume of the cytoplasm in certain types of cells is called ***endoplasmic reticulum (ER).***

ER is a single-membrane organelle, visible only in the electron microscopy. The electron micrograph, it is a maze of parallel internal membranes that encircle the nucleus and extend into many regions of the cytoplasm of cell.

The membranes of the ER form the framework or the cytoskeleton of the cytoplasm. These membranes usually consist of a series of tightly packed and flattened sack-like structures, which form interconnected compartments within the cytoplasm. The internal space formed by the membrane sheets is called the ***ER lumen***. In most cells the ER lumen forms a single internal compartment. Evidence also suggested that the ER membrane is continuous with the outer membrane of the cell nucleus, so that the compartment formed between the two nuclear membranes is connected to the ER lumen. The membranes of other organelles are not directly connected to the ER and appear to form distinct and separate compartments within the cytoplasm. Between the spaces of the membranes, the cytoplasm has a cytoplasmic matrix or ground substance, containing complex enzymes, synthetic products and storage materials. Enzymes catalyze many different types of chemical reactions. In some cases, the membranes serve a framework for systems of enzymes that carry out sequential biochemical reactions. Other ER enzymes are located within the ER.

The two surfaces of the membrane contain different sets of enzymes and represent of the cell with different synthetic capabilities. The two distinct regions of the ER can be seen in electron micrographs. Although these regions have different functions, their membranes are connected and their internal spaces are continuous. ***Rough ER*** has ribosomes attached to it and consequently appears rough in electron micrographs. One membrane face (the *cytosolic side*) is studied with dark particles, the ribosomes, whereas the other membrane face (the *lumen side*) appears to be bare. ***Smooth ER*** is more tubular in nature and does not have ribosomes bound to it, so its outer membrane surfaces have a smooth appearance.

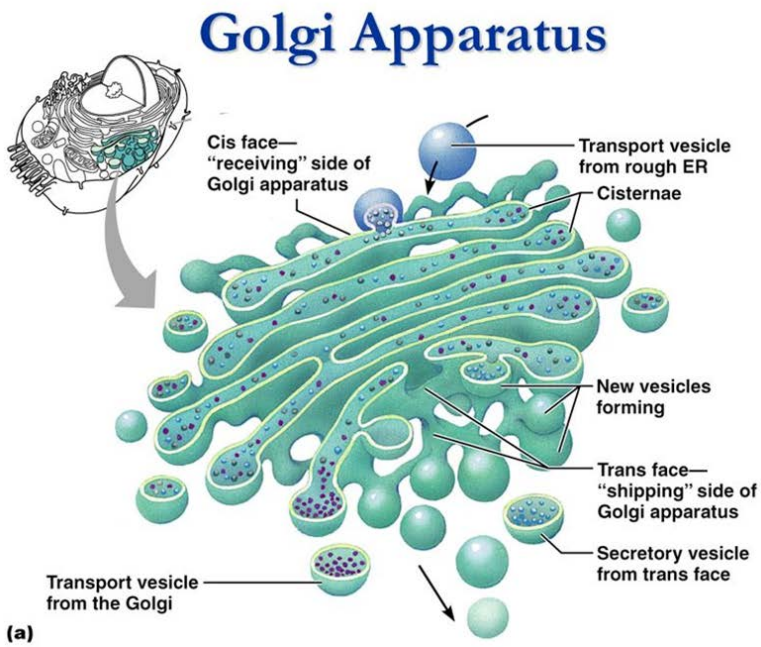
Functions of the Smooth ER

1.Synthesis and transport of lipids, carbocchadrates and steroids;
2.Smooth ER serves important function by localizing of detoxifying enzymes that breakdown chemicals such as carcinogens (cancer – causing molecules) and convert them to water-soluble products that can be excreted from the body.

Functions of the Rough ER.

1.Synthesis and transport of proteins.

## Golgi body (golgy apparatus)




The Golgi body is a single membranous organelle, is a differentiated portion of the endomembrane system of cytoplasm. Firstly, it was described by an Italian scientist ***Camillo Golgi*** in ***1898*** as reticular structure in the cytoplasm of nerve cells. This membranous component is spatially and temporally related to the endoplasmic reticulum on one side and, by way of secretory vesicles, may fuse with specific portions of the plasma membrane. Usually, The Golgi body is placed near the nucleus. It is found in both animal and plant cells.

Structure. The Golgi complex is morphologically very similar in both plant and animal cells. It consists of ***dictyosome units*** formed by stacks of 1) flattened disk-shaped single membrane-bound sacs called ***cisternae*** or ***saccules*** ; 2) clusters of tubules and vesicles and 3) lager vacuoles filled with an amorphous or granular content.

The cisternae are flattened, plate or saucer-like closed compartments. These are arranged in an orderly stack, much like a stack of pancakes. Typically, a Golgi stack contains fewer than eight cisternae. Depending on the cell type, an individual cell may contain from a few to several thousand stacks per cell.

The diameter of cisternae varies from 0.5 to 1.0 nm and in each stack, cisternae are separated by a space of 20 to 30 nm, which may contain rod-like elements or fibres. Each cisterna is bounded by a smooth unit membrane and is curved in a manner resembling a shallow cup.

The cisterna closest to the endoplasmic reticulum (ER) is usually convex and is said to be at the **cis face** or **proximal face** or **forming face**, while the cisterna at the opposite end of the stack is of concave shape and said to be at the **trans or distal or maturing face**. This polarization is called cis-trans axis of the Golgi apparatus.

Functionally the Golgi complex is also divided into four distinct compartments, the cis, medial, trans cisternal, and the trans Golgi network (TGN). Newly synthesized membrane, secretory and lysosomal proteins leave the ER and enter the Golgi through its cis face and then pass across the stack to the trans face.

The TGN or trans-Golgi network is also referred to as GERL (Golgi + smooth ER + lysosome). It is found to be involved in the origin of primary lysosome and melanin granules. It helps in processing, condensing and packaging of secretory material in endocrine and exocrine cells and in lipid metabolism. GERL is also a region of sorting of cellular secretory proteins.

Functions.

1. Participates in the processing, packing and distribution of the sub-stances synthesized in the RER.
2. Synthesizes large carbohydrate molecules (cellulose).
3. The chemical substances synthesized in the ER are subjected to cyclical changes in the GA: combines large carbohydrate molecules with proteins and secretes product (glycoproteins).
4. Participates in the condensation of secretory "products".
5. Participates in the formation of lysosomes.
6. GA is involved in the formation of plasma membrane, in dividing of plant cell (cytokinesis), in producing a cap-like acrosome on the head of the sperm. The hydrolases present in the acrosome facilitate fertilization by dissolving the egg membranes. Also it partakes in the synthesis of yolk in the egg cells.

## Lysosomes.

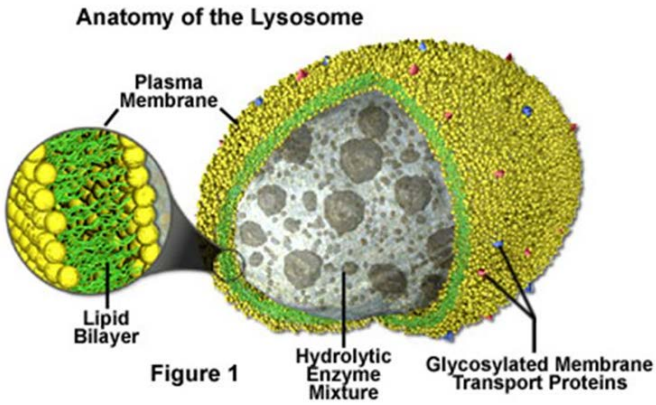


Figure 1

Lysosomes are single membrane cytoplasmic organelles, that contain numerous (about 50) hydrolytic enzymes and in which the main functions are intracellular and extracellular digestion. They were first discovered by a Belgian Biochemist ***Christian De Duve*** in ***1949*** and named in ***1955***. Lysosomes have been found both in animal and plant cells and in Protozoan.

Structure. Lysosomes are separated as a fraction that is intermediate between mitochondria and microsomes. The lysosomes are stable in the living cell. The lysosomal enzymes are enclosed within a membrane (accounting for their latency) and generally act at acid pH.

The ***primary lysosomes*** (storage granules) are dense particles of about 0,4 nm surrounded by a single membrane. The enzymatic content is synthesized by the ribosomes and accumulated in the ER. From there the enzymes penetrate into the Golgy body, undergo modification and are surrounded by a single membrane. Lysosomes are formed.

The ***secondary lysosomes*** (digestive vacuoles) result from the association of primary lysosomes with vacuoles containing phagocytized or pinocytized material. These called ***heterophagosomes***. ***Residual bodies*** are formed if the digestion is incomplete. They contain undigested material. These structures may be eliminated, but in most cases they remain in the cell as pigment inclusions and may be related to the aging process. The ***autophagic vacuole*** or ***cytolysosome*** is a special case in which parts of the cell are digested. This normal process is stimulated during starvation and by the pancreatic hormone glucagon.

Lysosomal enzymes are synthesized in the ER and then packaged at the gerl region of the Golgi complex to form the primary lysosomes. The mechanism of lysosome formation is yet unknown.

Functions.

1) Digestion of food or various materials taken by phagocytosis or pinocytosis;
2) Digestion of parts of the cell and foreign particles by a process called ***autophagy***.
3) Breakdown of extracellular material by the release of enzymes into the surrounding medium;
4) Autolysis of cell.

## Microbodies.

***Peroxisomes*** are the organelles rich with peroxidase, catalase, D-amino-acid oxidase, and urate oxidase. They are abundant in the liver, kidney and in many cell types of animals and plants. They have 0,6- 0,7 nm granules with a single membrane and a dense matrix. The peroxisome is related to the production and decomposition of $H_2O_2$ and to the β-oxidation of fatty acids and play a role in thermogenesis.

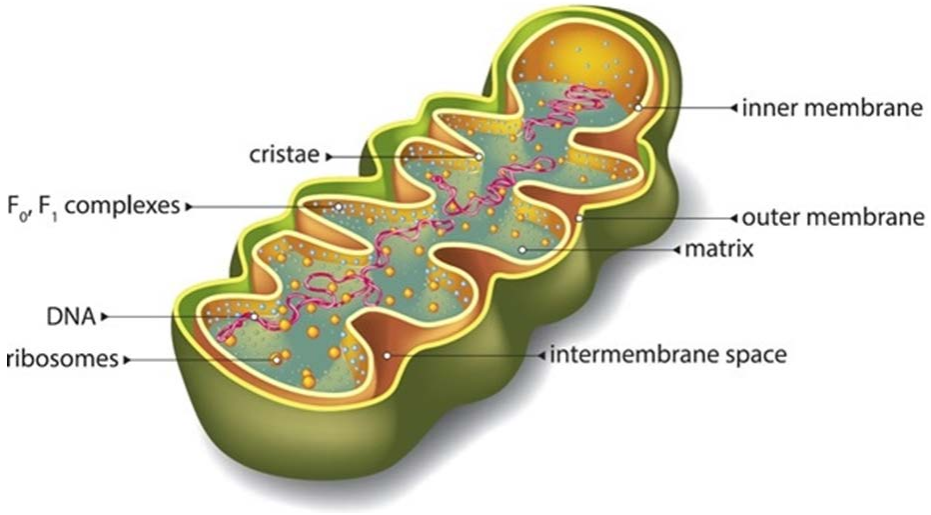
In plants, peroxisomes carry out the process of photorespiration, which involves the cooperation of chloroplasts and peroxisomes.

***Glyoxysomes*** are special plant organelles involved in the metabolism of stored lipids (fat metabolism).

## DOUBLE MEMBRANOUS ORGANELLES.

Energy transformation in cells takes place with the intervention of two main transducing systems (systems that produce energy transformations) represented by mitochondria and chloroplasts.

## Mitochondria.




Mitochondria are granular or filamentous organelles present in the cytoplasm of all eukaryotic cells (protozoa, animal and plant cells). They provide an energy-transducing system by which the chemical energy contained in foods tuffs is converted by oxidative phosphorylation into high-energy phosphate bonds (ATP).

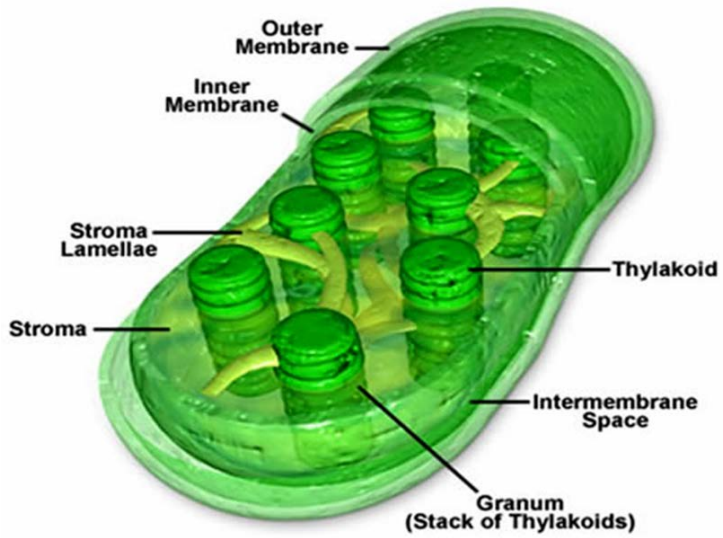
First observed at the end of the 19th century and described as "bioblasts" by ***Altman (1886),*** these structures were called "mitochondria" by ***Benda*** (***1897***). Altman predicted the relationship between mitochondria and cellular oxidation, and ***Warburg (1913)*** observed that respiratory enzymes were associated with cytoplasmic particles. In general, they are rod-shaped, with diameter of about 0,5 nm and variable length that may range up to 7 nm. Mitochondria are, in general, uniformly distributed throughout the cytoplasm, but there are many exceptions to this rule. In some cases, they accumulate preferentially around the nucleus or in the peripheral cytoplasm, during mitosis; mitochondria are concentrated near the spindle. The distribution of mitochondria within the cytoplasm should be considered in relation to their function as energy suppliers. Their orientation in the cell may be influenced by the organization of the cytoplasmic matrix and vacuolar system.

The number of mitochondria is also various in different cells (there are 1000 to 1600 mitochondria in a liver cell; 300000 in some oocytes). Green plants contain fewer mitochondria than animal cells.

Structure. A mitochondrion consists of two membranes and two compartments. An outer limiting membrane surrounds the mitochondrion. Within this membrane, and separated from in by a space of about 6 to 8 nm, is an inner membrane that projects into the mitochondrial cavity complex enfolding called ***mitochondrial cristae or crests***. This is generally homogeneous, but it may contain a filamentous material or small highly dense granules. The mitochondrial crests are incomplete septa or ridges that do not interrupt the continuity of the inner chamber. The shape and disposition of these crests vary in different cells and their number is related to the oxidative activity of the mitochondrion. The outer membrane is smooth and the inner membrane shows, on its inner surface, particles linked to the membrane, which contain a special ATP-ase. Within the mitochondrial matrix are small ribosomes (70S) and a circular DNA, different types of RNAs. The matrix is gel-like and contains a high concentration of soluble proteins and smaller molecules. Thus, mitochondria may synthesize the proteins, lipids. They are semiautonomous organelles.

Functions. Mitochondria are the “power house” of a cell, they bring about the chemical reactions which take place in tissue respiration and they also break down fats, proteins and carbocchadrates into smaller particles and transfer their chemical energy into complex energy-rich molecules of adenosine triphosphate (ATP).

## Plastids.




Eukaryotic plant cells have specialized organelles – the plastids – which are double-membranous and contain pigments and may synthesis and accumulate various substances.

In ***1883 Schimper*** first used the term “plastid” for special cytoplasmic organelles present in eukaryotic plant cells. There are three types of plastids: chloroplasts, chromoplasts and leucoplasts. *Leucoplasts* are colorless plastids are found in embryonic and germ cells, also found in meristemic cells and in those regions of the plant not receiving light. True leucoplasts are found in fully differentiated cells and never become green. The leucoplasts have also been characterized by the absence of thylakoids and ribosomes. They are three types: *amyloplats* produce starch, *proteinoplasts* accumulate protein, *elaioplasts* produce fats and essential oils.

Chromoplasts are colored plastids that contain less chlorophyll than the chloroplasts, but more carotinoid pigments, such as lycopene. Some plastids may store starch and protein at the same time. Yellow or orange chromoplasts occur in petals, fruits and roots of certain higher plants. The most important and most common plastids are the chloroplasts. They are characterized by the presence of pigments such as chlorophyll and carotenoids and by their fundamental role in photosynthesis. Chloroplasts are localized mainly in the cells of the leaves of higher plants and in algae. Their shape, size, number and distribution vary in different cells but are fairly constant for a given tissue. In higher plants they are discoid. The size and number are genetically controlled. Chloroplasts multiply by division, they are semiautonomous organelles.

Leukoplasts and chromoplasts have the similar structure as chloroplasts.

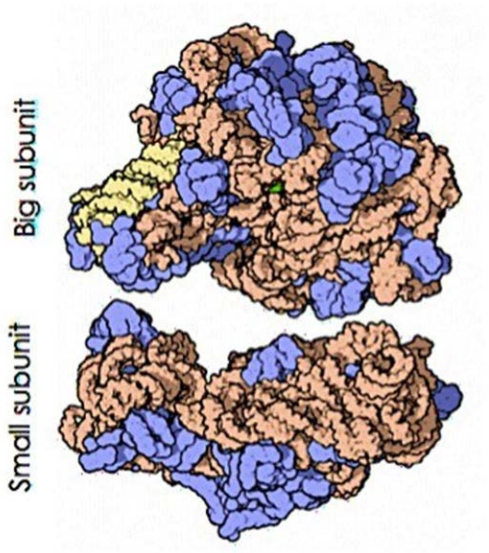
Structure. Under light microscope, many chloroplasts show small granules, called *grana*. There are three main components: the **envelope, stroma** and **thylakoids.** The envelope is made of a double limiting membrane (outer and inner). The inner membrane of mature chloroplast is not in continuity with the *thylakoids*. The stroma is a gel-fluid phase that contains 50% of the

chloroplast proteins. It has ribosomes and DNA. The thylakoids consist of flattened vesicles arranged as a membranous network. The outer surface of the thylakoid is in contact with the stroma, and its inner surface encloses an intra thylakoid space. Thylakoids may be stacked like a pile of coins, forming the grana or they may be unstacked (stroma thylakoids), forming a system of anastomosing tubules that are joined to the grana thylakoids. In thylakoids, chlorophyll, carotenoid molecules and a reaction center are assembled forming two photosystems, which are important for photosynthesis.

Function of chloroplasts: Photosynthesis.

## *Non-membranous eukaryotic organelles.*

### Ribosomes.

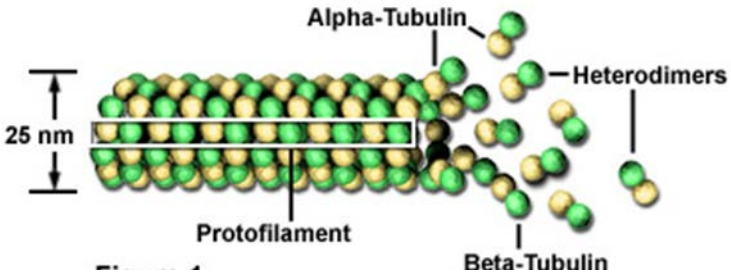



The ribosome is a non-membranous organelle, spherical particle and is composed of a large and a small subunit. Ribosomes were first observed by Palade in the electron microscope as dense particles or granules. Ribosomes are found in all cells and provide a scaffold for the ordered interaction of all molecules involved in protein synthesis.

Structure. Eukaryotic ribosomes sediment in sucrose gradients with a sedimentation coefficient of 80S (values of the sedimentation coefficients are not additive because they depend on factors such as the shape of the particles), prokaryotic ribosomes are smaller and sediment at 70S. Ribosomes are also found in the mitochondria and chloroplasts of eukaryotic cells. During protein synthesis several ribosomes become attached to one m-RNA molecule, forming a ***polyribosome or polysome***. The major constituents of ribosomes are r-RNA and proteins present in approximately equal amounts. R-RNA may provide some of the catalytic activities required for protein synthesis.

Function. Protein synthesis.

## Microtubules.




Structure. Microtubules are found in all eukaryotic cells – either free in the cytoplasm or forming part of centrioles, cilia and flagella. They are tubules 25 nm in diameter, several micrometers long, and with a wall 6 nm thick with 13 subunits. the stability of different microtubules varies. Cytoplasmic and spindle microtubules are rather labile, whereas those of cilia and flagella are more resistant to various treatments. The main component is a protein called ***tubulin***. The assembly of tubulin in the formation of microtubules is a specifically oriented and programmed process. Centrioles, basal bodies, and centromeres are sites of orientation for this assembly.

Functions.

1. They are related to the primitive forms of cell mobility.
2. They play a mechanical function and the shape of the cell and cell processes is dependent on microtubules (cell differentiation).
3. The polarity and directional gliding of cultured cells depend on microtubules.
4. Associated with transport of molecules, granules and vesicles within the cell.
5. They play a role in the contraction of the spindle and movement of chromosomes and centrioles.
6. They form the cilia and flagella.
7. They play a role in sensory transduction.

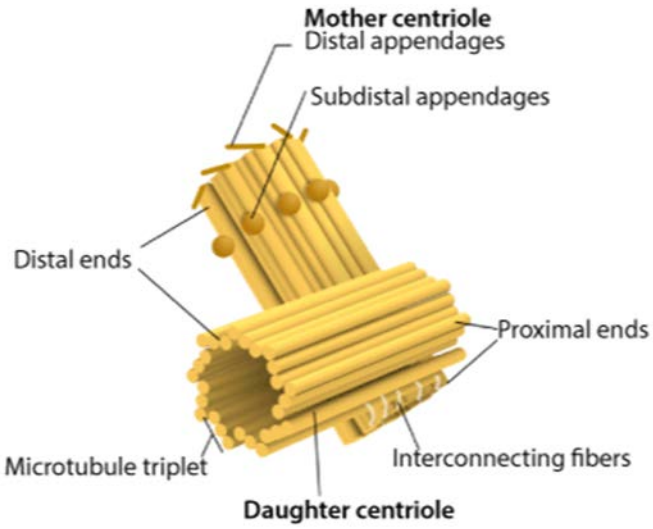
## Microfilaments.

Structure. The microfilaments ranging between 5 and 7 nm in width represent the active or motile part of the cytoskeleton. The contractile protein ***actin*** and ***myosin***, well as tropomyosin and other proteins found in muscle, are present in microfilaments.

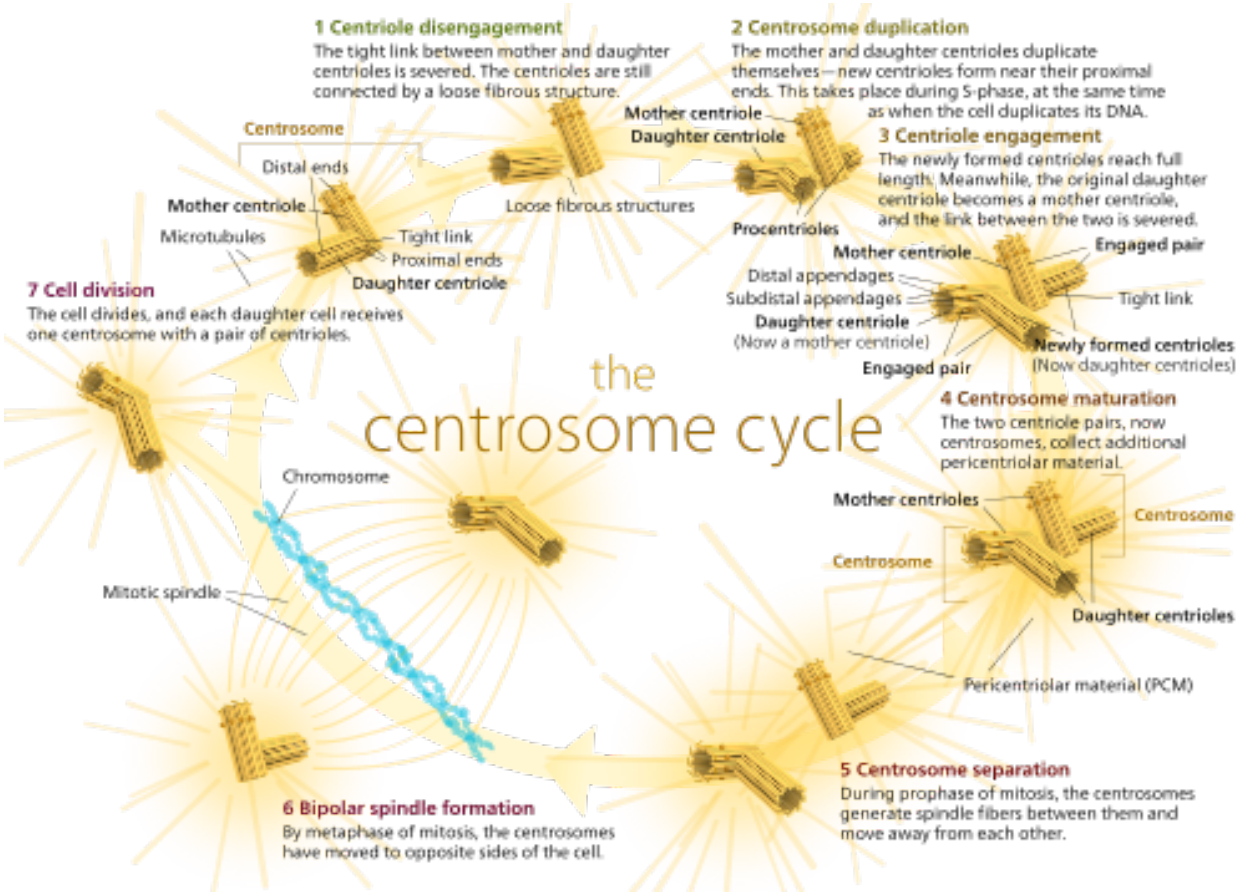
Function: They play the major role in cyclosis and amoeboid motion.

## Centrosome (cell center, centrosphere).

Lying near the nucleus is a small clear area of cytoplasm free from granules, it is known as centrosomes. The centrosome is extanuclear and firmly attached to the nuclear envelope. The centrosome's position often determines the polarity of the cell, with the cell axis passing through it and the nucleus.




The centrosome contains a minute dot-like centriole, at the S period of interphase, two pairs of centrioles exist.



The centriole as microtubular organelle has a triplet organization (nine triplets of microtubules). The centrosome is typically only for animal cell, in plant cells are absent.

Functions:

1.They play a role in forming the spindle during nuclear division.
2.They play a role in intracellular transport.
3.They play a role in cytoskeleton formation.

**THE QUESTIONS FOR SELF-CONTROL:**

***1. The history of discovery of the cell and the development of ideas about its structure (discovery of nucleus, cytoplasm).***
***2. The modern state of the cell theory.***
***3. The types of cell organization. Hypotheses of origin of eukaryotes.***
***4. The typical features of prokaryotic cells.***
***5. The typical features of eukaryotic cells.***
***6. Structural and functional organization of cell membrane.***
***7. Endoplasmic reticulum, structure and functions.***
***8. Golgi apparatus, structure and functions.***
***9. Lysosomes, their structure and functions.***
***10. Mitochondria and chloroplasts, their structure and biological role.***
***11. Ribosomes, microtubules, microfilaments, centrioles, their structure and functions.***

# *The structural organisation of hereditARy material IN CELLS of living organisms.*

The hereditary material in cells of all living organisms is nucleic acids: **DNA and RNA.**

The genetic material should be capable of the main **functions:**

1. It should be able to precisely duplicate itself and faithfully pass its copies into the progeny.
2. It should be able to occasionally develop inheritable changes (mutations) to allow adaptation and evolution to occur in the organisms.
3. It should be able to store information for the control of biological functions of the cells and to express its information.

## HISTORICAL OVERVEIW ON DISCOVERY OF THE NUCLEIC ACIDS.

Nucleic acids were discovered by F. Miescher in 1869 in the nuclei of human white blood cells. Their functions were unknown. This compound, later called nucleic acids, was isolated from the nuclei of many cell types.

Chemical analysis was done in 1910 identified two classes of nucleic acids — DNA and RNA. In 1924 microscopic studies using strains for DNA and proteins showed that both substances are in chromosomes. In 1928, an english bacteriologist Griffiths discovered the phenomenon "Transformation" and in 1944 O.T.Avery, C.Macleod and M.McCarty discovered transforming agent. Their experiments proved that the substance responsible for genetic transformation was DNA of the cell, hence DNA is the genetic material. This year (1944) and this discovery were beginning of Molecular Genetics.

A second pivotal experiment was reported by Alfred Hers hey and Marta Chase in 1952. Their experiments, reproduction of bacteriophage $T_2$ in the bacterium Escherichia coli were widely accepted as proof that DNA is the genetic material in all organisms.

James Watson and Francie Crick proposed the double helical structure for DNA molecule in 1953.

Astbury, Wilkins and Franklin in 1950-s suggested helical configuration for DNA.

In 1950 Chargaff formulated important generalization about DNA structure.

## DNA MOLECULE.

### Chemical composition of DNA molecule.

Chemical structure of DNA was explained by P.A.Levene (1869 — 1940). DNA is a polymer (a molecule containing repeating units). The basic subunit of DNA molecule is the deoxyribonucleotide (deoxyribotide, nucleotide). Each deoxyribonucleotide unit consists of three molecules:

- deoxyribose sugar (2'-deoxyribose);

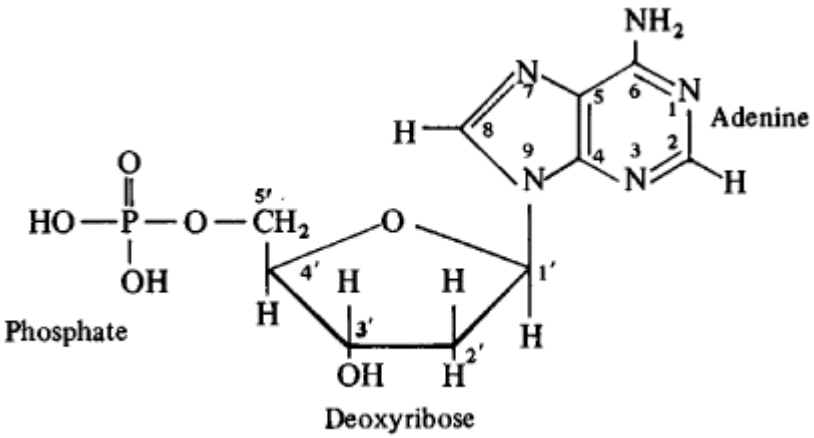
- phosphoric acid ($H_3PO_4$);

- nitrogen-containing base (purine or pyrimidine).

The deoxyribose sugar of DNA has 5 carbons. The carbon atoms are numbered 1', 2', 3', 4', 5'.

The nitrogenous base molecules are joined to the sugar molecules by glycosidic bonds. The glycosidic bond develops between the 1' carbon of the sugar and the nitrogen at the position 1 in case of pyrimidine base and at the position 9 in case of purine base.

The phosphate is joined to the 5' carbon atom of deoxyribose by ester bond.

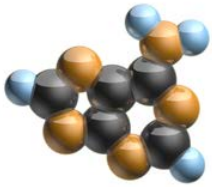



Four different bases are present in the DNA molecule:

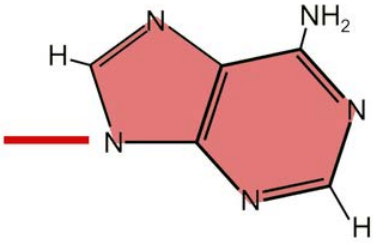
- the *purines*: adenine (A) and guanine (G);

- the *pyrimidines*, cytosine (C) and thymine (T).

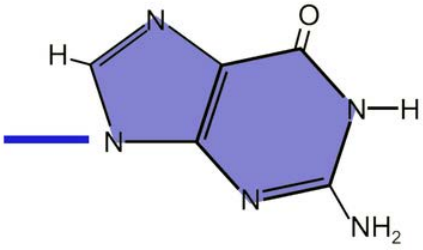
**Purines** are heterocyclic organic compounds containing a six-membered ring with two nitrogen atoms, which is fused to an imidazole ring.



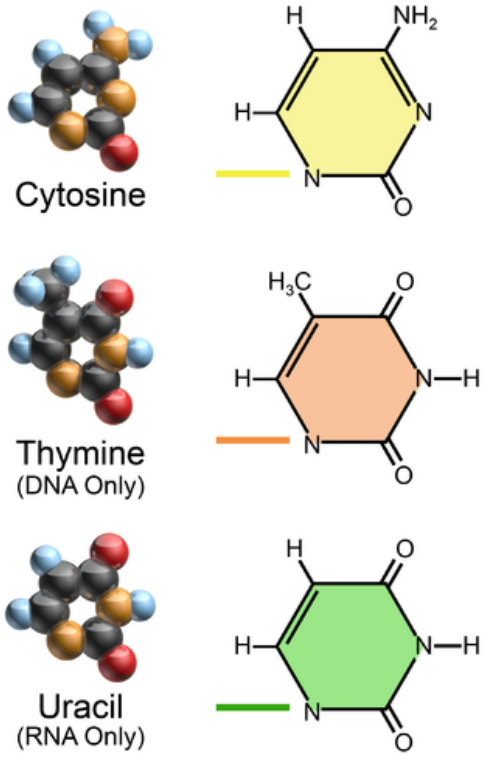
Adenine



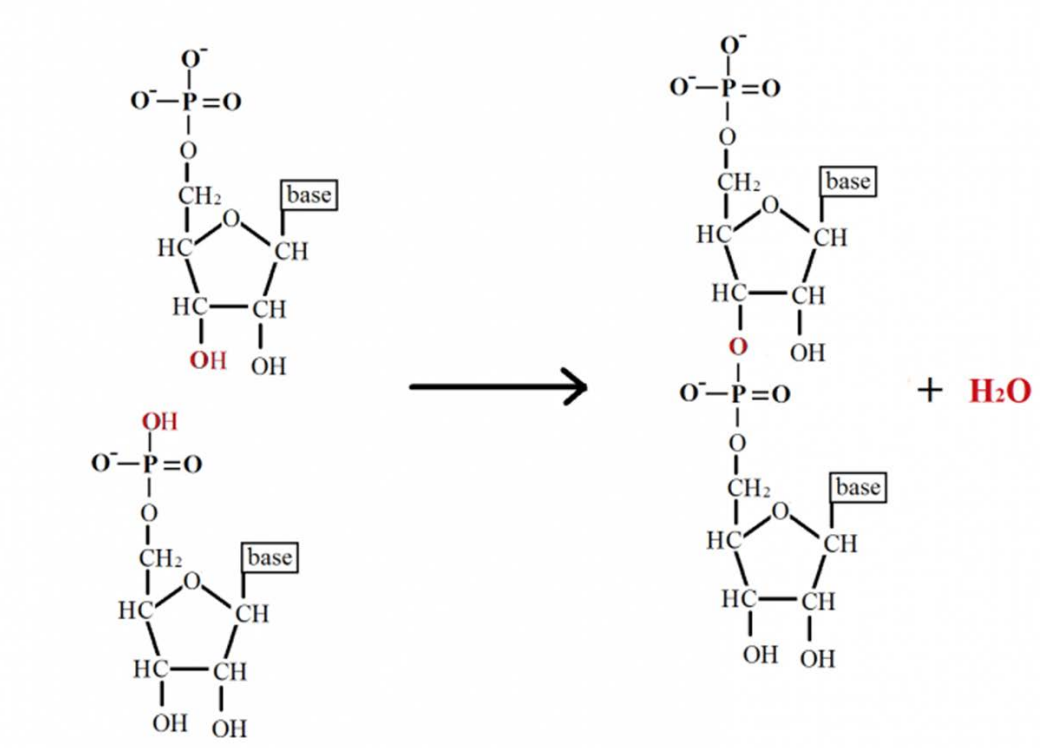

Guanine



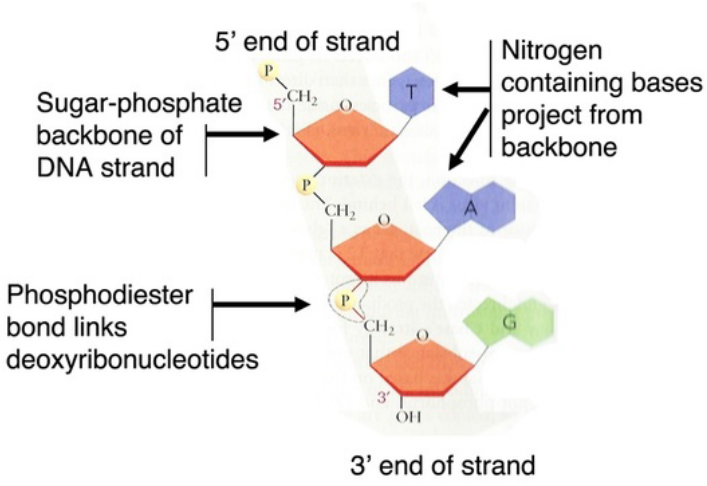

**Pyrimidines** are heterocyclic organic compounds, containing a six-membered ring with two nitrogen atoms. In pyrimidine, nitrogen atoms are found in the positions, 1 and 3 in the heterocyclic ring. Cytosine and thymine are the two nucleobases found in DNA. Uracil is found in RNA. While forming the double-stranded structure of nucleic acids, pyrimidines form hydrogen bonds with complementary purines in the process called complementary base pairing. Cytosine forms three hydrogen bonds with guanine and thymine forms two hydrogen bonds with adenine in DNA. In RNA, uracil forms two hydrogen bonds with adenine instead of thymine.



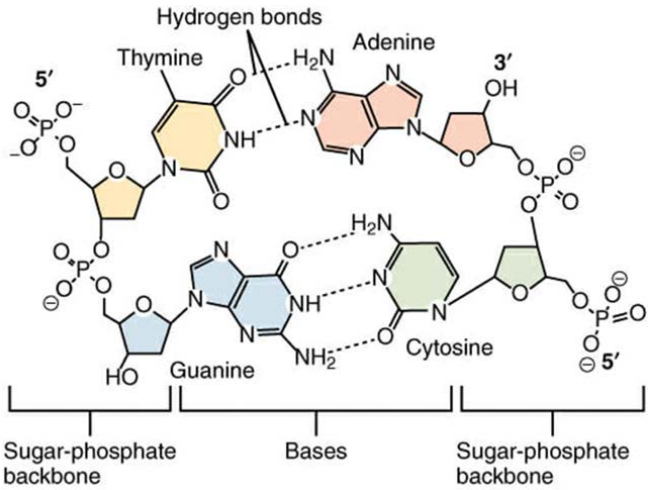

The backbone of the polymer is formed by linking the phosphate of one nucleotide to the deoxyribose of the adjacent nucleotide. The phosphate component carried by the 5'carbon atom of one nucleotide unit is joined by phosphodiester bond to the hydroxyl component of the 3' carbon atom of the sugar in the next nucleotide unit. These 3', 5' phosphodiester bonds provide a considerable stiffness to the polynucleotide.



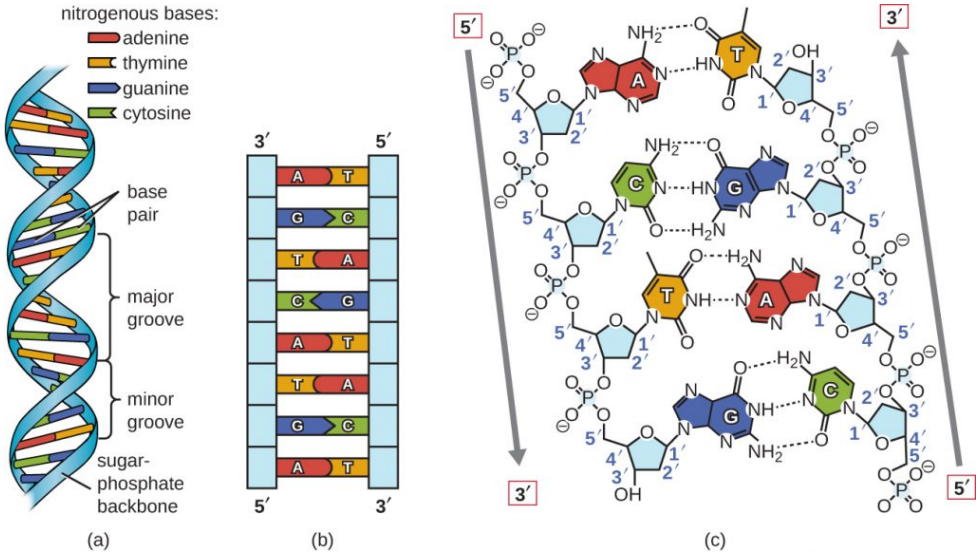
The resulting strand of nucleic acid has a free phosphate group at the 5′ carbon end and a free hydroxyl group at the 3′ carbon end. That ends are called 5’ end and 3’ end.




DNA usually exists as a double-stranded structure with two complementary strands. Two deoxyribonucleotide chains are held together by *hydrogen bonds.* Adenine of one chain is always joined to thymine of the other chain by two hydrogen bonds and cytosine of one chain is always linked to guanine of the other chain by three hydrogen bonds.




The two DNA strands are antiparallel, such that the 3′ end of one strand faces the 5′ end of the other. The resultant structure is exceeding stable, and the nucleotide sequence functions as the genetic template for the proteins of the cell.




*Watson and Crick proposed the double helix model for DNA. (a) The sugar-phosphate backbones are on the outside of the double helix and purines and pyrimidines form the "rungs" of the DNA helix ladder. (b) The two DNA strands are antiparallel to each other. (c) The direction of each strand is identified by numbering the carbons (1 through 5) in each sugar molecule. The 5' end is the one where 5' carbon is not bound to another nucleotide (there is free phosphate group); the 3' end is the one where 3' carbon is not bound to another nucleotide (there is free OH-group).*

## The characters of DNA double helix.

Watson and Crick proposed that DNA is made up of two strands that are twisted around each other to form a right-handed helix.

1. The distance between the two successive nucleotides is 0.34 nm (3.4 A).
2. Each turn of the double helix covers a distance of 3.4 nm (34A).
3. Each nucleotide in turned $36^0$ from the preceding one so that a complete turn of $360^0$ involves ten (10) base pairs.
4. The diameter of the double helix is approximately 2.0 - 2.2 nm (20—22 A) and this is possible only if the pairing takes place between a purine and a pyrimidine.

## Chargaff's rules.

1. The concentration of purine bases equals that of the pyrimidine bases; that is: [total purines]=[A]+[G]=[total pyrimidine]=[T]+[C].

2. The concentration of adenine and thymine are equal, as are the concentration of guanine and cytosine; that is [A]=[T] and [G]=[C]

3. The base ratio (A+G)/(T+C) = 1 may vary from one species to another, but is constant for a species. This ratio can be used to identify the source of DNA, and can help in classification.

4. The deoxyribose sugar and phosphate components occur in equal proportions.

## DNA polymorphism.

DNA polymorphism is an ability of DNA molecule to form different configurations. Now, there are six DNA-forms.

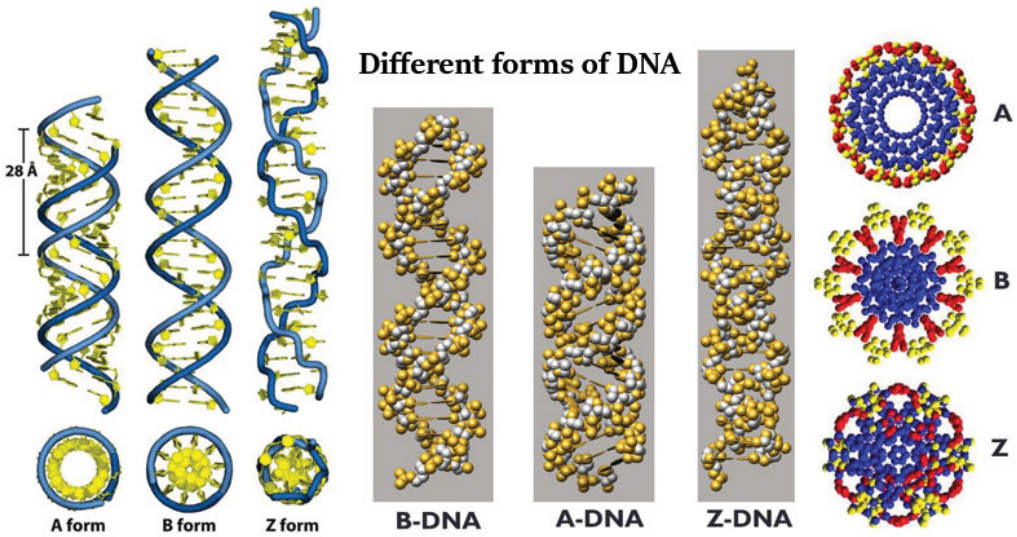
B-form was described by G.Watson and F.Crick and has standard structure.

A-form was found in environment, where the concentration of $K^+$ and $Na^+$ ions was high.

C-form is the same with B-form, but the number of base pairs in one turn in C-form less than in B-form.

D- and E-forms are extreme variants of one form, have few base pairs in one turn. These forms were found in DNA molecule, which don't contain guanine.

Z-form is formed only when purines and pyrimidines are present alternately in the chain. This form may be left-handed or right-handed and was discovered several years ago by Rich of the Massachusetts Institute of Technology (MIT). In this DNA the backbone of the strands follows a zigzag course. The Z-conformation is stabilized only in a solution with a high salt concentration or when the DNA is brominated or methylated. Z-form plays a role in the regulation of the activity. It is known, that when certain control sites in the genes are stabilized in the Z-configuration by methylation, a regulatory protein binds to the sites and keeps the gene turned off. Demethylation might switch the site to the B-conformation, causing the regulatory molecule to let go, and the gene is turned on. Thus, Z-configuration presumable has the regulatory function.



## Comparison geometries of the most common DNA forms

| Geometry attribute: | A-form | B-form | Z-form |
|---|---|---|---|
| Helix sense | right-handed | right-handed | left-handed |
| Repeating unit | 1 bp | 1 bp | 2 bp |
| Rotation/base pair (bp) | 32.7° | 34.3° | 60°/2 |
| Mean bp/turn | 11 | 10 | 12 |
| Inclination of bp to axis | +19° | −1.2° | −9° |
| Rise/bp along axis | 2.6 Å (0.26 nm) | 3.4 Å (0.34 nm) | 3.7 Å (0.37 nm) |
| Rise/turn of helix | 28.6 Å (2.86 nm) | 35.7 Å (3.57 nm) | 45.6 Å (4.56 nm) |
| Mean propeller twist | +18° | +16° | 0° |
| Nucleotide phosphate to phosphate distance | 5.9 Å | 7.0 Å | C: 5.7 Å, G: 6.1 Å |
| Diameter | 23 Å (2.3 nm) | 20 Å (2.0 nm) | 18 Å (1.8 nm) |

# GENETIC MATERIAL IN EUKARYOTIC CELLS

The DNA molecules in the mammalian chromosomes are exceedingly long, containing more than $10^8$ bases with a linear length of almost 1 mm. The main genetic material of eukaryotic cell is found into eukaryotic nucleus, where the DNA is packed in several chromosomes and not in a single chromosome as in prokaryotes. This is a chromosomal heredity. Also, there is cytoplasmic genetic material. It is a DNA of mitochondria and plastids (in plant cells). This is a cytoplasmic (non-chromosomal) heredity.

# RNA MOLECULE.

## Chemical composition of RNA molecule.

Chemically, RNA (ribonucleic acid) is very similar to DNA, but has many differences:

1) Although the sugar-phosphate core polymer is basically the same, the length of RNA molecules can vary considerably, from less then 100 bases in transfer RNA's to over 10000 bases in some messenger RNAs.

2) RNA has ribose sugar in place of deoxyribose of DNA;

3) The base thymine is replaced by uracil;

4) RNA is a single strand;

5) A few bases (especially in transfer RNA) may be derivatives of the major four. Lastly, even though RNA generally exists as a single strand, guanine and cytosine or adenine and uracil have the ability to form hydrogen-bonded base paires. It is thus possible at regions where the base sequence allows this for RNA to form intramolecular base pairs that result in a local helical structure.

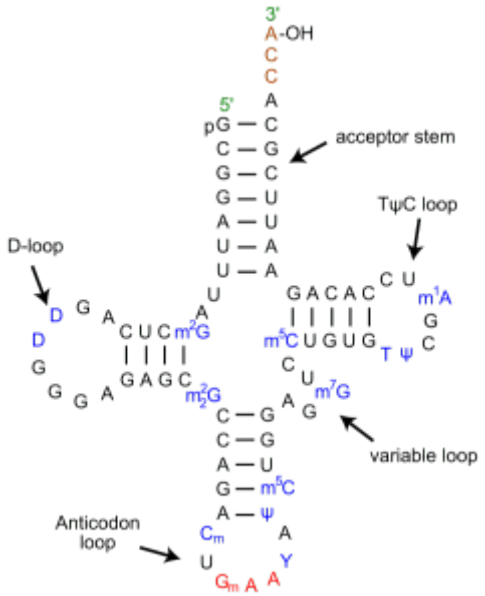
## The types of RNA molecules.

**m-RNA (messenger RNA)** is the DNA associated RNA. The DNA, that controls protein synthesis, is located in the chromosomes within the nucleus, whereas the ribosomes, on which the protein synthesis actually occurs, are placed in the cytoplasm. Therefore, some sort of agency must exist to carry instructions from the DNA to the ribosomes. The m-RNA directs the amino acid sequence in protein synthesis.

**t-RNAs (transfer RNA)** are small molecules of RNA (70-90 nucleotides and about 5 nm in length) and account for approximately 15% per cent of the total amount of RNA of the cell. The t-RNAs carry appropriate amino acids from the amino acid pool to the site of protein synthesis. A t-RNA molecule has the form of a clover leaf.

The two most important parts of a tRNA are its anticodon and the terminal 3' hydroxyl group, which can form an ester linkage with an amino acid. However, there are other aspects to a tRNA's structure such as the D-arm and T-arm, which contribute to its high level of specificity and efficiency.

The last three bases on the 3' end of tRNA are always CCA – two cytosines followed by one adenine base. This stretch is part of the acceptor arm of the molecule, where an amino acid is covalently attached to the hydroxyl group on the ribose sugar of the terminal adenine nucleotide. The acceptor arm also contains parts of the 5' end of the tRNA, with a stretch of 7-9 nucleotides from opposite ends of the molecule base pairing with each other.



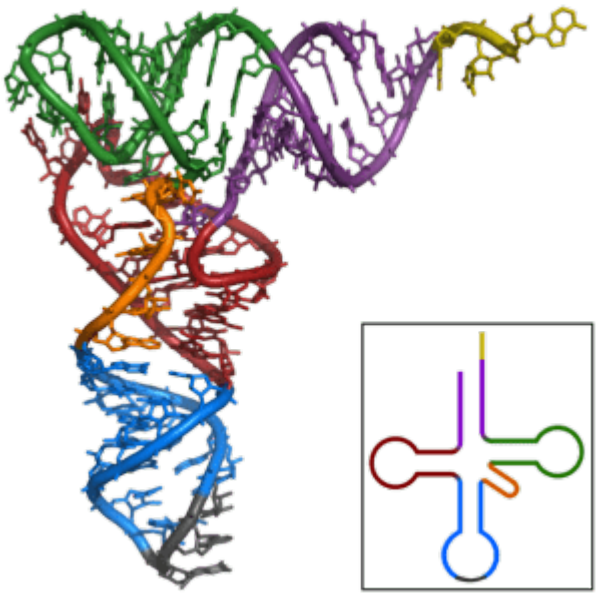

The anticodon loop, which pairs with mRNA, determines which amino acid is attached to the acceptor stem. The anticodon loop is recognized by aminoacyl tRNA synthetase (AATS), the enzyme that chemically links a tRNA to an amino acid through a high-energy bond. AATS 'reads' the anticodon and also recognizes the D-arm located downstream from the 5' end of the tRNA.

The D-arm is made of a double-stranded stem region formed by internal base pairing as well as a loop structure of unpaired nucleotides. The D-arm is a highly variable region and plays an important role in stabilizing the RNA's tertiary structure and also influences the kinetics and accuracy of translation at the ribosome.

The other structure that influences the role of tRNA in translation is the T-arm. Similar to the D-arm, it contains a stretch of nucleotides that base pair with each other and a loop that is single stranded. The paired region is called the 'stem' and mostly contains 5 base pairs. The loop contains modified bases and is also called the TΨC arm, to specify the presence thymidine, pseudouridine and cytidine residues (modified bases). tRNA molecules are unusual in containing a high number of modified bases as well as containing thymidine, usually seen only in DNA. The T-arm is involved in the interaction of tRNA with the ribosome.

Finally, a variable arm containing less than 20 nucleotides is situated between the anticodon loop and the T-arm. It plays a role in AATS recognition of tRNA, but could be absent in some species.

The secondary structure of tRNA containing the acceptor region, D- and T-arms and the anticodon loop is said to resemble a cloverleaf. After the RNA folds into its tertiary structure, it is L-shaped, with the acceptor stem and T-arm forming an extended helix and the anticodon loop and D-arm similarly making another extended helix. These two helices align perpendicularly to each other in a way that brings the D-arm and T-arm into close proximity while the anticodon loop and the acceptor arm are positioned on opposite ends of the molecule.



**r-RNA (ribosome RNA)**. The r-RNA is a component of the ribosomes. It also seems to play some general role in protein synthesis. A small amount of RNA is associated with the chloroplasts of plants and mitochondria of plants and animals.

## DNA Replication

A unique property of DNA is that it governs its own synthesis. This self duplication property of DNA is called replication.

It occurs in the nucleus during the S phase of the cell cycle when chromosomes are in their extended form and are not readily visible.

The stimulus which starts the process at this time and stops it at other times is not fully known.

Types of DNA Replication

***Semiconservative*** ( most of prokaryotes and eukaryotes).

In this type of replication, DNA is replicated each daughter duplex contains one parental and one newly synthesized strand.

***Conservative*** *(viruses)*

In this type of replication, the integrity of the whole parental double helix is conserved in the replication process. After one replication cycle, one of the daughter DNA molecules consists of two newly synthesized strands, while the other has both parental strands. Conservative replication would involve the duplication of only one of DNA strands which serves as the template. The newly formed strand then serves as a template for the synthesis of its own complementary strand.

***Dispersive*** *(viruses)*

This type of DNA replication is possible if the two strands break down along their length into small pieces. Each piece would then replicate and the pieces (old and new) would randomly join with another one to form the two DNA molecules, each having some old and some new pieces.

***EXPERIMENTAL EVIDENCE FOR SEMICONSERVATIVE REPLICATION.***

Meselson and Stahl in 1958 provided a strong experimental evidence which supported the semiconservative type of DNA replication. They grew E. Coli bacteria in a medium containing the heavy nitrogen isotope $^{15}N$ for many generations. This produced a population of bacterial cells that had uniformly $^{15}N$-labelled DNA, and this DNA was heavier that the DNA obtained from E. Coli grown in $^{14}N$-containing medium.

The bacterial cells with the heavier DNA were then transferred to a medium having the ordinary $^{14}N$ isotope. From the daughter cells of first generation, DNA was extracted, purified and centrifuged. It was that all the DNA molecules were hybrid $^{15}N$-$^{14}N$ , i. e. all were half heavy. This is what is expected in case of semiconservative mode of replication. Daughter cells were allowed to divide again. The second generation cells were found to have two types of DNA molecules, 50% half heavy with $^{14}N$-$^{15}N$ hybrid density and 50% light with $^{14}N$-$^{14}N$ density. This again conforms to the prediction based on semiconservative mode of DNA replication.

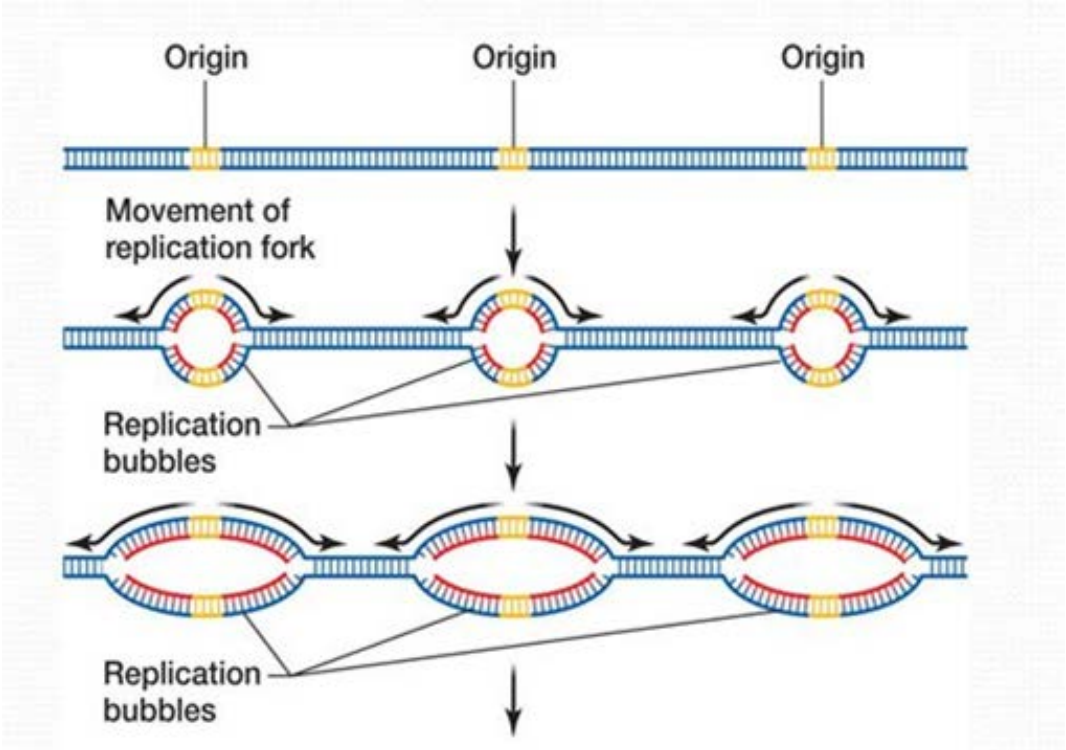
## Details of Semiconservative DNA Replication.

### Activation of Deoxyribonucleotides

The deoxyribonucleoside monophosphates (AMP, GMP, CMP, TMP) found floating free in the nucleus serve as the raw materials in DNA synthesis. These nucleotides are synthesized from precursor molecules available from digestion and metabolism. For incorporation into DNA, nucleotides are activated by union with ATP. This reaction is called phosphorylation and is catalyzed by an enzyme phosphorylase. It produces deoxyribonucleoside triphosphates, namely, ATP, GTP, CTP, and TTP.

### Exposure of Parent DNA Bases

The replication begins from a fixed origin of replication but then proceeds bidirectionally (with moving forks at both ends of the replication piece). The DNA strands start to separate at this specific point. Viral and prokaryotic DNA generally forms a single point of replication. Eukaryotic chromosomal DNA begins the process at many origins of replication. Unzipping of the double stranded DNA forms a Y – shaped structure called replication fork.



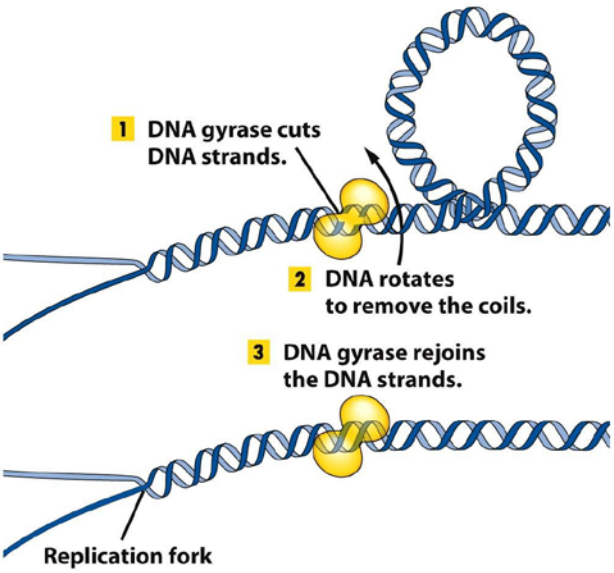
New DNA strands grow from and towards the fork. The DNA molecule is intricately coiled in its chromosome and its unwinding is not an easy job. The initiation of replication is began forming of supercoils in DNA molecule. The enzymes called topoisomerases help to convert rings (supercoils) of DNA from one topological form to another.

One topoisomerase, DNA gyrase (topoisomerases II) can induce twisting and coiling of the DNA, called supercoiling.

The supercoiled form may facilitate unwinding of the helix due to energy (ATP) of supercoiling.

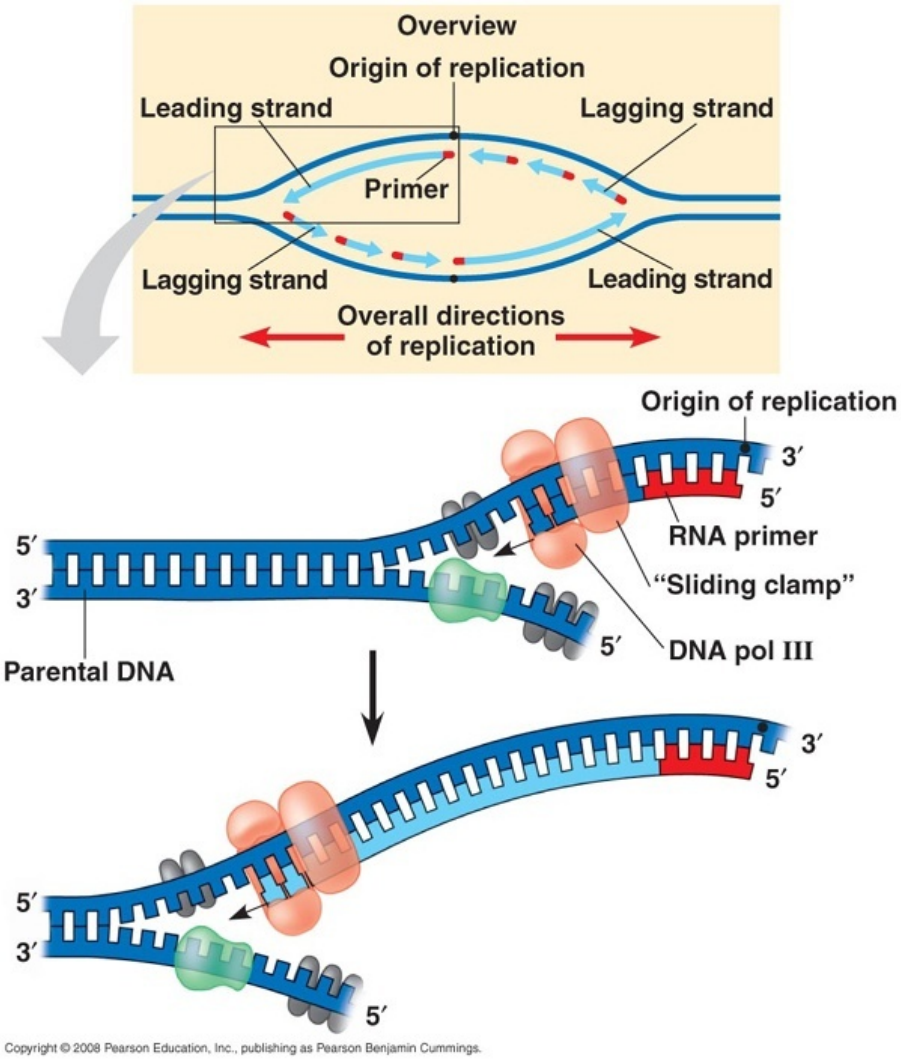
Enzymes helicases help in unwinding the helix. Other enzymes named topoisomerases I may cut and rejoin one strand of DNA to facilitate uncoiling.

The free single-stranded region would be subject to degradation, but it is protected by another protein termed the single-stranded DNA-binding (SSB) protein.




**Formation of RNA primer**

DNA polymerases cannot initiate DNA synthesis - they can only add on to an existing strand of DNA or RNA. The existing strand is said to "prime" DNA synthesis. During DNA replication, RNA primers (a short chain of RNA) are produced by RNA primase in order to provide a starting point for DNA polymerases to extend from. The primers are later removed and the gaps so left are filled with deoxyribonucleotides to make the DNA strand continuous.




**Base Pairing**

The deoxyribonucleoside triphosphates get joined by hydrogen bonds to the appropriate nitrogen bases pairing rule of Watson and Crick , A-T; T-A; C-G; G-C.

**Conversion to Deoxyribonucleoside Monophosphates**

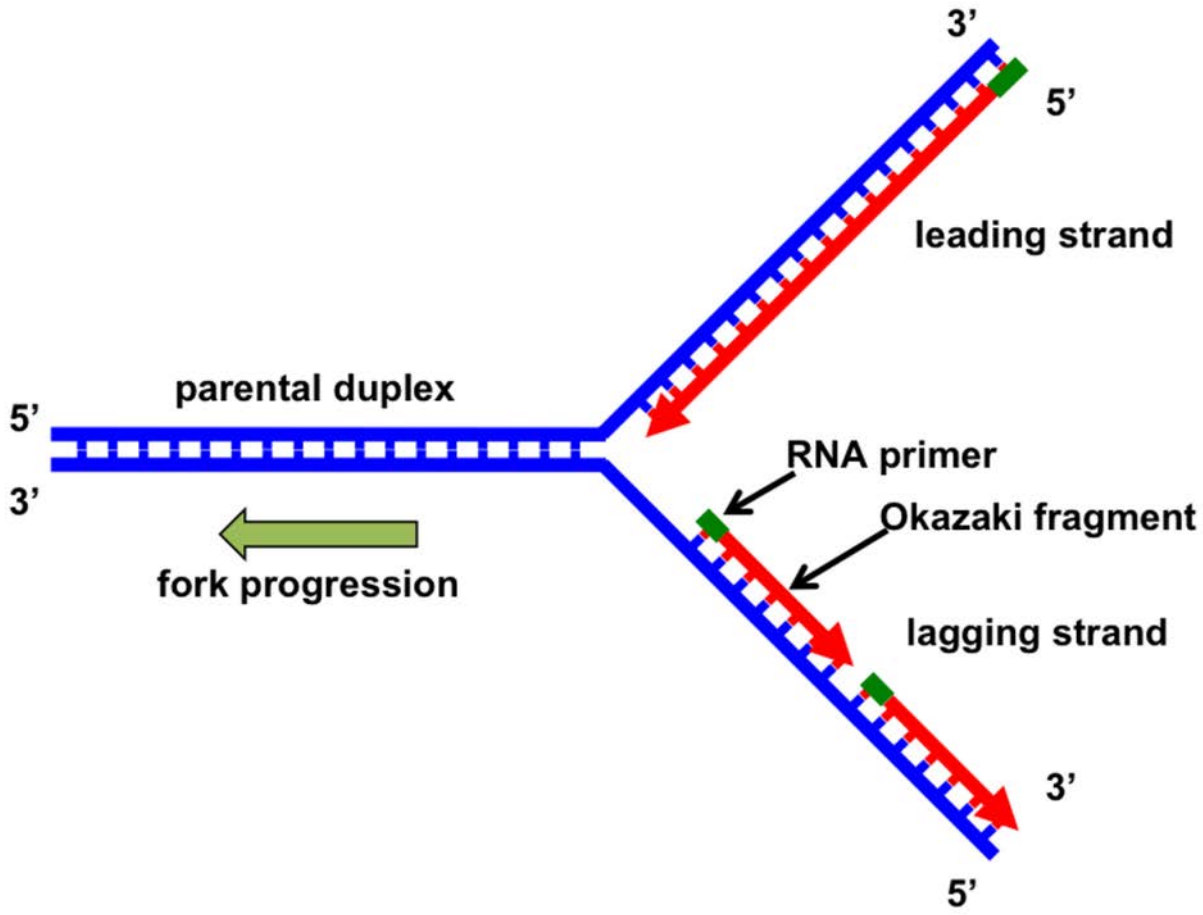
The deoxyribonucleoside triphosphates joined to each single DNA chain break off their inner high energy bonds and set free pyrophosphate (P~P) molecules. This changes them to deoxyribonucleoside monophosphates that are the normal components of DNA. Pyrophosphate undergoes hydrolysis by an enzyme pyrophosphatase and release energy and set free inorganic phosphate groups.

**Formation of New DNA Chains**

With the energy so released, the adjacent deoxyribonucleoside monophosphates joined to each single DNA chain become linked together, forming a new DNA chain. The process is catalyzed by an enzyme DNA polymerase and aided by metal ions $Mn^{2+}$ or $Mg^{2+}$.

There are three DNA polymerases (DNA-polymerase I, II, III). The enzyme first studied by Kornberg, now termed DNA polymerase I, is not the principal DNA replication enzyme, so here as enzyme termed DNA polymerase III probably is. All of the known DNA polymerases synthesize new chains only in the 5'- to 3'- direction, that is, from carbon 5'- end to carbon 3'- end of the sugar molecules in the DNA strands. Because the two DNA strands are anti parallel, the new strands must be formed on the old (parent) strands in opposite directions. One new strand is formed in a continuous stretch in the 5' – 3'- direction. This strand is called leading strand. On the other parent strand, short DNA segments are formed in the 5'- 3' – direction, starting from RNA primers. These DNA segments are known as Okazaki fragments (Japan scientist was the first, who saw these fragments).

They are later joined together, forming a continuous lagging strand. The Okazaki fragments are linked up by the enzyme DNA ligase (DNA synthetase) with polymerase I after replacing the RNA primers with deoxyribonucleotides. The Okazaki fragments have 100-200 b.p. in eukaryotes and 10-60 b.p. in prokaryotes. The process in which the Okazaki fragments are linked up is called maturation.




**Editing (Proofreading) and DNA repairs.**

The specificity of base pairing ensures accurate replication. However, sometimes wrong bases do get in. These are removed by DNA polymerase, which can go back for this purpose. The

abnormal regions of DNA resulting from mutation are cleaved by enzymes termed nucleases. The DNA polymerase I resynthesizes the missing segments of DNA strand, using the intact DNA strand as the template. The DNA ligase joins the new and old segments of the strand under repair. This makes the damaged DNA strand normal.

**Helix Formation**

Each daughter double DNA molecule becomes spirally coiled to from a double helix.

Differences between prokaryotes and eukaryotes in replication.

1. Eukaryotic DNA forms many origins of replication. Prokaryotic DNA forms a single point of replication.
2. The rate of replication in eukaryotes is approximately 50 b.p. per second. The rate of replication in prokaryotes, for examples E. coli ~ is 1700 b.p. per second.
3. Eukaryotic and prokaryotic organisms have some different enzymes in replication.
4. Eukaryotic organisms have the Okazaki fragments.

Similarities between prokaryotes and eukaryotes in replication

1. The mechanism of replication is the same in eukaryotes and prokaryotes.
2. DNA replicates always bidirectional in both eukaryotes and prokaryotes.

**THE QUESTIONS FOR SELF-CONTROL.**

***1. Hereditary material and its properties.***
***2. The chemical composition of nucleic acids. The structure of DNA and RNA nucleotides and their types.***
***3. The bonds between nucleotides in polynucleotide chain of DNA and RNA molecule.***
***4. The joining of DNA strands in double helix.***
***5. Functions of DNA and RNA in the cell.***
***6. The mechanism of DNA replication.***
***7. Gene as an elementary unit of heredity.***
***8. Nucleus as information center of eukaryotic cell.***

# *REALIZATION OF BIOLOGICAL INFORMATION IN CELL.*

## Historical overview on the gene discovery.

The concept of gene has been changing with progress in research in genetics and molecular biology. Mendel was the first to visualize a gene as unit of inheritance in 1865. He called it a factor. He postulated that the gametes brought from the parents distinct particulate factors which made their respective characters appear in the offspring.

The world "gene" was introduced by Johanson in 1909 for a single unit of heredity occupying a specific position (locus) in a chromosome.

In 1926 Morgan in article "Gene Theory" determined the Chromosome Theory. In which, the genes lay in a linear order in the chromosomes and were carried along with them to daughter cells in cell division. Behavior of chromosomes and genes was found to be parallel.

It later has been found that DNA is the hereditary material composed of a linear series of deoxyribonucleotide pairs. A gene is a segment of DNA having a limited number of nucleotide pairs in a unique sequence. Different genes have different sequence of nucleotide pairs. The specific sequence of bases in a gene forms the code that directs the cell to manufacture a particular protein. A gene undergo crossing over and can mutate also.

In 1948 Beadle and Tatum proposed their famous one gene-one enzyme (protein)hypothesis, and considered gene as a unit of hereditary material which codes for the formation of a single protein (enzyme). Gradually it was found that all proteins do not act as enzyme, some proteins have structural role in cells and some function as membrane receptors. Moreover, certain proteins are composed if more than one polypeptide chains. As a result of these findings, the Beadle and Tatum hypothesis has been replaced by one gene -one polypeptide chain principle.

In 1960-s, genes were found to code for r-RNA and t-RNA. Regulatory genes, such as the operators of prokaryotic DNA, were also discovered. These genes are never transcribed. Besides these, some viruses have overlapping genes which code for more than one polypeptide. Certain viruses and higher organisms have genes which code for long polyproteins. A eukaryotic gene may have noncoding introns between coding exons. These researches have led to the concept that a gene is a segment of DNA molecule that codes for a unit of function. The function may be to code for a polypeptide, a polyprotein, ribosomal and transfer RNA, or to regulate the activity of other functional units within the DNA.

## Properties of Gene.

The gene is a part of a DNA molecule coding for a definite polypeptide (or RNA).

Genes are characterized by the following properties:

1. ***Specificity*** — a unique sequence of nucleotides for every structural gene.
2. ***Integrity*** — being considered as a functional unit, the gene is indivisible (during protein synthesis).
3. ***Discretion*** — the gene includes subunits: a muton — a subunit responsible for mutations; a recon responsible for recombination. Their minimal length is equal to a pair of nucleotides.
4. ***Stability*** — genes are relatively stable. The frequency of spontaneous mutations of a gene is approximately 10–5 per a generation.
5. ***Lability*** — genes are able to modify, mutate.
6. ***Pleiotropy*** — multiple gene action (one gene is responsible for several characters).
7. ***Expressivity*** — the degree of phenotypic manifestation of the gene. It depends on environmental factors and effect of other genes.
8. ***Penetrance*** — frequency of gene manifestation: a ratio (per cents) of the number of individuals having the character to the number of individuals having the gene.

**The types of genes depending on how they are regulated:**

1. Constitutive (House Keeping Genes)

Constitutive genes are expressed continuously. It is because constitutive genes such as housekeeping genes code for products with housekeeping functions in cells, maintaining the basic cell processes or structure. They are expressed in all cell types of an organism.

2. Facultative genes (non-Constitutive Genes)

Facultative genes are not transcribed continually but only when needed.

**The types of genes depending on their role:**

1. Structural genes

A structural gene is a gene that encode for any RNA or protein product other than a regulatory factor. They are continuous in prokaryotes and split into introns and exons in eukaryotes.

2.Regulatory Genes.

These genes encode for repressor or activator proteins for regulating the expression of structural genes. The repressor blocks transcription of the structural gene, and activator activates it.

3. Regulatory sequences, such as enhancers, promotors, operators, terminators and so on. This sequences needed to bind to regulatory proteins and enzymes, that are provide transcription of structural genes.

**Genetic code.**

The information about proteins encoded in genes with genetic code.

Genetic code is a common system of coding of amino acids sequence in protein as the sequence of nucleotides in the nucleic acids.

## Characteristics of Genetic code.

The genetic code of DNA has certain fundamental characteristics:

1. Triplet Nature

The genetic code is a triplet code. Three adjacent nucleotides, termed codon, encode one amino acid.

2. No Overlapping

The adjacent codons do not overlap, they do not share any base. Each single base a part of only one codon.

3. No Punctuation

The genetic code is comma less. There are no "punctuation marks" (gaps) between the coding triplets.

Reading of the code begins at a fixed point and continues three nucleotides at a time, without a pause till the terminator codon, which marks the end of the message., is reached.

4. Universality

The genetic code is universal, that is a given codon in the DNA and m-RNA specifies the same amino acid in the protein-synthesizing systems of all organisms, from bacteria to man, also in viruses.

5. Non-ambiguity.

A particular codon will always code for the same amino acid.

6. Degeneracy

The genetic code is degenerate, that is one amino acid may be encoded by several codons

7. "Nonsense" or Terminator Codons

Three of the 64 codons, namely: UAA, UAG, UGA do not specify amino acids, but signal the end of the translation. They are called the nonsense or terminator codons (or stop codons). They stop synthesis of the polypeptide chain.

8. Polarity

The genetic code has polarity, that is, the code is always read in a fixed direction, i.e., in the 5′ → 3′ direction.

9. Initiation or Start Codons.

The codons AUG (in eukaryotes) and GUG (in prokaryotes) are called the initiation or start codons as they begin the synthesis of polypeptide chain.

**GENETIC CODE TABLE**

| first letter | second letter: U | second letter: C | second letter: A | second letter: G | third letter |
|---|---|---|---|---|---|
| U | UUU, UUC: Phe<br>UUA, UUG: Leu | UCU, UCC, UCA, UCG: Ser | UAU, UAC: Tyr<br>UAA stop<br>UAG stop | UGU, UGC: Cys<br>UGA stop<br>UGG Trp | U C A G |
| C | CUU, CUC, CUA, CUG: Leu | CCU, CCC, CCA, CCG: Pro | CAU, CAC: His<br>CAA, CAG: Gln | CGU, CGC, CGA, CGG: Arg | U C A G |
| A | AUU, AUC, AUA: Ile<br>AUG Met | ACU, ACC, ACA, ACG: Thr | AAU, AAC: Asn<br>AAA, AAG: Lys | AGU, AGC: Ser<br>AGA, AGG: Arg | U C A G |
| G | GUU, GUC, GUA, GUG: Val | GCU, GCC, GCA, GCG: Ala | GAU, GAC: Asp<br>GAA, GAG: Glu | GGU, GGC, GGA, GGG: Gly | U C A G |

## PROTEIN SYNTHESIS

Protein synthesis consist of several steps:

1. Transcription. It's a process of synthesis of pre-mRNA (precursor RNA) according to the complementary rules on the coding strand of DNA.

2. Processing (post-transcriptional modification or maturation of RNA). It's a process of formation of mRNA from pre-mRNA

3. Translation. It's a protein synthesis

4. Post-translational modification and protein folding

### Transcription.

Transcription is the first step in gene expression, in which information from a gene is used to construct a functional product such as a protein. The goal of transcription is to make a RNA copy of a gene's DNA sequence. For a protein-coding gene, the RNA copy, or transcript, carries the information needed to build a polypeptide (protein or protein subunit).

During transcription the pre-RNA (precursor or heterogeneous nuclear RNA) in eukaryotes and m-RNA in prokaryotes is formed.

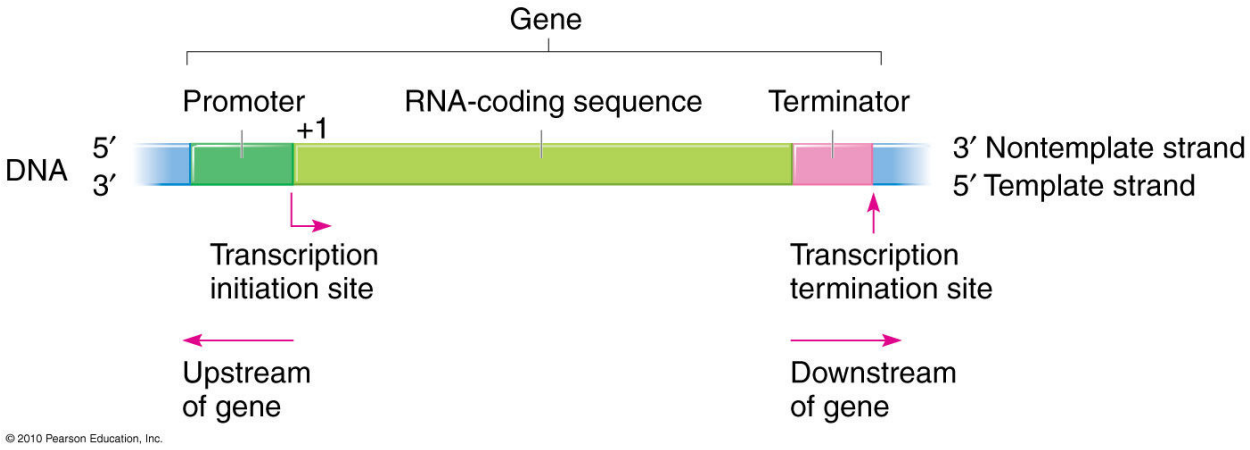
On a signal from the cytoplasm, a specific segment of the DNA double helix uncoils and splits into two chains. This is brought about by the enzyme **RNA polymerase**. Specifically, RNA polymerase builds an RNA strand in the 5' to 3' direction, adding each new nucleotide to the 3' end of the strand.

**Stages of transcription**

Transcription of a gene takes place in three stages: initiation, elongation, and termination. Here, we will briefly see how these steps happen in bacteria. You can learn more about the details of each stage (and about how eukaryotic transcription is different) in the stages of transcription article.

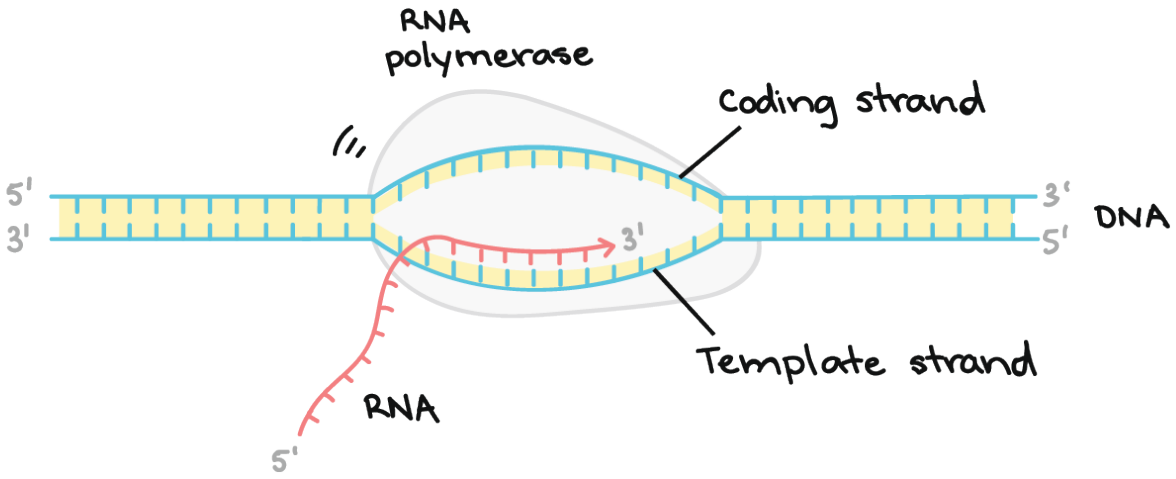
**Initiation.** RNA polymerase binds to a sequence of DNA called the promoter, found near the beginning of a gene. Each gene (or group of co-transcribed genes, in bacteria) has its own promoter. Once bound, RNA polymerase separates the DNA strands, providing the single-stranded template needed for transcription.

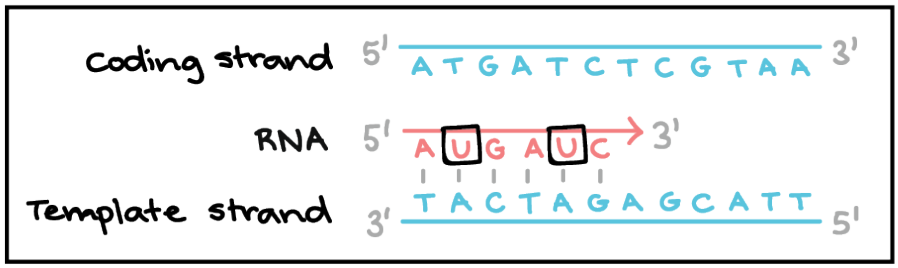
The promoter region comes before (and slightly overlaps with) the transcribed region whose transcription it specifies. It contains recognition sites for RNA polymerase or its helper proteins to bind to. The DNA opens up in the promoter region so that RNA polymerase can begin transcription.




**Elongation**. One strand of DNA, the template strand, acts as a template for RNA polymerase. As it "reads" this template one base at a time, the polymerase builds an RNA molecule out of complementary nucleotides, making a chain that grows from 5' to 3'. The RNA transcript carries the same information as the non-template (coding) strand of DNA, but it contains the base uracil (U) instead of thymine (T).

RNA polymerase synthesizes an RNA transcript complementary to the DNA template strand in the 5' to 3' direction. It moves forward along the template strand in the 3' to 5' direction, opening the DNA double helix as it goes. The synthesized RNA only remains bound to the template strand for a short while, then exits the polymerase as a dangling string, allowing the DNA to close back up and form a double helix.




In this example, the sequences of the coding strand, template strand, and RNA transcript are:

Coding strand: 5'-ATGATCTCGTAA-3'

Template strand: 3'-TACTAGAGCATT-5'

RNA: 5'-AUGAUC...-3' (the dots indicate where nucleotides are still being added to the RNA strand at its 3' end)

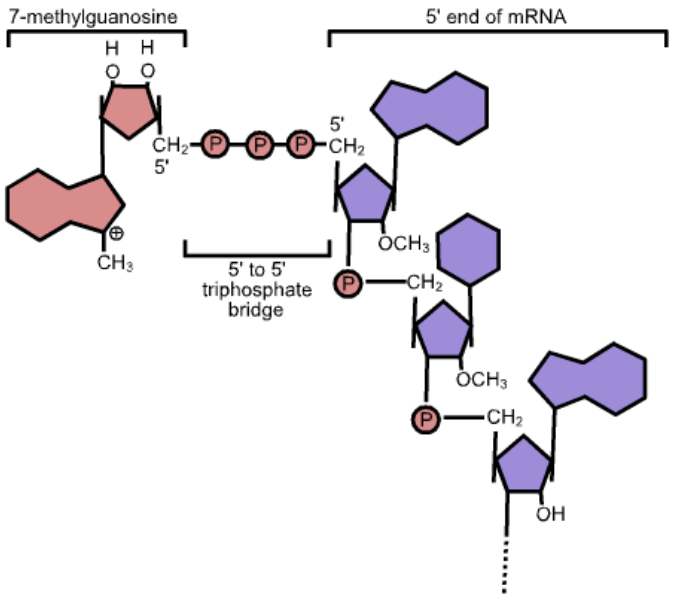
**Termination**. Sequences called terminators signal that the RNA transcript is complete. Once they are transcribed, they cause the transcript to be released from the RNA polymerase.

## PROCESSING (only in eukaryotes)

This is a process of formation of m-RNA from pre-RNA. It occurs when pre-RNA passes into the cytoplasm through the pores in the nuclear envelope. In the result of this process, the molecules of pre-RNA are subjected to modification in 5’ and 3’-ends and splicing.

1. Modification of 5’-end of pre-RNA.

A 7-methylguanosine cap is added to the 5′ end of the pre-mRNA while elongation is still in progress. The 5′ cap protects the nascent mRNA from degradation and assists in ribosome binding during translation.



2. Modification of 3'end of pre-RNA.

The pre-mRNA is cleaved off the rest of the growing transcript before RNA Polymerase II has stopped transcribing. This cleavage is done by an endonuclease-containing protein complex that binds to an AAUAAA sequence upstream of the cleavage site and to a GU-rich sequence downstream of the cut site. Immediately after the cleavage, Poly (A) Polymerase (PAP), which is also part of the protein complex, catalyzes the addition of up to 200 A nucleotides to the 3′ end of the just-cleaved pre-mRNA.

The modifications of 5' and 3'-ends of pre-RNA defend m-RNA of the activity of exonucleases and it's necessary for effective translation.

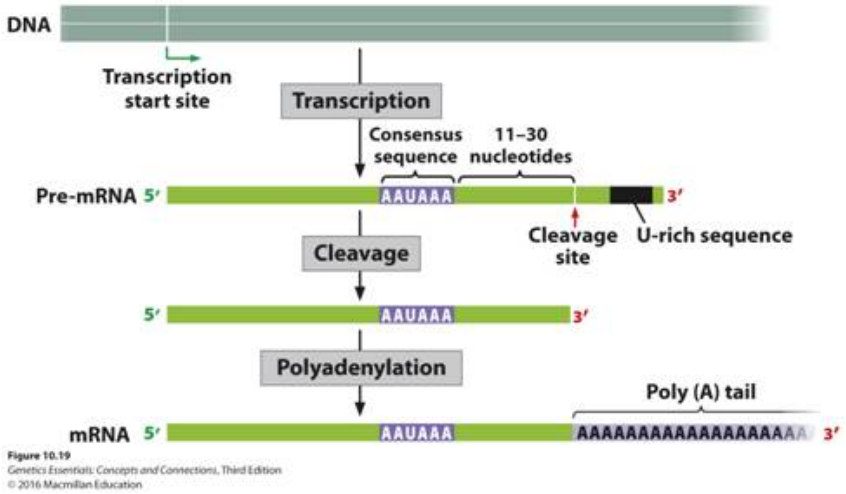


Figure 10.19
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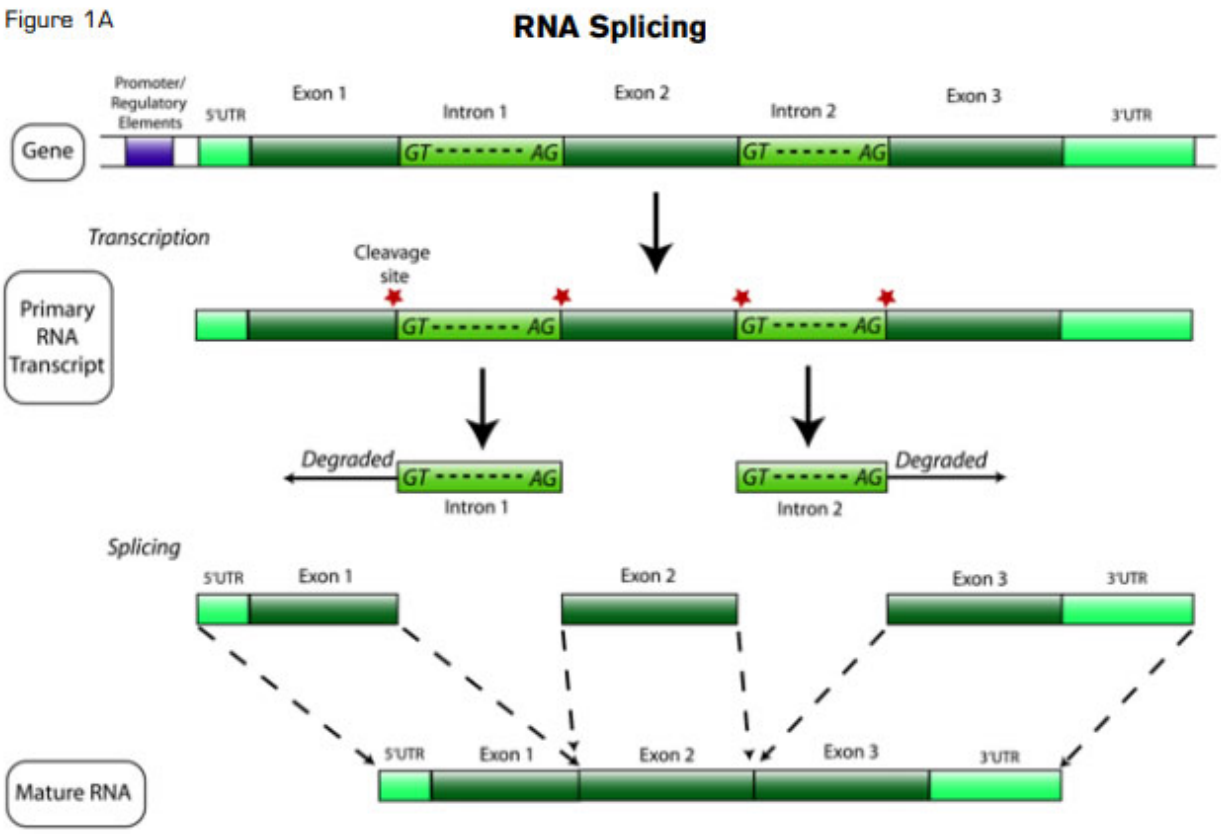
**SPLICING** pre-RNA is a removal of sequences of pre-RNA corresponding introns of DNA and joining of exons.

The molecule pre-RNA has molecular mass of mRNA more 10 but the molecular mass of mRNA is $2x10^{6}$.




TRANSLATION: synthesis of proteins.

Proteins are synthesized from mRNA templates by a process that has been highly conserved throughout evolution.

All mRNAs are read in the 5´ to 3´ direction, and polypeptide chains are synthesized from the amino to the carboxy terminus. Each amino acid is specified by three bases (a codon) in the mRNA, according to a nearly universal genetic code. The basic mechanics of protein synthesis are also the same in all cells: Translation is carried out on ribosomes, with tRNAs serving as adaptors between the mRNA template and the amino acids being incorporated into protein. Protein synthesis thus involves interactions between three types of RNA molecules (mRNA templates, tRNAs, and rRNAs), as well as various proteins that are required for translation.

**Transfer RNAs**

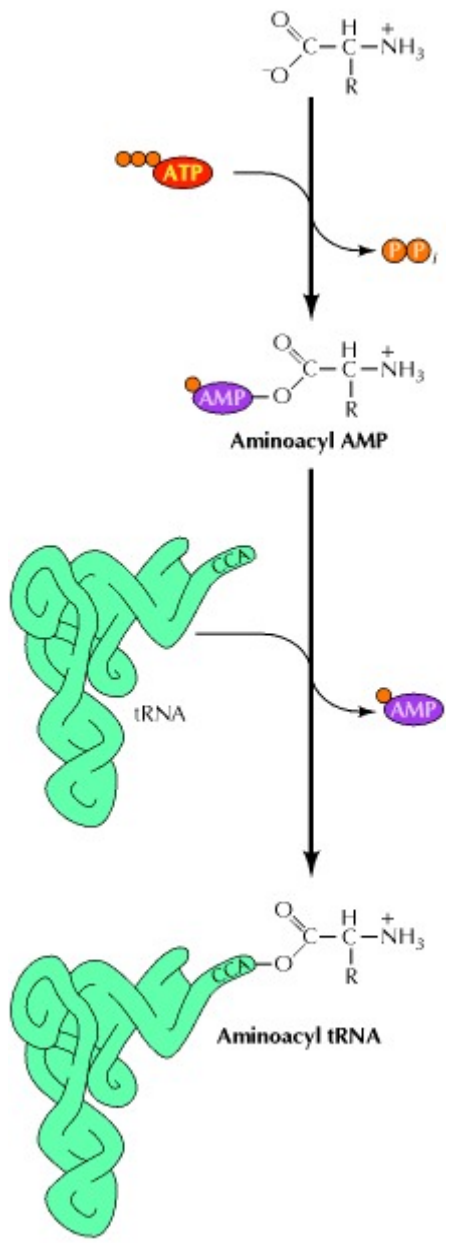
During translation, each of the 20 amino acids must be aligned with their corresponding codons on the mRNA template. All cells contain a variety of tRNAs that serve as adaptors for this process. As might be expected, given their common function in protein synthesis, different tRNAs

share similar overall structures. However, they also possess unique identifying sequences that allow the correct amino acid to be attached and aligned with the appropriate codon in mRNA.

The incorporation of the correctly encoded amino acids into proteins depends on the attachment of each amino acid to an appropriate tRNA, as well as on the specificity of codon-anticodon base pairing. The attachment of amino acids to specific tRNAs is mediated by a group of enzymes called aminoacyl tRNA synthetases, which were discovered by Paul Zamecnik and Mahlon Hoagland in 1957. Each of these enzymes recognizes a single amino acid, as well as the correct tRNA (or tRNAs) to which that amino acid should be attached.

The reaction proceeds in two steps.

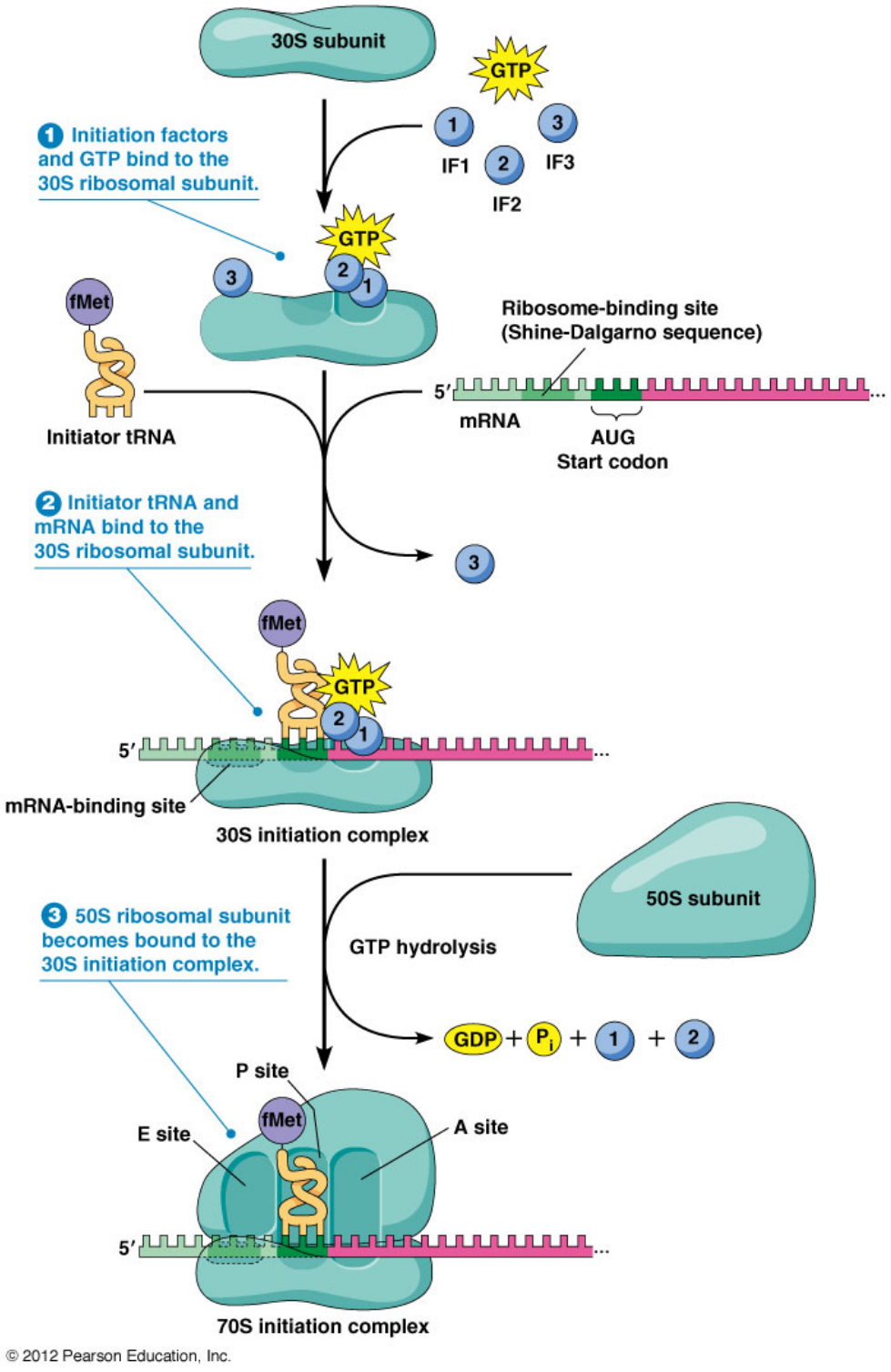
First, the amino acid is activated by reaction with ATP to form an aminoacyl AMP synthetase intermediate. The activated amino acid is then joined to the 3′ terminus of the tRNA. The aminoacyl tRNA synthetases must be highly selective enzymes that recognize both individual amino acids and specific base sequences that identify the correct acceptor tRNAs. In some cases, the high fidelity of amino acid recognition results in part from a proofreading function by which incorrect aminoacyl AMPs are hydrolyzed rather than being joined to tRNA during the second step of the reaction. Recognition of the correct tRNA by the aminoacyl tRNA synthetase is also highly selective; the synthetase recognizes specific nucleotide sequences (in most cases including the anticodon) that uniquely identify each species of tRNA.




**Initiation**

When translation begins, the small subunit of the ribosome and an initiator tRNA molecule assemble on the mRNA transcript. The small subunit of the ribosome has three binding sites: an amino acid site (A), a polypeptide site (P), and an exit site (E). The initiator tRNA molecule carrying the amino acid methionine binds to the AUG start codon of the mRNA transcript at the ribosome's P site where it will become the first amino acid incorporated into the growing polypeptide chain. Here, the initiator tRNA molecule is shown binding after the small ribosomal subunit has assembled on the mRNA; the order in which this occurs is unique to prokaryotic cells. In eukaryotes, the free initiator tRNA first binds the small ribosomal subunit to form a complex. The complex then binds the mRNA transcript, so that the tRNA and the small ribosomal subunit bind the mRNA simultaneously.

Once the initiation complex is formed on the mRNA, the large ribosomal subunit binds to this complex, which causes the release of IFs (initiation factors). The large subunit of the ribosome has three sites at which tRNA molecules can bind. The A (amino acid) site is the location at which the aminoacyl-tRNA anticodon base pairs up with the mRNA codon, ensuring that correct amino acid is added to the growing polypeptide chain. The P (polypeptide) site is the location at which the amino acid is transferred from its tRNA to the growing polypeptide chain. Finally, the E (exit) site is the location at which the "empty" tRNA sits before being released back into the cytoplasm to bind another amino acid and repeat the process. The initiator methionine tRNA is the only aminoacyl-tRNA that can bind in the P site of the ribosome, and the A site is aligned with the second mRNA codon. The ribosome is thus ready to bind the second aminoacyl-tRNA at the A site, which will be joined to the initiator methionine by the first peptide bond



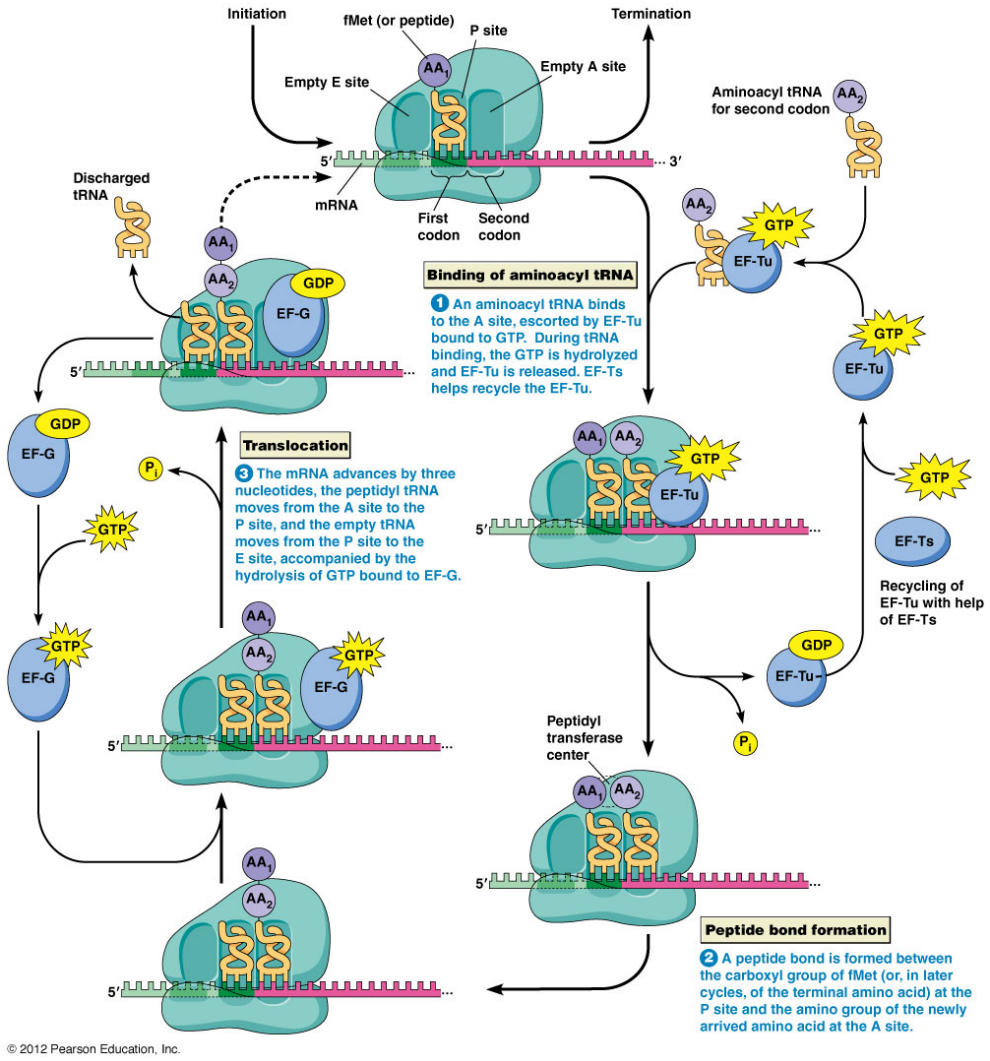

### Elongation

The next phase in translation is known as the elongation phase. First, the ribosome moves along the mRNA in the 5'-to-3'direction, which requires the elongation factor G, in a process called translocation. The tRNA that corresponds to the second codon can then bind to the A site, a step that requires elongation factors (in E. coli, these are called EF-Tu and EF-Ts), as well as guanosine triphosphate (GTP) as an energy source for the process. Upon binding of the tRNA-amino acid

complex in the A site, GTP is cleaved to form guanosine diphosphate (GDP), then released along with EF-Tu to be recycled by EF-Ts for the next round.

Next, peptide bonds between the now-adjacent first and second amino acids are formed through a peptidyl transferase activity. For many years, it was thought that an enzyme catalyzed this step, but recent evidence indicates that the transferase activity is a catalytic function of rRNA. After the peptide bond is formed, the ribosome shifts, or translocates, again, thus causing the tRNA to occupy the E site. The tRNA is then released to the cytoplasm to pick up another amino acid. In addition, the A site is now empty and ready to receive the tRNA for the next codon.

This process is repeated until all the codons in the mRNA have been read by tRNA molecules, and the amino acids attached to the tRNAs have been linked together in the growing polypeptide chain in the appropriate order. At this point, translation must be terminated, and the nascent protein must be released from the mRNA and ribosome




### Termination of Translation

At the end of mRNA chain there is “stop” or “terminator” codon (UAA, UAG, UGA). It is not read and joined by the anticodon of any tRNA-amino acid complex. A terminator factor joins and blocks the terminator codon. The linkage between the last tRNA and the polypeptide chain is

broken by some release factors. The ribosome jumps off the mRNA chain at the “stop” codon and dissociates into its two subunits. The completed polypeptide (amino acid chain) becomes free in the cytoplasm.

The ribosomes and the t-RNAs on release from the mRNA can function again in the formation of another polypeptide.

**Modification of Released Polypeptide.**

The just released polypeptide has primary structure, i.e., it is a straight linear molecule. It may lose some amino acids from the end with the help of an exopeptidase enzyme, and then coil and fold on itself to acquire secondary and tertiary structure. It may combine with other polypeptides to have quaternary structure.

Proteins have four levels of structure.

The sequence of its amino acids is the primary structure. This sequence is always written from the amino end (N-terminus) to the carboxyl end (C-terminus).

Protein secondary structure refers to common repeating elements present in proteins. There are two basic components of secondary structure: the alpha helix and the beta-pleated sheet. Alpha helices are tight, corkscrew-shaped structures formed by single polypeptide chains. Beta-pleated sheets are either parallel or anti-parallel arrangements of polypeptide strands stabilized by hydrogen bonds between adjacent –NH and –CO groups. Parallel beta-sheets have adjacent strands that run in the same direction (i.e., N-termini next to each other), while anti-parallel beta sheets have adjacent strands that run in opposite directions (i.e., N-terminus of one strand arranged toward the C-terminus of adjacent strand). A beta-pleated sheet may contain two to five parallel or antiparallel strands.

Tertiary structure is the full three-dimensional, folded structure of the polypeptide chain and is dependent on the suite of spontaneous and thermodynamically stable interactions between the amino acid side chains. Disulfide bond patterns, as well as ionic and hydrophobic interactions greatly impact tertiary structure.

Quaternary structure refers to the spatial arrangement of two or more polypeptide chains. This structure may be a monomer, dimer, trimer, etc. The polypeptide chains composing the quaternary structure of a protein may be identical (e.g., homodimer) or different (e.g., heterodimer).

The complete structure of a functioning protein involves more than polypeptide chains at the four levels of structure. Various covalent modifications often occur, either during or after assembly of the polypeptide chain. Most proteins undergo co- and/or post-translational modifications. Examples include phosphorylation (of serine, threonine or tyrosine residues), glycosylation, and ubiquitination.

Knowledge of these native modifications is extremely important because they may alter physical and chemical properties, folding, conformation distribution, stability, activity, and consequently, function of the proteins. The study of post-translational modifications (a different meaning from

the protein modification being discussed in the present article) is an important area of research; see related articles for a discussion of that topic.

### Comparing Eukaryotic and Prokaryotic Translation

The translation process is very similar in prokaryotes and eukaryotes. Although different elongation, initiation, and termination factors are used, the genetic code is generally identical. As previously noted, in bacteria, transcription and translation take place simultaneously, and mRNAs are relatively short-lived. In eukaryotes, however, mRNAs have highly variable half-lives, are subject to modifications, and must exit the nucleus to be translated; these multiple steps offer additional opportunities to regulate levels of protein production, and thereby fine-tune gene expression.

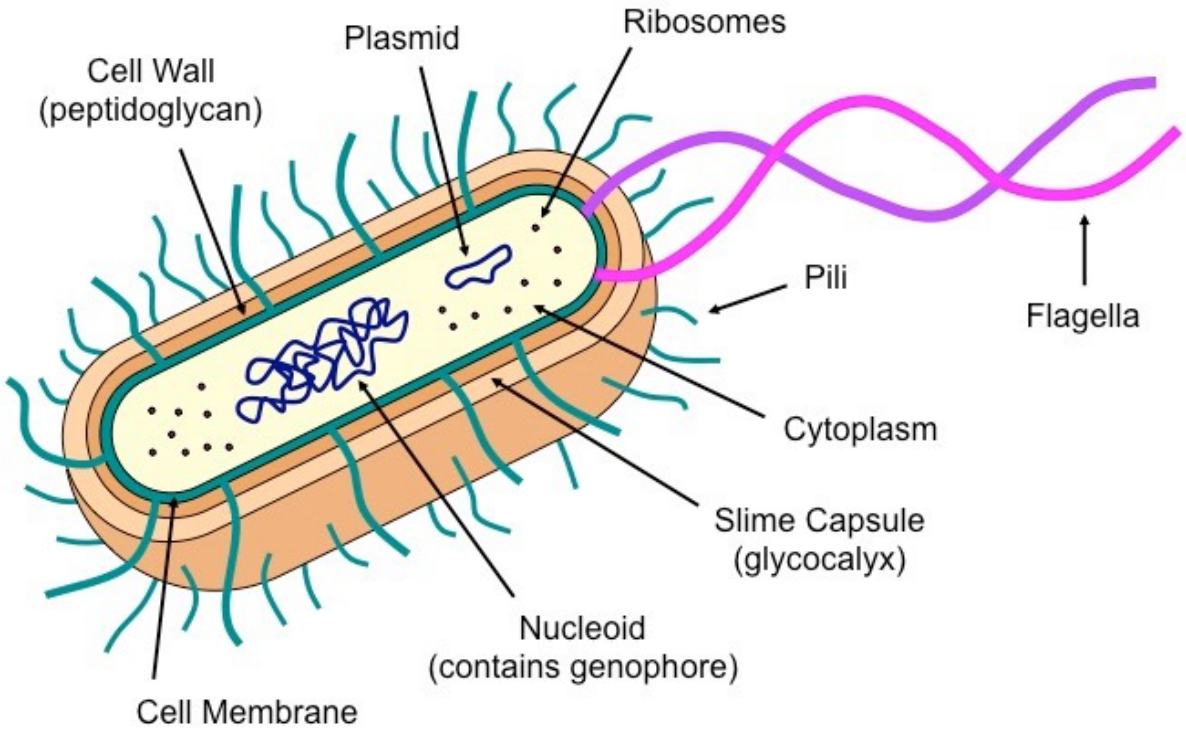
## GENETIC MATERIAL IN PROKARYOTIC CELLS

In bacteria the hereditary material is packed in a single chromosome. The latter is irregularly folded into a single circular DNA molecule the genophore. Genophore lies in region of prokaryotic cell which called nucleoid. Nucleoid is not nucleus, because it is not separated from the cytoplasm by a definitive nuclear membrane as in eukaryotic organisms.

As isolated the nucleoid contains in addition to DNA, small amounts of several proteins, which are thought to be responsible in some way for the multiply looped arrangement of the DNA. The degree of condensation of the isolated nucleoid (that is, its physical dimensions) is affected by a variety of factors, and some controversy exists about the state of the nucleoid which a cell. The total DNA (the genome) and hence the amount of information encoded, is much less than that of a eukaryotic cell; typically it contains several thousand genes, about 500 times fewer than a human cell.

In addition to the circular DNA there are plasmids in bacteria. Plasmids were discovered in1952 by William Hays and Joshus Zederberg. They are extra chromosomal DNA molecules present in most bacteria. Like the main chromosome , they are double stranded and circular, but are much smaller and of variable genetic constitution. They can replicate like and independently of the chromosome. A particular bacterial cell may carry single or multiple copies of one or more plasmids. The plasmids may be separated parts of main chromosome of the same or a foreign cell, and may combine with the main chromosome. A plasmid integrated to a chromosome is called episome. The plasmids an known to move around freely in the bacterial world and may pick up genes from one bacterium and transfer them to another.

This may be one method of providing variability to the asexually multiplying bacteria. The plasmids carry genes for sexuality, antibiotic resistance and some other traits, but not for any vital function. They are nonessential and a bacterium can survive without its plasmids. Not being a part of the main genome, the plasmids can be easily isolated and transferred to other bacteria or organisms for genetic engineering.




## GENETIC MATERIAL IN EUKARYOTIC CELLS

The DNA molecules in the mammalian chromosomes are exceedingly long, containing more than $10^8$ bases with a linear length of almost 1 mm. The main genetic material of eukaryotic cell is found into eukaryotic nucleus, where the DNA is packed in several chromosomes and not in a single chromosome as in prokaryotes. This is a chromosomal heredity. Also, there is cytoplasmic genetic material. It is a DNA of mitochondria and plastids (in plant cells). This is a cytoplasmic (non-chromosomal) heredity.

**THE QUESTIONS FOR SELF-CONTROL.**

***1. The definition of gene.***
***2. The properties of gene.***
***3. The functions of gene.***
***4. Structural organization of eukaryotic gene (Transcripton).***
***5. Genetic code.***
***6. The main properties of genetic code.***
***7. The main stages of protein synthesis: transcription, translation, and their biological significance.***
***8. The mechanism of transcription.***
***9. Processing.***
***10. Phenomenon of splicing.***
***11. Translation, its stages and mechanisms.***

***12. Organization of protein molecules.***

# *Chromosomal inheritance.*

**CHROMOSOMES** are deeply-staining rod-shaped nucleoprotein bodies microscopically observable in the nucleus during cell division, carry genes arranged in linear order.

Hofmeister discovered nuclear filaments in the nuclei of the pollen mother cells of *Tradescantia* in 1848. The nuclear filaments were called chromosomes by Waldeyer in 1888. The term "chromosome" means a coloured body (in Greak, chrome = colour, soma = body). It points to the fact that the chromosomes easily take up biological stains.

## The functions of chromosomes.

1. They carry hereditary characters from parents to offspring.
2. They held the cell grow, divide and maintain itself by directing the synthesis of structural proteins.
3. They control metabolism by directing the formation of necessary enzymatic proteins.
4. They undergo mutations and thus contribute to the evolution of the animal.
5. They guide cell differentiation during development..
6. They form nucleoli in daughter cells at nucleolar organizing sites.
7. They bring about continuity of life by replication.
8. They play a role in sex determination.

## Chemical composition of chromosomes.

The chromosomes may be in two structure-functional conditions, such as:

1) interphase condition (despiralized, decondensated);
2) metaphase condition ( condensated, spiralized).

In non-dividing cells the chromosomes are extremely long and thin and are dispersed throughout the nucleus. Individual chromosomes can not be seen, but some chromosomal material is remained in coil form and can be seen under light microscope and is known as chromatin.

**CHROMATIN** - is a specifically organized material of chromosomes, which stained with certain basic dyes and we can see it in forms of blocks, points and network during interphase under light microscope. It is the most important part of the nucleus controlling cellular activities. The chromosomes in these extended form are involved in the controlling of the synthesis of all materials of cell, but this function disappears during cell division. In the S-stage of interphase the chromosomes replicate and become double, each now consisting of two chromatids that remain joined together at one point called centromere. At the time of cell division the replicated

chromosomes condense and tightly coil up, and become distinct at the metaphase stage. At this time, they can be easily counted and individually distinguished by their size and position of the centromere. Chromatin is a stable ordered aggregate, where the DNA is associated with numerous protein molecules, metal ions, RNA and enzymes.

***DNA*.** There is a single long, double-stranded, linear DNA molecule in a chromosome. It's amount in all somatic cells of an organism is the same, the gametes have half of this amount.

***Proteins*.** The chromosomal proteins have nothing to do with the genetic potency of the chromosomes. They may be replaced with other proteins without in any way altering the function of the genes. The chromosomal proteins regulate gene action. They are of two types: simple basic histones and acid or neutral non-histones.

> ***Histones*** are low molecular weight proteins rich in the basic amino acids lysine and arginine. The DNA and histones are loosely bound together in 1:1 ratio to form deoxyribonuccleoproteins (DNP). This linkage maintains the helical form of the DNA molecule without disturbing its structure. The histones prevent RNA transcription. Five major types of histones are: H1, H2A, H2B, H3 and H4 occur in the chromosome of almost all eukaryotes. The positive charges enable histone molecules to bind to DNA, primarily by electrostatic attraction to the negatively charged phosphate groups in the sugar-phosphate backbone of DNA. Placing chromatin in a solution with a high salt concentration causes the histones to disassociate from the DNA. Histones also bind tightly to each other; both DNA-histone and histone-histone binding are important for chromatin structure. The histones from different organisms are remarkably similar to one another, with the exception of H1. In fact, the amino acid sequences of H3 and H4 molecules from widely different species are almost identifiable.
>
> ***Non-histone proteins*** are high-molecular weight proteins having amino acids tyrosine and tryptophan. They are believed to activate the genes to start RNA transcription.

***RNA.*** Some RNA remains associated with DNA along with proteins.

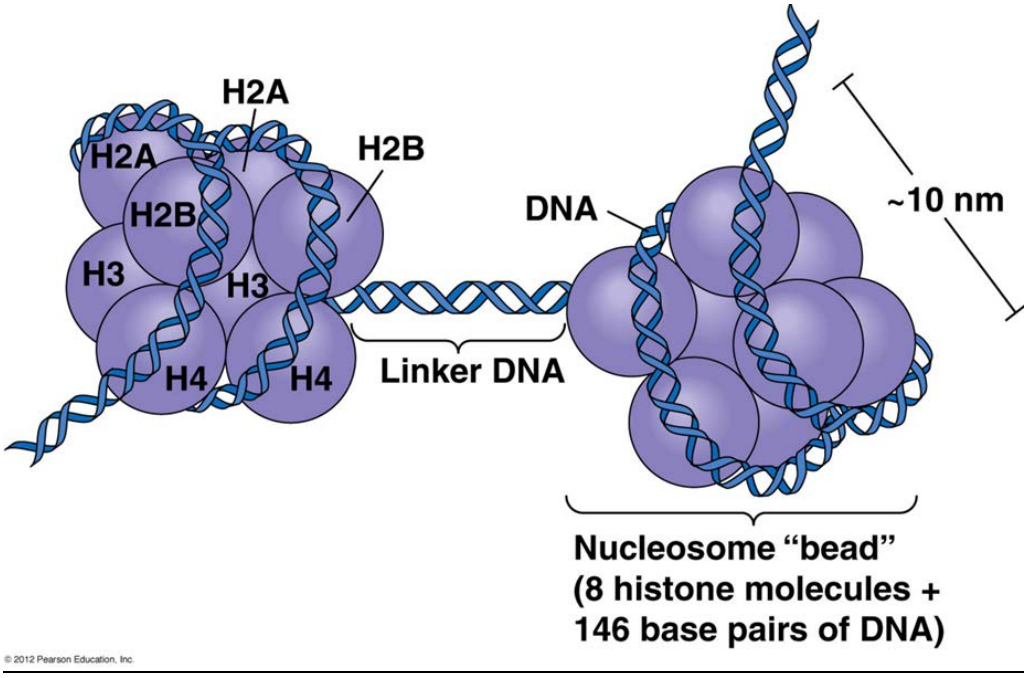
***Metal ions.*** The metal ions found in the chromosomes indicate $Mg^{2+}$, $Ca^{2+}$, $Fe^{2+}$. They keep the organization of chromosomes intact.

***Enzymes.*** The enzymes of the chromosomes are DNA polymerase; RNA polymerase, nucleoside triptophatase.

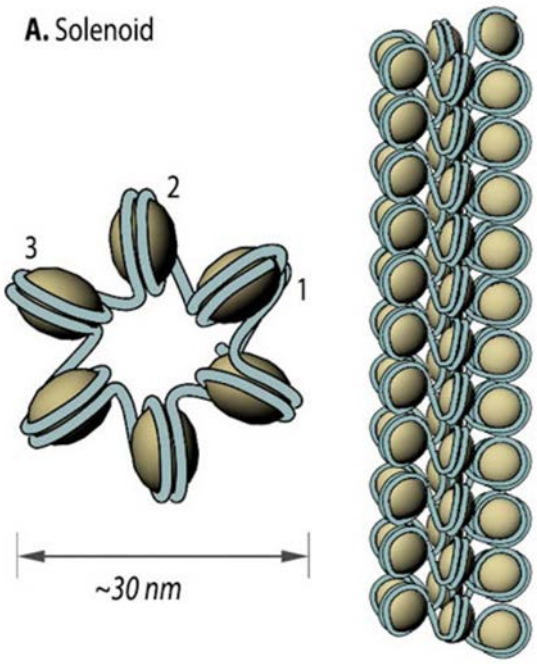
**Levels of chromatin packing**

The DNA molecule of a chromosome is folded and refolded in such a way that it is convenient to think of chromosomes as having several levels of organization, each responsible for a particular degree of shortening of the enormously long strand.

1. Ultra structural components of all chromosomes are *deoxyribonucleoproteins (DNP).* DNP consists of DNA and histones which loosely bound together in 1:1 ratio. They consist of the subunits - ***nucleosomes***. A nucleosome has a definitive composition - namely, two molecules each of H2A, H2B, H3, H4 and segment of DNA containing about 200 nucleotides pairs. The resulting structure called a core particle, consists of an octamer of pairs of H2A, H2B, H3 and H3 around which the remaining 145 - nucleotide pair length of DNA (core DNA) is wound in about 1-3/4 turns. Thus, a nucleosome is composed of a core particle, additional DNA (linker DNA) that links adjacent core particles and one molecule of H1; the H1 binds to the histone octamers and to the linker DNA, causing the linkers extending from both sides of the core particle to cross and draw near to the octamer, though some of the linker DNA does not come into contact with any histones. Linker DNA contains about 10-70 pairs of nucleotides. A nucleosome and a linker DNA are together referred to as a chromatosome. The resulting "beads-on-a-string" DNA structure 110 A (11nm) wide, roughly 6,5-7 times the width of free DNA.

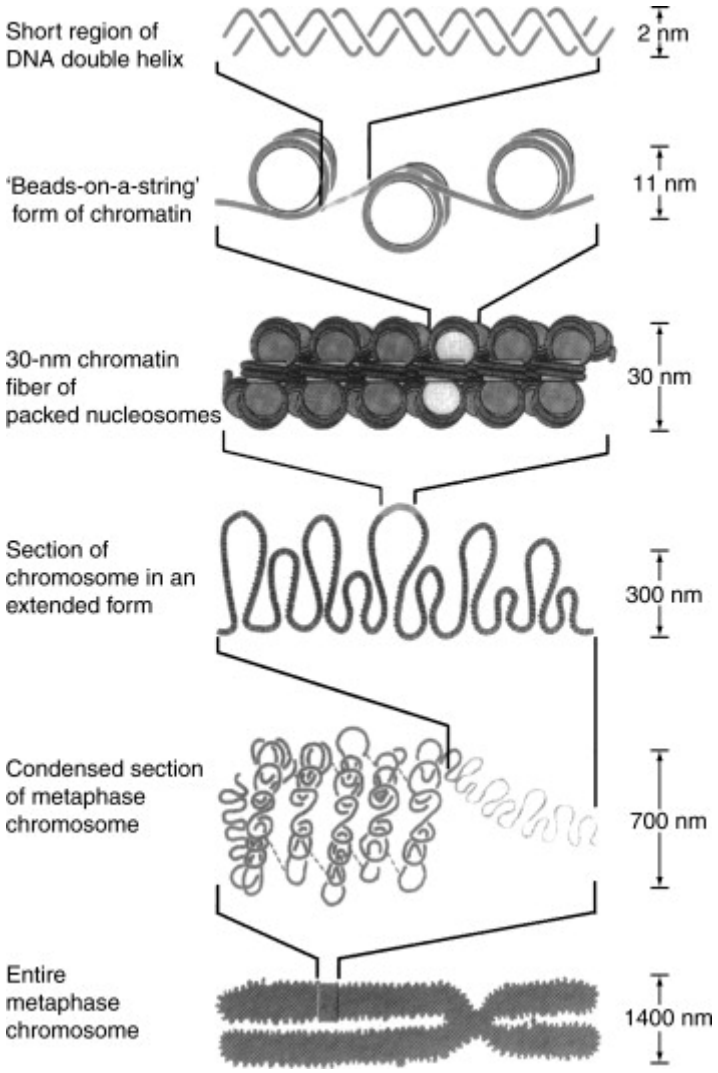



2. The second level of chromatin compaction – namely **chromatin fibers (solenoid).** The chromatin is further condensed by coiling into a 30-nm fiber, containing about six nucleosomes per turn. This structure formed by linker histones H1 which sit at the base of the nucleosome near the DNA entry and exit binding to the linker region of the DNA. H1 provide retraction adjacent nucleosomes.




3. The next level of organization is that in which the 30 nm fiber condenses into a **chromonema (loop domain)**. The chromonema is 300 nm fiber (shortening at 103 times). Chromonema is formed by 30-nm fibers, which are organized into large loops extending from a proteinaceous scaffold composed of nonhistone proteins. In interphase nuclei loop domains are folded into discrete higher-level chromatin complexes (chromomeres).

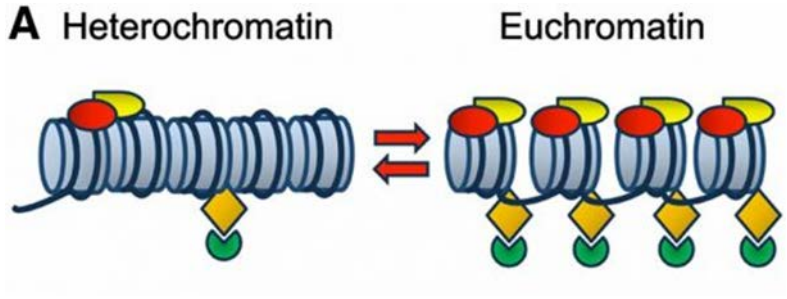
4. The last level of chromatin packing is the condensation of chromatin into metaphase chromosomes which is result in all shortening molecular double stranded DNA roughly in $10^4$ times. The chromonema fiber is packed into a chromatid of the compact metaphase chromosome with average diameter of 700 nm. The compaction of DNA and protein into chromatin and ultimately into chromosome greatly facilitates the distribution of the genetic material during nuclear division.




**Functional compartmentation of chromatin.**

The role of chromatin is to bundle DNA into more manageable volumes so they fit in the cell. Chromatin is also what makes the DNA stronger in order for meiosis and mitosis to happen. Furthermore, chromatin controls the DNA's expression as well as gene replication, and prevents damage to the DNA.

Chromatin has two types: euchromatin and heterochromatin.




**Euchromatin**

Euchromatin, which is composed of lightly packed material, is often found in the inner body of the nucleus. It is rich in the concentration of genes and is generally under active transcription. Euchromatin, where a high frequency of chromosomal crossing over takes place, makes up around 90 percent of the human genome. Found in both prokaryotes and eukaryotes, euchromatin appears to have light-colored bands when stained and viewed under an optical microscope. Euchromatin regions also appear as loops with about 40 to 100 kb regions of DNA inside it. The chromatin fiber in euchromatin is around 30 nm in diameter.

**Heterochromatin**

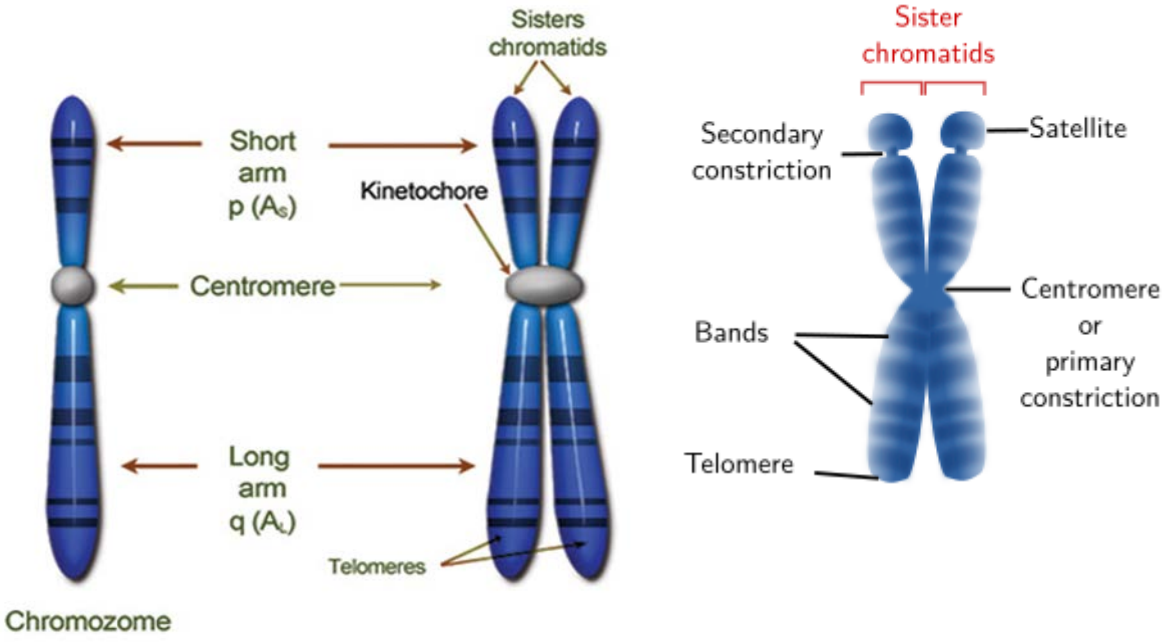
Heterochromatin, which is composed of tightly packed and transcriptionally repressed part of chromatin, is often found near the edge of the nucleus. The main roles of heterochromatin include the protection of the integrity of the chromosome and the regulation of the gene. The compact nature of heterochromatin prevents the crossover of chromosomes and other genetic events, which is why this type of chromatin is considered inactive both genetically and transcriptionally.

Heterochromatin has two types:

- ***Facultative heterochromatin*** is not a fixed attribute of the nucleus because it consists of the genome's inactive genes. These genes can be inactive either during some periods or in some other cells. Facultative heterochromatin is the result of genes that are silenced through a mechanism of gene regulation such as histone deacetylation. The formation of facultative heterochromatin is regulated, and is often associated with morphogenesis or differentiation of cell. An example of facultative heterochromatin is X chromosome inactivation in female mammals: one X chromosome is packaged as facultative heterochromatin and silenced (it called Barr body), while the other X chromosome is packaged as euchromatin and expressed.
- ***Constitutive heterochromatin*** has no genes inside the genome. A fixed attribute of the nucleus, it keeps its compact structure even during the cell's interphase. It is always transcriptionally inactive and contains highly repeated DNA sequences It present at centromeres and telomeres. In humans there is significantly more constitutive heterochromatin found on chromosomes 1, 9, 16, 19 and Y. Probably, its role is in maintenance of common nuclear structure, attachment of chromatin to nuclear envelope, mutual recognition of chromosomes during meiosis, division of neighboring structural genes, participation in processes of regulation of their activity. When genes are placed near a region of constitutive heterochromatin, their transcription is usually silenced. This is known as position-effect variegation

## Physical structure of chromosome.

A metaphase chromosome consists of two identical components, the chromatids, which lie side-by-side along their length and are held together at one point, the centromere. At this region each chromatid has a plate-like kinetochore where spindle microtubules join the chromosome during cell division. The centromere appears as a constriction in the chromosome. The parts of the chromatids on the two sides of the centromere are known as arms. The arms may be equal or unequal, depending upon the position of the centromere. The ends of the chromosomes are called telomeres. Besides the constriction at the centromere called primary constriction, a chromosome may have additional one termed secondary constriction. Part of the chromosome beyond the secondary constriction is called satellite. The chromosome bearing a satellite is known as sat chromosome Certain secondary constrictions are the sites for the formation of nucleoli during interphase. These are termed nucleolar organizers. The chromosomes having nucleolar organizers are called nucleolar chromosomes.

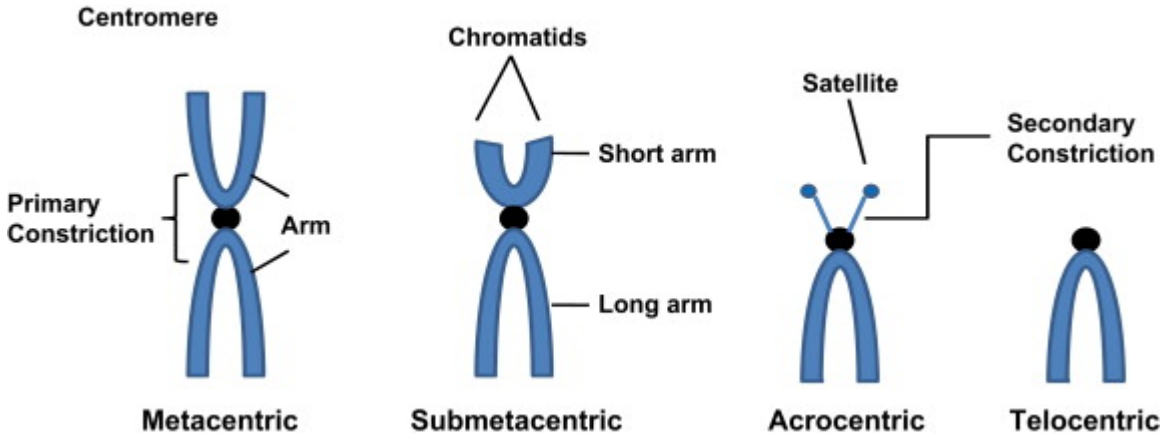



## The types of chromosomes.

The chromosomes are classified on the basis of the position and number of centromeres, and size of the arms.

1. *Metacentric.* The centromere is at the middle of the chromosome, and arms are equal. The chromosome looks V-shaped in anaphase.
2. *Submetacentric.* The centromere is near the centre of the chromosome, and the arms slightly unequal. The chromosome appears J- or L-shaped in anaphase.
3. *Acrocentric.* The centromere is near one end of the chromosome, and arms are very unequal.

4. *Telocentric.* The centromere is at one end of the chromosome, and the arms are on one side only. The chromosome remain rod shaped in anaphase also.




Certain animal groups have a particular type of chromosomes. For example, locusts have acrocentric chromosomes and amphibians have metacentric chromosomes. The human being has only metacentric, submetacentric and acrocentric chromosomes.

**Sex chromosomes and autosomes.**

In diploid organisms, with separate sexes, a specific pair of chromosomes determine the sex of the individual. They are called sex chromosomes. All other chromosomes are termed autosomes. The latter carry genes which control the somatic traits and have no bearing on the sex.

The two members of each pair of homologous autosomes are similar in size and shape, but this may not be true of sex chromosomes. In animals, including man and most insects, including fruit fly, one sex chromosome is smaller than the other in the males. The lager one is known as X chromosome and the smaller one as Y chromosome. The female in these animals has a pair of X chromosomes. Thus, the condition in the male may be briefly expressed as XY and that in the female as XX.

The similar and dissimilar sex chromosomes of females and males are described as homomorphic and heteromorphic respectively. Experiments have shown that the genes, which influence sex, lie in the X chromosomes. The Y chromosome usually bears a few or no genes. Though different in size and shape, the X and Y chromosomes act as homologous chromosomes in meiosis, they pair separate and pass into different gametes.

**Number of chromosomes.**

The number of chromosomes varies from two the least number an organism can have, to a few hundred in different species. All individuals of a species, however, have the same number of chromosomes in all body cells (but not in gametes). The somatic or body cells have a double set

of chromosomes, i.e. they have both the chromosomes of each homologous pair. This chromosome number is termed the diploid number or full number . It is indicated by the symbol 2n.

The diploid number of chromosomes in man is 46 chromosomes. A gamete, sperm or egg, has a single set of chromosomes, i.e., it has one chromosome of each homologous pair. This chromosome number is called the haploid number or reduced number (1n). It is denoted by the results from the union of sperm and ovum in sexual reproduction.

The complete set of chromosomes in a species or in an individual organism that detected in nucleus of somatic cell is called **karyotype**.

The karyotype is analyzed under a light microscope. Attention is paid to their length, the position of the centromeres, banding pattern, any differences between the sex chromosomes, and any other physical characteristics. The preparation and study of karyotypes is part of cytogenetics.

## Principles of chromosomes.

*I. Principle of constancy of chromosomal number.*

Each species of plants and animals has certain and constant number of chromosomes. It's a specific sign.

*II. Principle of forming of chromosomal pairs.*

Each chromosome has a pair. A pair of chromosomes having similar genes, which control the same characters are known as the homologous chromosomes. One member of a pair of homologous chromosomes comes from one parent (say father) and the other - from the other parent (say mother). In man there are 23 pairs of chromosomes, in fruitfully - 4 pairs.

*III. Principle of chromosomal individuality.*

The chromosomes of each homologous pair have typical size, shape and some similar gene sequences. Hence, the chromosomes have individuality and recognizable.

*IV. Principle of chromosomal continuity.*

Each chromosome has a ability to autoreproduction.

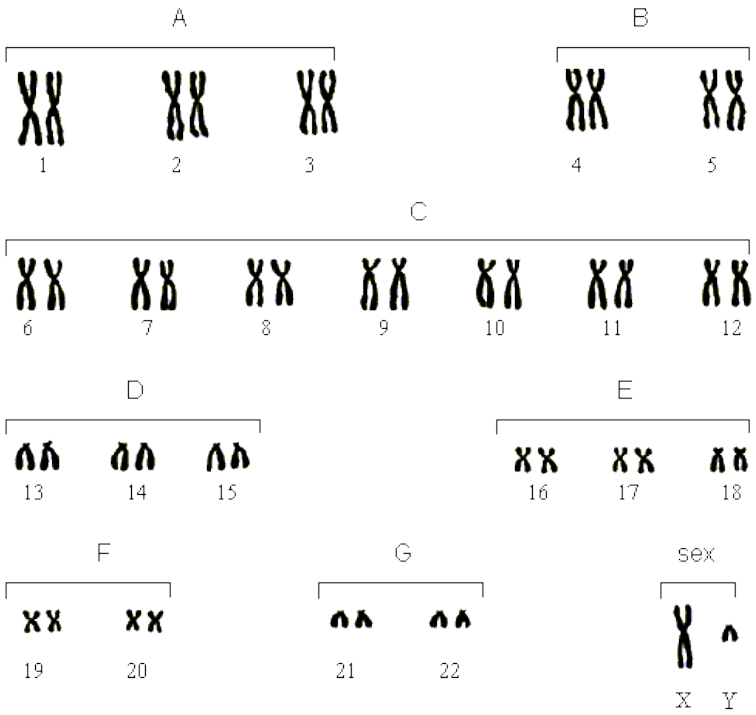
## Classification of human chromosomes.

The classification and nomenclature were determined by a convention of experts held at Denver, Colorado in 1960. In according to this recommendation:

1. The chromosomes are arranged in order of decrease of their size, except the sex chromosomes.
2. The chromosomes with similar sizes within one group are arranged in order of decrease of their centromere indexes. Centromere indexes - is expressed by length of short arm to length of all chromosomes ratio. It is expressed in percentages.
3. The autosomes are numbered 1 to 22.
4. The sex chromosomes of human and mammalies XX and XY are not numbered and placed at the end.

**DENVER CLASSIFICATION OF CHROMOSOMES (DENVER, 1960)**

| GROUP NUMBER | NUMBER OF CHROMOSOMES IN KARYOTYPE | DESCRIPTION OF CHROMOSOMES |
|---|---|---|
| A (I) | 1, 2, 3 | These are largest in size, metacentric, and near metacentric. |
| B (II) | 4, 5 | These are largest in size, submetacentric. |
| C (III) | 6 - 12 and X-chromosome | These are medium-sized, submetacentric. |
| D (IV) | 13, 14, 15 | These are short-sized and acrocentric |
| E (V) | 16, 17, 18 | These are short-sized, submetacentric. |
| F (VI) | 19, 20 | These are short-sized, metacentric. |
| G (VII) | 21, 22, Y-chromosome | These are smallest in size and acrocentric |




Any variation from the normal structure or number of chromosomes may lead to developmental abnormalities.

**THE QUESTIONS FOR SELF-CONTROL.**

***1. Nucleus as information center of eukaryotic cell.***

***2. Structure and functions of nuclear envelope and nucleolus.***

***3. Chromatin, structure and functions. Heterochromatin and euchromatin.***
***4. Levels of chromatin packing in eukaryotes and their characteristics.***
***5. Morphological structure of metaphase chromosome.***
***6. Morphological types of chromosomes.***
***7. Definition of karyotype. Human karyotype.***
***8. Cytogenetic method of studying heredity***
***9. Denver classification of human chromosomes.***

# *Mutations*

A mutation is the permanent alteration of the nucleotide sequence of the genes or any changes of structure or number of chromosomes.

Mutations are caused by mutagens. They are:

**Physical mutagens**

- Ionizing radiations such as X-rays, gamma rays and alpha particles cause DNA breakage and other damages.
- Ultraviolet radiations with wavelength above 260 nm are absorbed strongly by bases, producing pyrimidine dimers, which can cause error in replication if left uncorrected.
- Radioactive decay, such as 14C in DNA which decays into nitrogen.

**Chemicals – any chemical agents that may change the structure of DNA:**

- Hydroxylamine
- Base analogs (e.g., Bromodeoxyuridine (BrdU))
- Alkylating agents (e.g., N-ethyl-N-nitrosourea (ENU)). These agents can mutate both replicating and non-replicating DNA.
- Agents that form DNA adducts (e.g., ochratoxin A)
- DNA intercalating agents (e.g., ethidium bromide)
- DNA crosslinkers
- Oxidative damage
- Nitrous acid converts amine groups on A and C to diazo groups, altering their hydrogen bonding patterns, which leads to incorrect base pairing during replication.

**Biological agents**

- Virus – Virus DNA may be inserted into the genome and disrupts genetic function.
- Bacteria – some bacteria such as Helicobacter pylori cause inflammation during which oxidative species are produced, causing DNA damage and reducing efficiency of DNA repair systems, thereby increasing mutation.

## Classification of mutations.

***Mutations can be classified in two major ways:***

- Hereditary mutations are inherited from a parent and are present throughout a person's life in virtually every cell in the body. These mutations are also called germline mutations because they are present in the parent's egg or sperm cells, which are also called germ cells. When an egg and a sperm cell unite, the resulting fertilized egg cell receives DNA from both parents. If this DNA has a mutation, the child that grows from the fertilized egg will have the mutation in each of his or her cells.
- Acquired (or somatic) mutations occur at some time during a person's life and are present only in

certain cells, not in every cell in the body. These changes can be caused by environmental factors such as ultraviolet radiation from the sun, or can occur if an error is made as DNA copies itself during cell division. Acquired mutations in somatic cells (cells other than sperm and egg cells) cannot be passed to the next generation.

***According to the origin the mutations can be classified in two ways:***

a) spontaneous mutations;

b) induced mutations.

Mutations arise spontaneously at low frequency owing to the chemical instability of purine and pyrimidine bases and to errors during DNA replication. Natural exposure of an organism to certain environmental factors, such as ultraviolet light and chemical carcinogens (e.g., aflatoxin B1), also can cause mutations.

A common cause of spontaneous point mutations is the deamination of cytosine to uracil in the DNA double helix. Subsequent replication leads to a mutant daughter cell in which a T-A base pair replaces the wild-type C-G base pair. Another cause of spontaneous mutations is copying errors during DNA replication. Although replication generally is carried out with high fidelity, errors occasionally occur.

In order to increase the frequency of mutation in experimental organisms, researchers often treat them with high doses of chemical mutagens or expose them to ionizing radiation. Mutations arising in response to such treatments are referred to as induced mutations. Generally, chemical mutagens induce point mutations, whereas ionizing radiation gives rise to large chromosomal abnormalities.

***Based on their phenotype:***

a) Recessive mutations requiring two copies of the mutated allele to manifest the phenotype
b) Dominant, i.e. one or two copies of the mutated allele produces the phenotype
c) Semidominant, i.e. one mutant allele produces an intermediate phenotype

***Based on the effect of mutation on the gene structure:***

a) small-scale mutations (gene mutation, point mutation);

b) large scale mutations (chromosome mutation)

**Small-scale mutations (gene mutation, point mutation)**

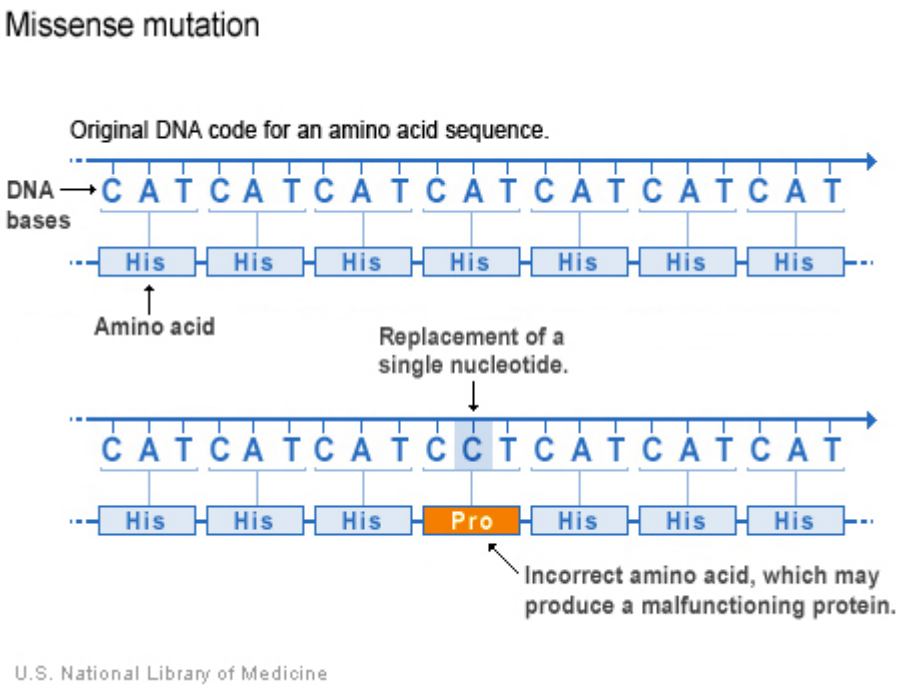
Small-scale mutations affect a gene in one or a few nucleotides. (If only a single nucleotide is affected, they are called point mutations.)

Small-scale mutations include:

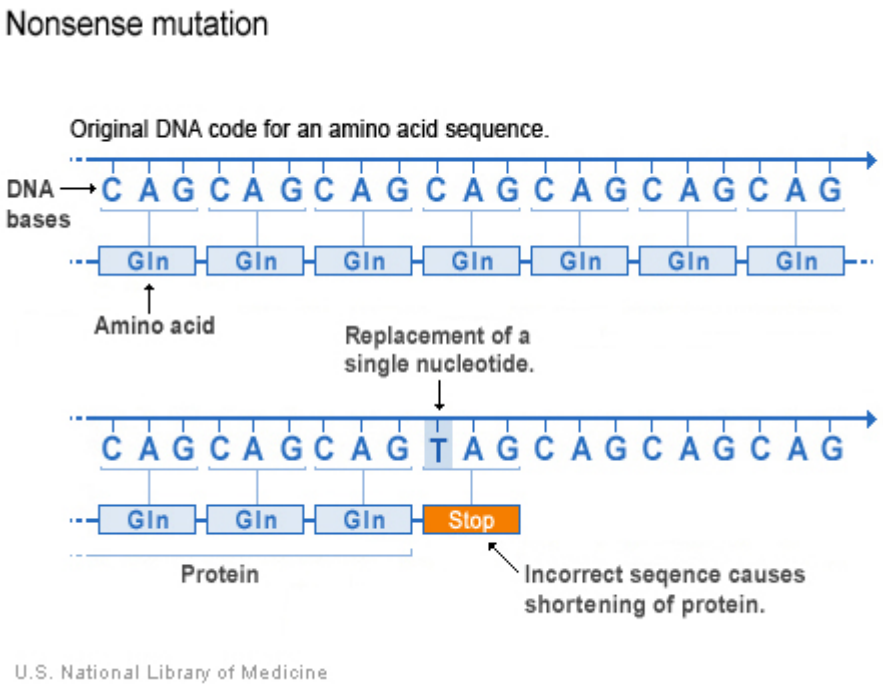
- **Insertions** add one or more extra nucleotides into the DNA. They are usually caused by transposable elements, or errors during replication of repeating elements. Insertions in the coding region of a gene may alter splicing of the mRNA (splice site mutation), or cause a shift in the reading frame (frameshift), both of which can significantly alter the gene product. Insertions can be reversed by excision of the transposable element.
- **Deletions or/Deficiency** remove one or more nucleotides from the DNA. Like insertions, these mutations can alter the reading frame of the gene. In general, they are irreversible.
- **Substitution mutations**, often caused by chemicals or malfunction of DNA replication, exchange a single nucleotide for another. These changes are classified as:
  - ***transitions*** exchanges a purine for a purine (A ↔ G) or a pyrimidine for a pyrimidine, (C ↔ T). A transition can be caused by nitrous acid, base mispairing, or mutagenic base analogs such as bromodeoxyuridine.
  - ***transversions*** exchanges a purine for a pyrimidine or a pyrimidine for a purine (C/T ↔ A/G). An example of a transversion is the conversion of adenine (A) into a cytosine (C).

Point mutations that occur within the protein coding region of a gene may be classified as:

**Missense mutation**. One nucleotide in gene is exchanged to another which results in a substitution of one amino acid to another in a protein.



**A nonsense mutation** is also a change in one DNA base pair. Instead of substituting one amino acid for another, however, the altered DNA sequence prematurely signals the cell to stop building a protein. This type of mutation results in a shortened protein that may function improperly or not at all.

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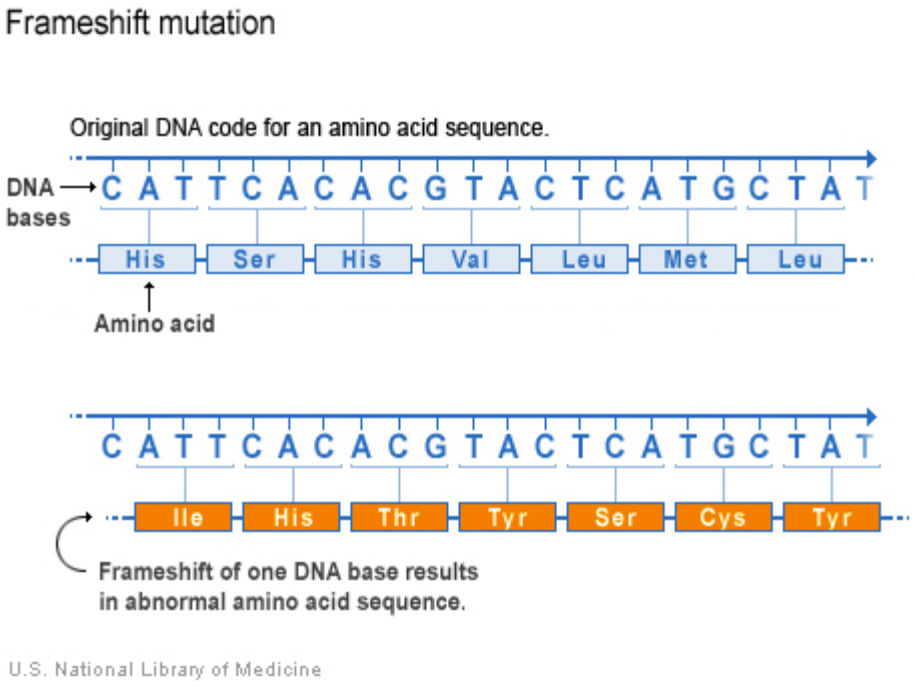
**Frameshift mutation**. This type of mutation occurs when the addition (insertion) or loss (deletion) of DNA bases changes a gene's reading frame. A reading frame consists of groups of 3 bases that each code for one amino acid. A frameshift mutation shifts the grouping of these bases and changes the code for amino acids. The resulting protein is usually nonfunctional. Insertions, deletions, and duplications can all be frameshift mutations

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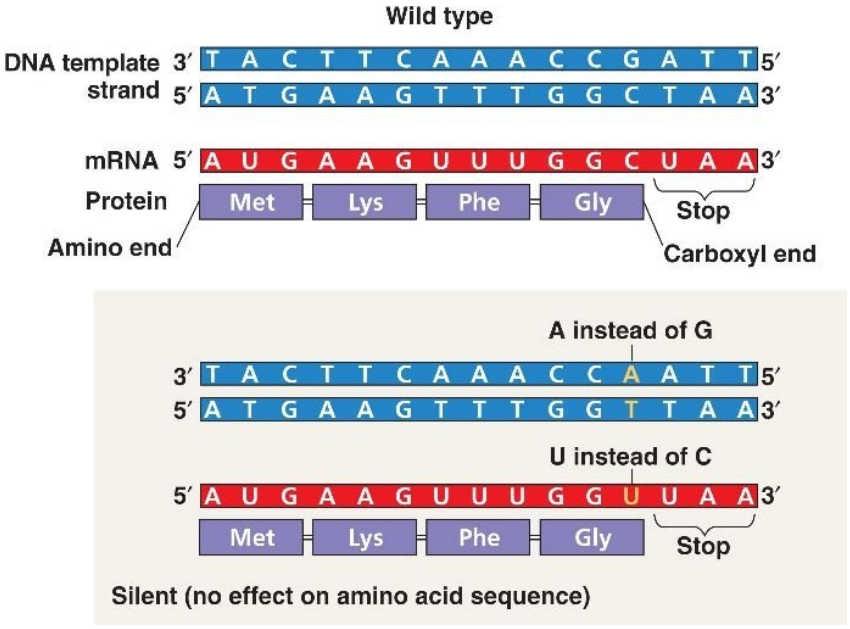
**Silent mutations** are mutations in DNA that do not have an observable effect on the organism's phenotype. It may be result by degeneracy of gene code.




## DNA Repair Mechanisms

Because DNA is the repository of genetic information in each living cell, its integrity and stability are essential to life. DNA, however, is not inert; rather, it is a chemical entity subject to assault from the environment, and any resulting damage, if not repaired, will lead to mutation and possibly disease.

DNA repair processes exist in both prokaryotic and eukaryotic organisms, and many of the proteins involved have been highly conserved throughout evolution. In fact, cells have evolved a number of mechanisms to detect and repair the various types of damage that can occur to DNA, no matter whether this damage is caused by the environment or by errors in replication.

### *Nucleotide Excision Repair*

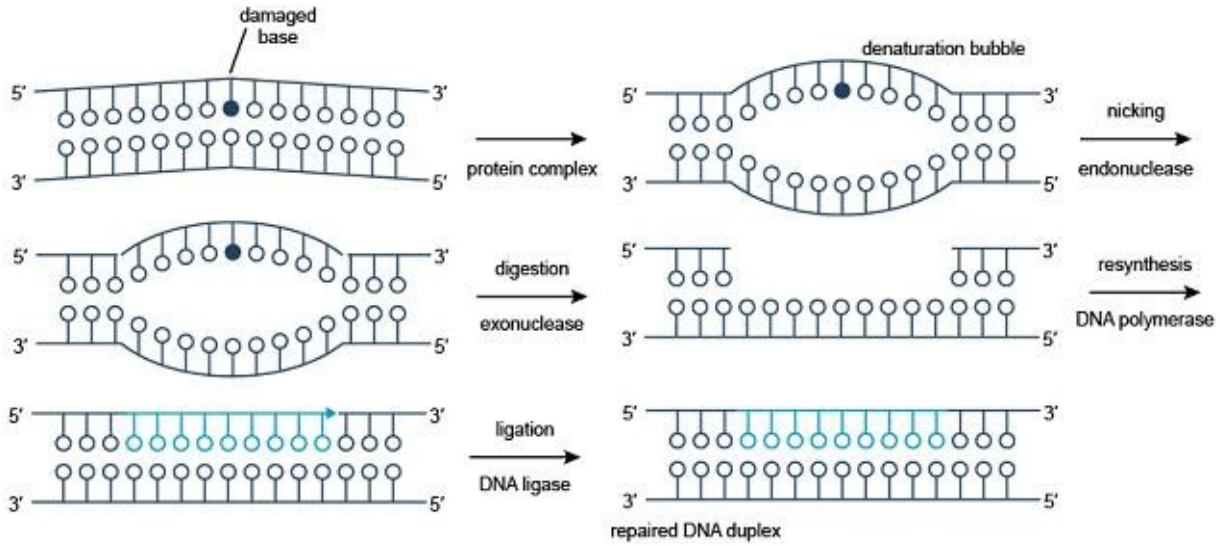
While base excision repair is a specialised type of repair that identifies damages to DNA bases, nucleotide excision repair (NER) is a generic type of excision repair mechanism.

NER detects damages based on the overall structure integrity of the DNA double helix. This allows NER to be able to support and repair multiple types of DNA damage.

Eukaryotic NER is carried out by at least 18 protein complexes via several discrete steps:

1) NER recognises distortion in the double helix with the aid of damage surveillance protein complexes.
2) Helicases opens the region around the damaged DNA base, creating a bigger distortion. This process helps to further verify the occurrence of the DNA damage.
3) Upon confirmation of damage, incision is performed by endonuclease enzymes to remove the region of damage base(s) and its surrounding neighbours.

4) With the removal of damaged bases, DNA polymerase synthesize the correct nucleotide to fill the region.
5) Lastly, DNA ligase helps to close and seal the bond.



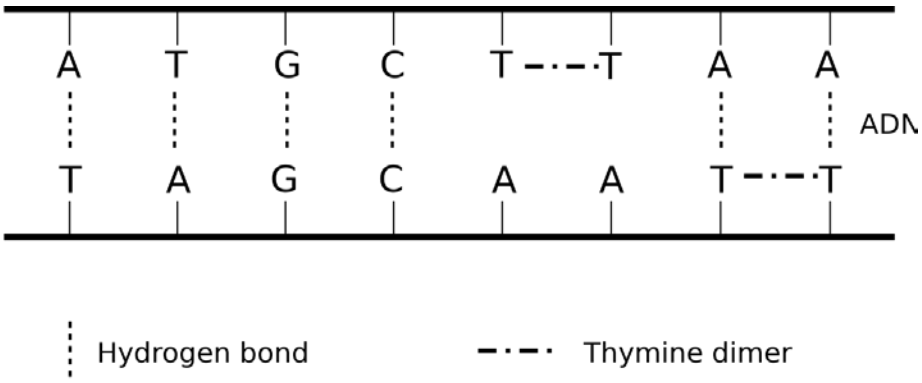

***Photoreactivation***.

Kelner discovered that the UV effect on genetic material can be reversed before the genetic material is permanently affected. This can be done by exposing the cells after UV treatment to visible light. This repair phenomenon is called photoreactivation. This effect is observed is lower organisms like bacteria, protozoans, algae, etc.

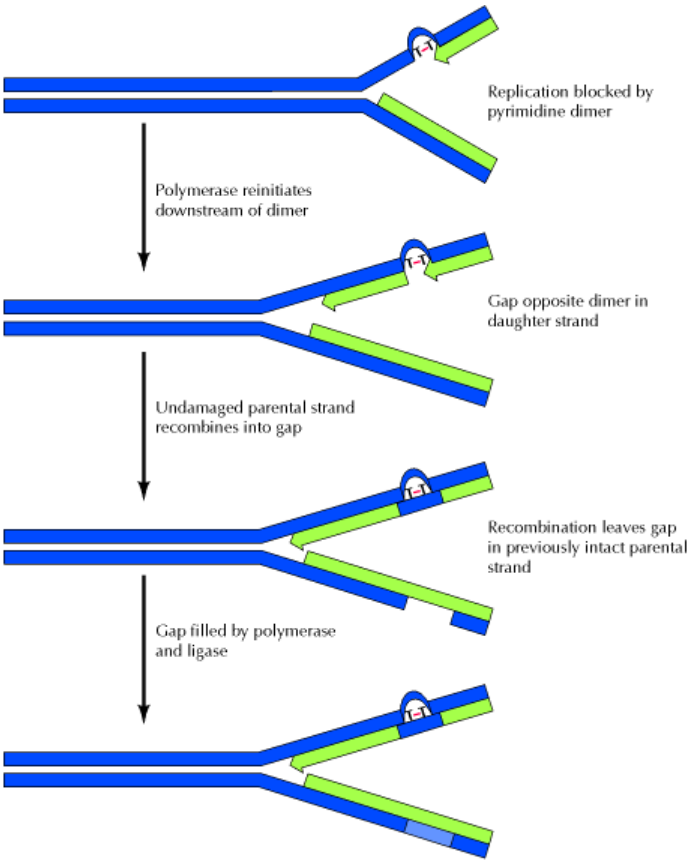
According to Setlow and Setlow, there are specific enzymes which split the dimers of pyrimidine bases produced by UV, thus restoring the normal DNA helix. The enzyme responsible for converting the thymine dimer into two thymine monomers is known as the photoreactivating enzyme. The enzyme absorbs a photon of visible light before breaking the dimer and hence is so named, although it can bring about the conversion of a dimer into monomers in the dark. The enzyme is present in all organisms including man.

**Post-replication repair.**

The post-replication repair mechanism has been studied in Escherichia coli, and is equally applicable in higher organisms. Photoreactivation and excision repair mechanisms, although efficient, still leave some thymine dimers in the DNA strand. Thymine dimers are produced when adjacent thymidine residues are covalently linked by exposure to utraviolet radiation.




The presence of a thymine dimer blocks replication, but DNA polymerase can bypass the lesion and reinitiate replication at a new site downstream of the dimer which is referred as postdimer initiation. The result is a gap opposite the dimer in the newly synthesized DNA strand. In recombinational repair, this gap is filled by recombination with the undamaged parental strand. Although this leaves a gap in the previously intact parental strand, the gap can be filled by the actions of polymerase and ligase, using the intact daughter strand as a template. Two intact DNA molecules are thus formed, and the remaining thymine dimer eventually can be removed by excision repair. It is otherwise known as daughter strand gap or sister strand gap repair system because only the gaps formed opposite to dimers, rather than the dimers themselves, are repaired. Since recombination repair occurs after DNA replication, in contrast with excision repair, it has been called as postreplicational repair.




The repair mechanisms are the result of biological adaptation during the evolutionary process. If repair mechanism did not exist, there would not have been any life on the earth which is being constantly exposed to sunlight and gaseous pollutants. Photoreactivation seems to be the first developed repair mechanism followed by the excision and the post-replication repair mechanisms. More than one repair system ensures the neutralization on harmful effects of UV and other mutagens. In case one mechanism fails, the other can take over.

## Large-scale mutations (chromosome mutation)

A chromosome mutation is an unpredictable change that occurs in a chromosome. These changes are most often brought on by problems that occur during meiosis or by mutagens (chemicals, radiation, etc.).

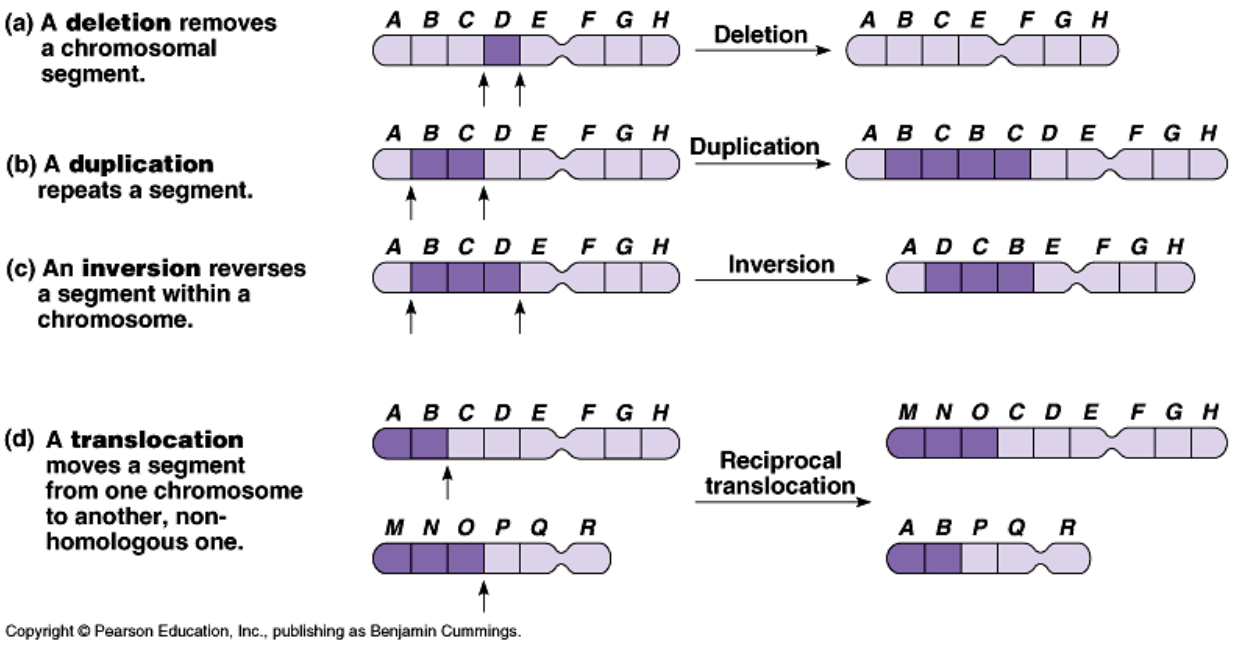
Chromosome mutations can result in changes in the number of chromosomes in a cell or changes in the structure of a chromosome. Unlike a gene mutation which alters a single gene or larger segment of DNA on a chromosome, chromosome mutations change and impact the entire chromosome. Large-scale mutations in chromosomal structure include:

### Chromosome Structure Changes

Duplications and breakages of chromosomes are responsible for a type of chromosome mutation that alters chromosome structure. These changes affect protein production by changing the genes on the chromosome. Chromosome structure changes are often harmful to an individual leading to developmental difficulties and even death. Some changes are not as harmful and may have no significant effect on an individual.

There are several types of chromosome structure changes that can occur. Some of them include:

- ***Deletion***: This mutation results from the breakage of a chromosome in which the genetic material becomes lost during cell division. The genetic material can break off from anywhere on the chromosome.
- ***Duplication***: Duplications are produced when extra copies of genes are generated on a chromosome.
- ***Inversion***: In an inversion, the broken chromosome segment is reversed and inserted back into the chromosome. If the inversion encompasses the centromere of the chromosome, it is called a pericentric inversion. If it involves the long or short arm of the chromosome and does not include the centromere, it is called a paracentric inversion.
- ***Translocation***: The joining of a fragmented chromosome to a non-homologous chromosome is a translocation. The piece of chromosome detaches from one chromosome and moves to a new position on another chromosome. There are two main types of translocations:
    - *Reciprocal translocation*: Segments from two different chromosomes have been exchanged.
    - *Robertsonian translocation*: An entire chromosome has attached to another at the centromere. So two acrocentric chromosomes form one metacentric or submetacentric.

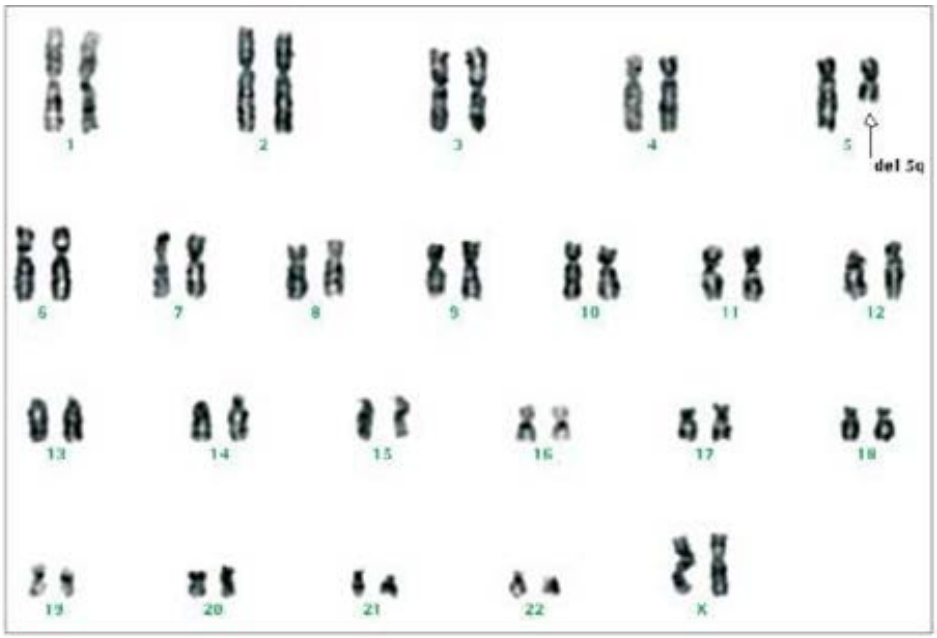




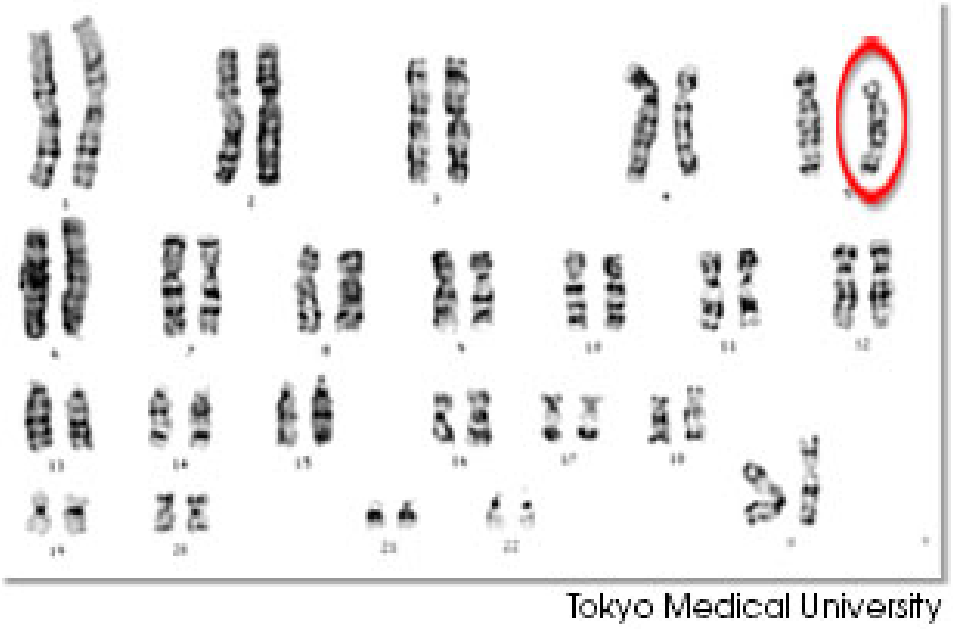

## Examples of Chromosome Structure Changes

### *5q minus (5q-) syndrome*

Deletion of a region of DNA from the long (q) arm of chromosome 5 is involved in a condition called 5q minus (5q-) syndrome. This deletion occurs in immature blood cells during a person's lifetime and affects one copy of chromosome 5 in each cell. 5q- syndrome is a type of bone marrow disorder called myelodysplastic syndrome, in which immature blood cells fail to develop normally. Individuals with 5q- syndrome often have a shortage of red blood cells (anemia) and abnormalities in blood cells called megakaryocytes, which produce platelets, the cells involved in blood clotting. Affected individuals also have an increased risk of developing a fast-growing blood cancer known as acute myeloid leukemia.



***Cri-du-chat (cat's cry) syndrome*** is caused by a deletion of the end of the short (p) arm of chromosome 5. This chromosomal change is written as 5p- (5p minus). The signs and symptoms of cri-du-chat syndrome are probably related to the loss of multiple genes in this region. Researchers are working to determine how the loss of these genes leads to the features of the disorder. They have discovered that in people with cri-du-chat syndrome, larger deletions tend to result in more severe intellectual disability and developmental delays than smaller deletions. Researchers have also defined regions of the short arm of chromosome 5 that are associated with particular features of cri-du-chat syndrome. A specific region designated 5p15.3 is associated with a cat-like cry, and a nearby region called 5p15.2 is associated with intellectual disability, small head size (microcephaly), and distinctive facial features.

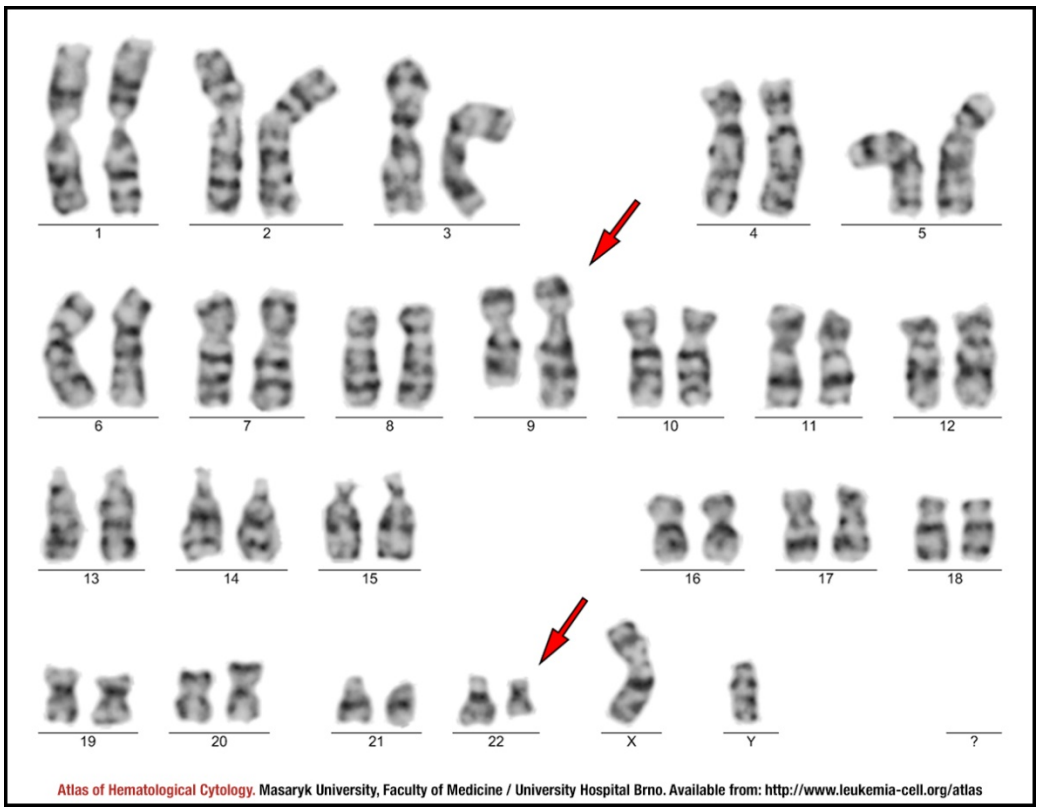



***Chronic myeloid leukemia***

A rearrangement (translocation) of genetic material between chromosomes 9 and 22 causes a type of cancer of blood-forming cells called chronic myeloid leukemia. This slow-growing cancer leads to an overproduction of abnormal white blood cells. Common features of the condition include excessive tiredness (fatigue), fever, weight loss, and an enlarged spleen.

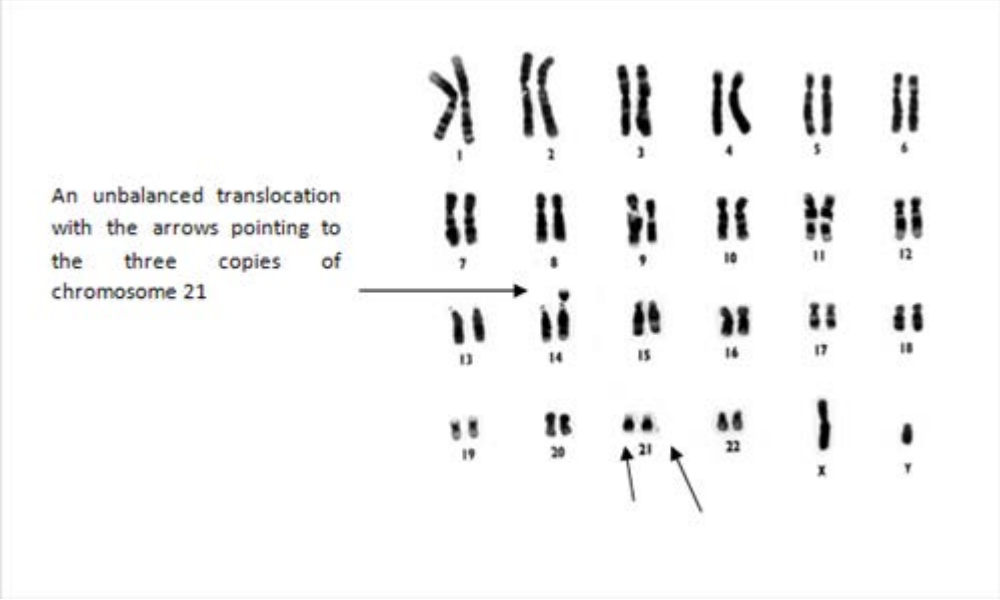
The translocation involved in this condition, written as t(9;22), fuses part of the ABL1 gene from chromosome 9 with part of the BCR gene from chromosome 22, creating an abnormal fusion gene called BCR-ABL1. The abnormal chromosome 22, containing a piece of chromosome 9 and the fusion gene, is commonly called the Philadelphia chromosome. The translocation is acquired during a person's lifetime and is present only in the abnormal blood cells. This type of genetic change, called a somatic mutation, is not inherited.

The protein produced from BCR-ABL1 gene signals cells to continue dividing abnormally and prevents them from self-destructing, which leads to overproduction of the abnormal cells.




***Translocation Down syndrome***

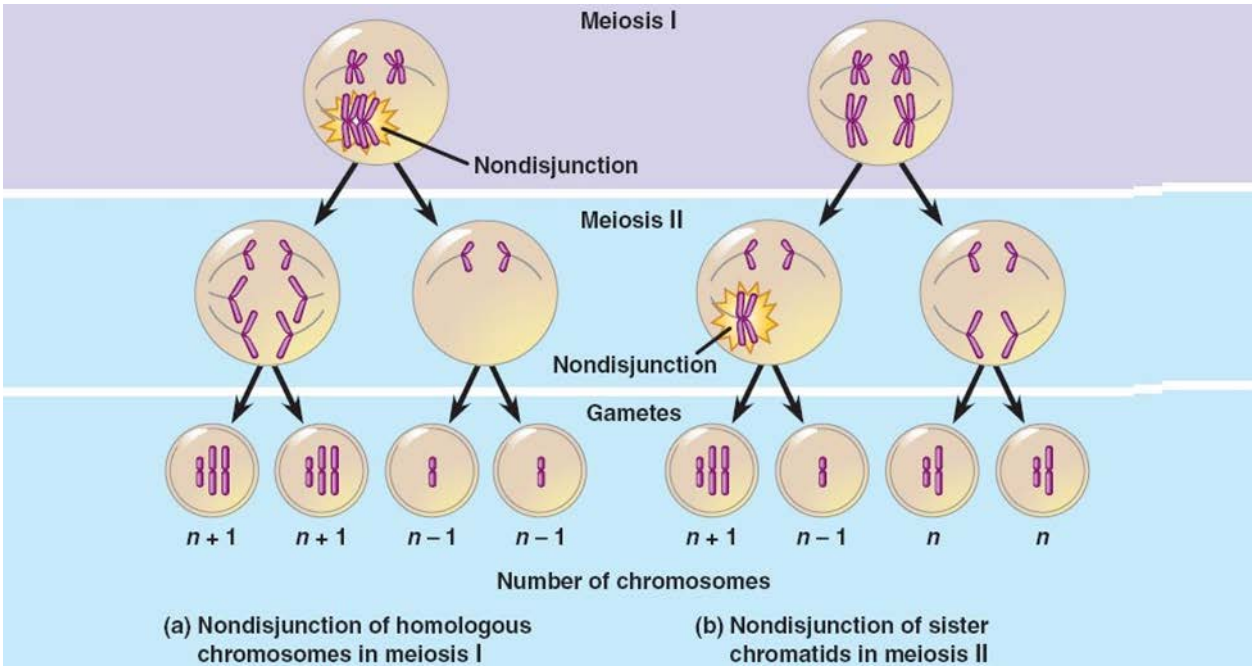
Down syndrome occurs when part of chromosome 21 becomes attached (translocated) to another chromosome during the formation of reproductive cells (eggs and sperm) or very early in fetal development. Affected people have two copies of chromosome 21 plus extra material from chromosome 21 attached to another chromosome, resulting in three copies of genetic material from chromosome 21. Affected individuals with this genetic change are said to have translocation Down syndrome.



## Chromosomal Number Mutations (Aneuploidy)

Whole single chromosomes are lost or gained from cells entering or undergoing meiosis which results in the change in chromosome number.

These changes arise from nondisjunction.




**Nondisjunction** is the failure of homologous chromosomes or sister chromatids to separate properly during cell division.

There are three forms of nondisjunction:

1) failure of a pair of homologous chromosomes to separate in meiosis I
2) failure of sister chromatids to separate during meiosis II
3) failure of sister chromatids to separate during mitosis.

Nondisjunction results in daughter cells with abnormal chromosome numbers (**aneuploidy**). Two common types of aneuploidy have their own special names:

- ***Monosomy*** is when an organism has only one copy of a chromosome that should be present in two copies (2n-1).
- ***Trisomy*** is when an organism has a third copy of a chromosome that should be present in two copies (2n+1)

Aneuploidy also includes cases where a cell has larger numbers of extra or missing chromosomes, as in nullisomy (2n - 2), polysomy (2n + 2 or 2n +3 and etc.).

However, if there is an entire extra chromosome set (e.g., 3n, 4n…), this is not formally considered to be aneuploidy. Organisms with more than two complete sets of chromosomes are said to be **polyploid**. If a mutation causes a cell to have three haploid sets, it is called *triploidy*. If the cell

has four haploid sets, it is called *tetraploidy*.

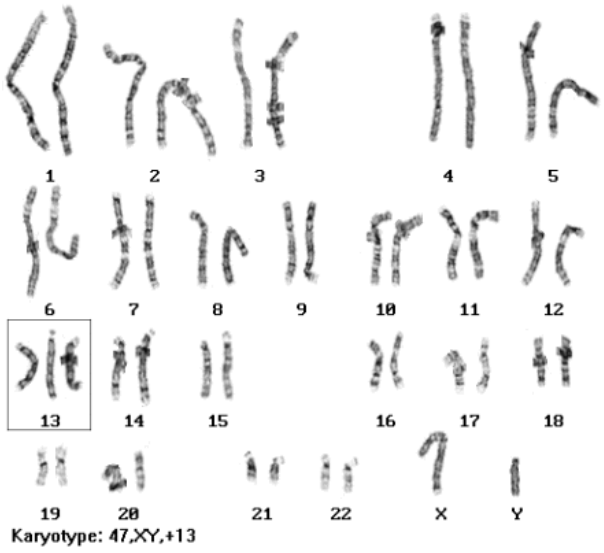
If a mutation causes a cell with haploid set of  chromosome instead of normal diploid, it is called **haploidy**.

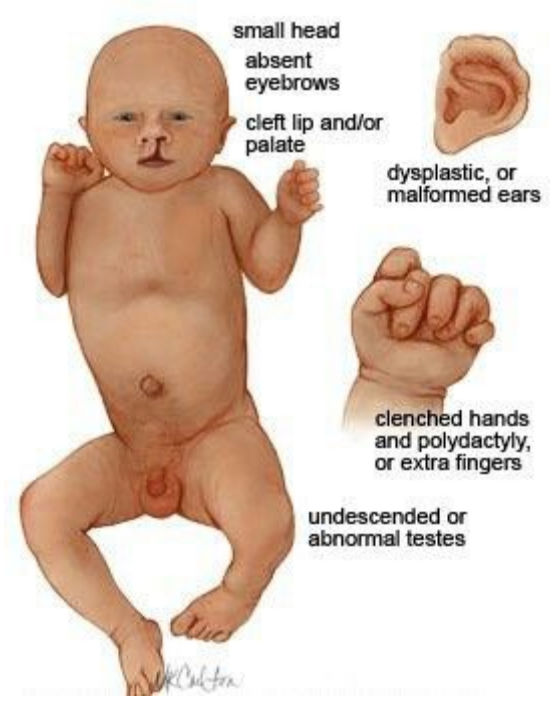
## EXAMPLES OF CHROMOSOMAL NUMBER MUTATIONS (ANEUPLOIDY)

***Patau syndrome (47, XY +13 or 47, XX, +13).***

Patau syndrome is the result of trisomy 13, meaning each cell in the body has three copies of chromosome 13 instead of the usual two. A small percentage of cases occur when only some of the body's cells have an extra copy; such cases are called mosaic Patau.

Infants with trisomy 13 have distinct clinical features, including growth retardation, cleft lip/palate, eye anomalies (microphthalmia, anophthalmia), scalp defects, structural brain anomalies (holoprosencephaly, absence of corpus callosum, olfactory bulbs), and postaxial polydactyly. Congenital heart defect is common and is reported in almost 80% of infants. Septal defects are the most common abnormalities. About half of the infants die within the first month of life and only 5–10% of babies survive beyond the first year of life.

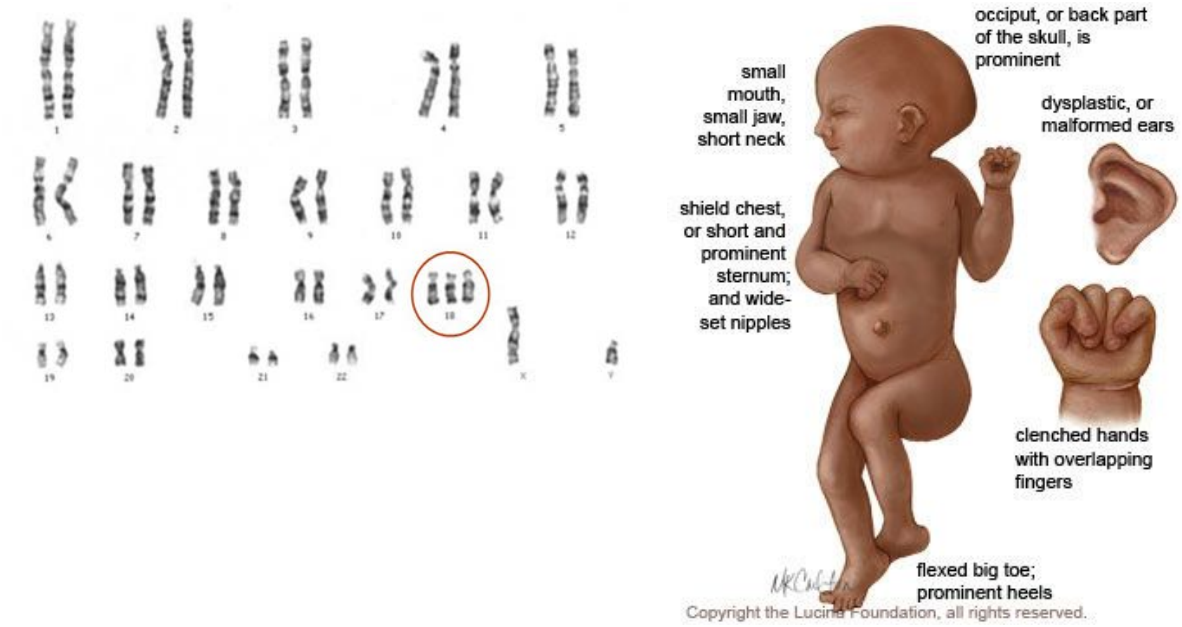



***Edwards syndrome (47, XY +18 or 47, XX, +18).***

Trisomy 18, also called Edwards syndrome, is a chromosomal condition associated with abnormalities in many parts of the body. Individuals with trisomy 18 often have slow growth before birth (intrauterine growth retardation) and a low birth weight. Affected individuals may have heart defects and abnormalities of other organs that develop before birth. Other features of

trisomy 18 include a small, abnormally shaped head; a small jaw and mouth; and clenched fists with overlapping fingers. Due to the presence of several life-threatening medical problems, many individuals with trisomy 18 die before birth or within their first month. Five to 10 percent of children with this condition live past their first year, and these children often have severe intellectual disability.




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

This condition is most often caused by trisomy 21. Trisomy 21 means that each cell in the body has three copies of chromosome 21 instead of the usual two copies.

Down syndrome is a chromosomal condition that is associated with intellectual disability, a characteristic facial appearance, and weak muscle tone (hypotonia) in infancy. All affected individuals experience cognitive delays, but the intellectual disability is usually mild to moderate.

People with Down syndrome may have a variety of birth defects. About half of all affected children are born with a heart defect. Digestive abnormalities, such as a blockage of the intestine, are less common.

Individuals with Down syndrome have an increased risk of developing several medical conditions. These include gastroesophageal reflux, which is a backflow of acidic stomach contents into the esophagus, and celiac disease, which is an intolerance of a wheat protein called gluten. About 15 percent of people with Down syndrome have an underactive thyroid gland (hypothyroidism). The thyroid gland is a butterfly-shaped organ in the lower neck that produces hormones. Individuals with Down syndrome also have an increased risk of hearing and vision problems. Additionally, a small percentage of children with Down syndrome develop cancer of blood-forming cells (leukemia).

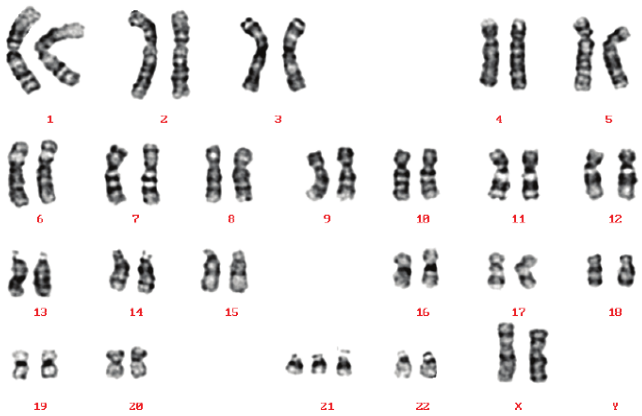
Delayed development and behavioral problems are often reported in children with Down syndrome. Affected individuals' speech and language develop later and more slowly than in children without Down syndrome, and affected individuals' speech may be more difficult to understand. Behavioral issues can include attention problems, obsessive/compulsive behavior, and

stubbornness or tantrums. A small percentage of people with Down syndrome are also diagnosed with developmental conditions called autism spectrum disorders, which affect communication and social interaction.

People with Down syndrome often experience a gradual decline in thinking ability (cognition) as they age, usually starting around age 50. Down syndrome is also associated with an increased risk of developing Alzheimer disease, a brain disorder that results in a gradual loss of memory, judgment, and ability to function. Approximately half of adults with Down syndrome develop Alzheimer disease. Although Alzheimer disease is usually a disorder that occurs in older adults, people with Down syndrome usually develop this condition in their fifties or sixties.

*Some common physical features of Down syndrome include:*

- A flattened face, especially the bridge of the nose
- Almond-shaped eyes that slant up
- A short neck
- Small ears
- A tongue that tends to stick out of the mouth
- Tiny white spots on the iris (colored part) of the eye
- Small hands and feet
- A single line across the palm of the hand (palmar crease)
- Small pinky fingers that sometimes curve toward the thumb
- Poor muscle tone or loose joints
- Shorter in height as children and adults





***Klinefelter syndrome (47,XXY)***

Klinefelter syndrome is a chromosomal condition in boys and men that can affect physical and intellectual development. It is caused by an extra copy of the X chromosome. Boys and men

with Klinefelter syndrome have the usual single Y chromosome plus two copies of the X chromosome, for a total of 47 chromosomes in each cell (47,XXY).

Some people with features of Klinefelter syndrome have an extra X chromosome in only some of their cells; other cells have one X and one Y chromosome. In these individuals, the condition is described as mosaic Klinefelter syndrome (46,XY/47,XXY). Boys and men with mosaic Klinefelter syndrome may have milder signs and symptoms than those with the extra X chromosome in all of their cells, depending on what proportion of cells have the additional chromosome.

Several conditions resulting from the presence of more than one extra sex chromosome in each cell are sometimes described as variants of Klinefelter syndrome. These conditions include 48,XXXY syndrome and 49,XXXXY syndrome (both described above). The features of these disorders tend to be more severe than those of Klinefelter syndrome and affect more parts of the body.

Most commonly, affected individuals are taller than average are unable to father biological children (infertile); however the signs and symptoms of Klinefelter syndrome vary among boys and men with this condition. In some cases, the features of the condition are so mild that the condition is not diagnosed until puberty or adulthood, and researchers believe that up to 75 percent of affected men and boys are never diagnosed.

Boys and men with Klinefelter syndrome typically have small testes that produce a reduced amount of testosterone (primary testicular insufficiency). Testosterone is the hormone that directs male sexual development before birth and during puberty. Without treatment, the shortage of testosterone can lead to delayed or incomplete puberty, breast enlargement (gynecomastia), decreased muscle mass, decreased bone density, and a reduced amount of facial and body hair. As a result of the small testes and decreased hormone production, affected males are infertile but may benefit from assisted reproductive technologies. Some affected individuals also have differences in their genitalia, including undescended testes (cryptorchidism), the opening of the urethra on the underside of the penis (hypospadias), or an unusually small penis (micropenis).

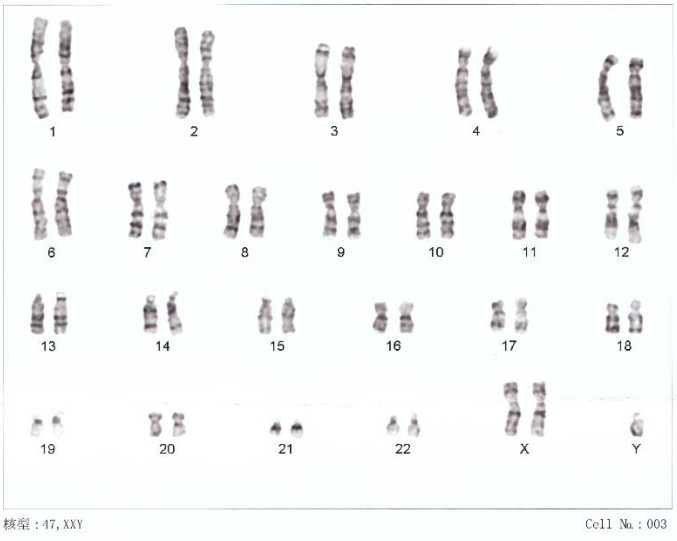
Other physical changes associated with Klinefelter syndrome are usually subtle. Older children and adults with the condition tend to be somewhat taller than their peers. Other differences can include abnormal fusion of certain bones in the forearm (radioulnar synostosis), curved pinky fingers (fifth finger clinodactyly), and flat feet (pes planus).

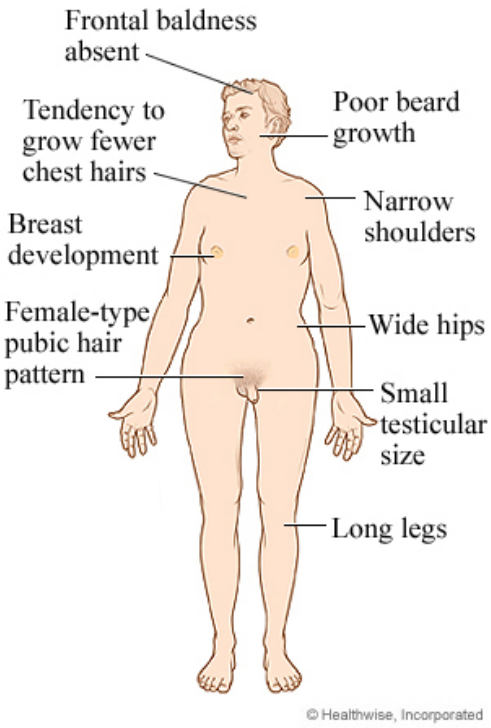
Children with Klinefelter syndrome may have low muscle tone (hypotonia) and problems with coordination that may delay the development of motor skills, such as sitting, standing, and walking. Affected boys often have learning disabilities, resulting in mild delays in speech and language development and problems with reading. Boys and men with Klinefelter syndrome tend to have better receptive language skills (the ability to understand speech) than expressive language skills (vocabulary and the production of speech) and may have difficulty communicating and expressing themselves.

Individuals with Klinefelter syndrome tend to have anxiety, depression, impaired social skills, behavioral problems such as emotional immaturity and impulsivity, attention-deficit/hyperactivity disorder (ADHD), and limited problem-solving skills (executive functioning). About 10 percent of boys and men with Klinefelter syndrome have autism spectrum disorder.

Nearly half of all men with Klinefelter syndrome develop metabolic syndrome, which is a

group of conditions that include type 2 diabetes, high blood pressure (hypertension), increased belly fat, high levels of fats (lipids) such as cholesterol and triglycerides in the blood. Compared with unaffected men, adults with Klinefelter syndrome also have an increased risk of developing involuntary trembling (tremors), breast cancer (if gynecomastia develops), thinning and weakening of the bones (osteoporosis), and autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis. (Autoimmune disorders are a large group of conditions that occur when the immune system attacks the body's own tissues and organs.)




***Turner syndrome (45,X0)***

Turner syndrome results when one normal X chromosome is present in a female's cells and the other sex chromosome is missing or structurally altered. The missing genetic material affects development before and after birth, leading to short stature, ovarian malfunction, and other features of Turner syndrome.

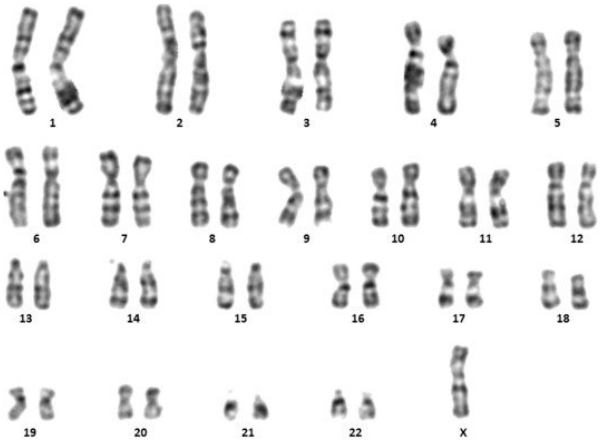
About half of individuals with Turner syndrome have monosomy X (45,X), which means each cell in an individual's body has only one copy of the X chromosome instead of the usual two sex chromosomes. Turner syndrome can also occur if one of the sex chromosomes is partially missing or rearranged rather than completely absent.

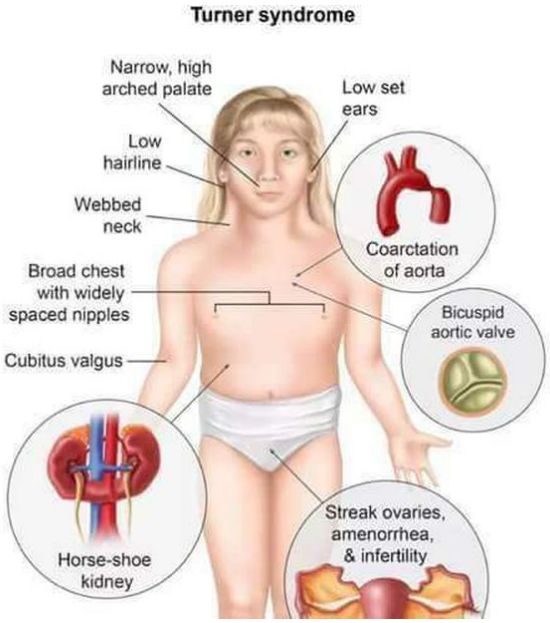
The most common feature of Turner syndrome is short stature, which becomes evident by about age 5. An early loss of ovarian function (ovarian hypofunction or premature ovarian failure) is also very common. The ovaries develop normally at first, but egg cells (oocytes) usually die prematurely and most ovarian tissue degenerates before birth. Many affected girls do not undergo puberty unless they receive hormone therapy, and most are unable to conceive (infertile). A small percentage of females with Turner syndrome retain normal ovarian function through young adulthood.

About 30 percent of females with Turner syndrome have extra folds of skin on the neck (webbed neck), a low hairline at the back of the neck, puffiness or swelling (lymphedema) of the

hands and feet, skeletal abnormalities, or kidney problems. One third to one half of individuals with Turner syndrome are born with a heart defect, such as a narrowing of the large artery leaving the heart (coarctation of the aorta) or abnormalities of the valve that connects the aorta with the heart (the aortic valve). Complications associated with these heart defects can be life-threatening.

Most girls and women with Turner syndrome have normal intelligence. Developmental delays, nonverbal learning disabilities, and behavioral problems are possible, although these characteristics vary among affected individuals.




**THE QUESTIONS FOR SELF-CONTROL.**

1. Characteristics of mutations.
2. Classification of mutations
3. Gene mutations. Characteristics. Classification. Examples.
4. Chromosomal Mutations. Characteristics of Structural Chromosomal Mutations. Classification. Examples
5. Characteristics of Chromosomal number Mutations. Classification. Examples.

# *Cell Division*

Cell is the structural and functional unit of life. One of the important properties of the living cells is their capacity to grow and divide. When cells grow to the maximum size, they usually, divide into two daughter cells. Remak and Virchow stated that cells always arose only from pre-existing cells. Hence, the process by which new cells are formed from the pre-existing cells is called **cell division.**

The life of Metazoans begins with a single cell (zygote) and multicellularity is achieved through repeated cell divisions. In multicellular organisms, there are two types of cells; the **somatic cells** or the body cells (which form the body of the organism) and the **reproductive cells** (such as *gamete* producing cells and *spore* producing cells).

The new *somatic* cells arise by **mitosis** (equational division) and the *reproductive cells* arise by **meiosis** (reduction division) Mitosis helps *in growth* and *development* of an organism. When body cells are destroyed, their *replacement* takes place only through cell divisions. Also cell divisions are necessary for *reproduction.* Meiosis produces gametes in sexual reproduction and spores in asexual reproduction.

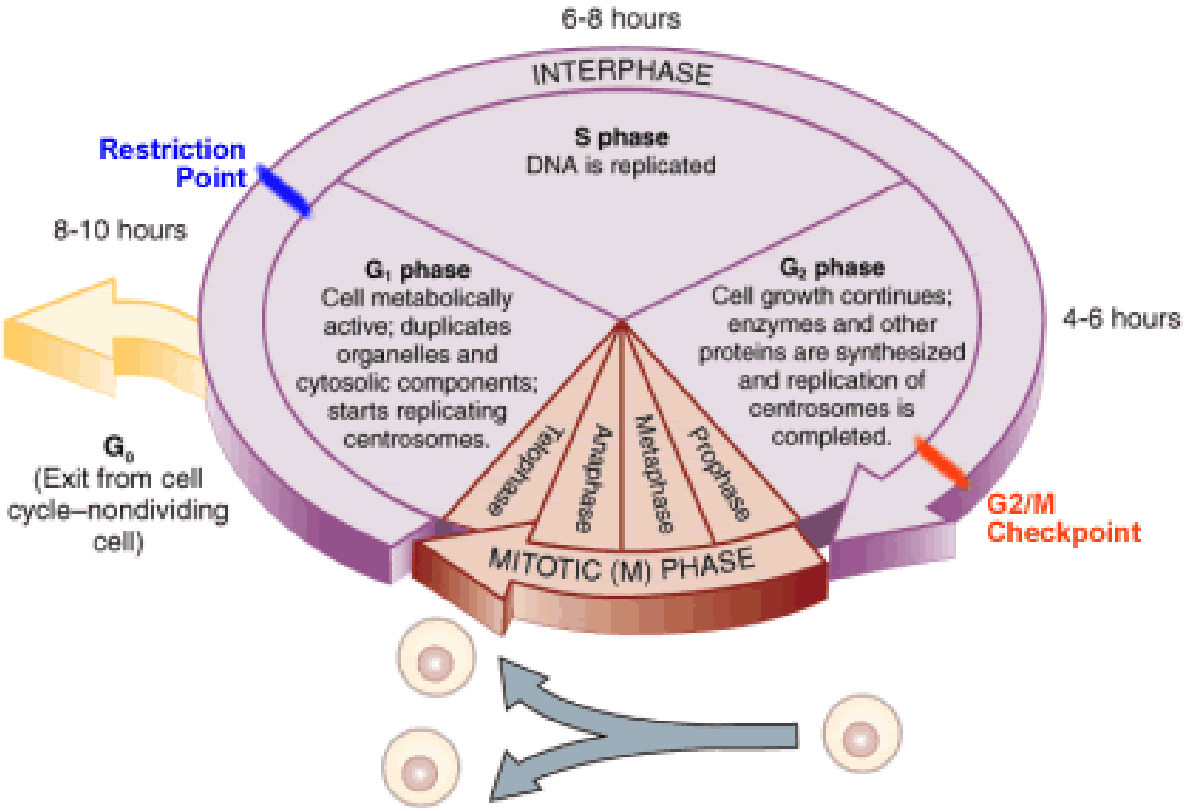
All eukaryotic organisms, plants as well as animals, show great regularity as well as similarity in the cell divisions. Generally cell increases in size before dividing. This is mainly due to the synthesis of proteins, RNA and DNA. This is followed by division of the cell nucleus *(karyokinesis)* and finally the division of the cell cytoplasm *(cytokinesis).* All these events collectively form a cell cycle. Sometimes the cell cycle is identical the life cycle of a cell.

The **life cycle of a cell** is cell ontogenesis, i.e. it refers to the period from the appearance of the cell, which arises after the previous division, to its own division or its death.

The **cell cycle** also called generation time is the sequence of events in the life of a cell, which starts immediately after one cell division and ends with the completion of the next division.

The cell cycle of eukaryotic cells is classified into

1. Interphase:
    a. G1
    b. S
    c. G2
2. Mitotic phase or M-phase(i.e. the cell division):
    a. karyokinesis
    b. cytokinesis




The *M-phase* refers to the period, from the beginning to the end of a cell division.

- *Karyokinesis* – the division of the parent nucleus into daughter nuclei.
- *Cytokinesis* – the division of the cytoplasm. It occurs after karyokinesis and divides the parent cell into daughter cells.
- *Interphase*– the interval between two mitotic phases or the preparatory phase during which cell is metabolically very active and prepares itself for the division.

As no visible changes occur in the nucleus, this phase was thought to be a resting phase, in the earlier days. It lasts for a very long period in the mitotic cycle. Recent investigations have revealed intensive activity in both the nucleus and cytoplasm, during this phase.

Three important processes occur in Interphase;

1. Replication of chromosomal DNA, synthesis of RNA and the basic nuclear proteins-histones;
2. Synthesis of energy rich compounds (ATP), which provides energy for mitosis;
3. Division of the centriole in animal cells.

On the basis of DNA synthesis, interphase is sub-divided into following three stages.

• ***G1 (Gap 1)***: It starts immediately after the previous division, but it is before the synthesis phase. Therefore G1 is called post- division gap phase or first growth phase or pre-synthetical gap phase. During this phase, transcription of t-RNA, r-RNA, and m-RNA occurs. These are required for the synthesis of many types of proteins. As a result, the cell grows in volume. Besides, various substances and enzymes required for DNA synthesis are assembled.

• ***S-phase (Synthesis phase)***: The important event in this phase is replication of DNA. The amount of DNA increases in two times. This results in doubling of the chromosomal threads. Also in this phase replication of centrioles occurs.

• ***G2 (Gap 2)***: It is the last part of interphase and follows after the synthesis phase, but occurs just before the new cell division. Hence G2 is called post- synthetical gap phase or pre-division gap phase or second growth phase. G2-phase: in this phase synthesis of protein of spindle fibers and energy rich compounds (ATP), which provides energy for cell division, taking place.

The total duration of a cell cycle varies greatly in different organisms and under different conditions, e.g. it may be as short as 20-30 minutes in the bacterium Escherichia coli or may take 12-24 hours as in most higher plants and animals.

The time required for completion of each phase in the cell cycle varies greatly. In general, actual cell division (M-phase) occupies only a short span of the total cycle while major span is occupied by the interphase. Normally, time duration of S and G2 phases is more or less equal. The duration of G1 is longer in cells, which are not divided frequently, and is very short in cells, which are divided repeatedly in close succession.

Therefore, there are two types of interphases.

Autosynthetical interphase is the interval between two divisions, when the cell prepares itself for the own division. It is characteristic for cells, which are divided repeatedly in close succession.

Heterosynthetical interphase is the time interval after the previous division, when the cell growths, develops, acquires the differentiation and begins to work in structure of the entire organisms. In future the cell is not divided until its death e.g. neurons.

Significance of cell cycle:

1. In multicellular organisms, the ‘cycling type' of cells (dividing cells) help in reproduction, growth and replacement of dead cells, healing of wounds, etc.
2. The interphase allows time for synthesis and growth of dividing cell.
3. Properly controlled and regulated cell cycle results in normal and proportionate growth of organisms. Loss of control over cell cycle can lead to cancerous growth.

## Mitosis

Mitosis is the characteristic division of the body cells, hence called somatic division.

***Mitosis is equational division, dividing the mother cell into two daughter cells which are identical to one another and also to the original mother cell in every respect. In mitosis, the chromosomes of the mother cell are distributed equally to the two daughter cells.***

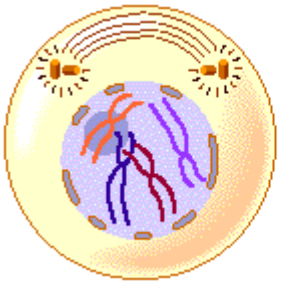
Karyokinesis: it involves a series of changes in the nucleus, which are visible under the microscope. This is a continuous process but, for convenience, it has been divided into 4 phases:

1) Prophase
2) Metaphase
3) Anaphase and
4) Telophase.

### *Prophase*

The centrioles, which have already undergone replication in the interphase, begin their journey in the opposite direction and reach the opposite poles of the cell. Between the centrioles long filamentous fibers, the primary spindle fibers are extended. A number of short fibers are also radiated from the centrioles. They are known as astral rays. A centriole with astral rays is called an aster. The asters along with the primary spindle fibers constitute the mitotic spindle or the mitotic apparatus (achromatic apparatus). During early prophase, the chromatin network becomes visible as separate threads or chromosomes. At this stage, each chromosome appears as a very fine, long single thread, chromonema and is described as the monad. The nuclear envelope and nucleolus are prominently visible.

As the prophase progresses, chromosomes become shorter and thicker (due to condensing of their coils). In each chromosome, the chromonema splits lengthwise into two identical threads or chromonemata. A substance called nuclear matrix accumulates around each chromonema. As a result, chromosomes become more. A chromonema surrounded by the matrix is called a *chromatid.* At this, each chromosome is shorter, thicker and rod-liked consists of two identical sister chromatids joined together by a spherical body called *centromere (kinetochore).* By the end of prophase, nuclear envelope and nucleolus disappear completely. The chromosomes are plunged into the cytoplasm.

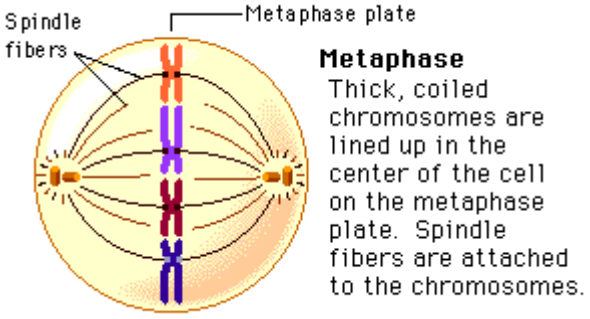


**Prophase**
The chromosomes appear condensed, and the nuclear envelope is not apparent.

### *Metaphase*

The chromosomes move to the equator of the cell. The chromosomes arrange in a plane along the equator of the cell in such order that in each chromosome, the two chromatids are

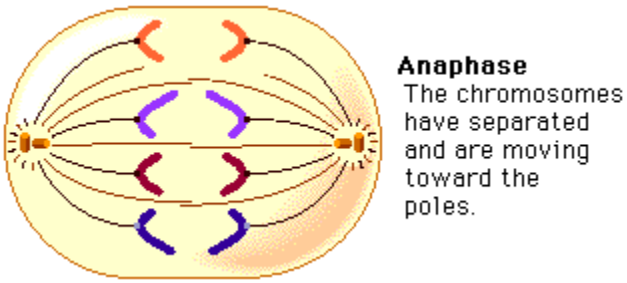
facing the opposite poles. This results in the formation of ***equatorial plate (metaphasic plate).*** The chromatids are still attached at the centromere. The spindle fibers are now attached to the centromere and are known as *"chromosomal fibers" or "half spindle fibers"*. By the time this phase comes to a close, the chromosomal coiling and condensation are completed and the mitotic spindle comes into full existence.




### *Anaphase*

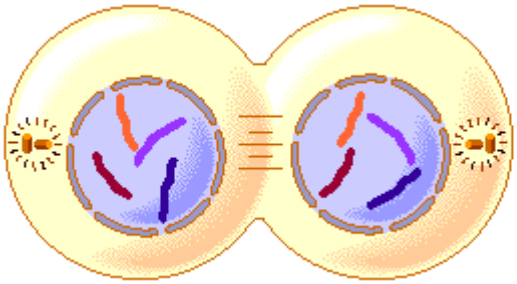
The centromere of each chromosome divides longitudinally into two. As a result, each chromosome is now completely divided into two identical halves (sister chromatids) called daughter chromosomes. The centromere of each daughter chromosome remains connected to the pole on its respective side by a chromosomal fiber.

Two groups of daughter chromosomes are pulled away from each other and begin to move to the opposite poles. Their movement depends on the contraction of the spindle fibers. During the journey towards the poles, the daughter chromosomes acquire shapes like 'L' or 'V'. The centromeres of the daughter chromosomes face the poles and their arms face the equatorial plane during their journey towards the poles. With the arrival of the daughter chromosomes at the poles, this phase concludes. The poles move apart, as the daughter chromosomes make their journey towards their perspective poles. The rate of movement of the daughter chromosomes is very slow. It is only 0.2 to 0.5 μm per minute.




### *Telophase*

After arriving at the poles, the daughter chromosomes are described as chromosomes. They gradually start loosing their condensation. The nucleolus and nuclear membrane reappear. Except for the centrioles, the mitotic apparatus undergoes dissolution and disappears gradually. Thus with the formation of daughter nuclei, the nuclear division comes to an end.



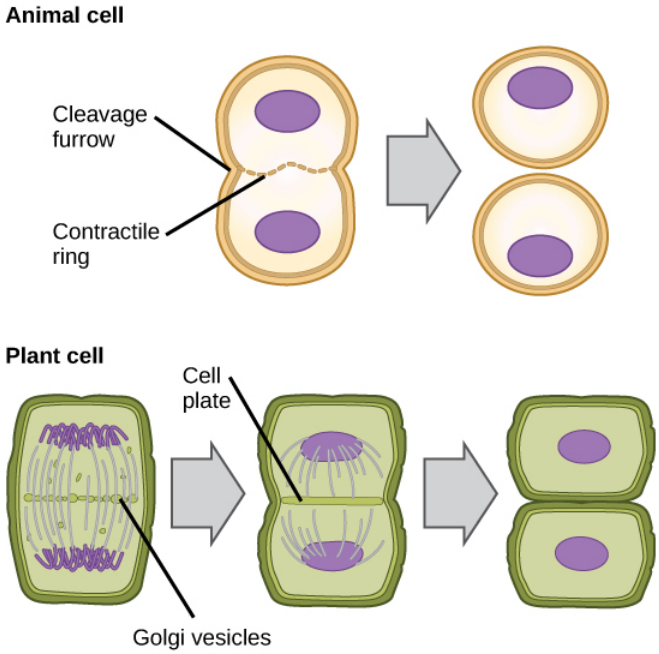
**Telophase**
The chromosomes are at the poles, and are becoming more difuse. The nuclear envelope is reforming. The cytoplasm may be dividing.

*Each daughter nucleus has the same number of chromosomes as that of the mother cell. The original structure of each chromosome is also retained unchanged in both the daughter nuclei. In other words, the two daughter nuclei are identical in structure and characters. They are also exact copies of the original parent nucleus.*

### Cytokinesis

In plant cells, cytokinesis usually begins with centrifugal formation of cell plate along the equatorial plane and is followed by new wall formation. It divides the mother cell into two equal daughter cells.

In animal cells, cytokinesis takes place by the cleavage constriction of the cell cytoplasm. It begins peripherally and progresses centripetally.




**The content of genetic material in the cell changes during the cell cycle.**

Keys used to denote it are: «n» is a haploid set of chromosomes and «c» is the number of DNA copies in haploid set of chromosomes.

G1 period: the content of genetic material is 2n2c.

S period: 2n4c.

G2 period: 2n4c.

Prophase: 2n4c

Metaphase: 2n4c

Anaphase: 4n4c.

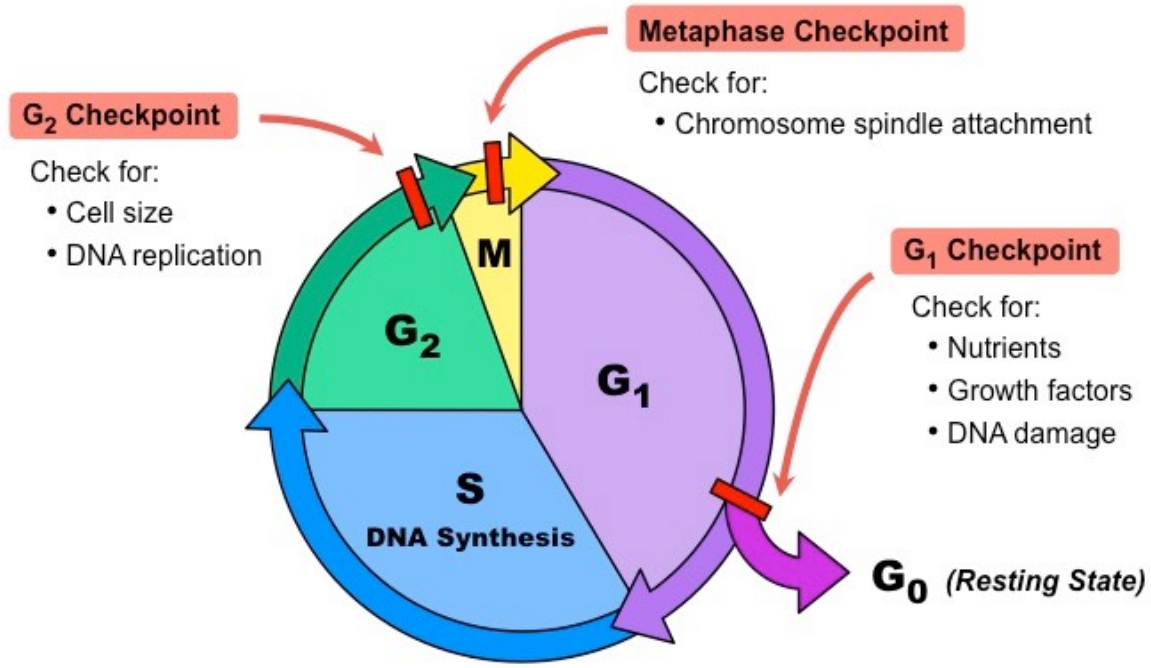
Telophase: 2n2c.

## SIGNIFICANCE OF MITOSIS

1. It is an equational division, which maintains equal distribution of chromosomes after each cell cycle.
2. The resulting daughter cells inherit identical chromosomal material (hereditary material) both in quantity (i.e. number) and quality (i.e. genetic make up or characters).
3. Mitosis maintains constant number of chromosomes in all body cells of an organism.
4. Newly formed cells through mitosis replace dead cells. It thus helps in the repair of the body.
5. In plants and some animals, it is involved in the asexual reproduction.
6. It plays an important role in regeneration.
7. It participates in the growth and development of organisms.

## Regulation of the Cell Cycle

Cell cycle checkpoints are control mechanisms in eukaryotic cells which ensure proper division of the cell. Each checkpoint serves as a potential point along the cell cycle, during which the conditions of the cell are assessed, with progression through the various phases of the cell cycle occurring when favorable conditions are met. Currently, there are three known checkpoints: the G1 checkpoint, also known as the restriction or start checkpoint or (Major Checkpoint); the G2/M checkpoint; and the metaphase checkpoint, also known as the spindle checkpoint.




***G1 (restriction) checkpoint***

A major cell cycle regulatory point in many types of cells occurs late in G1 and controls progression from G1 to S.

A decision point in late G1, called the restriction point in animal cells. The passage of animal cells through the cell cycle is regulated primarily by the extracellular growth factors that signal cell proliferation, rather than by the availability of nutrients. In the presence of the appropriate growth factors, cells pass the restriction point and enter S phase. Once it has passed through the restriction point, the cell is committed to proceed through S phase and the rest of the cell cycle, even in the absence of further growth factor stimulation.

On the other hand, if appropriate growth factors are not available in G1, progression through the cell cycle stops at the restriction point. Such arrested cells then enter a quiescent stage of the cell cycle called G0, in which they can remain for long periods of time without proliferating. G0 cells are metabolically active, although they cease growth and have reduced rates of protein synthesis. As already noted, many cells in animals remain in G0 unless called on to proliferate by appropriate growth factors or other extracellular signals.

For example, skin fibroblasts are arrested in G0 until they are stimulated to divide as required to repair damage resulting from a wound. The proliferation of these cells is triggered by platelet-derived growth factor, which is released from blood platelets during clotting and signals the proliferation of fibroblasts in the vicinity of the injured tissue.

DNA damage also slows the progression of cells through S phase and arrests cell cycle progression at a checkpoint in G1. This G1 arrest may allow repair of the damage to take place before the cell enters S phase, where the damaged DNA would be replicated. In mammalian cells, arrest at the G1 checkpoint is mediated by the action of a protein known as p53, which is rapidly induced in response to damaged DNA. Interestingly, the gene encoding p53 is frequently mutated in human cancers. Loss of p53 function as a result of these mutations prevents G1 arrest in response to DNA damage, so the damaged DNA is replicated and passed on to daughter cells instead of being repaired. This inheritance of damaged DNA results in an increased frequency of mutations

and general instability of the cellular genome, which contributes to cancer development. Mutations in the p53 gene are the most common genetic alterations in human cancers, illustrating the critical importance of cell cycle regulation in the life of multicellular organisms.

***G2 checkpoint***

This checkpoint arrests cells in G2 in response to damaged or unreplicated DNA.

G2 checkpoint senses unreplicated DNA, which generates a signal that leads to cell cycle arrest. Operation of the G2 checkpoint therefore prevents the initiation of M phase before completion of S phase, so cells remain in G2 until the genome has been completely replicated. Only then is the inhibition of G2 progression relieved, allowing the cell to initiate mitosis and distribute the completely replicated chromosomes to daughter cells.

Progression through the cell cycle is also arrested at the G2 checkpoint in response to DNA damage, such as that resulting from irradiation. This arrest allows time for the damage to be repaired, rather than being passed on to daughter cells.

***Metaphase checkpoint***

Another important cell cycle checkpoint that maintains the integrity of the genome occurs toward the end of mitosis. This checkpoint monitors the alignment of chromosomes on the mitotic spindle, thus ensuring that a complete set of chromosomes is distributed accurately to the daughter cells. For example, the failure of one or more chromosomes to align properly on the spindle causes mitosis to arrest at metaphase, prior to the segregation of the newly replicated chromosomes to daughter nuclei. As a result of this checkpoint, the chromosomes do not separate until a complete complement of chromosomes has been organized for distribution to each daughter cell.

## AMITOSIS.

Amitosis (amitotic division, direct cell division) is a direct division of nucleus with following cell division without formation chromosomes and mitotic spindle apparatus. Amitosis is studded now. It occurs in cells of mammalian embryonic envelopes, and cells of malignant tumors, usually in Protozoa.

In amitosis the nucleus elongates and becomes dumb-bell-shaped, then it divides into two nuclei, the cytoplasm constricts into two parts, half going to each nucleus thus two daughter cells are forms.

A main distinction between amitosis and mitosis is the genetic material is distributed not equivalent between cells.

## ENDOMITOSIS

This is a closed mitosis. It is a process in which the number of chromosomes increases in the nucleus without destruction of nuclear membrane, nucleolus and forming of mitotic spindle apparatus. And then the division of cytoplasm is not occurred.

Endomitosis occurs at intensively functioning cells (e.g. the cells of liver). Endomitosis leads to polyploidy and polythenia.

**THE QUESTIONS FOR SELF-CONTROL.**

***1. Cell cycle, its division into periods.***
***2. Types of cell division.***
***3. Mitotic cycle.***
***4. Morphological description of stages of mitotic cycle.***
***5. Interphase, its stages and their biological role.***
***6. Characteristics of mitotic phases.***
***7. Genetic description of stages of mitotic cycle and mitotic phases.***
***8. The change of morphological and genetical structure of chromosomes during mitotic cycle.***
***9. The significance of morphological changes of chromosomes.***
***10. Cytokinesis.***

## Meiosis

***Meiosis*** is a special type of cell division of cells in which, the diploid number of chromosomes is reduced to haploid in the daughter cells.

In meiosis, chromosomes divide once while the nucleus (and in some cases the cytoplasm also) divides twice. Four haploid daughter cells result from one diploid mother cell. These differ from each other as well as from the mother cell.

Meiosis occurs in the gonads of sexually reproducing animals, only at the time of gamete formation. Through meiotic division, the primary spermatocytes (2n) and primary oocytes (2n) produce the germ cells sperms (n) and eggs (n) respectively. Because of the reducing nature of this division, the germ cells receive only a haploid number (n) or half the number of chromosomes.

As the germ cells have only a haploid number of chromosomes, the zygote, a product of the fusion of two gametes, regains the diploid number of chromosomes (2n). Thus as the diploid number is restored, the sexually reproducing organisms maintain a constant chromosomal number.

For example, the diploid number of chromosomes in man is 46. The sperms and eggs produced through meiotic division receive only half that number and when they fuse, the diploid number is restored again in the zygote. Thus the chromosomal number remains constant in man generation after generation. The chromosomes having the same gene sequence are known as *homologous chromosomes*. In the diploid cells, they occur in pairs. Offspring receive one homologous chromosome from each parent (paternal and maternal chromosomes).

Meiosis consists of two divisions — meiosis I and meiosis II. Each division has four phases: prophase, metaphase, anaphase and telophase.

## Meiosis I (reduction division)

**Prophase-I:** This is the longest phase in meiosis and involves some very important events. Prophase-I is sub-divided into five sub-phases:

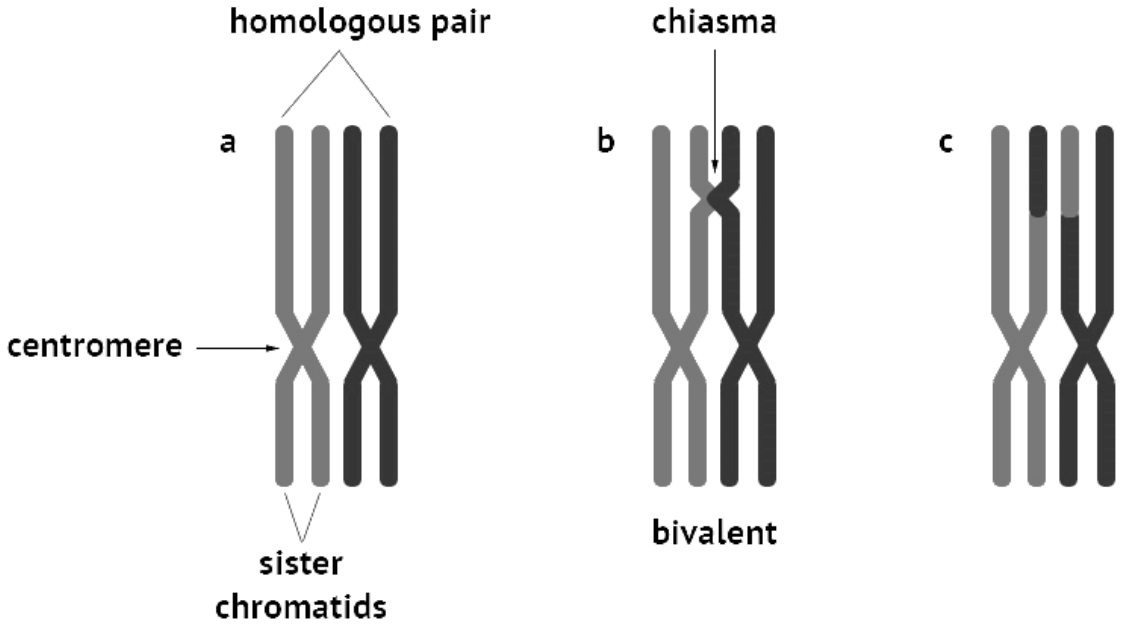
- ***Leptotene:*** Due to gradual coiling and condensation the chromonema gradually acquires the shape of a chromosome. As replication of chromonema already takes place in the Interphase, each chromosome consists of two chromatids joined at the centromere. Only partial condensation of chromosomes is completed by the end of Leptotene. Beaded structures, chromomeres appear on chromosomes, i.e. each thread like chromosome The chromosomal ends or telomeres remain in contact with the nuclear membrane. The nuclear envelope and the nucleolus are prominently visible. The thin chromosomes are scattered in the nucleus.
- ***Zygotene:*** An important change occurs in this phase. From the two sets the homologous chromosomes (one paternal and the other maternal) are attracted towards each other and form pairs. In each pair, the two homologues lie parallel to each other all along their lengths. This pairing is called synapsis or synaptic pairing. The complex structure thus produced is known as synaptonemal complex. The mechanism of pairing up of the chromosomes, the homologous chromosomes recognizing each other and the perfect alignment of the pairing chromosomes is not known. Due to synapsis of homologous chromosomes, the genes lie apposed-gene for gene. As the chromosomal complex consists of two homologous chromosomes, it is also known as a "bivalent". As four chromatids are present in a bivalent, it is also known as a "tetrad".
- ***Pachytene:*** Though Leptotene and Zygotene last for a few hours, Pachytene phase is often extended for days and weeks. During this period the homologous chromosomes are held closely together in the synaptonemal complex. Due to increased condensation, the chromosomes are more clearly visible now. Now, the chromatids of the two homologous chromosomes often exchange exactly equal segments between them. This phenomenon is called crossing over.

Thus, **crossing over** *is a mutual exchange of equal quantity (segments) of chromosomal material between two non-sister chromatids.* It never takes place between sister chromatids.

***Crossing over has great evolutionary significance:***

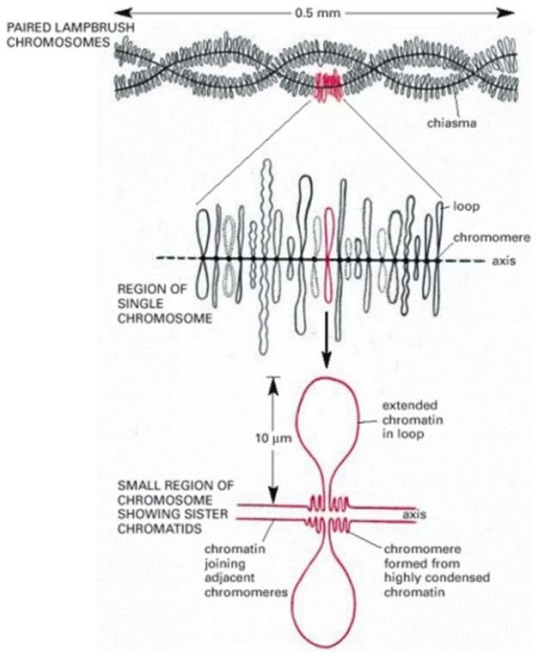
1) *The gametes produced through meiosis receive new combination of characters (genes);*
2) *Therefore, when the gametes fuse, individuals with new combination of characters are produced in each generation;*
3) *It forms the genetic basis for variations and plays important role in evolution.*

The regions where crossing over takes place are called chiasmata (singular chiasma). The number of chiasmata is formed corresponds to the length of the chromosomes.

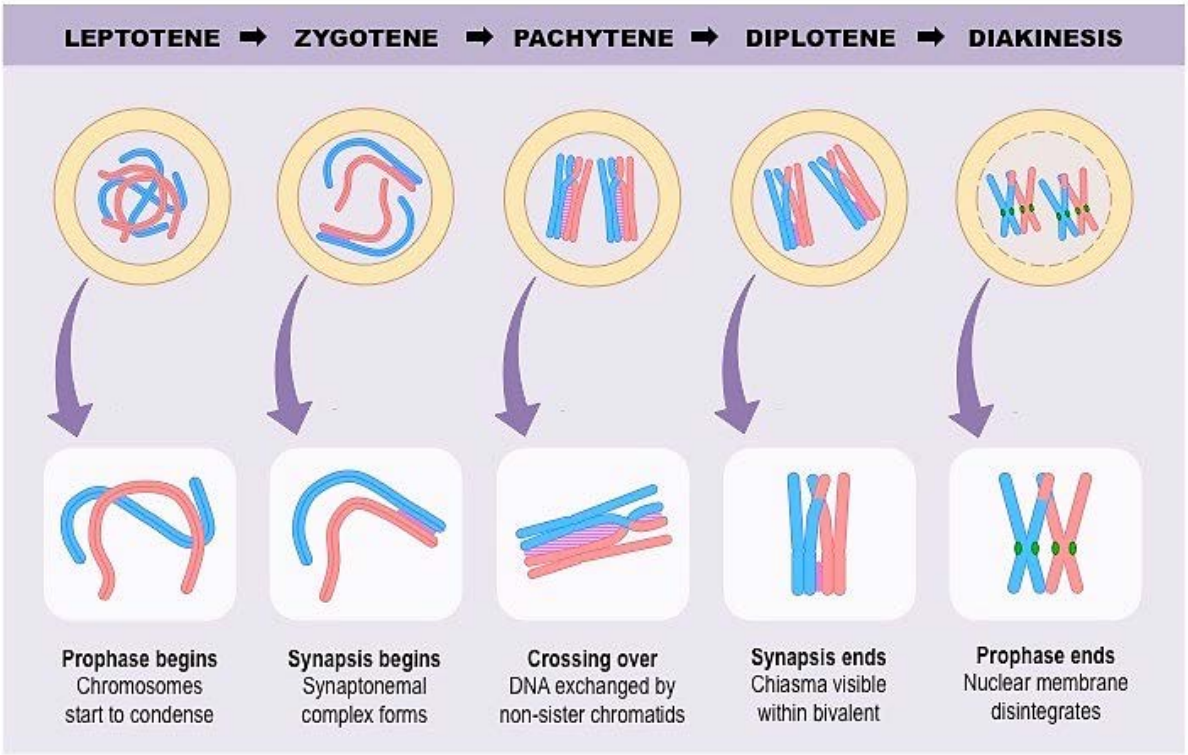



- ***Diplotene****:* In each pair, the homologous chromosomes start repelling each other. As a result, they begin to separate, but chromosomes stay bound in the area of chiasm (crossings). The separation of the homologues begins at the centromeres and proceeds towards the ends. This causes progressive shifting of the chiasmata towards the ends of the chromatids. This is called terminalization of chiasma.

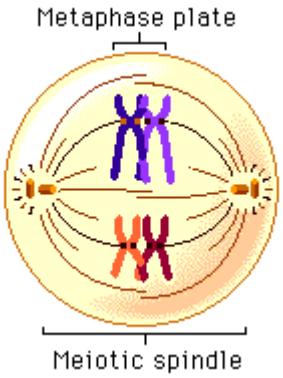
During the development of most of the primary oocytes in mammals, when they are in the diplotene stage, i.e. the chromosomes are paired and exchanging segments, they go into the restng phase, which is known as the ***dictyotene stage***. This stage is extremely long and the bulk of cell growth occurs. The chromosomes of primary oocytes become dispersed and the final configuration is called lampbrush - chromosomes**.** Scientists have observed intense activity of RNA synthesis on the loops of the lampbrush - chromosomes. The RNA synthesized during this phase continues to participate in protein synthesis beyond the stage of oogenesis.



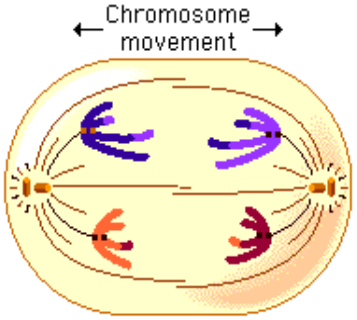

***- Diakinesis:*** This is the last phase of prophase - I. Chromosomes are still in pairs and in contact with each other by terminal chiasma. They re-appear through condensation, if they have undergone dispersion during Diplotene. Hence, the chromosomes become shorter, thicker and more prominent. By the end of prophase-I, the nuclear envelope and the nucleolus disappear completely and the pairs of chromosomes are seen scattered in the hyaloplasm. There is a formation of achromatic apparatus in the cytoplasm.




**Metaphase - I:** The homologous chromosomes, still in pairs, move on to the equatorial region. By this time, the mitotic spindle formation is completed. The bivalents or paired chromosomes are arranged along the equatorial plane in such way that in each pair, the two homologues are facing the opposite poles. In every pair, the centromere of each chromosome (homologue) is connected to the pole on its respective side only. As the paired chromosomes are arranging themselves along the equatorial plane, the base is being laid down naturally and automatically for an important phenomenon of free and independent assortment of chromosomes.



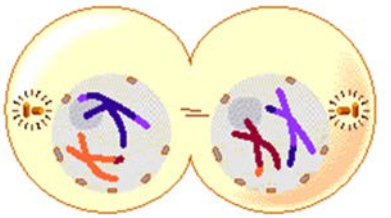

**Anaphase-I:** Now the homologous chromosomes are separated from each other and move to the opposite poles, due to the contraction of spindle fibers. Thus, the homologous [maternal and paternal] chromosomes move to opposite poles.




*The main difference between the mitotic and meiotic divisions can be seen at this stage. In the mitotic division, the chromosomes divide into two chromatids (daughter chromosomes) and move to the opposite poles. But in the*

*case of meiotic division, the entire chromosomes (having two chromatids) journey to the poles.*

**Telophase-I:** Usually the chromosomes disperse into chromatin, after reaching the poles and the nucleolus and nuclear membrane reappear. The achromatic apparatus disappear, except for the centrosomes. After cytokinesis two daughter cells with haploid number of chromosomes (having two chromatids) are produced.



Hence, **M-I is called reduction division**

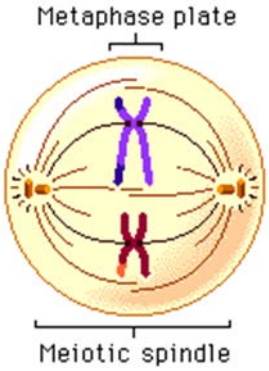
**Interkinesis:** The time interval between M-I and M-II is called interkinesis. The main peculiarity of this period is the absence of DNA replication.

## Meiosis II (equational division)

Second meiotic division is similar to mitosis, i.e. it is an equational division in which there is division of the chromosomes. The two haploid daughter nuclei formed at the end of M-I divide during M-II and produce in all 4 haploid nuclei.

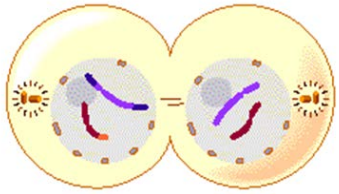
***Prophase-II:*** Except for some minor differences, it resembles mitotic prophase. Chromosomes are undergo recondensation. The chromosomes appear "X" - shaped and the chromatids are attached at the centromere only. The nucleolus and nuclear envelope disappear. A new achromatic spindle takes shape.

***Metaphase - II:*** The chromosomes move on to the equatorial plane. The chromosomes are arranged in a such way that their centromeres lie on the equatorial plane with the arms bent towards the poles. The chromosomal number is haploid. The spindle fibers get attached to the centromeres.



***Anaphase - II:*** The chromatids travel towards the poles, due to the contraction of the spindle fibers.

***Telophase - II:*** After reaching the poles, the chromatids are known as chromosomes. They lose condensation and disperse' as chromatin filaments. The nucleolus and nuclear envelope reappear and the mitotic spindle disappear. Thus, four daughter nuclei result at the end of Telophase - II**.** As a result of cyticinesis four daughter cells are produced. These cells carry only haploid number of chromosomes.



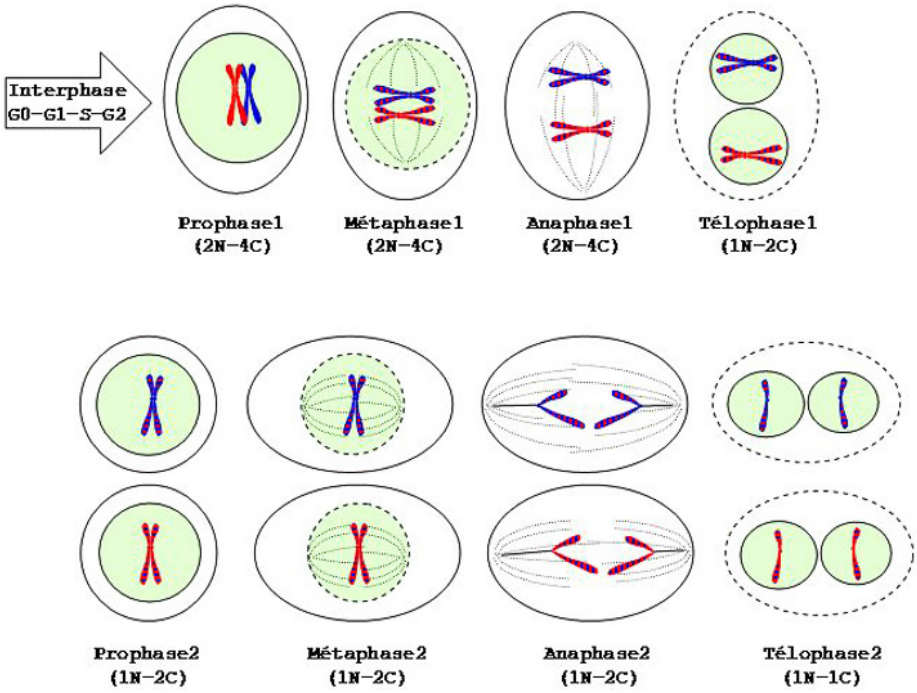
**The content of genetic material in the cell changes during meiosis.**

**Meiosis I**

- Prophase: 2n4c
- Metaphase: 2n4c
- Anaphase: 2n4c.
- Telophase: n2c.

**Meiosis II**

- Prophase: n2c
- Metaphase: n2c
- Anaphase: 2n2c.
- Telophase: nc.




## SIGNIFICANCE OF MEIOSIS

1. In sexually reproducing animals, meiotic division necessarily occurs during gamete formation, reducing the chromosomal number to half. This ensures constancy of the chromosomal number, generation after generation.
2. Due to crossing over and random assortment of chromosomes in the period of Anaphase-I and Anaphase-II recombination occur, resulting in variations.

**THE QUESTIONS FOR SELF-CONTROL.**

***1. Meiosis and its cytological and genetical mechanisms.***

***2. Biological role of meiosis.***

***3. Changes of morphological and genetical chromosome structures during meiosis.***

# *GAMETOGENESIS*

Gametogenesis is the process whereby a haploid cell (n) is formed from a diploid cell (2n) through meiosis and cell differentiation.

Gametogenesis in the male is known as **spermatogenesis** and produces spermatozoa. Gametogenesis in the female is known as **oogenesis** and result in the formation of ova. In this article we shall look at both spermatogenesis and oogenesis.

Both the process is basically similar, though minor differences exist. Both involve three important phases:

1) **multiplication phase**, in which the germ cells of the gonads multiply by mitosis;
2) **growth phase**, in which the germ cells growth is size;
3) **maturation phase**, in which meiosis takes place to produce the gametes.

But spermatogenesis include additional phase – **spermiogenesis**.

## Spermatogenesis

Males start producing sperm when they reach puberty, which is usually from 10-16 years old. Sperm are produced in large quantities (~200 million a day) to maximise the likelihood of sperm reaching the egg. Sperm are continually produced as males need to be ready to utilise the small window of fertility of the female.

Sperm production occurs in the testes of the male, specifically in the seminiferous tubules. The tubules are kept separate from the systemic circulation by the blood-testis barrier.

The blood-testis barrier is formed by Sertoli cells and is important in preventing hormones and constituents of the systemic circulation from affecting the developing sperm, and also in preventing the immune system of the male from recognising the sperm as foreign – as the sperm are genetically different from the male and will express different surface antigens. Sertoli cells also have a role in supporting the developing spermatozoa.

Spermatogenesis starts at puberty. Normal spermatogenesis is provided by Sertoli or nurse cells, and by Leydig cells. During spermatogenesis, the developing sperms keep their heads embedded in the Sertoli cells to draw nourishment from them. And Leydig cells produce the male hormone testosterone, that induces spermatogenesis.

Spermatogenesis consist of 4 phases:

1) **In multiplication phase** the germ cells; which are called ***spermatogonia*** proliferated by mitotic divisions from the primary germ cells of the germinal epithelium lining the seminiferous tubules. The spermatogonia have nuclei which contain diploid number of chromosomes. The spermatogonia increase their population by repeated mitotic divisions so that, each newly-formed

spermatogonium possesses the same number of chromosomes (2n2c). One of these cells will be used to replenish the pool of spermatogonia – these cells are A1 spermatogonia. This replenishment of spermatogonia means that males are fertile throughout their adult life. The other cell – type B spermatogonium – will eventually form mature sperm. Type B spermatogonia replicate by mitosis several times to form identical diploid cells linked by cytoplasm bridges and enter to next phase.

2) **Growth phase**. This phase is similar with interphase. During this phase growth of cells and replication of DNA occur. Now the germ cells are named the **primary spermatocytes**. The amount of heredity material is 2n4c.

3) **Maturation phase**. The primary spermatocytes then enter into the maturation phase, where each cell divides by meiosis. Meiosis consists of two divisions. In the result of firth meiotic division each primary spermatocyte divides into two cells of equal size, which are called **secondary spermatocytes**. The amount of heredity material of this cells is n2c.

The secondary spermatocytes soon undergo the second meiotic division and produce **spermatids**. The amount of heredity material of this cells is nc.

4) The spermatids undergo **spermiogenesis** (remodelling and differentiation into mature spermatozoa) as they travel along the seminiferous tubules until they reach the epididymis.

During spermiogenesis, the spermatids begin to form a tail by growing microtubules on one of the centrioles, which turns into basal body. These microtubules form an axoneme. Later the centriole is modified in the process of centrosome reduction. The anterior part of the tail (called midpiece) thickens because mitochondria are arranged around the axoneme to ensure energy supply. Spermatid DNA also undergoes packaging, becoming highly condensed. The DNA is packaged firstly with specific nuclear basic proteins, which are subsequently replaced with protamines during spermatid elongation. The resultant tightly packed chromatin is transcriptionally inactive. The Golgi apparatus surrounds the now condensed nucleus, becoming the acrosome.

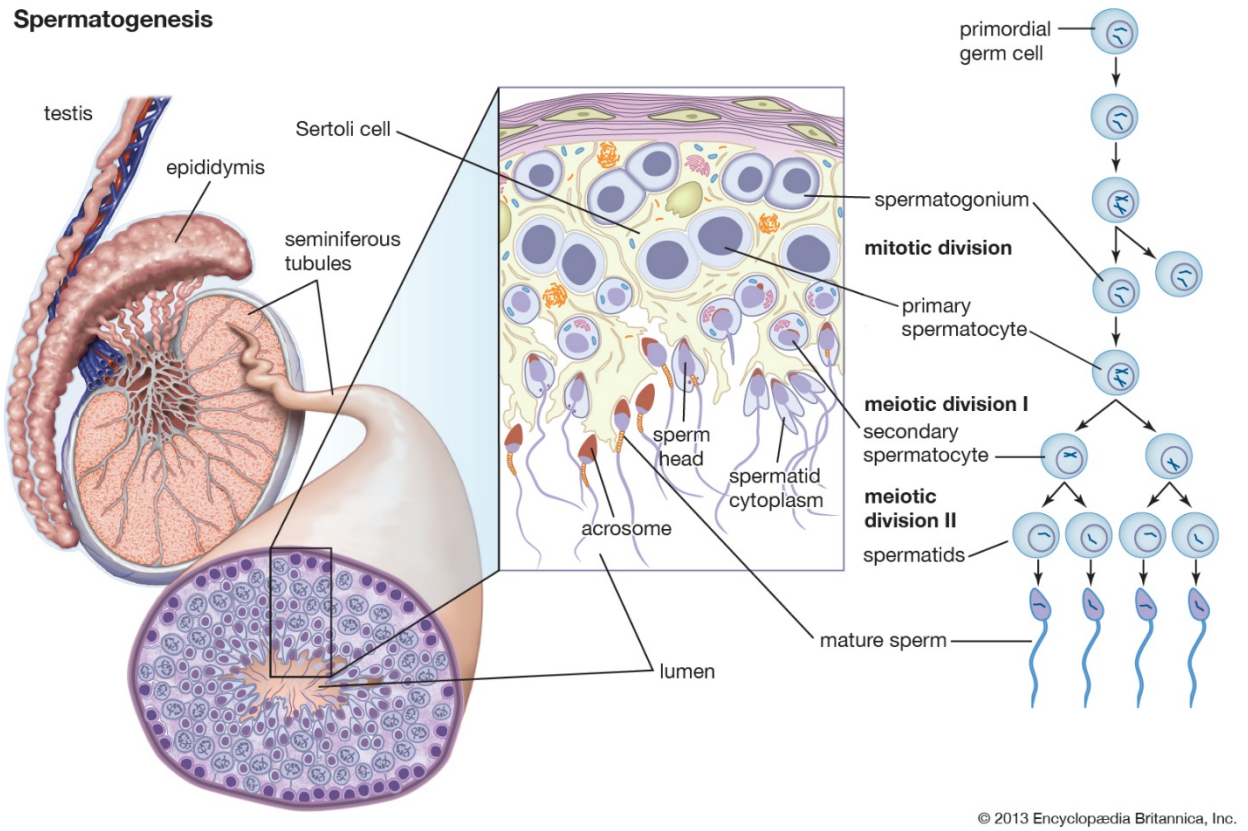
Maturation then takes place under the influence of testosterone, which removes the remaining unnecessary cytoplasm and organelles. The excess cytoplasm, known as residual bodies, is phagocytosed by surrounding Sertoli cells in the testes. The resulting spermatozoa are now mature but lack motility, rendering them sterile. The mature spermatozoa are released from the protective Sertoli cells into the lumen of the seminiferous tubule in a process called *spermiation*.

The non-motile spermatozoa are transported to the epididymis in testicular fluid secreted by the Sertoli cells with the aid of peristaltic contraction. While in the epididymis the spermatozoa gain motility and become capable of fertilization. However, transport of the mature spermatozoa through the remainder of the male reproductive system is achieved via muscle contraction rather than the spermatozoon's recently acquired motility.

From the seminiferous tubule they travel to the rete testis, which acts to “concentrate” the sperm by removing excess fluid, before moving to the epididymis where the sperm is stored and undergoes the final stages of maturation.

Spermatogenesis takes approximately 70 days, therefore in order for sperm production to be

continuous and not intermittent, multiple spermatogenic processes are occurring simultaneously within the same seminiferous tubule, with new groups of spermatogonia arising every 16 days (spermatogenic cycle). Each of these populations of spermatogenic cells will be at different stages of spermatogenesis.




## OOGENESIS

It is a process of maturation of female gametes, from oogonia to mature ovum. It takes place in the ovaries.

Oogenesis consist of 3 phases:

1) **In multiplication phase** the germ cells; which are called ***oogonia*** proliferated by mitotic divisions from the primary germ cells. The oogonia have nuclei which contain diploid number of chromosomes (2n2c). It is commonly believed that, when oocytogenesis is complete, no additional primary oocytes are created, in contrast to the male process of spermatogenesis, where gametocytes are continuously created. In other words, primary oocytes reach their maximum development at ~20 weeks of gestational age, when approximately seven million primary oocytes have been created; however, at birth, this number has already been reduced to approximately 1-2 million.

2) **Growth phase**. By the 3 to 7-th month of embryonic period, all the oogonia stop to divide and are transformed into primary oocytes. During this phase growth of cells and replication of DNA occur. The amount of heredity material of primary oocytes is 2n4c.

Each primary oocyte is surrounded by a layer of flattened ovarian epithelial cells, known as the follicular cells. They provide nourishment of oocyte and secret the steroid hormone estradiol. A primary oocyte, together with follicular cells forms the primary ovarian follicle or primordial follicle. As a rule, each follicle contains one oocyte.

3) **Maturation phase**. The primary oocytes enter into the maturation phase, where each cell divides by meiosis. But in dictyotene phase the meiosis is arrested until puberty. At this time hypophysis starts to secret follicle stimulating hormone which stimulates the growth of ovarian follicles. The primary oocyte from the dictyotene stage completes the first meiotic division. The result of this division is the formation of the two daughter cells that have amount of heredity material n2c. One cell is large, receives abundant cytoplasm of the mother cell and is known as the ***secondary oocyte*** (n2c) the other cell is small carrying scanty amount of cytoplasm and persists as ***the first polar body.***

During this time follicular cells secret fluid with estradiol. Because of this the follicle is enlarged and become mature follicle named as Graafian follicle. It appears beneath the surface of the ovary. When a fully formed Graafian follicle ruptures on the surface of the ovary, it is called ***ovulation.***

The secondary oocyte, surrounded by the zona pellucida and the corona radiata, is shed from the ovary and enters the uterine tube through the fimbriated end.

The secondary oocyte immediately enters in the process of second meiotic division. But at the metaphase II division is stopped again until a sperm fuses with the oocytes.

If fertilization happens the secondary oocyte completes the second meiotic division and results in the formation of two unequal daughter cells, each having nc. The large cell presenting abundant cytoplasm is known as ***the mature ovum*** *(nc)*, and the small cell forms ***the second polar body***, which appears in the per vitelline space. The first polar body also divided into two second polar bodies. Thus, the result of two meiotic divisions is: ***one mature ovum and three second polar bodies.*** All polar bodies are small cells. They have no role in oogenesis they eventually degenerate.

In absence of fertilization, the secondary oocyte does not complete the second meiotic division and degenerates as such within 24-48 hours after ovulation.

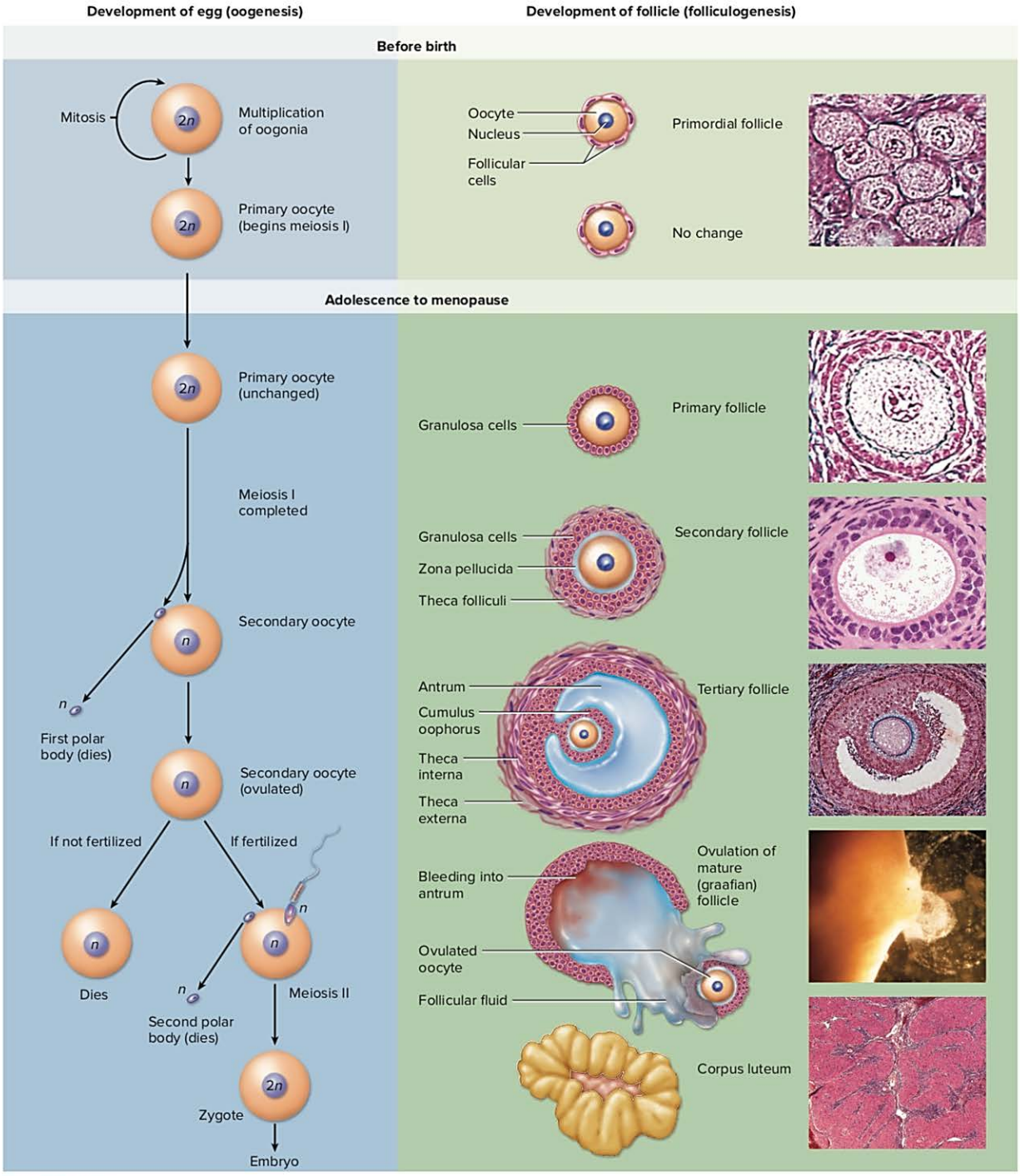



**FIGURE 28.11** **Oogenesis (Left) and Corresponding Development of the Follicle (Right).** AP|R

**(1, 2):** © Ed Reschke/Getty Images; **(3):** © McGraw-Hill Education/Al Telser, photographer; **(4):** © Ed Reschke/Getty Images; **(5):** © Petit Format/Science Source

**Comparison of human oogenesis and spermatogenesis:**

| Spermatogenesis | Oogenesis |
|---|---|
| 1. It occurs in the testes | 1. It occurs in the ovaries |
| 2. Growth phase is short that spermatocytes are only twice the size spermatogonia. Spermatozoons are minute, yolkless, motile. | 2. Growth phase is very long that oocytes are much larger than oogonia. Oocytes accumulate yolk for nutrition of future embrio. Ova are much larger then spermatozoons, often with yolk and non-motile. |
| 3. One spermatocyte forms 4 similar spermatids | 3. One oocyte forms 1 ovum and 3 polar bodies. |
| 4. Spermatogenesis is a continuos process. | 4. Oogenesis is discontinuos process. It has 2 pauses (at dictyotene and metaphase II) |
| 5. Spermatogenesis consist of 4 phases. | 5. Oogenesis consist of 3 phases. |
| 6. Spermatogenesis appears in the seminiferous tubules of testes at purberty. | 6. Oogenesis starts in the ovaries (prophase of meiosis I) in the 3-th month of embryonic period. |

## DISTINCTIONS GAMETES FROM SOMATIC CELLS.

1. Gametes have a haploid number of chromosomes.

2. Gametes have particular nuclear-cytoplasmic ratio.

3. Gametes have low metabolism.

## MORPHOLOGICAL STRUCTURE OF OVUM AND SPERMATOZOON.

### SPERMATOZOON

The sizes of a human spermatozoon are 52–70 μm.

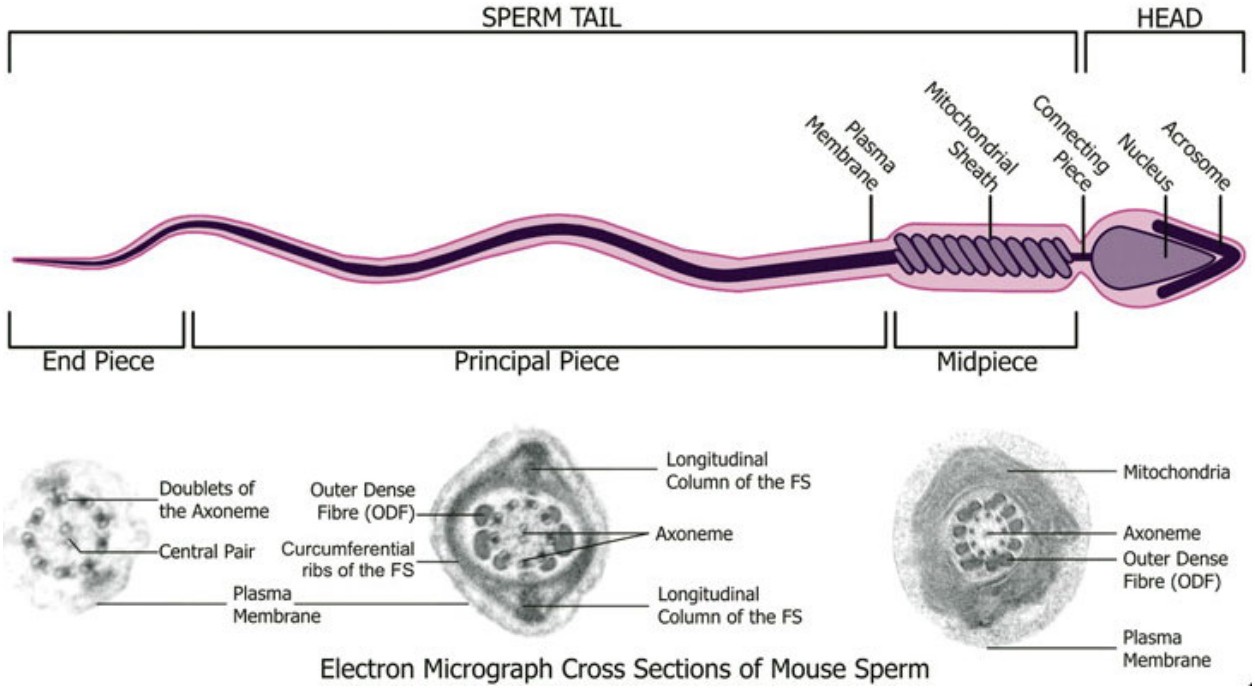
A spermatozoon consists of four parts: head, neck, middle piece and tail.

**Head.** The head varies in form in different species. It is flat and oval in human sperm. It is composed of a large nucleus and a small acrosome. The nucleus is very compact and consists only of condensed chromosomes. Acrosome lies at the tip of the nucleus. It is formed from the Golgi apparatus. It contains hydrolytic enzymes, and it used to contact and penetrate the egg in fertilization.

**Neck**. The neck is very short and contains centrosome. Centrosome provides formation of microtubules of tail and plays a role in first division of zygote.

**Middle piece.** The middle piece contains many mitochondria which produce ATP needed for movement of sperm cell. The middle piece is the power house of a sperm. The amount of energy is limited. If a sperm fails to contact an ovum within a specific period, it depletes its energy and dies.

**Tail.** The tail is very long, it formed of cytoplasm. The spermatozoon swim about by vibrating their tail in a fluid medium in search of ova.



Electron Micrograph Cross Sections of Mouse Sperm

## OVUM

The ovum is a rounded, non-motile cell. Its size varies in different animals depending upon the amount of yolk in it. It has abundant cytoplasm called ***ooplasm***, having a large nucleus at the center and surrounded by a plasma membrane. The ovum shows polarity. Its side which contacts with polar bodies is called ***animal pole***. The opposite site is termed ***vegetal pole***.

The human's ovum is enclosed by two additional egg coats:

1) inner thin, transparent, noncellular ***zona pellucida*** composed of protein and sugars, and probably secreted by ovum (primary membrane). This membrane prevents of polyspermy. Also it holds the cells during cleavage together.

2) outer thick ***corona radiata*** formed of radially elongated follicular cells (secondary membrane).

A narrow ***periviteline space*** exists between the zone pellucida and plasma membrane.

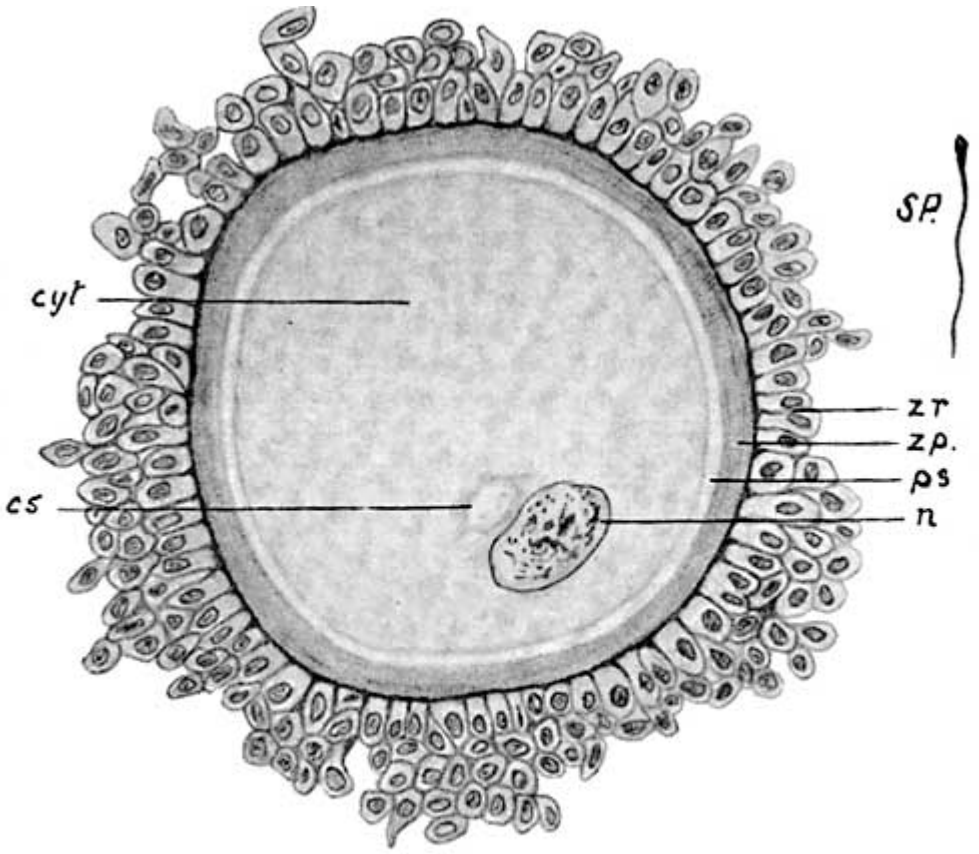



FIG. 2.—OVUM, MATURE, SEMI-DIAGRAMMATIC, WITH SPERMATOZOON (SP) AT SAME MAGNIFICATION (× 300) TO SHOW RELATIVE SIZES.

*Cyt*, body of ovum; *cs*, centrosome; *n*, nucleus; *ps*, perivitelline space; *zp*, zona pellucida; *zr*, zona radiata. (Frazer, 1940, p.5)

The eggs of many animals have tertiary membrane. It is a protective membrane, which may be soft and jellylike or hard and calcified, like shells. This membrane formed by epithelial cells of genital tract and needed to protect embryo in external environment.

# *REPRODUCTION OF ORGANISMS*

## Types of reproduction and their characteristic.

Reproduction is a universal organism property of all living things, which provides reproduction of their own selves and is based on transmission of genetic information from generation to generation. Reproduction on the molecular level is DNA replication, on the subcellular level — doubling of some organelles, on the cellular one — amitosis, mitosis. Cell division is the basis of organisms' reproduction.

Forms of organisms' reproduction.

- asexual reproduction
- sexual reproduction

**The characteristic of asexual reproduction:**

- 1 parental individual takes part in reproduction;
- somatic cells are a source of genetic information;
- genotypes of daughter cells are identical to parental ones;
- the number of individuals increases fast;
- it provides the existence of a species in constant environmental conditions

### Types of Asexual reproduction

1. Asexual reproduction of unicellular organisms:

a) *division* (fission) in two longitudinal fission (euglenas) transverse fission (infusoria);
b) *schizogony* is a multiple fission. At first, the nucleus divide into several parts, then the cytoplasm do (malaria parasite);
c) *budding* — a bud forms on the mother cell, it grows and then separates from the mother individual (yeast, suctorians).

2. Asexual reproduction in multicellular organisms:

A. In plants — *by vegetative organs*: the root, stem, leaves.
B. Animals:
    a) *budding* (hydra);
    b) *fragmentation* — division of the body by constrictions into several parts (ciliates and ringworms);
    c) *polyembryony* — division of the zygote into several parts, each form a separate organism (flukes).

3. Sporogenesis: in special organs (sporogonia) spores are formed, they give rise to a new organism (water-plants, mushrooms, mosses, lycopodia, horsetails, ferns).

**Characteristic of sexual reproduction:**

- 2 parental individuals take part in reproduction;
- parental gametes are a source of genetic information;
- genotypes of daughter cells differ from the parental ones due to combinative variation;
- it promotes the adaptability of organisms to changing environmental conditions

## Types of Sexual reproduction

- Unicellular:
  - conjugation;
  - copulation.
- Multicellular:
  - with fertilization;
  - without fertilization (parthenogenesis).

***Conjugation***. It involves temporary pairing of two parents which exchange their male pronuclei and then separate. It is found in Paramecium and other ciliates.

***Copulation***. It involves the complete and permanent fusion of two gametes (in the case of multicellular organisms) or two unicellular organism (Flagellata) to form the zygote.

Copulation is two kinds with regard to the structure of the fusing gametes:

- *Isogamy*. The fusing gametes are similar morphologically as well as physiologically as in Monocystis, a protozoan. Such gametes are known as isogametes.
- *Anisogamy*. The fusing gametes are different in form, size and behavior as in frog, rabbit and humans. Such gametes are known as anisogametes. The type of anisogamy is *oogamy*. The fusing gametes are different in form and size. One gamete is big and non-active (ovum) but another gamete is small and active (sperm cell).

Reproductive unites in sexual reproduction.

The reproductive unites in sexual reproduction are specialized cells called gametes. The gametes are generally of two kinds: male gametes called microgametes and female gametes termed macrogametes or ova. The male gametes are minute and mobile so that they may swim to the female gametes for fertilization. The female gametes are usually large, non-mobile and often have a store of food to nourish the developing embryo. The male and female gametes are in the most of animals produced by male and female parents respectively. Such animals are said to be unisexual or dioecious. .However in some animals, such as liver fluke, earthworm and leech, both kinds of gametes are produced by a single, individual. Such animals are said to be bisexual or hermaphrodite.

***Parthenogenesis.***

It is a modification of sexual reproduction in which an egg develops into a complete off-spring without fertilization. It is found in the invertebrates such as aphids, bees, crustaceous. It may be complete and incomplete.

*Complete parthenogenesis*. There is no biparental sexual reproduction, no males at all, females develop exclusively by parthenogenesis (of some rotifers).

*Incomplete parthenogenesis*. Some animals have both sexual and parthenogenic individuals. In honeybee; unfertilized eggs develop into male bees (drones) with haploid cells, and fertilized eggs give rise to female ( queen bees and worker bees) with diploid cells.

**THE QUESTIONS FOR SELF-CONTROL.**

***1. Gametogenesis, its biological significance.***
***2. Spermatogenesis, stages and cytogenetic description.***
***3. Oogenesis, stages and cytogenetic description.***
***4. The peculiarities of spermatogenesis and oogenesis in human.***
***5. Morpho-functional organization of spermatozoa.***
***6. Morpho-functional organization of ova.***
***7. Reproduction and its biological role.***
***8. The types of reproduction.***
***9. The evolution of reproduction.***
***10. Asexual reproduction, types, mechanisms. Significance of asexual reproduction.***
***11. Sexual reproduction, types, mechanisms.***
***12. Significance of sexual reproduction.***
***13. The differences between asexual and sexual reproduction.***

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