خلية وبيولوجيا جزيئية واجنه (Z)

BGS 113

الجزء النظـــــرى

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د/ عبيـــــــده فوزي

كلية التربية – التعليم الاساسى: شعبة العلومE

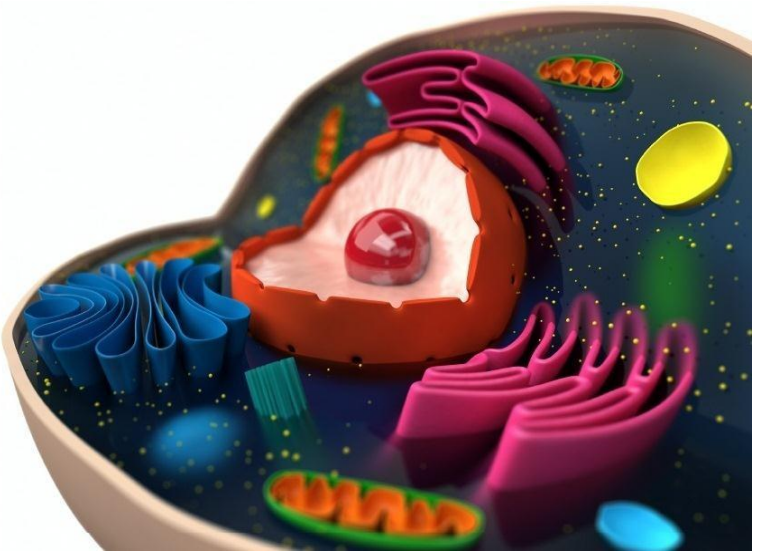
الفرقة الاولى

الفصل الدراسي الاول

2023-2024

PART (1)

CYTOLOGY



Cell is the building unit of a living organism and cytology is the science that studies cell properties, structure and components.

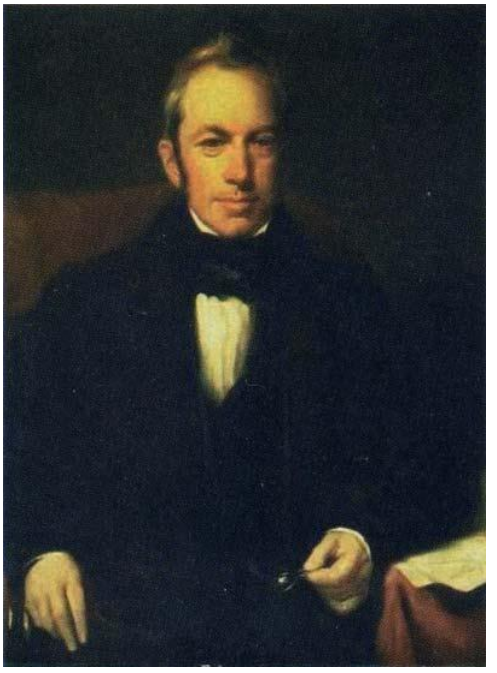
Cytology can be considered as the base science on which all branches of life sciences are constructed. The emergence of this science has been facilitated by the invention of microscope, which has been contributed in exploring interesting details about life that were previously unknown

Robert Hooke

The earliest phase of cytology began with the English scientist Robert Hooke's microscopic investigations of cork in 1665. He observed dead cork cells and introduced the term "cell" to describe them.

In 1831, the Scottish scientistRobert

Brown had described the cell nucleus.



Robert Brown

In 1839 two Germans, the botanist Matthias Schleiden and the physiologist Theodor Schwann were among the first to clearly state that cells are the fundamental particles of bothplants and animals, they have founded what is known as “the cell theory”.

Theodor Schwann **Matthias Schleiden**

The cell theory had a wide impact on a large number of life bra

knowledge, as this theory included that every cellarises from th

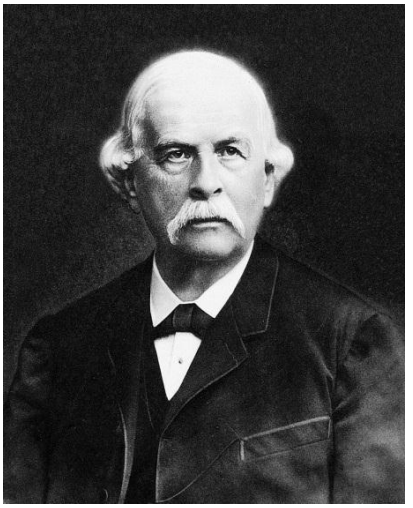
previous cell. In 1846, researchers Dujardin, Schulteze, Purkinj

Mohlhad described the protoplasm.

Purkinji

Dujardin

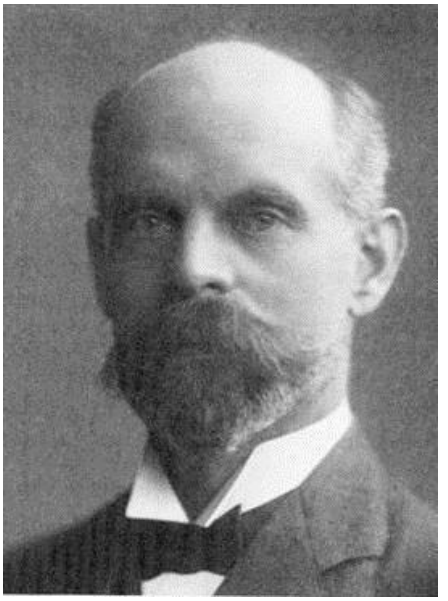
In 1855, the German pathologist Virchow and the Swiss embryologist Kölliker had demonstrated that an organism develops from the fusion of two cells, the sperm and the egg, through a process called fertilization.



Albert von Kölliker

Rudolf Virchow

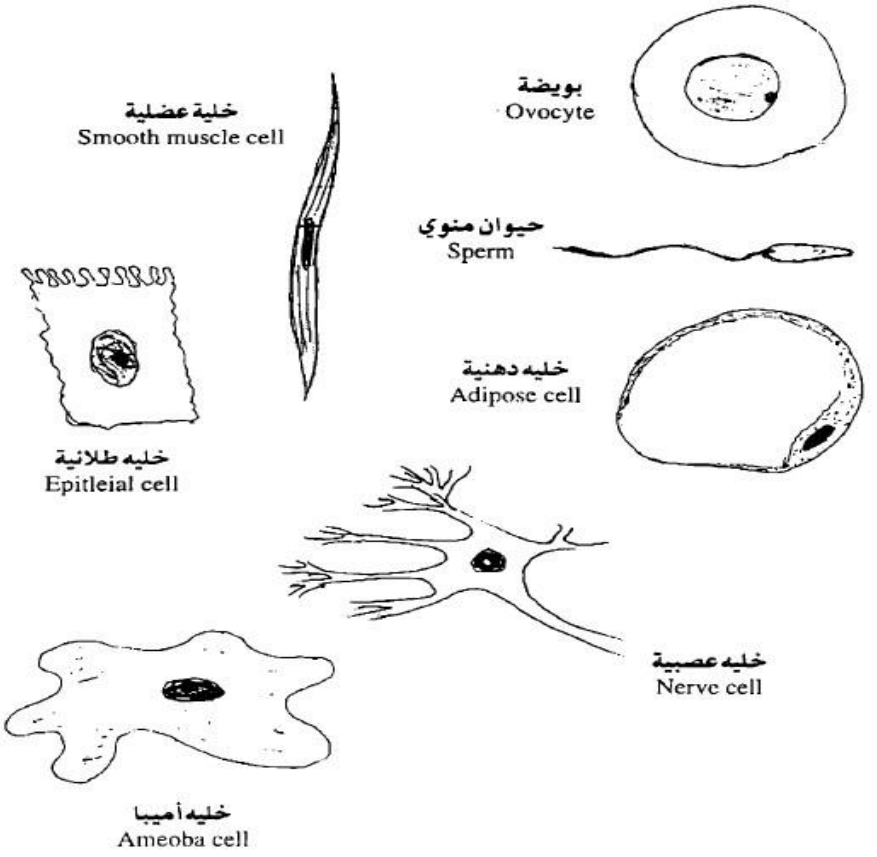
In 1895, the British scientist, Ernest Overton, described the plasma membrane and developed a primitive concept of its supposed structure.



Ernest Overton

Through the diligent work of a large number of scientists and researchers around the world, it became known to us now how cell divides, furthermore, we had all the details concerned with the distribution of chromosomes and the separation of their pairs,as well as, the full information on meiotic division. Moreover, chemists have succeeded to isolate the chemical components of most parts of the cell and studied it in a large scale.

Cells Shapes and Sizes



- Most animal cells are between 10 to 100 microns in size.

- The size and shape of cells in living organisms varies greatly. And the difference reaches its deepest when we find that there are thousands of shapes, types and sizes of cells in a single organism originating from one cell.

- It seems that this difference in the size and shape of cellsis due to important reasons such as age, location of cells,their embryonic development and function, which is of great importance in determining the size and shape of the cell.

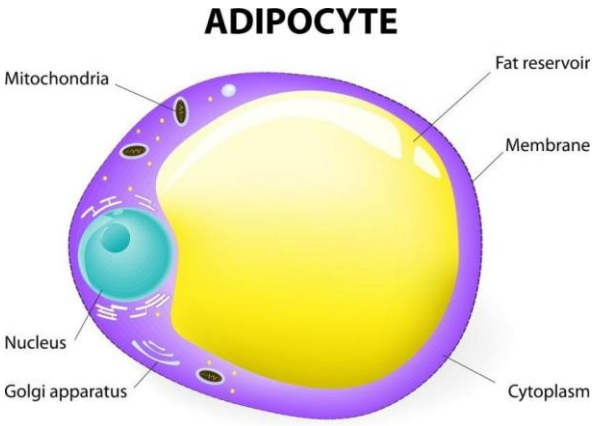
For example, red blood cells have a disc shape that helps them pass through narrow blood vessels.

Red Blood Cells

Neurons are characterized by their large size and the presence of many dendrites protruding from the cell body in addition to the presence of a long protrusion that connects with other neurons located far away in another location and thus can transmit thousands of neurons through their dendritic appendages associated with thousands of axons of other nerve cells.

Fat cells and egg are among the largest cells in size due to the presence of a lot of nutrients stored in these cells.

Egg cell



Fat Cell

Thus, the fusiform shape of the smooth muscles, the cylindricalshape of the skeletal and cardiac muscles, the caudal fusiform of the spermatozoa, and the ciliated cells in the lining of the trachea, intestine, and ovarian canals serve the function of these cells, as well as amebic cells and white blood cells adapting different forms to serve their function.

Cell Structure

Chemistry of the cellular components

Chemical definition of the cell: A cell is a massive assembly of anumber of different molecules that are organized in a high- precision manner that enables the cell to perform its different lifeactivities

- Different molecules that make a cell are collectively called

  protoplasm.

- Protoplasm is composed of:

  - Water: 70-80%
  - Carbohydrates
  - Lipids
  - Proteins
  - Electrolytes

Cell Structure

- The cell is mainly composed of the following

structures:

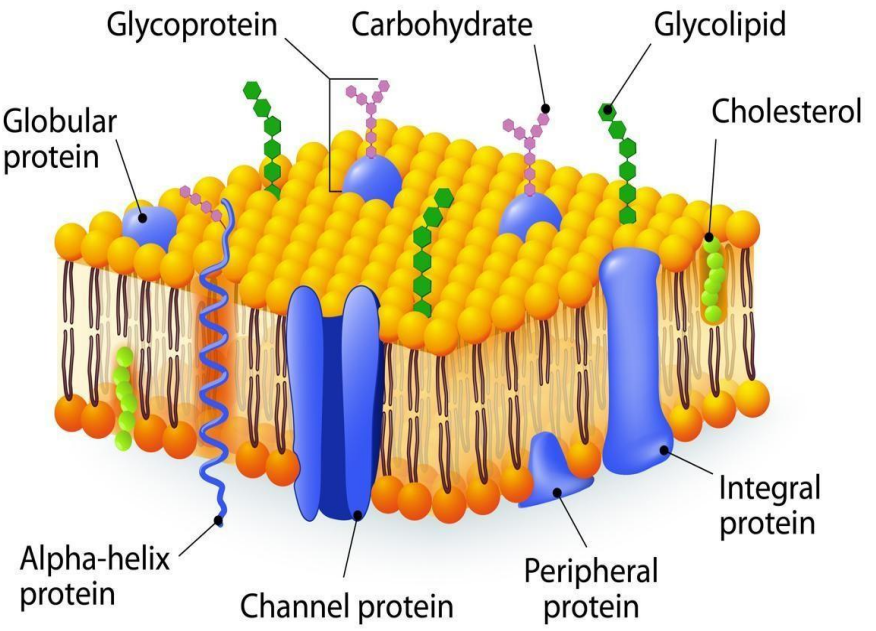
1. Cell Membrane
2. Cytoplasm and its organelles
3. Nucleus

Cell Membrane

Structure

The cell membrane, also known as the plasma membrane, is a double layer of phospholipids and proteins that surrounds the cell and separates the cytoplasm (the contents of the cell) from the surrounding environment.

CELL MEMBRANE



Function of Cell Membrane

- Supports the cell and maintains its shape.
- Contributes to the transport of substances to and from the cell.
- It forms a selective barrier between the inside and outside of thecell, controlling the entry and exit of different molecules and ions between the external and internal environment in what is known as selective

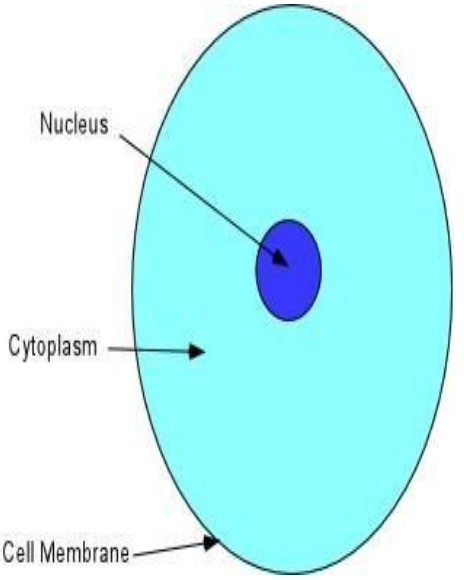
permeability.

- The receptors on its outer surface contribute to cell assemblyand tissue formation.
- Cell membrane proteins act as receptors for various chemical messages as the receptors of hormones.
- The cell membrane transmits chemical or electrical signals.
- It prevents toxic substances from entering the cell and maintainsits integrity.

Cytoplasm

Structure

The cytoplasm is a viscous (gel-like) substance surrounded by the cell membrane. It consists of a cytosol that contains water, proteins, fats, nucleic acids, inorganic salts, and sugars in smaller quantities as well as cytoplasmic inclusions and a number of cellular organelles with different functions.



Cytoplasm

Function

- If the cell is devoid of cytoplasm it will not be able to maintain its shape and it will be vacuole and flat and organelles will not remain suspended in the cell solution without supporting the cytoplasm.
- Most of the enzymatic reactions and the metabolic activity of the celloccur in the cytoplasm.
- The cytoplasm helps to move materials, such as hormones, around the cell and also dissolves cellular waste.

Cytoskeleton

Structure

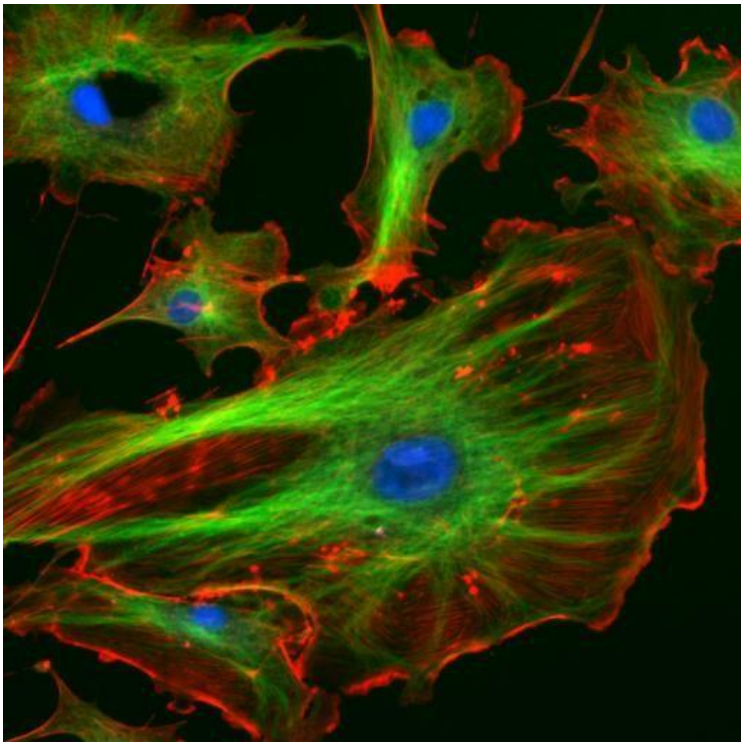
The cytoskeleton is a complex, dynamic network of interlinking protein filaments present in the cytoplasm of all cells.

Cytoskeleton Contains three main kinds of cytoskeletal filaments: microfilaments, microtubules, and intermediate filaments.

Cytoskeleton

Function

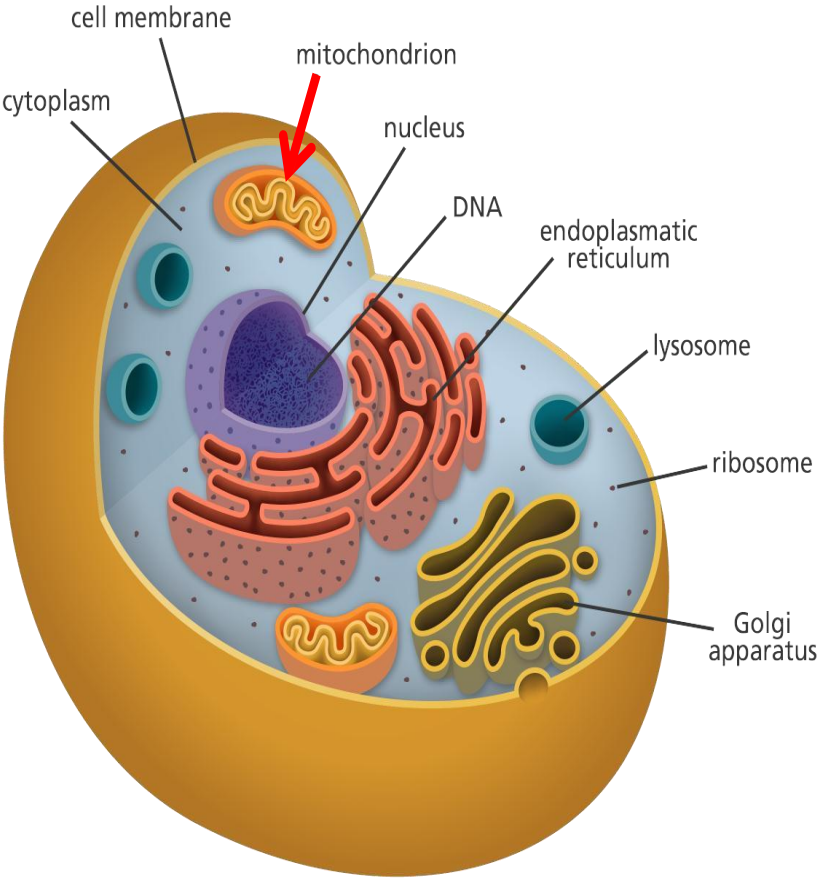
- Giving the cell a fixed support and shape.
- Muscle contraction and relaxation.
- Movement of organelles and their fixation.



Actin filaments are shown in red, microtubules in green a nd nucleusin blue

Mitochondria

mitochondrion

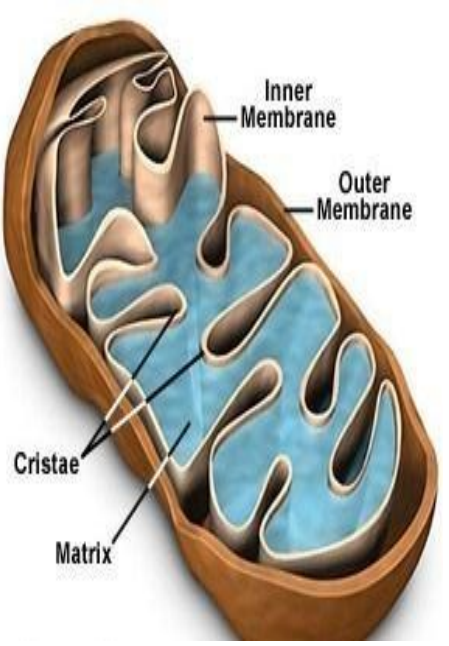


Mitochondria

Structure

The mitochondria are a double-membrane structure, consisting of two separate inner and outer membranes with intermembranous space between them, while the inner membrane surrounds the matrix of the mitochondria.

The inner membrane bends forming cristae which increase the area of the inner surfaceof the mitochondria.
Mitochondria contain ribosomes and DNA.



Mitochondria

Function

Mitochondria are the houses of energy production, as they are the respiratory centers of the cell because they contain respiratory enzymes that oxidize organic materials and provide the cell with energy in the form of the ATP.

- Mitochondria play a role in many other cellular activities. For example, mitochondria regulate the process of self-destructing cells (apoptosis). It is also necessary for the production of substances such as cholesterol and iron group, heme, (a component of hemoglobin, the molecule that carries oxygen in the blood).

- Important to maintain proper concentration of calcium ions within the various compartments of the cell.

Cell Structure

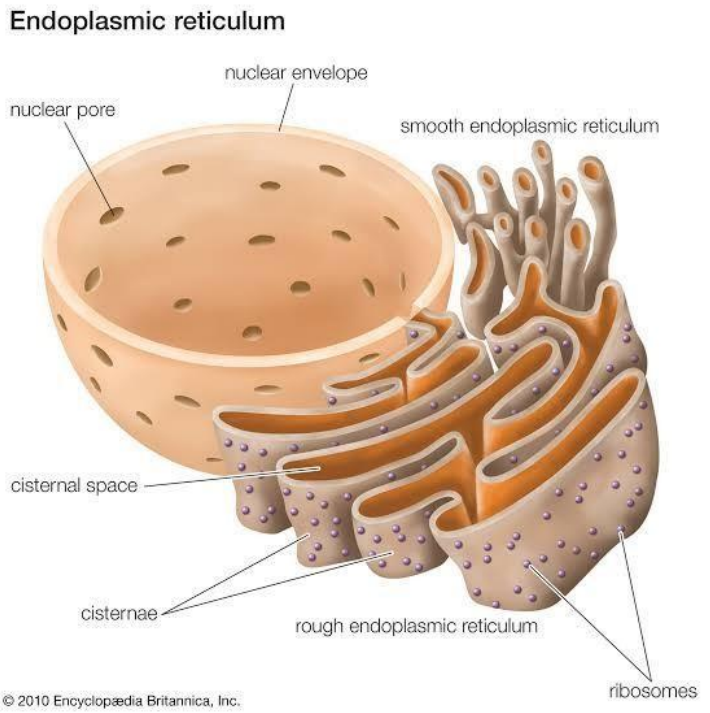
Endoplasmic Reticulum

Structure

- Network of tubular and vesicular structureswhich are interconnected with one another.
- Some parts are connected to the nuclear membrane, while others are connected tothe cell membrane.
- Two types: Smooth SER (lacks ribosomes) and rough RER (studded with ribosomes)

Function

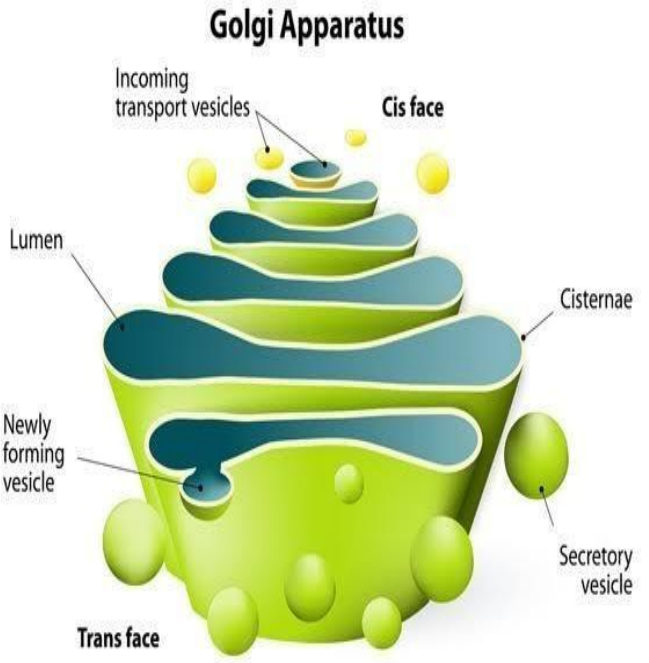
- Gives internal support to the cytoplasm.
- RER synthesize secretory proteins and membrane proteins.
- SER synthesize lipids for cell membrane.
- In liver cells SER detoxify drugs and poisons.
- In muscle cells SER store calcium ions.



Golgi Apparatus

Structure

- Discovered by Italian Scientist Camillo Golgi.
- Formed by stacks of 5-8 membranous sacs.
- Sacs are usually flattened and are called the cisternae.
- Has two ends: cis face situated near the endoplasmic reticulum and trans face situated near the cell membrane.



Golgi Apparatus

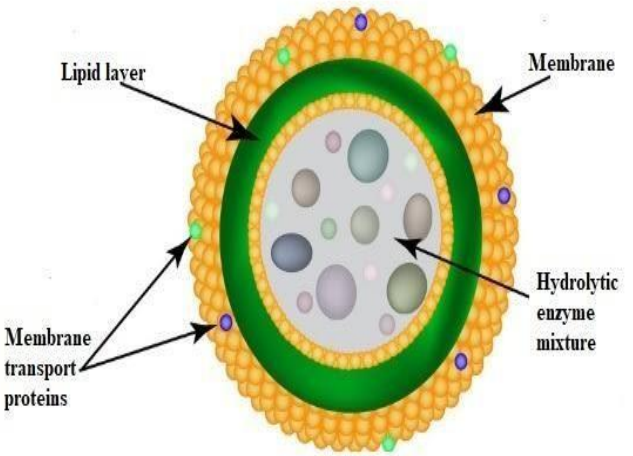
Function

- Modifies, sorts and packs materials synthesized in the cell.
- Delivers synthesized materials to various targets inside the cell and outside the cell.
- Produces vacuoles and secretory vesicles.
- Forms plasma membrane and lysosomes.

Lysosomes

Structure

- Small, spherical, single membrane sac.
- Found throughout the cytoplasm.
- Filled with hydrolytic enzymes.
- Occur in most animal cells and in few type of plant cells.



Lysosome

Lysosomes

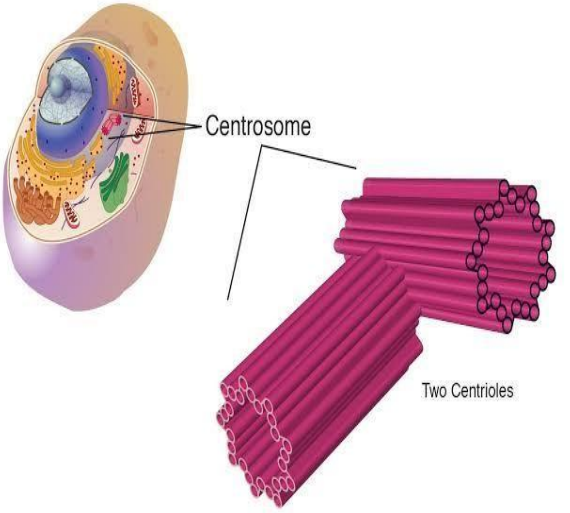
Function

- Help in digesting of large molecules.
- Protect cell by destroying foreign invaders like bacteria and viruses.
- Degradation of worn out organelles.
- In dead cells perform autolysis.

Centrosome

Structure

- Centrosome is the membrane bound organelle present near the nucleus.
- Consists of two structures called centrioles.
- Centrioles are hollow, cylindrical structures made of microtubules.
- Centrioles are arranged at right angles to each other.



Centrosome

Function

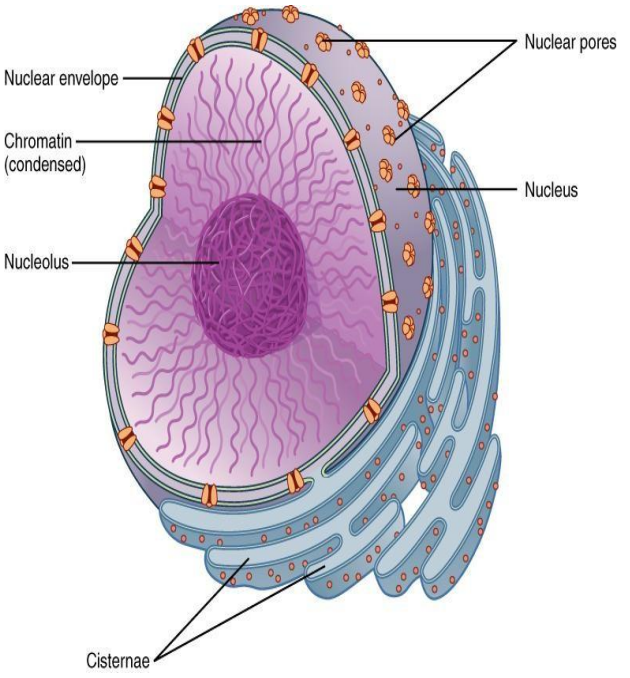
- Forms spindle fibres which help in the movement of chromosomes during cell division.
- Help in the formation of cilia and flagella.

Nucleus

Structure

- Dense spherical body with diameter 10-25µm located near the center ofthe cell.
- Most of cells having only one nucleus and few types have more than one nucleus (skeletal muscle cells)

Nucleus



Nucleus

Structure

- Nucleus is covered by double layer called nuclear membrane.
- Nuclear membrane has pores of diameter 80-100 nm.
- Colorless dense sap present inside the nucleas known as nucleoplasm.
- Nucleoplasm contains round shaped nucleolus and network of chromatin fibres.

Nucleus

Structure

- Fibres are composed of deoxy ribonucleic acid (DNA) and proteinhistone.
- These fibres condense to form chromosomes during cell division.
- Chromosomes contain segments of DNA called genes.

Nucleus

Function

- Nucleus controls all the cell activities like metabolism, protein synthesis, growth and cell division.
- Nucleus stores hereditary information in genes.
- Nucleolus is responsible for ribosomal RNA (rRNA) synthesis and ribosome biogenesis.

Cell Division

- Cell division takes place within the body at two levels, each of which produces two different types of cells.

- The difference is limited only for the number of chromosomes contained in the cells.

- According to this explanation, the body has two types of cells:somatic cells and sexual cells.

- **Somatic cells:** It refers to the cells that make up various organs of the body, and their reproduction leads to the growth of the body, whether in the fetal stage, or after birth and during the growth of theindividual. These cells are produced through a process of cell division called mitosis or indirect division.

- **Sexual cells:** It refers to the cells responsible for determining the gender of the individual, and the number of its chromosomes is half the number of chromosomes in the somatic cells. These cells are sperms in male, which results from the division of testicular cells, and eggs in female, which results from the division of ovarian cells.This division is called meiotic division through which the number of resulting chromosomes from mother cell division is reduced to half.

Division Processes

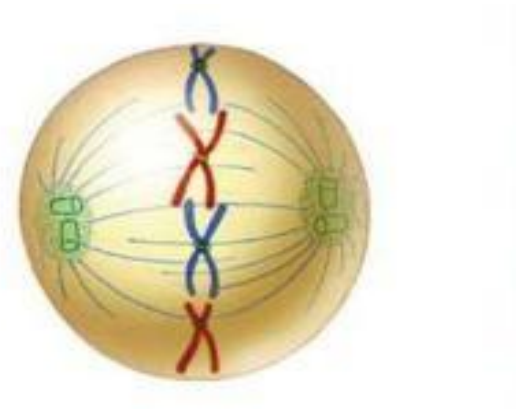
Mitotic Division

- Mitotic division occurs in somatic cells for the purpose of growth or compensation of damaged tissues.
- Before mitotic division, the cell enters a phase called the interphase.
- During which the cell size is doubled as well as the number of chromosomes that appear as undifferentiated chromatin filaments,moreover the centrioles are doubled.
- In this stage, the role of nucleus is involved in protein synthesis.
- After interphase, the mitotic division passes through several phases.

1- Prophase

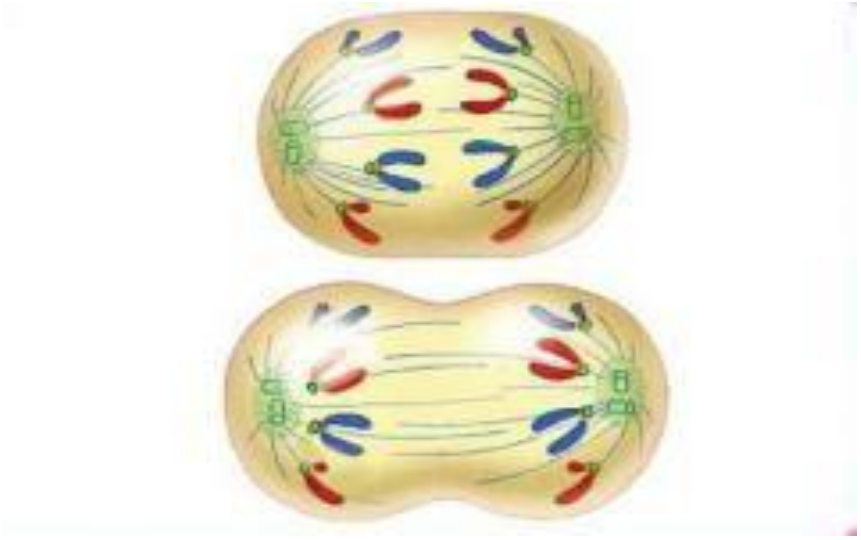
- Nuclear envelope begins to dissolve and disappear.
- Chromatin filaments differentiate into chromosomes and each chromosome consists of two sister chromatids that are linked by ajunction called the centromere.
- Spindle fibres are formed and made of proteins and microtubules.
- The two pairs of centrioles gradually move away from each other due to the elongation of the microtubules until each pair of centrioles settles at one of cell poles.

2- Metaphase



- The nuclear membrane disappears completely.
- The chromosomes are arranged at the midline of the cell.
- Centrioles settle at the poles of the cell and spindle fibres that extend between them are connected to the chromosomes at centromere point.

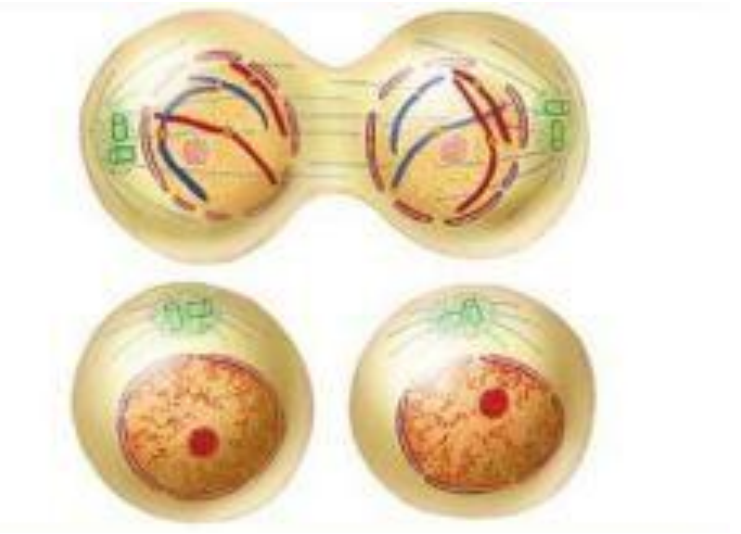
3- Anaphase



- The centromere is separated in each chromosome and thus the sister chromatids forming the chromosome are separated from each other, andat this stage the chromatids are considered complete chromosomes called daughter chromosomes.
- Spindle filaments contract, pulling the chromatids with them to the cell

  poles, thus collecting at each pole the full number of chromosomes.
- The cytoplasm begins to divide.

4-Telophase



- The nuclei begin to appear at the ends of the cell and a nuclear envelope forms around each of them.
- The nucleoli appear again.
- The chromatin fibers forming the chromosomes begin to disintegrate.
- At the end of telophase, cytoplasmic division is completed, and two identical cells are formed, each containing the full number of chromosomes as the original parent cell.

Meiotic Division

- Meiotic division occurs in cells that reproduce sexually to producegametes.
- Before the start of meiosis, the cell enters interphase stage through which the cell size increases and the chromosomes that appear as chromatin filaments are doubled. The nucleus is surrounded by a nuclear envelope, the nucleolus is visible, and the centrioles are doubled.
- It differs from mitotic division in that it consists of two stages instead of one stage, through which meiosis passes through several phases.

First Meiotic Division

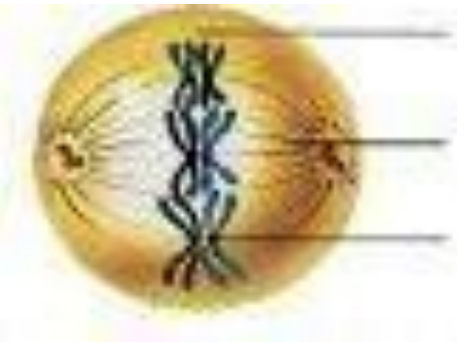
Prophase I

- Chromosomes are condensed, attached to the nuclear envelope, and arranged in pairs with each of two homologous chromosomes adjacentto each other.
- Since each chromosome consists of two chromatids, the two homologous chromosomes can be described as a tetrad (4 sister chromatids).

Prophase I

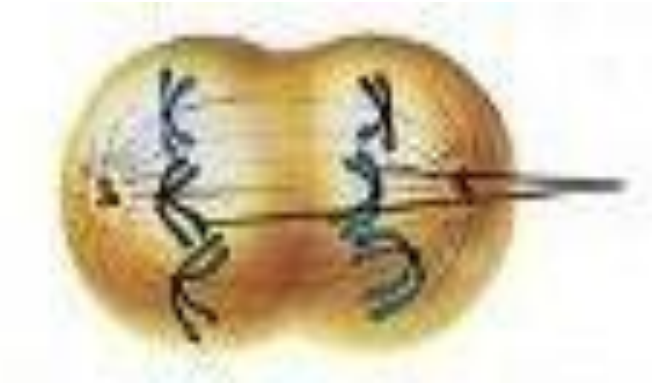
- An interlacing occurs between the chromatids of adjacent chromosomes,which allows the occurrence of genetic recombination, that is a transfer of a part of the genetic material from the first chromosome to the secondone and vice versa in what is known as the process of **crossing over**.
- Chromosomes get thicker and separate from the nuclear envelope.
- The nucleus and the nuclear envelope disappear.
- Centriole pairs begin to move towards the poles of the cell.

Metaphase I



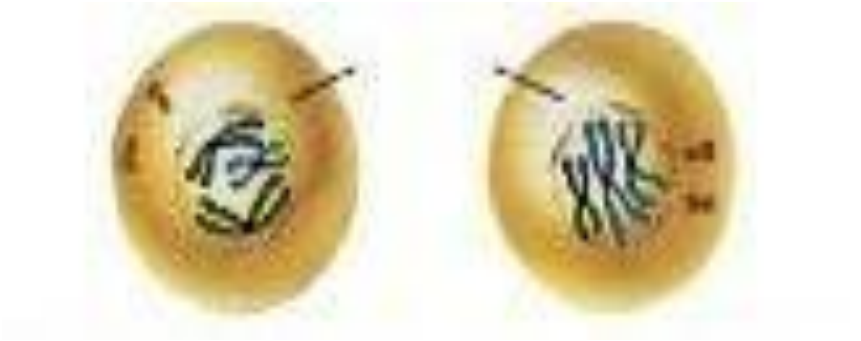
- The tetrads are arranged in the midline of the cell.

Anaphase I



- The microtubules of the spindle filaments contract, as each chromosomeseparates from its homologous one and begins to move toward the polesof the cell.
- The separation occurs for homologous chromosomes, not for chromatids as it does in mitosis.

Telophase I



- Half of the original number of chromosomes meet at each pole of the cell.
- The division of the cytoplasm begins.
- At the end of this phase the cell divides into two cells, each cell contains half the number of chromosomes of the parent cell.
- Each cell enters the second stage of meiosis without replicating thegenetic material.

Second Meiotic Division

Prophase II

- The nucleolus and the nuclear membrane disappears.
- The spindle filaments appear, and the chromosomes begin to move toward the midline of the cell.

Metaphase II

- Chromosomes are arranged in the midline of the cell.

Anaphase II

- Sister chromatids that make up the chromosomes separate from each other and begin to move toward the poles of the cell.
- The cell begins to elongate.
- The chromatids gather at the end of the anaphase at the poles.
- The chromatids in this case are complete chromosomes called daughter chromosomes.

Telophase II

- The nucleoli appear at the poles of the cell.
- The cytoplasm divides and meiosis results in four cells, each containing half the number of chromosomes in the parent cell.

Questions

First Question:

A - Which of the following is NOT a function of cell division?

1- growth of multicellular organisms

2- repair of multicellular organism

3- reproduction of multicellular organisms

4- aerobic cellular respiration of multicellular organisms

B- Which of the following is not part of the cell theory?

1- All living things are made of cells

2- Cells come from existing cells

3- Cells are the basic units of structure and function in all living things

4- All cells contain the same organelles

C- Which cell feature is responsible for making proteins?

1- lysosomes

2- ribosomes

3- Mitochondria

4- vacuoles

D- What cell feature is responsible for powering the cell?

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1- endoplasmic reticulum

2- golgi apparatus

3- Mitochondria

4- lysosomes

E- What cell feature contains digestive enzymes which breaks things down?

1- lysosomes

2- ribosomes

3- Vacuoles

4- Mitochondria

F- What cell feature is responsible for storing water?

1- mitochondria

2- lysosome

3- Vacuole

4- endoplasmic reticulum

G- Which of the following is the function of the cytoskeleton?

1- Helps a cell keep its shape

2- Contains DNA

3-Surrounds the cell 4 Helps make proteins

H- Which organelle would not be found in animal's cells?

A. Smooth ER

B. Chloroplast

C. Mitochondria

D. Ribosome

Eukaryotic cell contains

A. Only ribosomes

B. Membrane bound organelles

C. DNA floating in cytoplasm

D. Just cytoplasm

*** The paired chromatids are separating and being pulled to opposite ends of the cell.**

Metaphase

Anaphase

Prophase

*** In humans, a zygote is a _____ cell having _____ chromosomes.**

diploid; 46

diploid; 23

haploid; 46

- * During _____ , the chromosomes first become visible.

A) interphase

B) prophase

C) metaphase

E) telophase

* Mention the most important function of the following -:

1- Golgi apparatus

2- Endoplasmic Reticulum (E.R.)

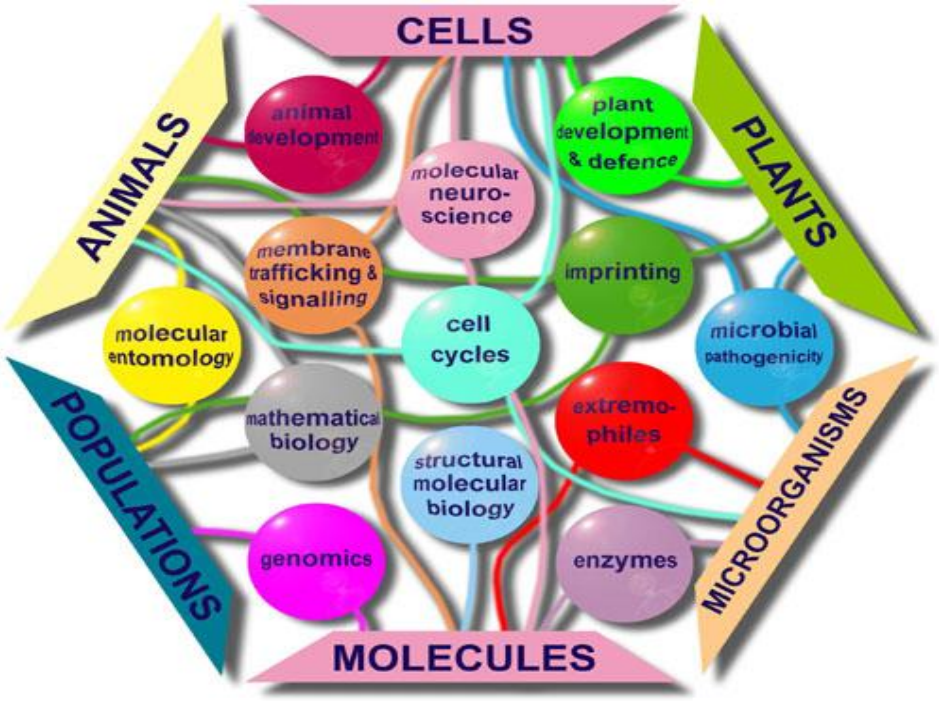
3- Cell membrane

PART (1)

Molecular biology

Molecular biology:definition

Molecular biology is the study of molecular underpinnings of the process of replication, transcription and translation of the genetic material.



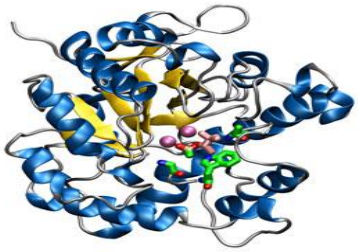
This field overlaps with other areas of biology and chemistry, particularly genetics and biochemistry. Molecular biology chiefly concerns itself with understanding the interactions between the various systems of a cell, including the interactions between DNA, RNA and protein biosynthesis as well as learning how these interactions are regulated.

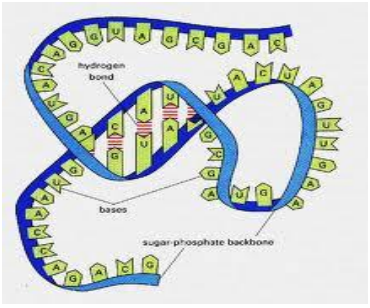
Much of the work in molecular biology is quantitative, and recently much work has been done at the interface of molecular biology and

computer science in bioinformatics and computational biology.

Since the late 1950s and early 1960s, molecular biologists have learned to characterize, isolate, and manipulate the molecular components of cells and organisms includes DNA, the repository of genetic information; RNA, a close relative of DNA; and proteins, the major structural and enzymatic type of molecule in cells.

Components involve in molecular biology





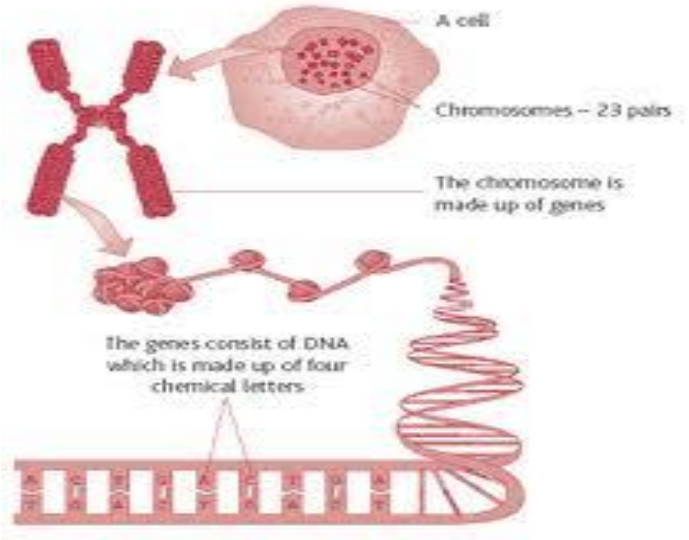
DNA RNA Protein

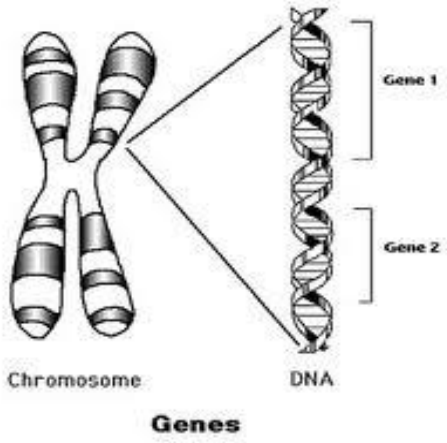
Gene : Unit of heredity

The DNA segments that carries genetic information are called genes.

It is normally a stretch of DNA that codes for a type of protein or for an RNA chain that has a function in the organism.

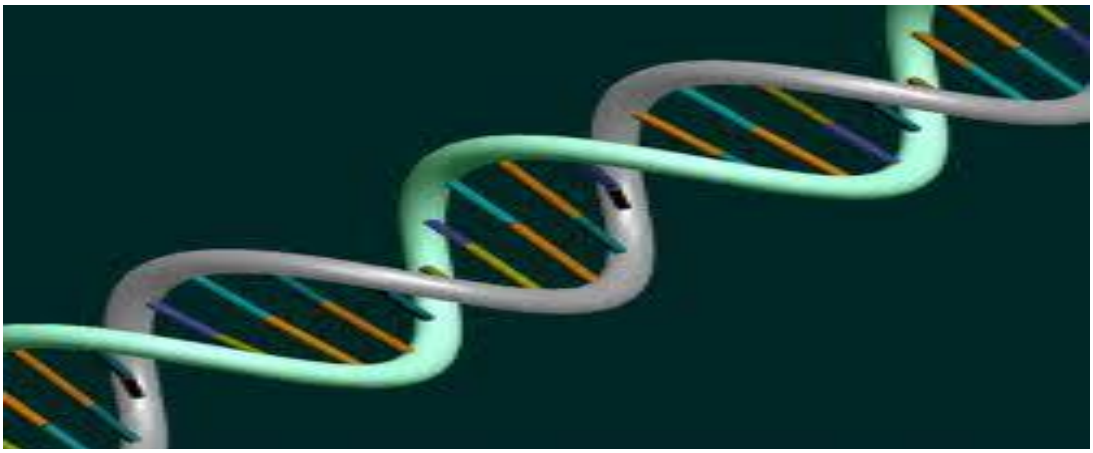
Genes hold the information to build and maintain an organism's cells and pass genetic traits to offspring.





Deoxyribonucleic acid (DNA)

DNA is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms and some viruses.



DNA structure:

Chemically, DNA consists of two long polymersof simple units called nucleotides, with backbones made of base, sugars and phosphate groups.

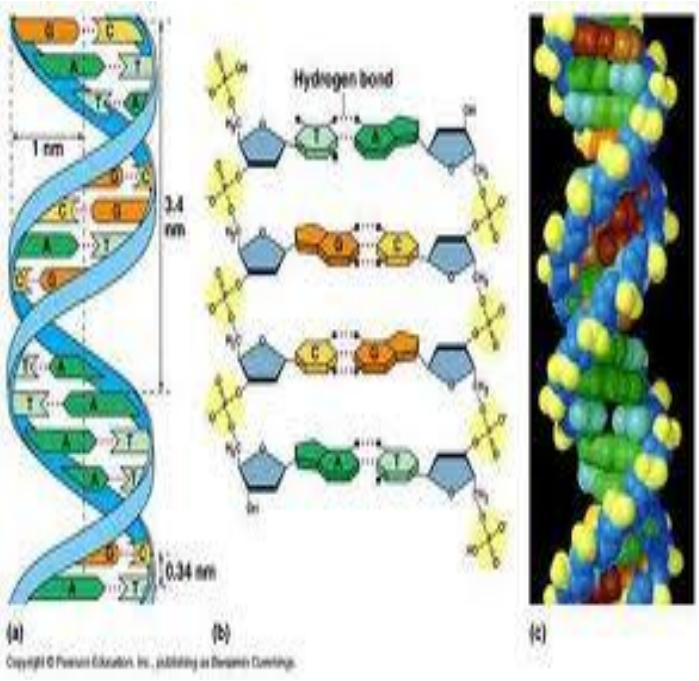
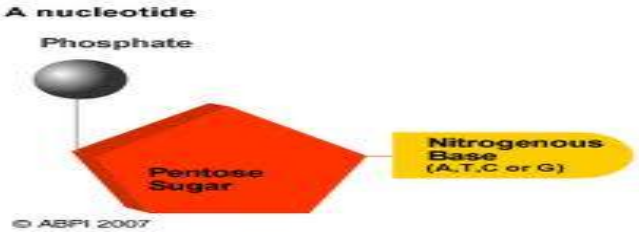


Fig : DNA double helix

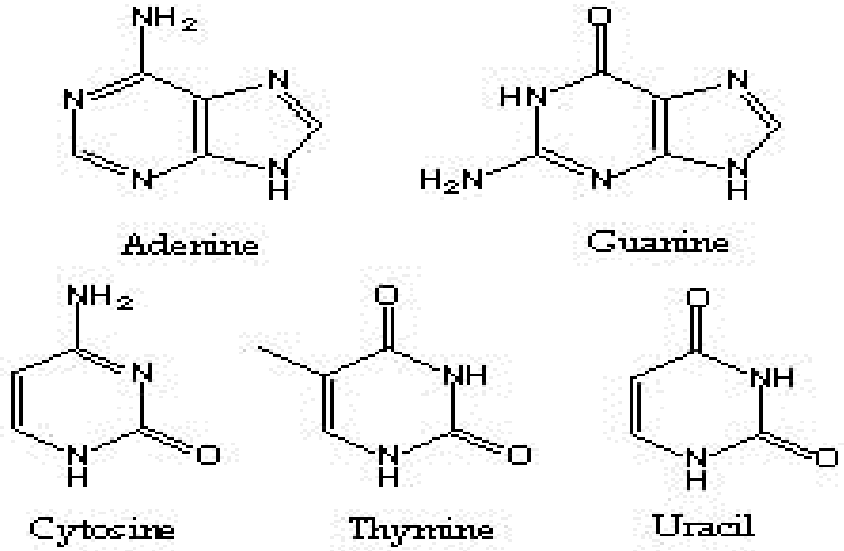
- **Sugar +Base = nucleoside**

Phosphate+ sugar + Base = nucleotide

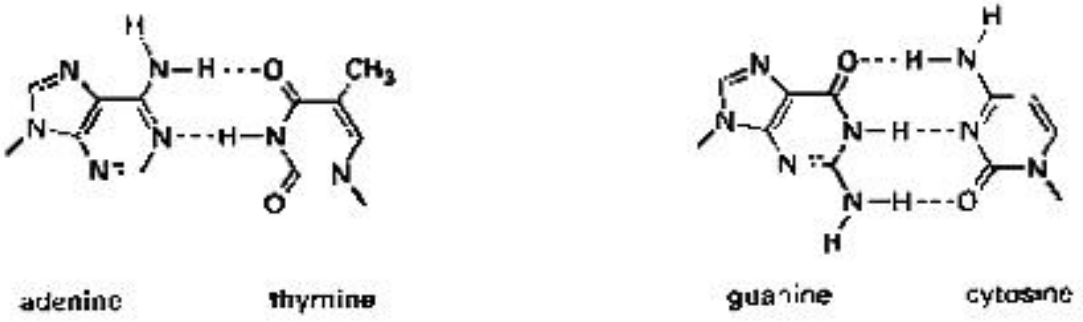


Bases

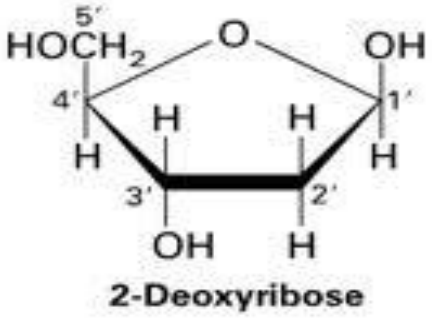
Types:- **adenine** and **guanine** (fused five- and six-membered heterocyclic compounds) – **Purines**



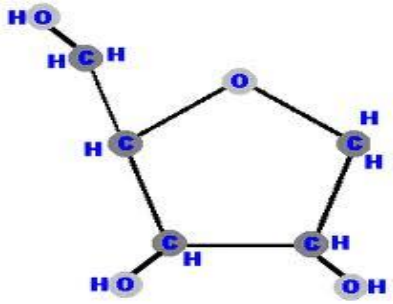
- cytosine & thymine (six-membered rings)- Pyrimidines.
- A fifth pyrimidine base, called uracil (U), usually takes the place of thymine in RNA and differs from thymine by lacking a methyl group on its ring.
- PAIRING : A =T and A=U G≡C
- The DNA double helix is stabilized by hydrogen bonds between the bases attached to the two strands.



- One major difference between DNA and RNA is the sugar, with the 2-deoxyribose in DNA being replaced by the alternative pentose sugar ribose in RNA.

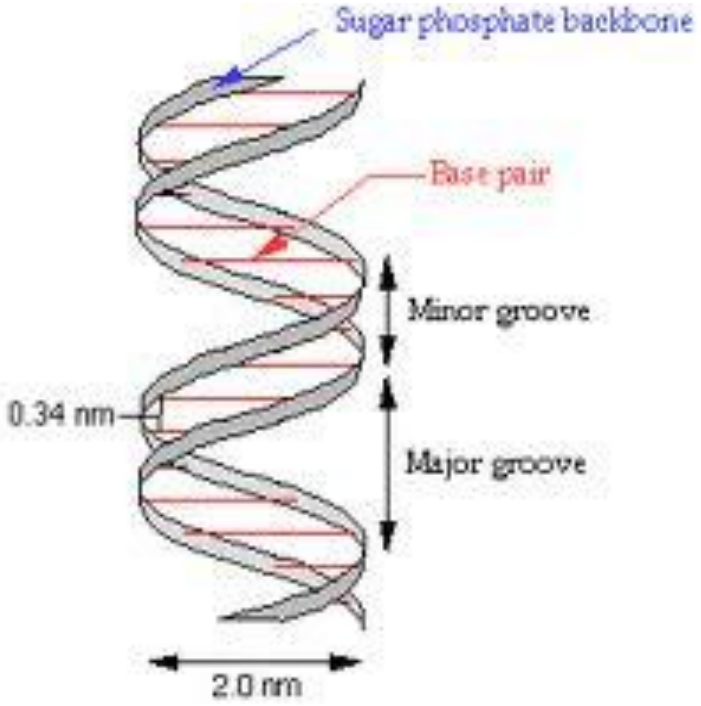


2-Deoxyribose



Ribose

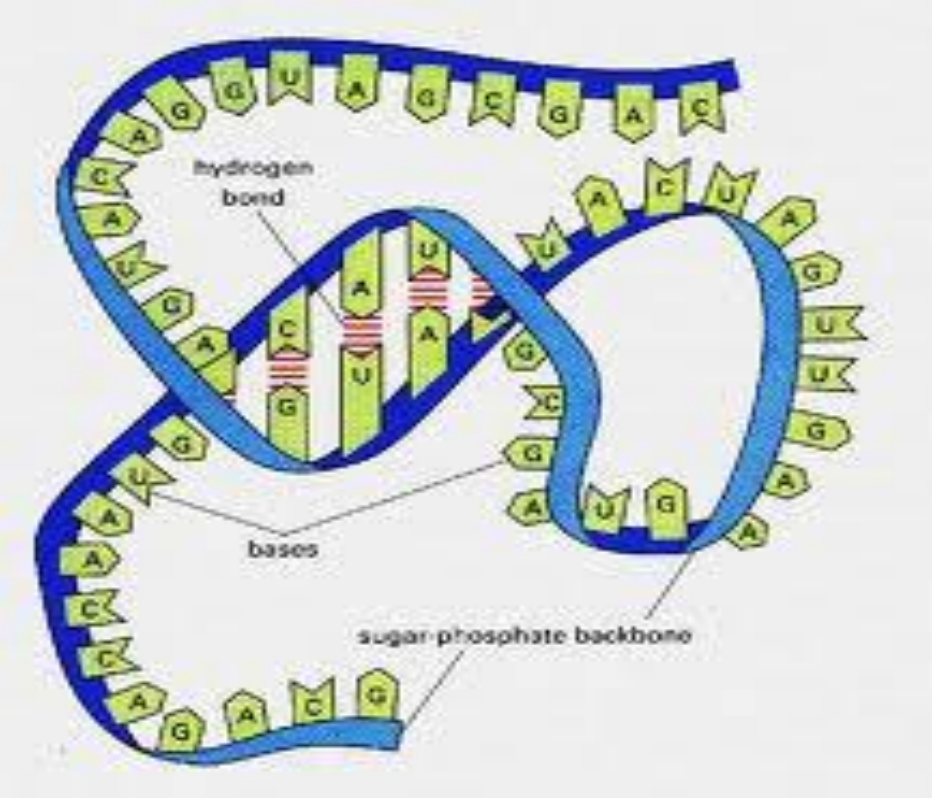
Size:



The DNA chain is 22 to 26 Ångströms wide (2.2 to 2.6 nanometres), and one nucleotide unit is 3.3 Å (0.33 nm) long.

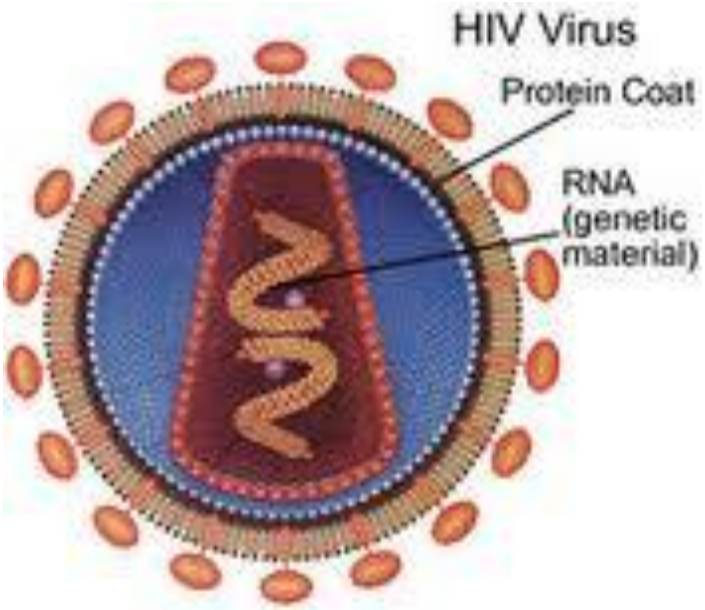
Ribonucleic acid (RNA)

- **RNA** is a biologically important type of molecule that consists of a long chain of nucleotide units.
- Each nucleotide consists of a nitrogenous base, a ribose sugar, and aphosphate.



Double-stranded RNA

- Double-stranded RNA (dsRNA) is RNA with two complementary strands, similar to the DNA found in all cells.
- dsRNA forms the genetic material of some viruses (double-stranded RNA viruses).



Types of RNA

Type	Abbr	Function	Distribution
Messenger RNA	mRNA	Codes for protein	All organisms
Ribosomal RNA	rRNA	Translation	All organisms
Transfer RNA	tRNA	Translation	All organisms

in post-transcriptional modification

Small nuclear RNA	snRNA	Splicing and other functions	Eukaryotes and archaea
Y RNA		RNA processing, DNA replication	Animals
Telomerase RNA		Telomere synthesis	Most eukaryotes

Regulatory RNAs

Antisense RNA	aRNA	Transcriptional attenuation / mRNA degradation / mRNA stabilisation / Translation block	All organisms

Messenger RNA

- mRNA carries information about a protein sequence to the ribosomes, the protein synthesis factories in the cell.
- It is coded so that every three nucleotides (a codon) correspond to one amino acid.

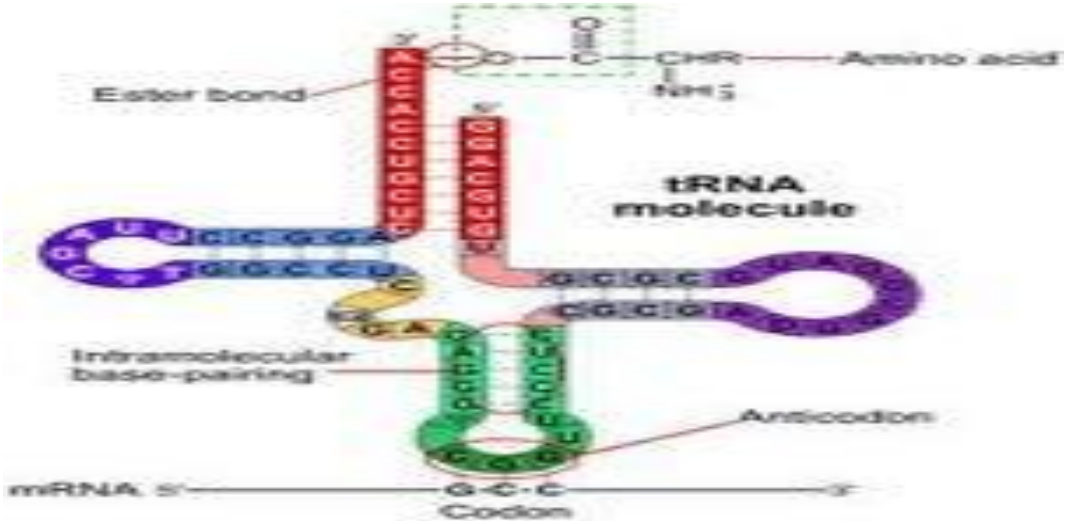
- In eukaryotic cells, once precursor mRNA (pre-mRNA) has been transcribed from DNA, it is processed to mature mRNA. This removes its introns—non-coding sections of the pre-mRNA.
-

-
- The mRNA is then exported from the nucleus to the cytoplasm, where it is bound to ribosomes and translated into its corresponding protein form with the help of tRNA.

- In prokaryotic cells, which do not have nucleus and cytoplasm compartments, mRNA can bind to ribosomes while it is being transcribed from DNA.

Transfer RNA

Transfer RNA (tRNA) is a small RNA chain of about 80 nucleotides that transfers a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation.

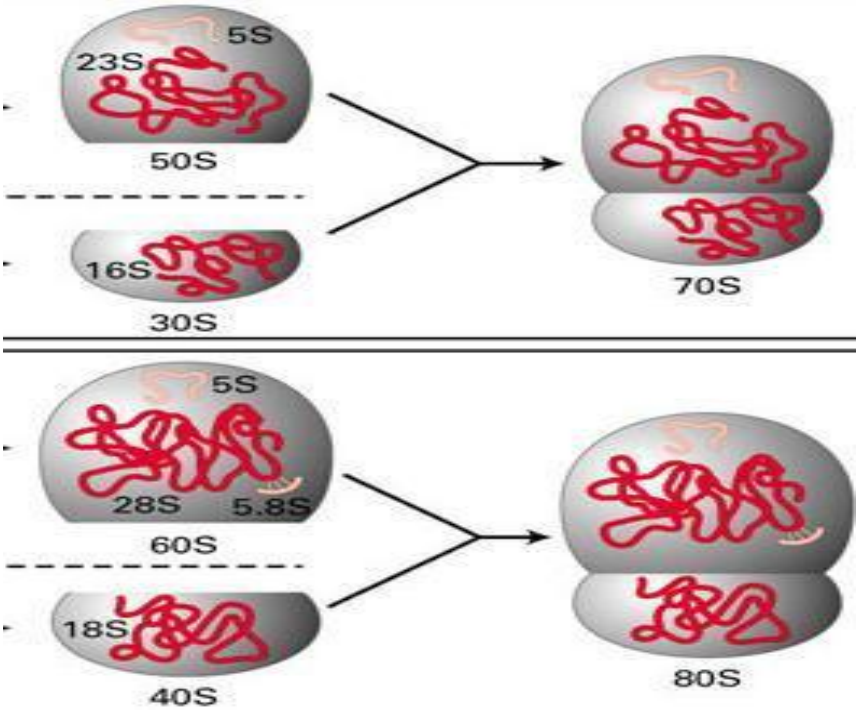


- It has sites for amino acid attachment and an anticodon region for codon recognition
- that site binds to a specific sequence on the messenger RNA chain through hydrogen bonding.

Ribosomal RNA

- Ribosomal RNA (rRNA) is the catalytic component of the ribosomes.
- Eukaryotic ribosomes contain four different rRNA molecules: 18S, 5.8S, 28S and 5S rRNA.
- rRNA molecules are synthesized in the nucleolus.
- In the cytoplasm, ribosomal RNA and protein combine to form a nucleoprotein called a ribosome.
- The ribosome binds mRNA and carries out protein synthesis. Several ribosomes may be attached to a single mRNA at any time.

- rRNA is extremely abundant and makes up 80% of the 10 mg/ml RNA found in a typical eukaryotic cytoplasm.



Difference between RNA & DNA

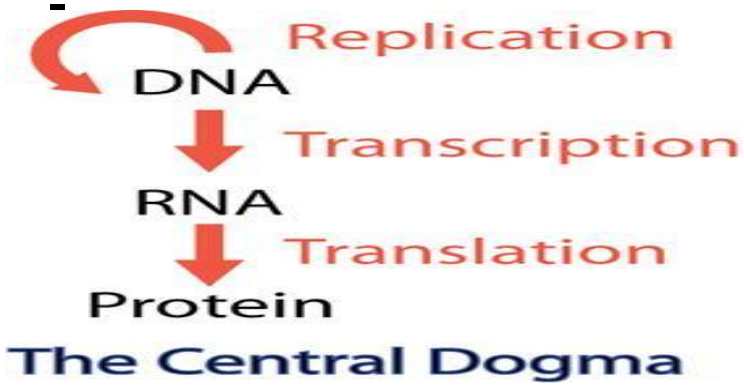
RNA	DNA
RNA nucleotides contain ribose sugar	DNA contains deoxyribose
RNA has the base uracil	DNA has the base thymine
presence of a hydroxyl group at the 2' position of the ribose sugar.	Lacks of a hydroxyl group at the 2' position of the ribose sugar.
RNA is usually single-stranded	DNA is usually double-stranded

Protein

Proteins (also known as polypeptides) are made of amino acids arranged in a linear chain and folded into a globular form.
The sequence of amino acids in a protein is defined by the sequence of a gene, which is encoded in the genetic code.
genetic code specifies 20 standard amino acids.

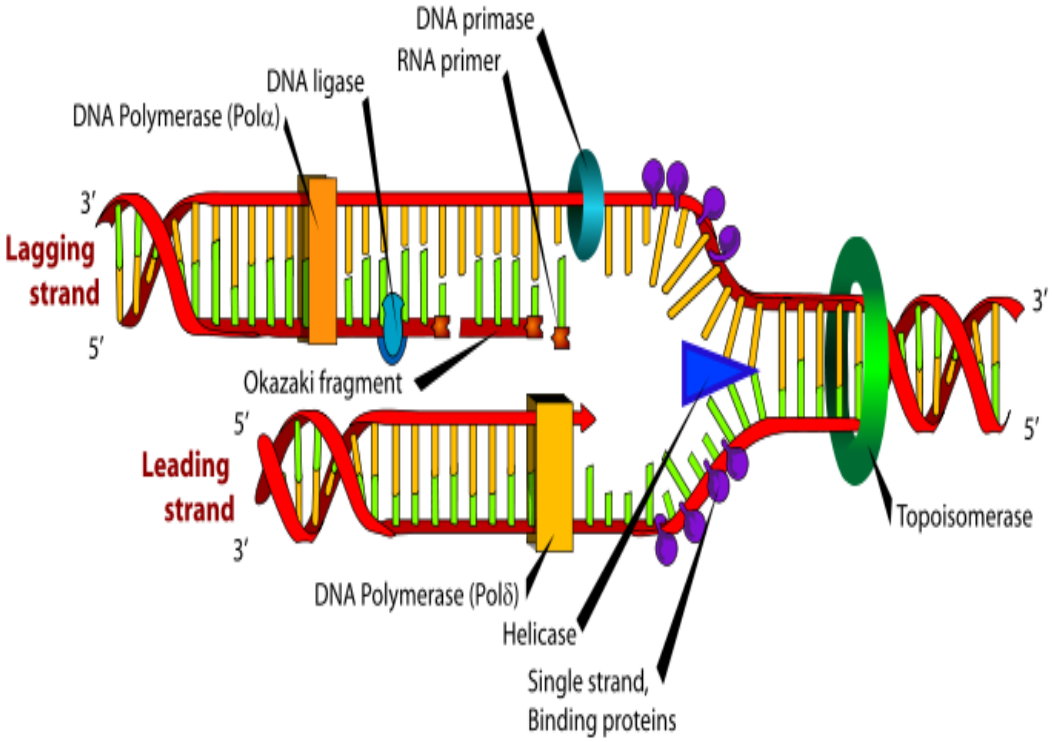
Basic players in molecular biology: DNA, RNA, and proteins. What they do is this :

-



DNA replication

DNA replication, the basis for biological inheritance, is a fundamental process occurring in all living organisms to copy their DNA.
In the process of "replication" each strand of the original double-stranded DNA molecule serves as template for the reproduction of the complementary strand.
Two identical DNA molecules have been produced from a single double-stranded DNA molecule.



In a cell, DNA replication begins at specific locations in the genome, called "origins".-

Unwinding of DNA at the origin, and synthesis of new strands, forms a replication fork.

In addition to DNA polymerase, the enzyme that synthesizes the new DNA by adding nucleotides matched to the template strand, a number of other proteins are associated with the fork and assist in the initiation and continuation of DNA synthesis.

Cellular proofreading that ensure near perfect fidelity for DNA replication.

Transcription

- Transcription, is the process of creating an equivalent RNA copy of a sequence of DNA.
- Transcription is the first step leading to gene expression.-

DNA —transcription→ RNA.

DNA ←reverse transcription— RNA.

- During transcription, a DNA sequence is read by RNA polymerase, which produces a complementary, antiparallel RNA strand.
- Transcription results in an RNA complement that includes uracil (U) instead of thymine (T).

Transcription process

The stretch of DNA transcribed into an RNA molecule is called a transcription *unit* and encodes at least one gene.

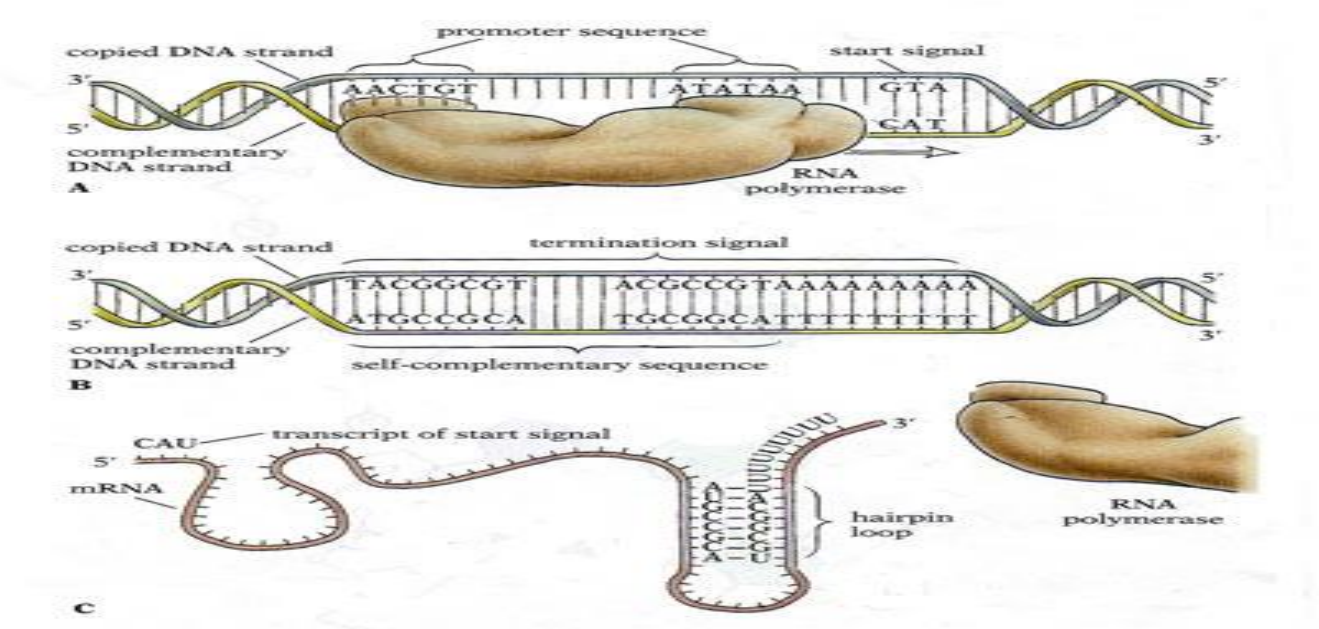
If the gene transcribed encodes for a protein, the result of transcription is messenger RNA (mRNA).

This mRNA will be used to create that protein via the process of translation.

Alternatively, the transcribed gene may encode for either rRNA or tRNA, other components of the

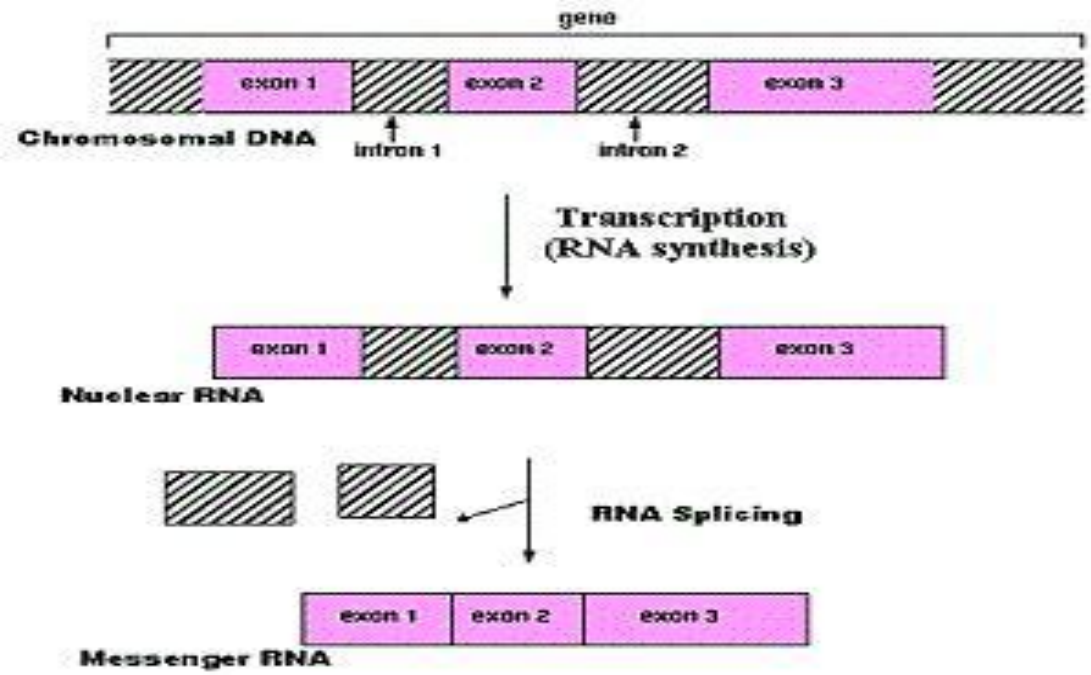
protein-assembly process, or other ribozymes.

A DNA transcription unit encoding for protein (the *coding sequence*) and *regulatory sequences* that direct and regulate the synthesis of that protein.



- DNA is read from 3' → 5' during transcription.
- the complementary RNA is created from the 5' → 3' direction.
- only one of the two DNA strands, called the template strand, is used for transcription because RNA is only single-stranded.

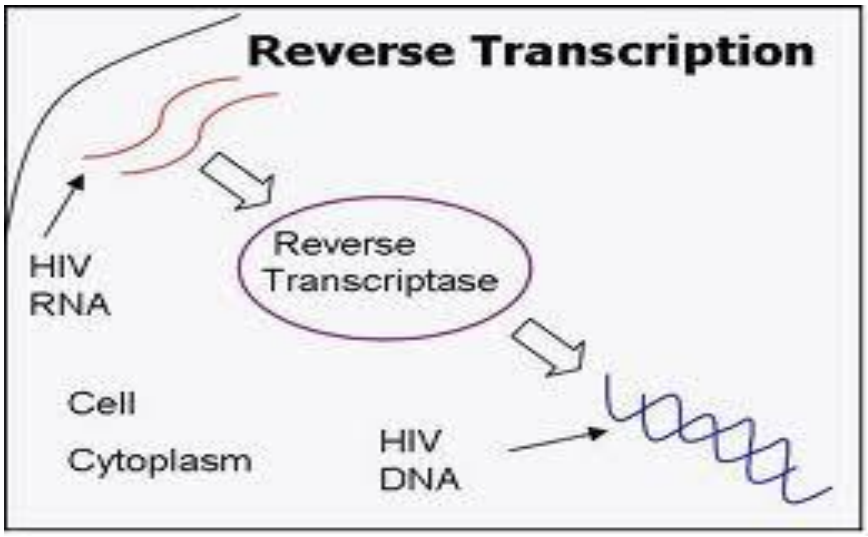
- The other DNA strand is called the coding strand.

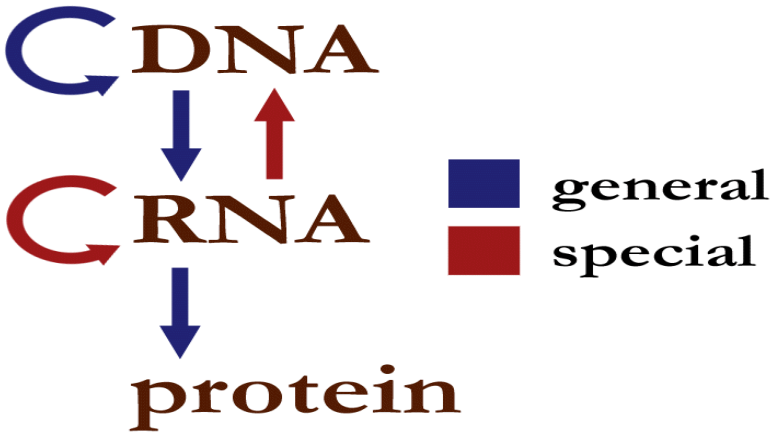


RNA synthesis and processing

Reverse transcription

Reverse transcribing viruses replicate their genomes by reverse transcribing DNA copies from their RNA; These DNA copies are then transcribed to new RNA. Retrotransposans also spread by copying DNA and RNA from one another.

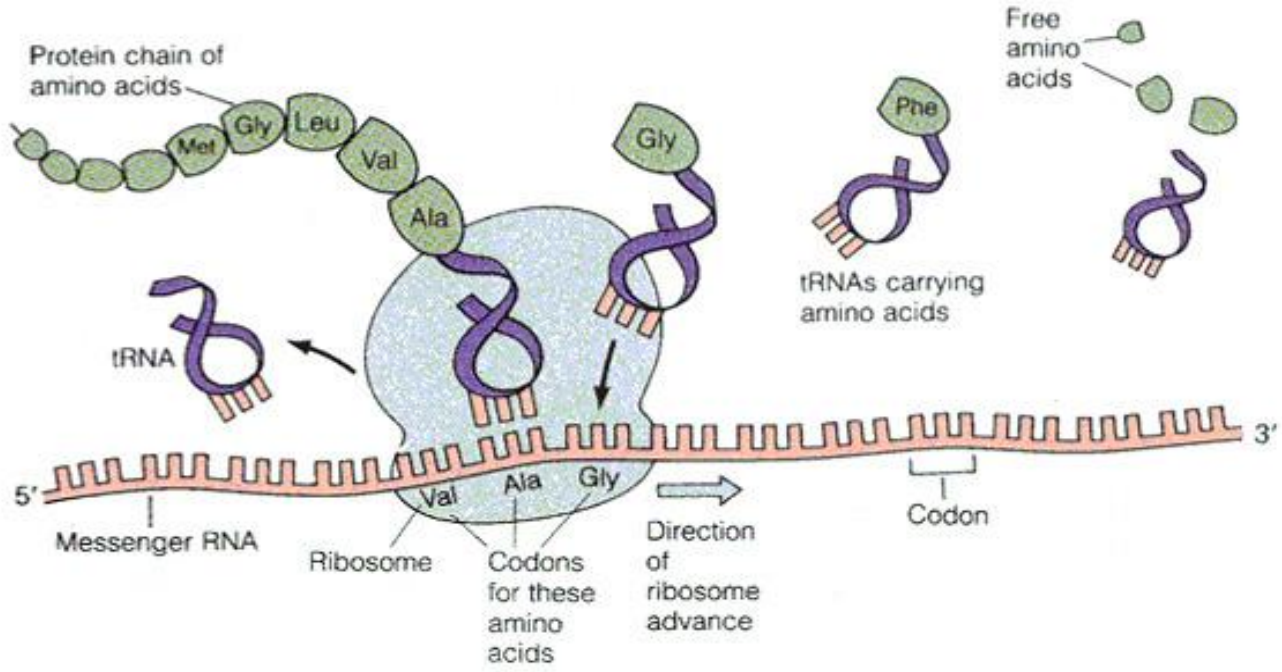




Translation

- Translation is the first stage of protein biosynthesis .
- In translation, (mRNA) produced by transcription is decoded by the ribosome to produce a specific amino acid chain, or polypeptide, that will later fold into an active protein.
- Translation occurs in the cell's cytoplasm, where the large and small subunits of the ribosome are located, and bind to the mRNA.

- **<u>Translation process</u>**



- The ribosome facilitates decoding by inducing the binding of <u>tRNAs</u> with <u>complementary</u> <u>anticodon</u> sequences to mRNA.

- The tRNAs carry specific amino acids that are chained together into a polypeptide as the mRNA passes through and is "read" by the ribosome.

- the entire ribosome/mRNA complex will bind to the outer membrane of the <u>rough endoplasmic reticulum</u> and release the nascent protein polypeptide inside for later vesicle transport and secretion outside of the cell.

What is Genome ?

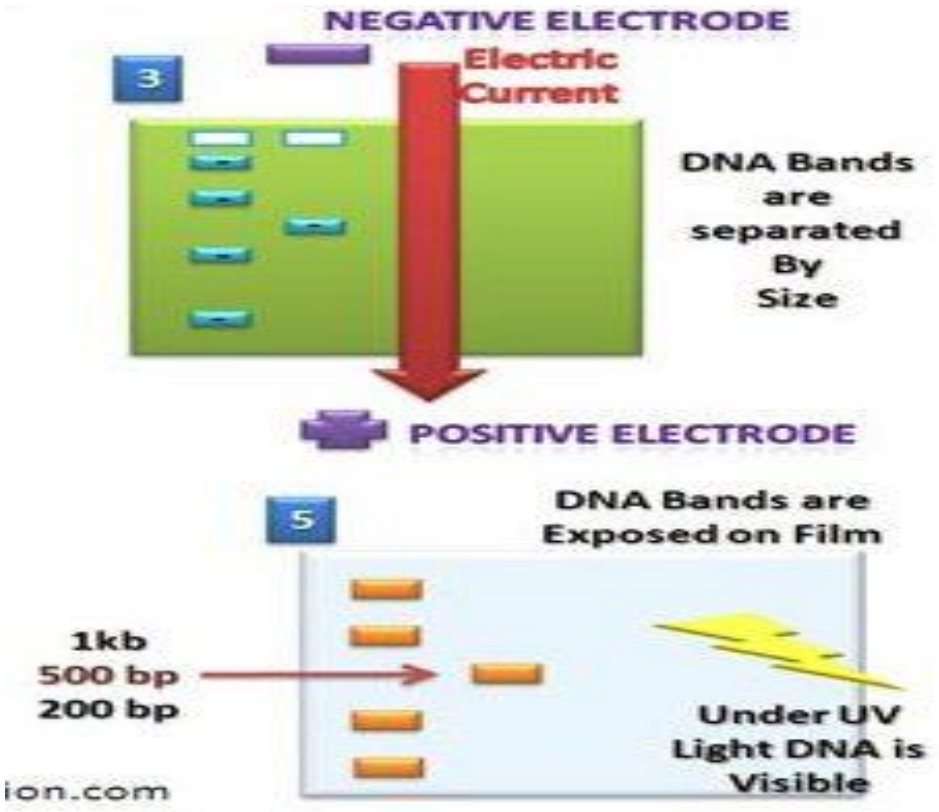
- **Genome** is the entirety of an organism's hereditary information.
- It is encoded either in DNA or, for many types of virus, in RNA.
- The genome includes both the genes and the non-coding sequences of the DNA.

Why Genome analysis ?

- The prediction of genes in uncharacterised genomic sequences.
- To obtain the complete sequences of as many genomes as possible.
- For Genetic modification.
- Genetic modification to develop new varieties at a faster rate like BT cotton and BT brinjal.

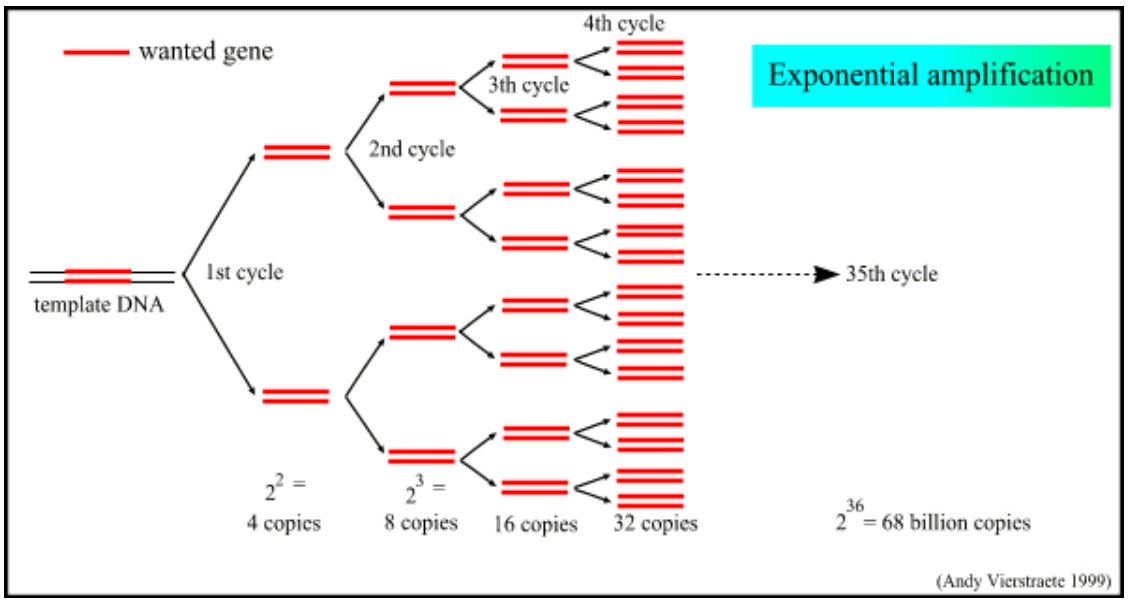
Tools used in Molecular Biology

❖ Gel electrophoresis



- The basic principle is that DNA, RNA, and proteins can all be separated by means of an electric field.
- In agarose gel electrophoresis, DNA and RNA can be separated on the basis of size by running the DNA through an agarose gel.
- Proteins can be separated on the basis of size by using an SDS-PAGE gel, or on the basis of size and their electric charge by using what is known as a 2D gel electrophoresis.
- The polymerase chain reaction
- is an extremely versatile technique for copying DNA.

- PCR allows a single DNA sequence to be copied (millions of times), or altered in predetermined ways.
- PCR has many variations, like reverse transcription PCR (RT-PCR) for amplification of RNA, and real-time PCR (QPCR) which allow for quantitative measurement of DNA or RNA molecules.



The process follows the principle of DNA replication

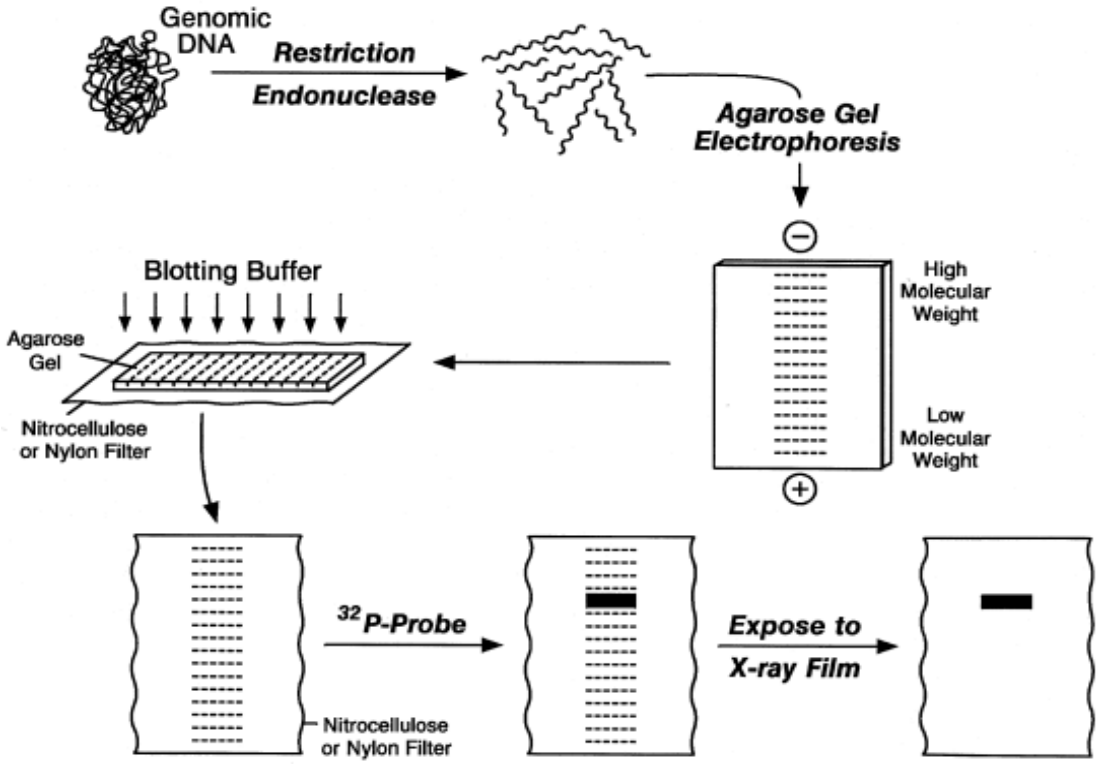
PRIMER

- A **primer** is a strand of nucleic acid that serves as a starting point for DNA synthesis.

- These primers are usually short, chemically synthesized oligonucleotides, with a length of about twenty bases. They are hybredized to a target DNA, which is then copied by the polymerase.

- minimum primer length used in most applications is 18 nucleotides.
- Replication starts at the 3'-end of the primer, and copies the opposite strand.
- In most cases of natural DNA replication, the primer for DNA synthesis and replication is a short strand of RNA .

Macromolecule blotting & probing



Southern blotting

- Southern blot is a method for probing for the presence of a specific DNA sequence within a DNA sample.
- DNA samples are separated by gel electrophoresis and then transferred to a membrane by blotting via capillary action.

- The membrane is then exposed to a labeled DNA probe that has a complement base sequence to the sequence on the DNA of interest.
- less commonly used due to the capacity of other techniques, such as PCR.
- Southern blotting are still used for some applications such as measuring transgene copy number in transgenic mice, or in the engineering of gene knockout embryonic stem cell lines.

Northern blotting

- The northern blot is used to study the expression patterns of a specific type of RNA molecule as relative comparison among a set of different samples of RNA.
- RNA is separated based on size and is then transferred to a membrane then probed with a labeled complement of a sequence of interest.
- The results may be visualized through a variety of ways depending on the label used. Most result in the revelation of bands representing the sizes of the RNA detected in sample.
- The intensity of these bands is related to the amount of the target RNA in the samples analyzed.

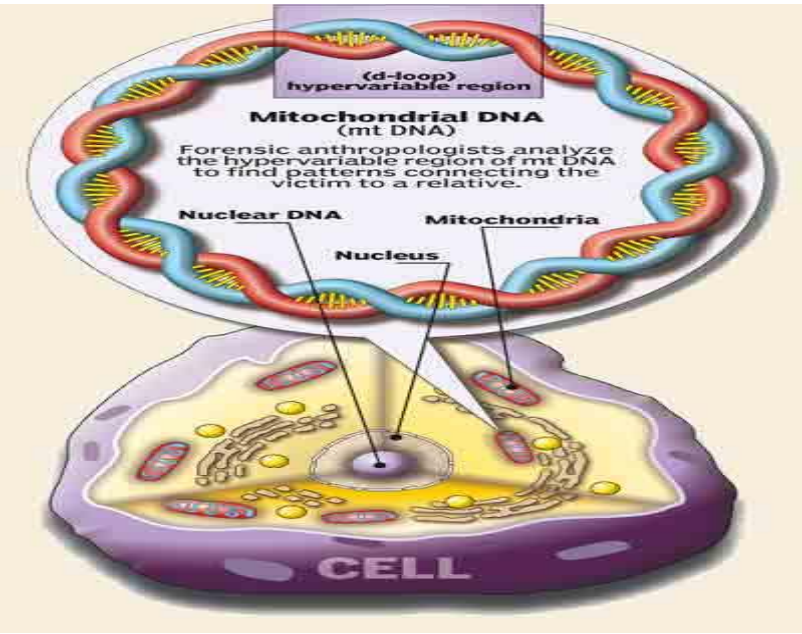
- It is used to study when and how much gene expression is occurring by measuring how much of that RNA is present in different samples.
- one of the most basic tools for determining at what time, and under what conditions, certain genes are expressed in living tissues.

Western blotting

- In western blotting, proteins are first separated by size, in a thin gel sandwiched between two glass plates in a technique known as SDS-PAGE sodium dodecyl sulphate polyacrylamide gel electrophoresis.
- The proteins in the gel are then transferred to a nitrocellulose, nylon or other support membrane.
- This membrane probed with solutions of antibodies. Antibodies specifically bind to the protein of interest & visualized by a variety of techniques, including colored products, chemiluminescence, or autoradiography.
- Antibodies are labeled with enzymes. When a chemiluminescent substrate is exposed to the enzyme it allows detection.
- Using western blotting techniques allows not only detection but also quantitative analysis.
-

Molecular markers

- Molecular marker are based on naturally occurring polymorphism in DNA sequence(i.e. base pair deletion, substitution ,addition or patterns).
  - Genetic markers are sequences of DNA which have been traced to specific locations on the chromosomes and associated with particular traits.
- It can be described as a variation that can be observed.
- A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism, SNP), or a long one, like mini satellites.



Some commonly used types of genetic markers are

- RFLP (or Restriction fragment length polymorphism)

- AFLP (or Amplified fragment length polymorphism)
- RAPD (or Random amplification of polymorphic DNA)
- VNTR (or Variable number tandem repeat)
- Micro satellite polymorphism, SSR (or Simple sequence repeat)
- SNP (or Single nucleotide polymorphism)
- STR (or Short tandem repeat)
- SFP (or Single feature polymorphism)
- DArT (or Diversity Arrays Technology)
- RAD markers (or Restriction site associated DNA markers)

There are 5 conditions that characterize a suitable molecular marker

- Must be polymorphic
- Co-dominant inheritance
- Randomly and frequently distributed throughout the genome
- Easy and cheap to detect
- Reproducible

Molecular markers can be used for several different applications including

- Germplasm characterization,

- Genetic diagnostics,
- Characterization of transformants,
- Study of genome
- Organization and phylogenic analysis.
- Paternity testing and the investigation of crimes.
- Measure the genomic response to selection in livestock

RFLP (Restriction fragment length polymorphism)

RFLPs involves fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs. A RFLP occurs when the length of a detected fragment varies between individuals and can be used in genetic analysis.

Advantages:

- Variant are co dominant
- Measure variation at the level of DNA sequence, not protein sequence.

Disadvantage:

- Requires relatively large amount of DNA

AFLP (Amplified fragment length polymorphism)

In this analysis we can amplify restricted fragments and reduces the complexity of material to be analyzed (approx 1000 folds).it can be used for *comparison b/w closely related species only.*

Advantages:

- Fast -Relatively inexpensive
- Highly variable

Disadvantage:

- Markers are dominant
- Presence of a band could mean the individual is either homozygous or heterozygous for the Sequence - can't tell which?

RAPD (Random amplification of polymorphic DNA)

Random **A**mplification of **P**olymorphic **D**NA. It is a type of PCR reaction, but the segments of DNA that are amplified are random.

Advantages:

- Fast
- Relatively inexpensive
- Highly variable

Disadvantage:

- Markers are dominant
- Presence of a band could mean the individual is either homozygous or heterozygous for the Sequence - can't tell which?
- Data analysis more complicated

Micro satellite polymorphism, SSR or Simple sequence repeat

Microsatellites, Simple Sequence Repeats (SSRs), or Short Tandem Repeats (STRs), are repeating sequences of 1-6 base pairs of DNA.

Advantages:

- Highly variable

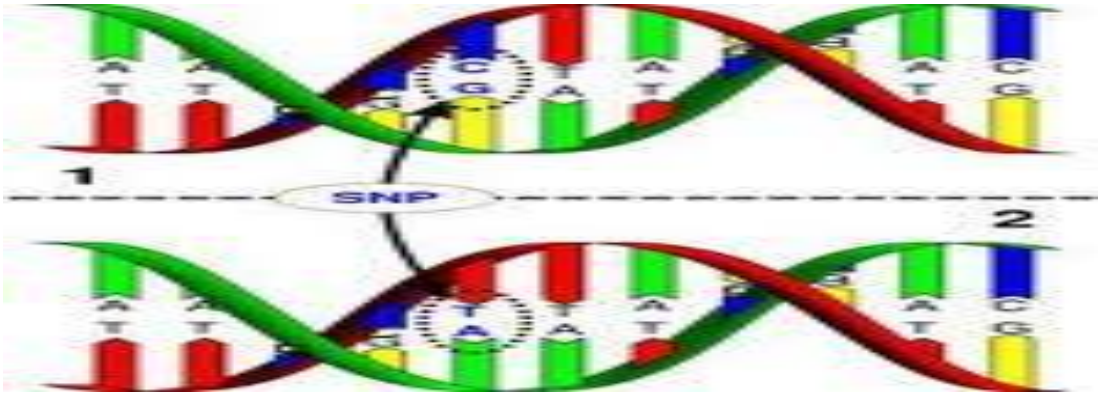
- Fast evolving
- Co dominant

Disadvantage:

- Relatively expensive and time consuming to develop

SNP

- A single-nucleotide polymorphism (SNP, pronounced *snip*) is a DNA sequence variation occurring when a single nucleotide — A, T,C, or G — in the genome (or other shared sequence) differs between members of a species or paired chromosomes in an individual.
- Used in biomedical research ,crop and livestock breeding programs.



STR

- A short tandem repeat (STR) in DNA occurs when a pattern of two or more nucleotides are repeated and the repeated sequences are directly adjacent to each other.
- The pattern can range in length from 2 to 16 base pairs (bp) (for example $(CATG)_n$ in a genomic region) and is typically in the non-coding intron region

- Used in forensic cases.
- used for the genetic fingerprinting of individuals

PRINCIPLES OF DNA ISOLATION & PURIFICATION

DNA can be isolated from any nucleated cell.

DNA is a giant anion in solution.

Sources of DNA include

- Blood
- Buccal cells
- Cultured cells (plant and animal)
- Bacteria
- Biopsies

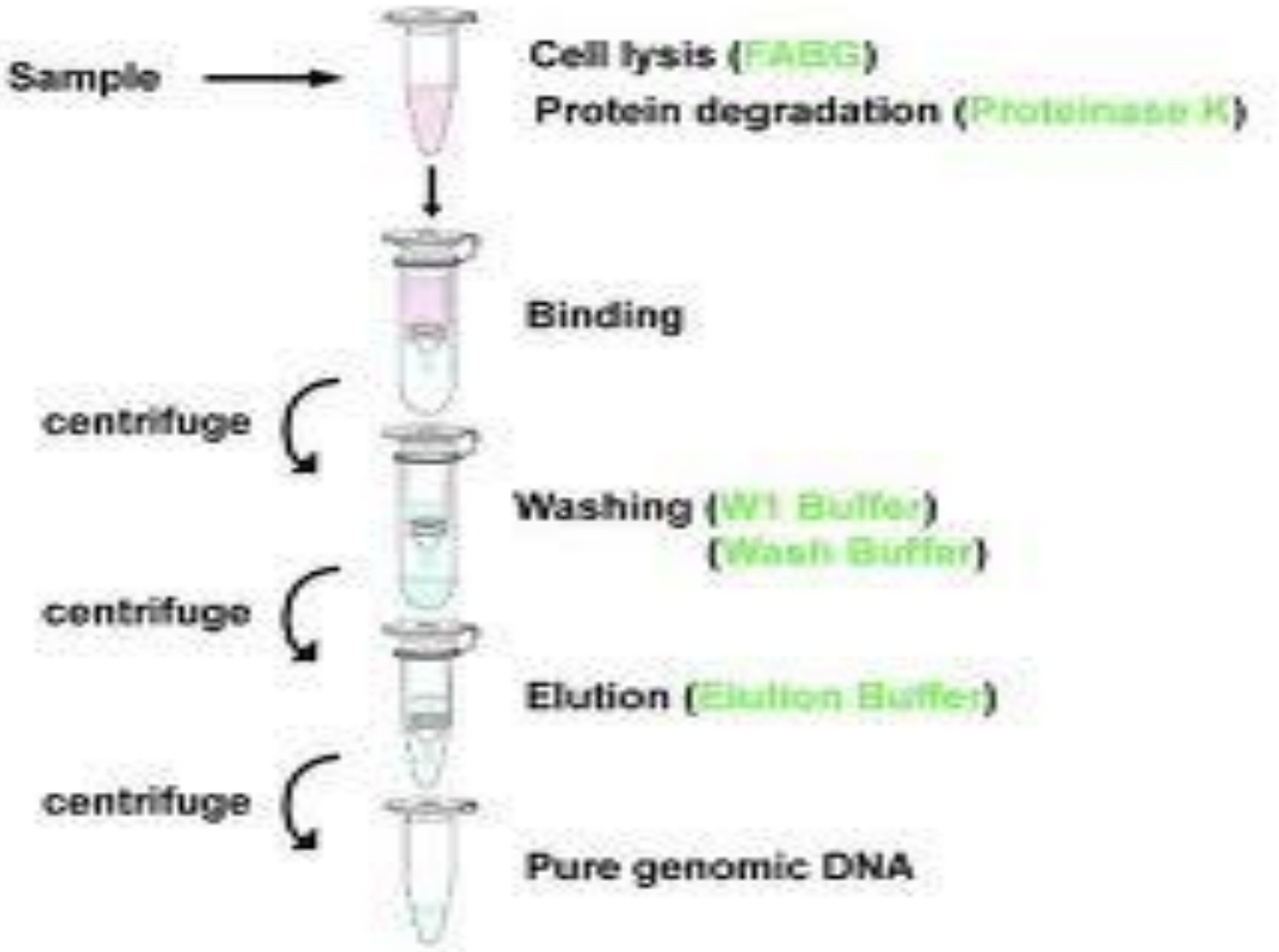
Forensic samples i.e. body fluids, hair follicles, bone & teeth roots.

DNA isolation

is a routine procedure to collect DNA for subsequent molecular analysis. There are three basic steps in a DNA extraction:

- **Cell disruption**:- This is commonly achieved by grinding or sonicating the sample. Removing membrane lipids by adding a detergent.

- **Isolation of DNA**:- Removing proteins by adding a protease (optional but almost always done).
- **Precipitating the DNA** :-usually ice-cold ethanol or isopropanol is used. Since DNA is insoluble in these alcohols, it will aggregate together, giving a *pellet* upon centrifugation. This step also removes alcohol soluble salt.



Basic rules

- **Blood** – first lyse (explode) the red blood cells with a gentle detergent such as Triton-X-100.
- **Wash cells** – haemoglobin (and other pigments) inhibits restriction enzymes and TAQ polymerase.
- Work on **ice** to slow down enzymatic processes.

- Wear **gloves** to protect your samples from you!!
- **Autoclave** all solutions and store in fridge (except SDS and organic solvents!)
- Keep all pellets & supernatants until you have the DNA you want.

Getting to the DNA

- Cells – **lyse** all cells in presence of :
- **NaCl** so that DNA is stabilised and remains as a double helix,
- **EDTA** which chelates Mg++ and is a co-factor of DNAse which chews up DNA rapidly.
- **anionic detergent SDS** which disrupts the lipid layers, helps to dissolve membranes & binds positive charges of chromosomal proteins (*histones*) to release the DNA into the solution.
- Include a **protease** *(proteinase K*) to digest the proteins
- incubate the solution at an **elevated temperature** (56^{o}C to inhibit degradation by DNAses) for 4-24 hrs.
-

Getting rid of the protein

- **Organic solvent extraction** using equal volume phenol:chloroform (24:1)
- Protein at the interface after centrifugation (10000 rpm at 10^{o} c for 10 min.)

Precipitating the DNA

- add 2.5 - 3 volumes **ice-cold 95% ethanol** to the DNA & leave at -20^{o}C overnight.
- Centrifuge sample at 10000 rpm ,10 min., 4^{0}C.
- **Wash** DNA pellet to remove excess salt in 70% EtOH and air-dry.
- **Resuspend** in sterile distilled water(pH7.4)
- Store at 4^{o}C or frozen at -20^{o}C long term.

Quantifying the DNA

- The amount of DNA can be quantified using the formula:

$$\text{DNA concentration } (\mu g/ml) = \frac{OD_{260} \times 100 \text{ (dilution factor)} \times 50\ \mu g/ml}{1000}$$

- Nucleic acids have a peak absorbance in the ultraviolet range at about 260 nm
- 1 A260 O.D. unit for dsDNA = 50 µg/ml
- 1 A260 O.D. unit for ssDNA = 33 µg/ml
- 1 A260 O.D. unit for RNA = 40 µg/ml

DNA purity

- The purity of the DNA is reflected in the OD260:OD 280 ratio and must be between 1.6 and 2.00.

< 1.6 – protein contaminated
> 2.0 – chloroform / phenol contaminated

- Repurify sample.

Summary

- Sample for DNA extraction
- Lysis of cells at elevated temperature + detergent + enzyme in salt buffer
- Removal of cellular proteins
- Precipitation of nucleic acids with ethanol
- Quantitation and purity measurement of DNA

Future aspects

- For agricultural development and environment protection.
- To ensure food security for ever growing human population.

PART (3)

Embryology

Embryology

Almost all higher animals start their lives from a single cell, the fertilized ovum (*zygote*). The zygote has a dual origin from two gametes- a spermatozoon from the male parent and an ovum from the female parent. The time of fertilization represents the starting point in the life history.**Embryology** deals with the study of development of animals from the fertilized egg to the formation of all major organ systems. Embryology is the study of the origin and development of an organism. But the term development is used in various senses in biology, but in its broadest sense it deals with the complex changes which an individual organism undergoes in its life cycle from fertilization to death.

Historical background of Embryology

Theories of Embryology

1-Preformartion theories

During seventeenth century a Dutch scientist theorized that sex-cells contained preformed embryos or miniatures of the adult. Other biologists thought that the ovum contained a

transparent, highly folded, small and unobservable miniature of the adult, which was in some way stimulated to growth by the seminal fluid. Some other microscopists, such as, *Leeuwen hoek* (1632-1723), believed that preformed miniature organism presented in the head of the sperm and not in egg and that was called *homunculus* or *animalculs* (animalcule means spermatozoon).

2- Theory of Epigenesis

We may now turn to the other great theory of development: epigenesist. This theory assumes that embryonic development and differentiation originate in a homogeneous mass of living matter in which there is no preformed materials, neither tissue nor organs. In the mid-eighteenth century, the theory of epigenesis maintained that the egg contains the material from which the embryo is gradually built.

3- Cell theory

After the formation of the cell theory, embryological research entered an entirely new avenue. It was discovered that the ovum was a single cell and that fertilization consisted of the

union of the ovum and spermatozoon in the formation of the zygote.

4- Recapitulation Theory

This theory was postulated by *Ernst Haeckel* (1868) and *Muller* (1864). It is based on the contribution of evolutionary theory. This theory states that higher animals in their development passes through stages which are similar to the adult stages of lower animals which were their ancestors. In other words the ancestral characters reappear in the developmental stages of an individual. Thus the recapitulation theory states that the embryonic stages of a higher animal resemble the adult stages of its ancestor.

The value of Embryology

1- The recent techniques make possible the diagnosis and/or treatment of genetic disease and birth defects before a baby is born.

2- Examination of a small amount of the amniotic fluid that surrounds an embryo makes it possible to determine the sex of a

baby before it is born and to detect the presence of genetic conditions that could lead to defective child.

3- The application of ultrasound and new x-ray imaging techniques allows the diagnosis of many anatomical defects in fetuses. Some of these can be dealt with by means with intrauterine surgery.

5- Embryology is a key that helps unlock such secretes as heredity, the determination of sex and organic evolution. A general conception of how man, like other animals, develops from a single cell should in the cultural background of every educated mind.

6-From embryology we can learn in one short, uninterrupted story how each individual grows into an adult. We can see this process going on in the laboratory under our very eyes.

Special fields in embryology

Over the years, the science of embryology has evolved in response to new modes of thought and the availability of new

technique. Embryology has many sub-branches in it they are:

1- Descriptive Embryology

The term is applied to the methods of study concerned with the direct observation and description of embryological development. Between 1880 and 1890, the new techniques of serial sections and of making three-dimensional wax plate reconstructions from them provided the basis for descriptive embryology. More than a century later, the availability of supercomputers and the appropriate soft ware allows the construction of three-dimensional digitized images of embryos. Digitized images contain much more information than wax plate reconstructions and can be assembled in a fraction of the time.

2- Experimental Embryology

In experimental embryology experiments are used for studying the developmental stages. It helps to understand the fundamental developmental mechanisms. In experimental embryology the various parts of developing embryo are removed, transplanted, parts exchanged or the environmental conditions altered. This helps to understand induction, gradient system, etc.

3- Comparative Embryology

In comparative embryology the embryological development of different animals are studied and compared. It also gives some ideas on the developmental stages of certain animals in whose case the study of development is difficult. In recent years, comparative embryology has undergone a resurgence through the investigations of taxonomists, who have recognized that valuable clues to taxonomic relationships among species can be found by studying their embryonic development.

4- Chemical Embryology

During the 1930s and 1940s newly emerging chemical and biochemical techniques led to the establishment of chemical embryology, which provided descriptive information about chemical and physiological events in the embryo. More recent biochemical and molecular studies are revolutionizing our understanding of the manner in which different components of embryos intract and how the basic body pattern of the embryo is laid down.

5- Teratology

Teratology is the branch of embryology concerned with the study of malformations. Drawings and images of abnormal individuals are among the oldest biological records. In ancient times the birth of a malformed infant was often assumed to portend events to come. In the middle Ages, the writing on teratology seemed to degenerate into contests to discover who could assemble the most bizarre malformations. During this century, work in teratology has taken on an entirely new aspect. With birth defects having moved well up among the top 10 causes of death in countries with advanced levels of medicine, great effort is being spent to identify and eliminate genetic and environmental factors that cause congenital defects.

The Normal Sequence of Events in Embryology

1- Gametogenesis 2-Fertilization

3- Cleavage and blastula formation

4- Gastrulation 5- Organgenesis

1-Gametogenesis

The embryogenesis is started from the time of

differentiation and organization of haploid male and female sex cells, *viz*., *sperm* and *ova* respectively, from diploid somatic cells of each parent during a process called gametogensis. The gametogenesis include spermatogenesis and oogenesis. The spermatogenesis is a process which occurs in male gonads (testes) and produces small-sized, motile, haploid sex cell, the *sperms* or *spermatozoa*. The oogenesis occurs in female gonads (ovaries) and produce a large, non-motile, nutrient- *polar bodies* or *polocytes*.

2-Fertilization

Fertilization is the initial event in development in sexual reproduction. Union of male and female gametes. Provides for recombination of paternal and maternal genes. Restores the diploid number. Activates the egg to begin development.

The process of fertilization involves a number of independent biological and physiological events

1-The nucleus and cytoplasm of spermatozoon fuse with the nucleus and cytoplasm of the egg

2- The cortical reaction in the egg cytoplasm to elevate a

fertilization membrane outside the plasma membrane

3- Activation of egg to start its rapid cleavage by mitosis.

3-Cleavage and blastula formation

During third phase of embryogenesis, the *cellulation*, *segmentation* or *cleavage*, no growth of egg-cytoplasm (*ooplasm*) takes place, but, rate of synthesis of some macromolecules such as DNA and proteins is increased is increased at the expense of reverse food substances of egg (*viz*., glycogen, fats and yolk); and the fertilized egg undergoes repeated and successive mitotic cell divisions and produces a compact heap of cells or *blastomeres*, called *morula*. The blastomeres of morula soon get arranged in a hollow spherical body, a *blastula*, with a layer of blastomeres, called blastoderm, surrounding a fluid-filled central space or cavity, called *blastocoel*. The conversion of a fertilized egg into a multicellular blastula is called *blastulation*.

4-Gastrulation

Following blastulation a part of the blastoderm disappears from the surface of the blastula by *morphogenetic movements* of

cells and is enclosed by the reminder of the blastoderm. The part of the blastoderm that remains on the surface becomes *ectoderm*; the part of migrating into the interior becomes *endoderm* and *mesoderm*. In this way a simple spherical body becomes converted into two or three layered embryo known as the *gastrula*. The process involved in gastrula formation is called *gastrulation*, (gastrula, diminutive from the greek word gaster , meaning stomach) and the cellular layers of gastrula are known as the *primary germinal layer*. The germinal layers are complex rudiments from which various organs of the animal's body are derived.

The fully formed gastrula has a cavity called *archenteron*, which is lined by endoderm. The opening leading from this cavity to the exterior is called the *blastopore*. During later development, the archenteron or part of it eventually gives rise to the cavity of the alimentary canal.

The fate of blastopore (the opening from the outside into the archenteron) differs in the three main groups of metozoa:

1-In Coelenterate it becomes the mouth.

2-In Protostomia (including Annelida , Mollusca, Arthropoda and allied groups), it becomes subdivided into two opening, one of which becomes the mouth and other the anus.

3-In Deuterostomia (including Echinodermata and Chordata), only the anus is formed.

5-Organogenesis

During the fifth phase of development, the *organogenesis* or organs formation, the continuous masses of cells of the three germinal layers split up into smaller groups of cells, called the *primary organ rudiments*, each of which is destined to produce a certain organ or part of the adult animal body. The primary organ rudiments, further, subdivide into *secondary organ rudiments* which are rudiments of the subordinate and simpler organs and parts. With the appearance of primary and secondary organ rudiments the embryo begins to show some similarity to the adult animal, or to the larva, if the development includes a larval stage.

Gametogenesis

The reproductive cells, which unite to initiate the

development of a new individual, are known as *gametes*- the *ova* of the female and the *spermatozoa* of the male. The gametes themselves and the cells that give rise to them constitute the individual's *germ plasm*. The other cells of the body, which take no direct part in the production of gametes, are called somatic cells or, collectively, the *somatoplasm*. The somatoplasm can thus be regarded as the material that protects and nourishes the germ plasm.

Gametogenesis (*oogenesis* in the female and *spermatogenesis* in the male) is a broad term that refers to the processes by which germ plasm is converted into highly specialized sex cells that are capable of uniting at fertilization and producing a new being. Commonly, gametogenesis is divided into four major phases:

1-The origin of the germ cells and their migration to the gonads

2- The multiplication of the germ cells in the gonads through the process of mitosis

3- Reduction of the number of chromosomes by one-half by meiosis

4- The final stages of maturation and differentiation of the

gametes

Into spermatozoa or ova.

Primordial germ cells

The cells which are destined to develop into gametes are called primordial germ cells. The germ cells either arise from the germinal epithelium of grads (*germinal epithelial origin*) or may arise outside the gonad at an early period of embryonic development and then migrate to garads (extra gonadal origin).

The origin of primordial germ cells and their migration to the gonads.

Primordial germ cells of birds, reptiles, and mammals arise in the epiblast of the early embryo and then take up temporary residence in the extraembryonic tissue before returning to the body of the embryo proper. In birds they are recognizable in the *germinal crescent*, which is located well beyond the future head region of the embryo.

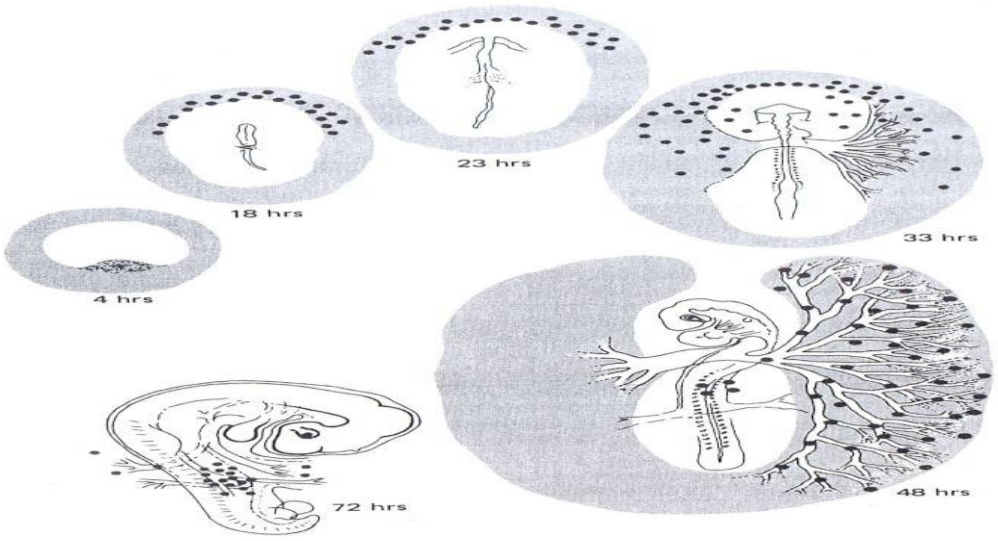
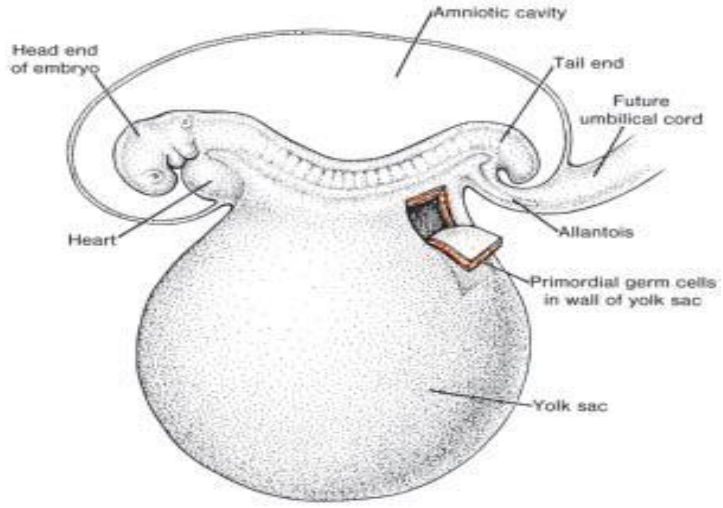


FIGURE 3-3
The migration of primordial germ cells (*dark circles*) in the avian embryo: 4 hours—no identifiable germ cells before the primitive streak is formed; 18 and 23 hours—passive accumulation of primordial germ cells in the anterior germinal crescent; 33 hours—active penetration into blood islands and their entry into the circulation; 48 hours—circulation of germ cells and their early egress into the gonadal primordia; 72 hours—colonization of the gonads. (*Redrawn from Nieuwkoop and Sutasurya, 1979.*)

In mammals the germ cells originate in the endoderm of adjoining region of the yolk sac in human before migrating into the gonds (tests or ovary)



Primordial germ cells in vertebrates migrate to the gonads by to principal mechanisms. In birds and reptiles, they pass through the walls of local blood vessels and enter the circulation. From the bloodstream they are apparently able to recognize the blood

vessels of the gonads, because there they penetrate the walls of the blood vessels and settle down in the gonads.

Spermatogenesis

The transition from mitotically active primordial germ cells to mature spermatozoa is called *spermatogenesis*, and it involves a sweeping series of structural transformation. Although is a wide variety in the morphology of mature, the overall process of spermatogenesis is much the same throughout the vertebrate classes. This process can be broken down into three principal phases: (1) mitotic multiplication, (2) meiosis, and (3) spermiogenesis.

Mitosis of sperm-forming cells occurs throughout life, and the mitotically active cells within the seminiferous tubules are known as *spermatogonia*. These cells are concentrated near the outer wall of the seminiferous tubules. Spermatogonia have been subdivided into two main populations. *Type-A spermatogonia* represent the stem-cell population. Within this population is a group of noncycling dark A cells that may be

long-term reserve cells. Some of these cells become mitotically active pale A cells, which ultimately give rise to *type-B spermatogonia*. These are cells that have become committed to leaving the mitotic cycle and which go on to finish the process of spermatogenesis.

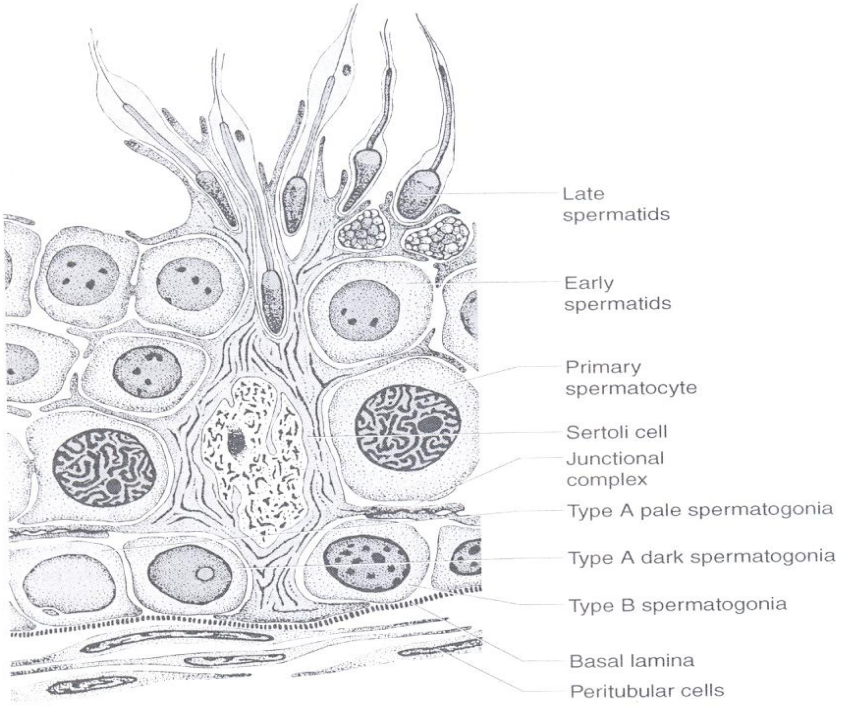
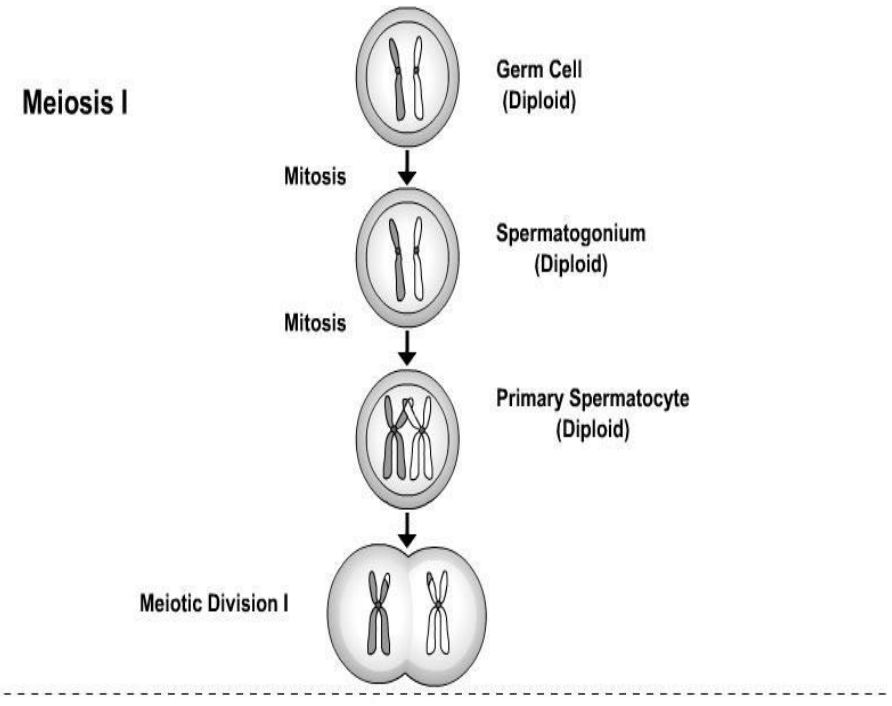
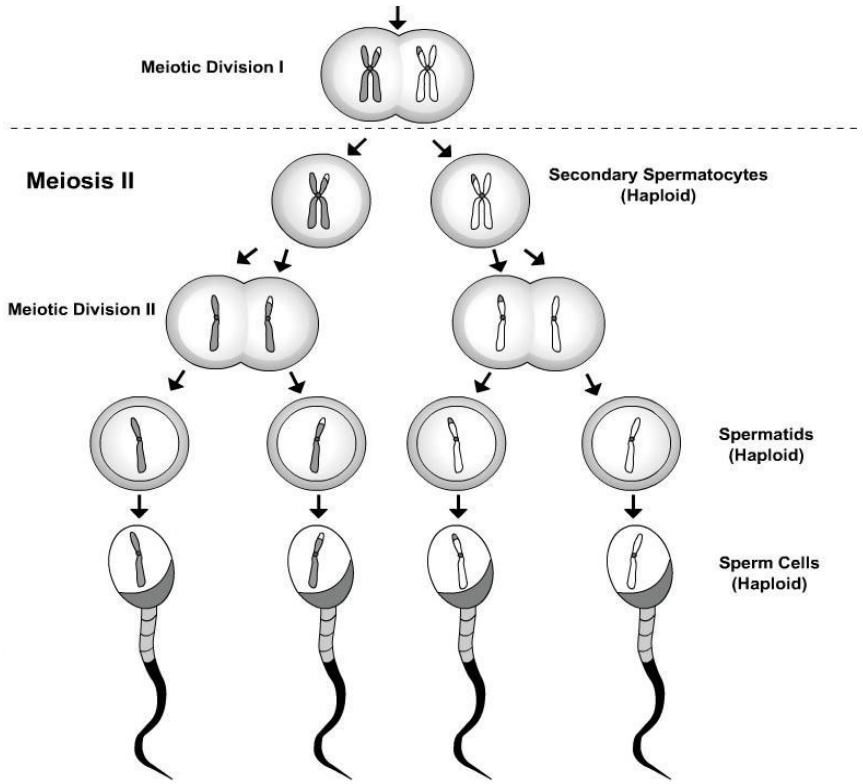


Figure 1.24 Sertoli cells and maturing spermatocytes. Spermatogonia, spermatocytes, and early spermatids occupy depressions in basal aspects of the cell; late spermatids are in deep recesses near the apex.

During the first meiotic division each *primary spermatocytes* divides into two equal daughter cells. With the onset of the second meiotic division these cells are known as *secondary spermatocytes*. In the human the first meiotic division lasts for several weeks, whereas the second one is completed in about 8 hours. Four haploid *spermatids* result from the meiotic phase of spermatogenesis.





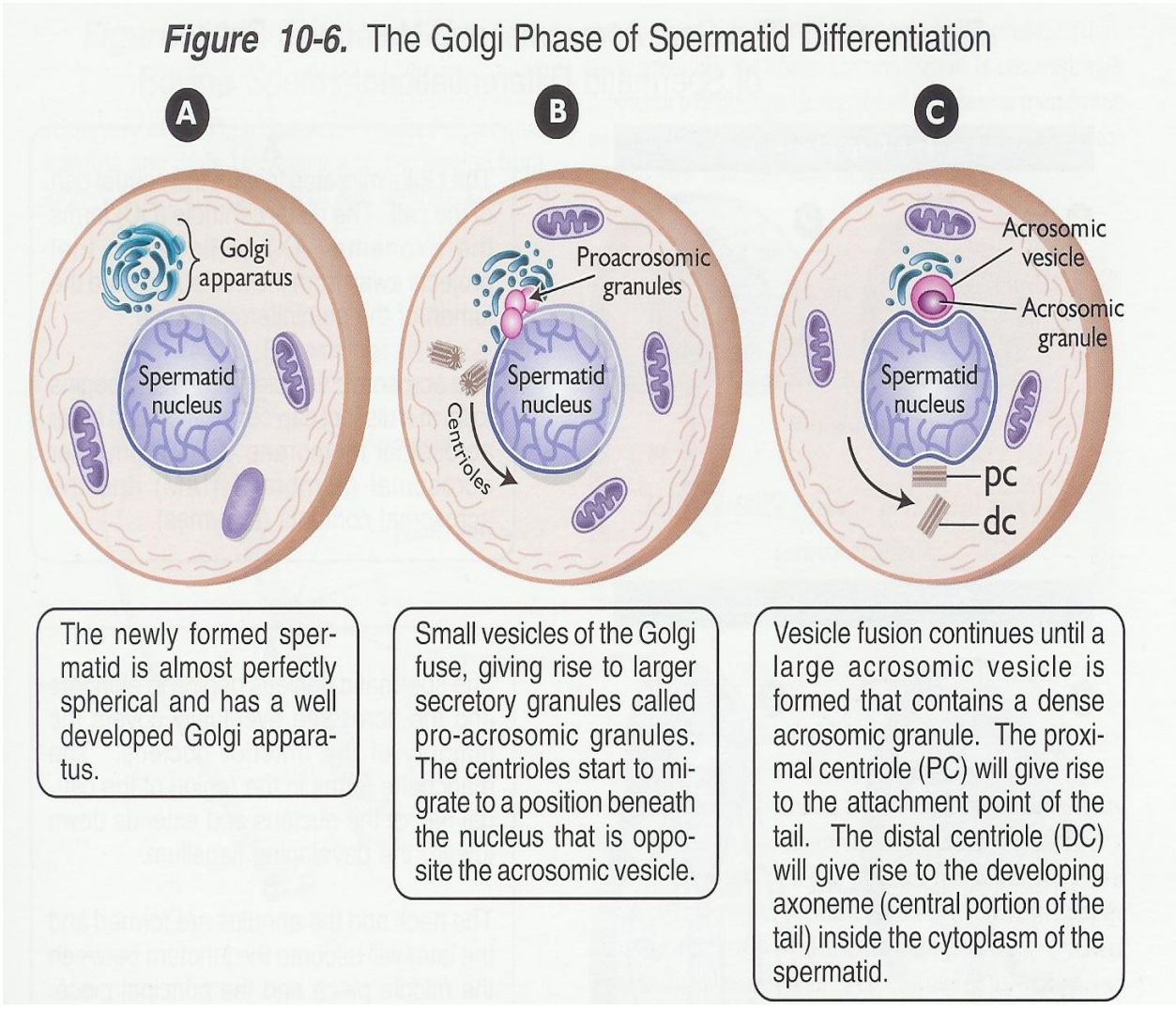
Although they no longer divide, the spermatids undergo a profound transformation from relatively ordinary looking cells to extrermely specialized *spermatozoa*.

The third phase in spermatogenesis is called spermiogenesis.

The cytoplasm streams away from the nucleus, which will become the sperm head, leaving only a thin layer covering the nucleus. At the apical end the developing sperm head, the Golgi complex forms proacrosomal granules, which fuse to form the *acrosome* . within the cytoplasm the centrioles become more conspicuous and appear to be a point of anchorage for the

developing flagellum. The distal centriole moves away from the proximal one, and microtubules from it become continuous with microtubules in the flagellum. Mitochondria begin to form a spiral investment around the proximal part of the flagellum. As spermiogenesis continues, the remaining cytoplasm becomes aggregated into a remnant, or residual bodies, which sloughed off and phagocytized by the Sertoli cells.

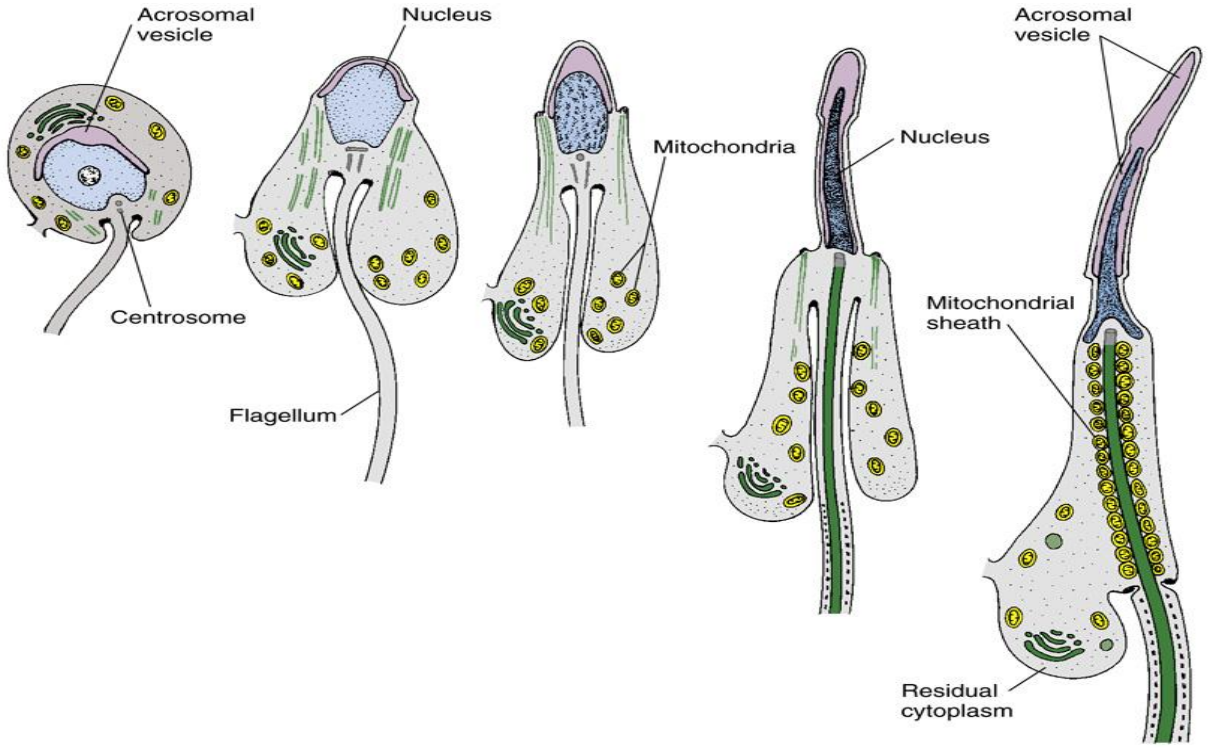
Figure 10-6. The Golgi Phase of Spermatid Differentiation



The newly formed spermatid is almost perfectly spherical and has a well developed Golgi apparatus.

Small vesicles of the Golgi fuse, giving rise to larger secretory granules called pro-acrosomic granules. The centrioles start to migrate to a position beneath the nucleus that is opposite the acrosomic vesicle.

Vesicle fusion continues until a large acrosomic vesicle is formed that contains a dense acrosomic granule. The proximal centriole (PC) will give rise to the attachment point of the tail. The distal centriole (DC) will give rise to the developing axoneme (central portion of the tail) inside the cytoplasm of the spermatid.



This leaves the mature spermatozoon stripped of all nonessential parts. It consists of,

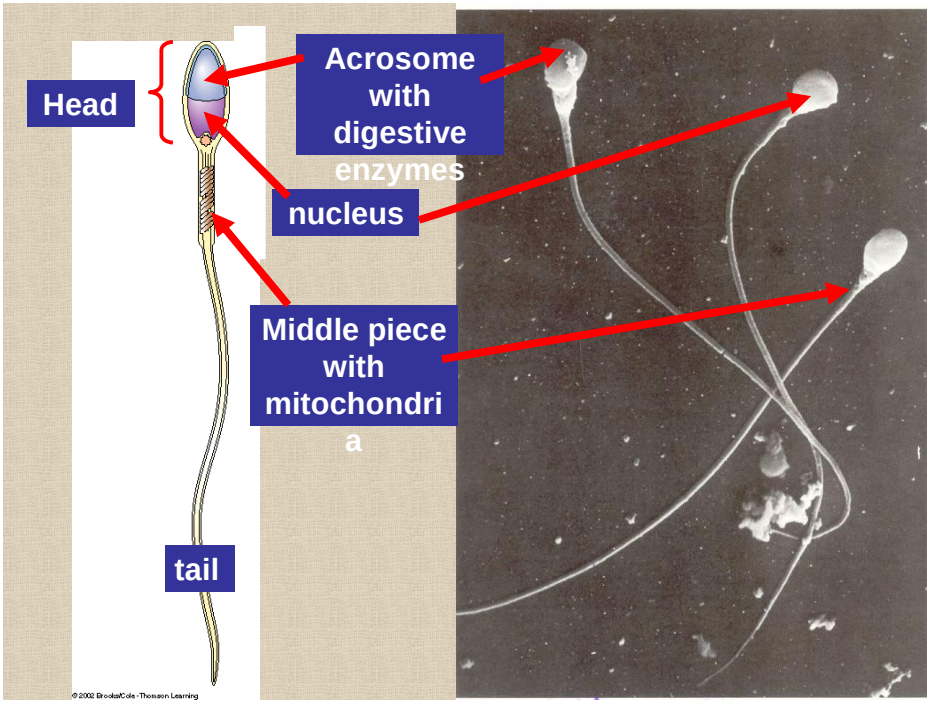
1- A head containing the nucleus and acrosome

2- A neck containing the proximal centriole

3- A middle piece containing the proximal part of the flagellum, the centrioles, and the mitochondrial helix, which acts as an energy source.

4- The tail, a highly specialized flagellum (Fig.).

During spermatogenesis, the cells are also closely associated with Sertoli cells, which lie at regular intervals along the seminiferous tubule (Fig.). Sertoli cells serve a wide variety

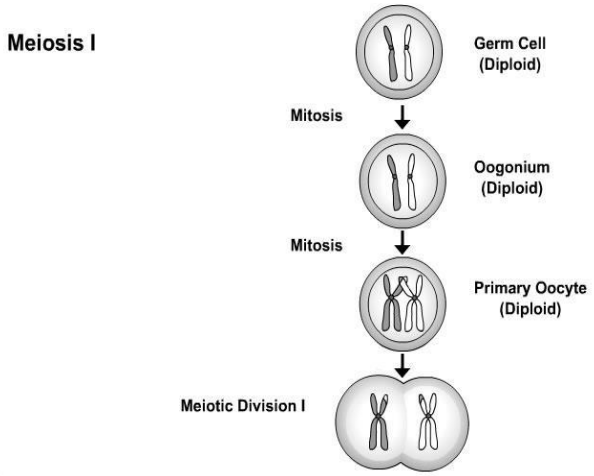


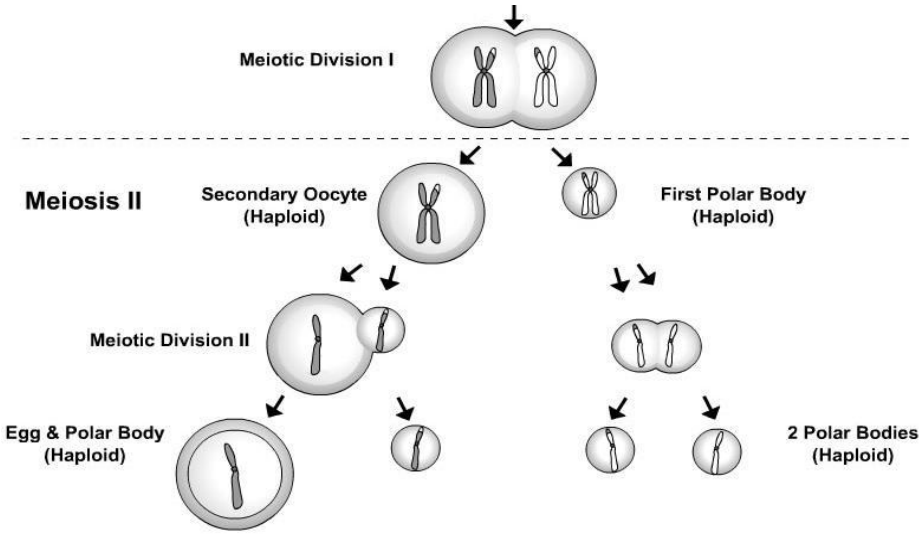
Oogenesis

The goal of oogenesis is to produce one egg with massive amounts of cytoplasm

1-The primary oocyte divides by Meiosis Division I to produce a secondary oocyte.The other nucleus resulting from Division I is a throw-away nucleus known as a polar body.

2- The secondary oocyte divides by Meiosis Division II to produce the egg cell and a polar body. The earlier polar body also divides to form two polar bodies.





Oogenesis in mammals

Maturation of Oocytes Begins Before Birth

1- Once primordial germ cells have arrived in the gonad of a genetic female, they differentiate into **oogonia**

These cells undergo a number of mitotic divisions and, **by the end of the third month**, are arranged in clusters surrounded by a layer of flat epithelial cells, known as **follicular cells,** originate from surface epithelium covering the ovary.

The majority of oogonia continue to divide by mitosis, but some of them arrest their cell division in prophase of meiosis I and form **primary oocytes**

During the next few months, oogonia increase rapidly in number, and **by the fifth month**, the total number of germ cells in the ovary reaches **7 million**. At this time, cell death begins,

and many oogonia as well as primary oocytes become atretic.

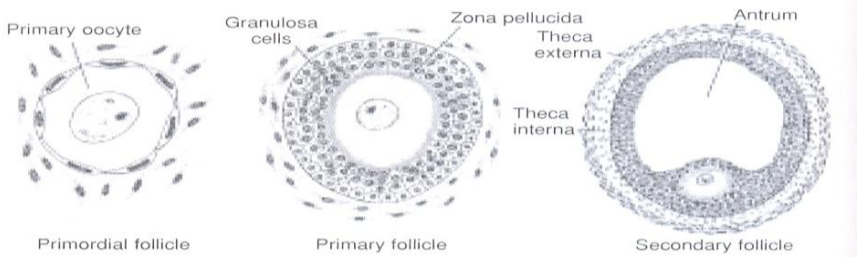


Figure 2.1 From the pool of primordial follicles, every day some begin to grow and develop into secondary (preantral) follicles, and this growth is independent of FSH. Then, as the cycle progresses, FSH secretion recruits primary follicles to begin development into secondary (antral, Graafian) follicles. During the last few days of maturation of secondary follicles, estrogens, produced by follicular and thecal cells, stimulate increased production of LH by the pituitary (Fig. 2.13), and this hormone causes the follicle to enter the preovulatory stage, to complete meiosis I, and to enter meiosis II where it arrests in metaphase approximately 3 hours before ovulation.

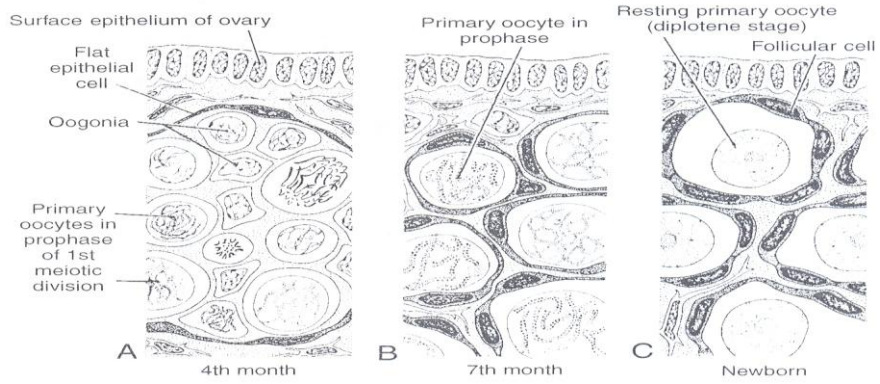


Figure 1.17 Segment of the ovary at different stages of development. **A.** Oogonia are grouped in clusters in the cortical part of the ovary. Some show mitosis; others have differentiated into primary oocytes and entered prophase of the first meiotic division. **B.** Almost all oogonia are transformed into primary oocytes in prophase of the first meiotic division. **C.** There are no oogonia. Each primary oocyte is surrounded by a single layer of follicular cells, forming the primordial follicle. Oocytes have entered the diplotene stage of prophase, in which they remain until just before ovulation. Only then do they enter metaphase of the first meiotic division.

By the seventh month, the majority of oogonia have degenerated except for a few near the surface. All surviving primary oocytes have entered prophase of meiosis I, and most of them are individually surrounded by a layer of flat epithelial cells known as a **primordial follicle**.

Near the time of birth, all primary oocytes have started prophase of meiosis I, and enter the **diplotene stage,** a resting stage during prophase. **Primary oocytes remain in prophase**

and **do not finish their first meiotic division before puberty is reached,** apparently because of **oocyte maturation inhibitor** (OMI), a substance secreted by follicular cells.

The total number of primary oocytes at birth is estimated to vary from **700,000 to 2 million**. During childhood most oocytes become atretic; only approximately **400,000** are present **by the beginning of puberty**, and fewer than **500 will be ovulated**.

Some oocytes that reach maturity late in life have been dormant in the diplotene stage of the first meiotic division **for 40 years** or more before ovulation.

Whether the diplotene stage is the most suitable phase to protect the oocyte against environmental influences is unknown. The fact that the risk of having **children with chromosomal abnormalities** increases with maternal age indicates that primary oocytes are vulnerable to damage as they age.

At puberty, Each month, 15 to 20 follicles begin to mature, passing through three stages:

1) **primary** or **preantral**

2) **secondary** or **antral** (also called **vesicular** or **Graafian)** the longest stage

3) **preovulatory. (** 37 hours before ovulation **)**

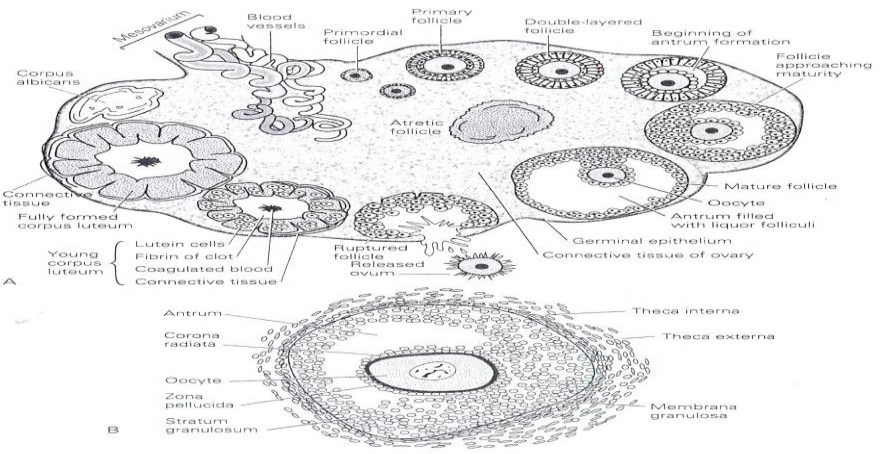
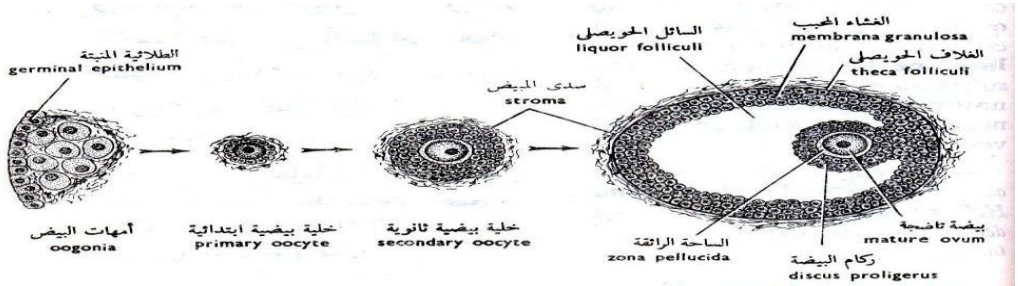
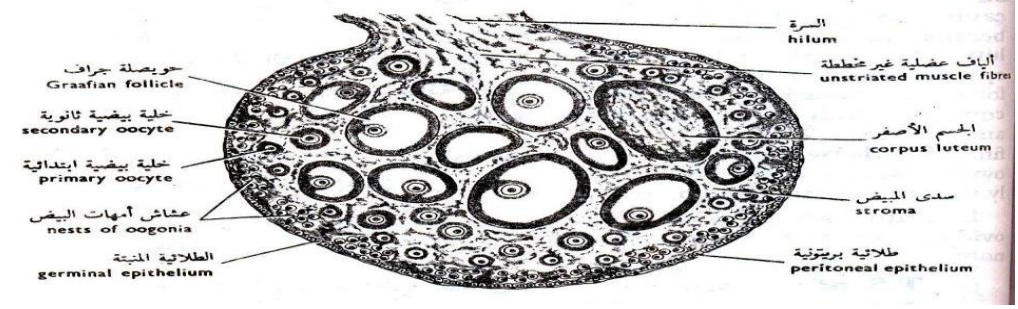


FIGURE 3-21
(A) Schematic diagram of ovary showing sequence of events in origin, growth, and rupture of ovarian (Graafian) follicle and in formation and retrogression of corpus luteum. Follow clockwise around ovary, starting at mesovarium. (B) Drawing of a secondary follicle.



مراحل تكوين حويصلة جراف
Developmental stages of a Graafian follicle



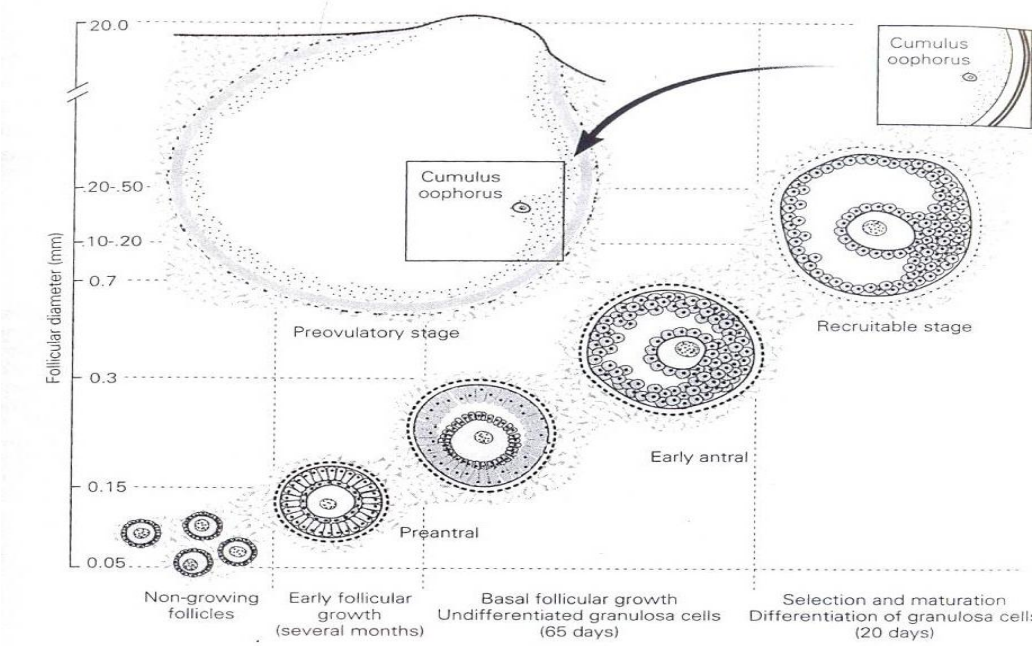


FIGURE 3-22
Representation of the growth and development of the human oocyte. (*After A. Gougeon, 1993.*)

As the **primary oocyte** begins to grow, surrounding follicular cells change from flat to cuboidal and proliferate to produce a stratified epithelium of **granulosa cells,** and the unit is called a **primary follicle**

Granulosa cells rest on a basement membrane separating them from surrounding stromal cells that form the **theca folliculi.**

Granulosa cells and the oocyte secrete a layer of glycoproteins on the surface of the oocyte, forming the **zona pellucida**.

Theca folliculi organize into an inner layer of secretory cells, the **theca interna,** and an outer fibrous capsule, the **theca externa.** Also, small, finger-like processes of the follicular cells

extend across the zona pellucida and interdigitate with microvilli of the plasma membrane of the oocyte. These processes are important for transport of materials from follicular cells to the oocyte. As development continues, fluid-filled spaces appear between granulosea cells. Coalescence of these spaces forms the **antrum,** and the follicle is termed a **secondary (vesicular, Graafian) follicle.** Initially, the antrum is crescent shaped, but with time, it enlarges. (Granulosa cells surrounding the oocyte remain intact and form the **cumulus oophorus.** At maturity, the **secondary**

follicle may be **25 mm** or more in diameter.

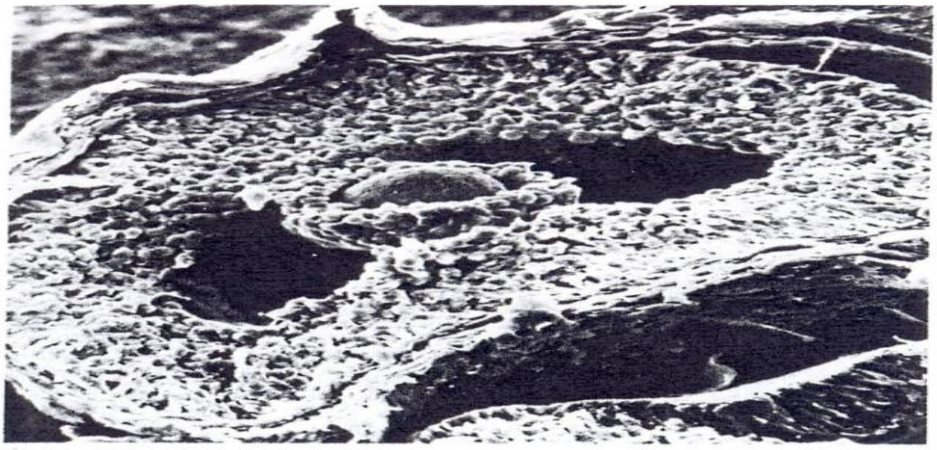
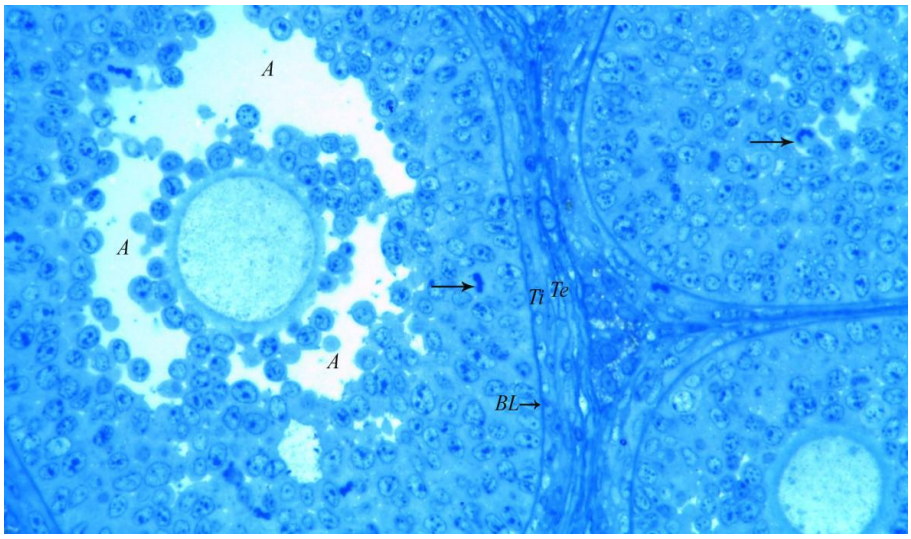


FIGURE 3-23
Scanning electron microscope of a mature follicle in the rat, showing the spherical oocyte (*center*) surrounded by smaller cells of the corona radiata, which projects into the antrum. x840. (*Courtesy of P. Bagavandoss.*)



With each ovarian cycle, a number of follicles begin to develop, but usually only one reaches full maturity. The others degenerate and become atretic.

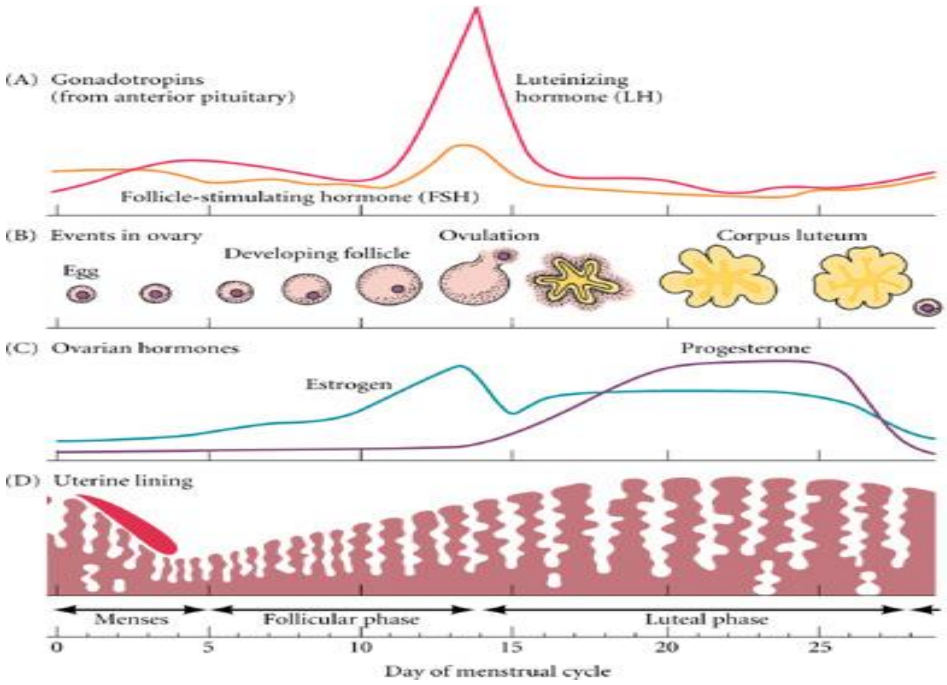
When the secondary follicle is mature, a surge in **luteinizing hormone (LH)** induces the preovulatory growth phase. **Meiosis I is completed**, resulting in formation of two daughter cells of unequal size, each with 23 double structured chromosomes.

- One cell, the **secondary oocyte,** receives most of the cytoplasm; the other, the **first polar body**, receives practically none. The first polar body lies between the zona pellucida and the cell membrane of the secondary oocyte in the perivitelline space.

The cell then enters **meiosis II** but arrests in metaphase approximately 3 hours before ovulation. Meiosis II is completed only if the oocyte is fertilized; otherwise, the cell degenerates approximately 24 hours after ovulation. The first polar body also undergoes a second division.

Ovarian Cycle

At puberty, the female begins to undergo regular monthly cycles. These **sexual cycles** are controlled by the hypothalamus. **Gonadotropin-releasing hormone (GnRH)** produced by the hypothalamus acts on cells of the anterior pituitary gland, which in turn secrete **gonadotropins.** These hormones, **follicle-stimulating hormone (FSH)** and **luteinizing hormone (LH),** stimulate and control cyclic changes in the ovary.



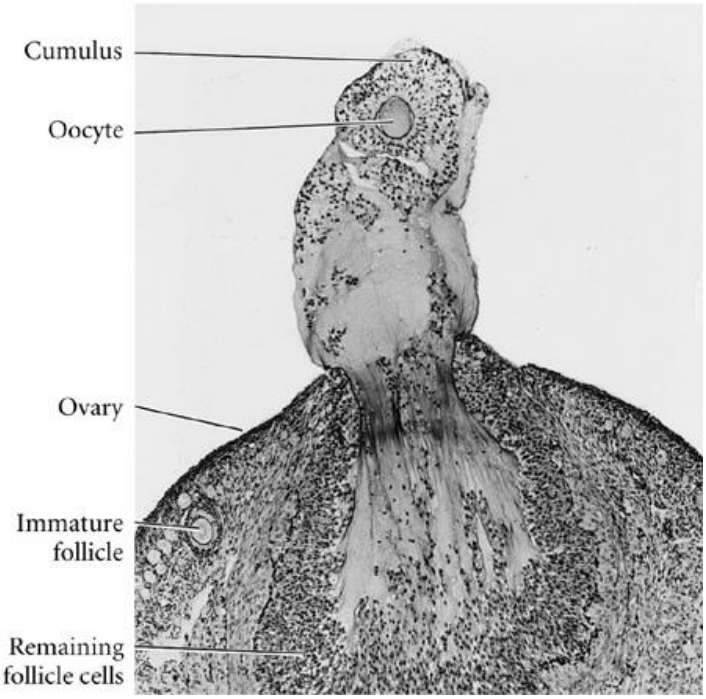
At the beginning of each ovarian cycle, 15 to 20 primary (preantral) stage follicles are stimulated to grow under the influence of FSH. Under normal conditions, only one of these follicles reaches full maturity. The others degenerate and become atretic. When a follicle becomes atretic, the oocyte and surrounding follicular cells degenerate and are replaced by connective tissue, forming a **corpus atreticum.** FSH also stimulates maturation of **follicular (granulosa)** cells surrounding the oocyte.

OVULATION

In the days immediately preceding ovulation, under the influence of FSH and LH, the secondary follicle grows rapidly to a diameter of 25 mm. Increase in LH causes the primary

oocyte to complete meiosis I and the follicle to enter the preovulatory stage. Meiosis II is also initiated, but the oocyte is arrested in metaphase approximately 3 hours before ovulation. In the meantime, the surface

of the ovary begins to bulge locally, and at the apex, an avascular spot, the **stigma,** appears. The high concentration of LH increases collagenase activity, resulting in digestion of collagen fibers surrounding the follicle. The muscular contractions in the ovarian wall extrude the oocyte, which together with its surrounding granulosa cells from the region of the cumulus oophorus, breaks free **(ovulation)** and floats out of the ovary.



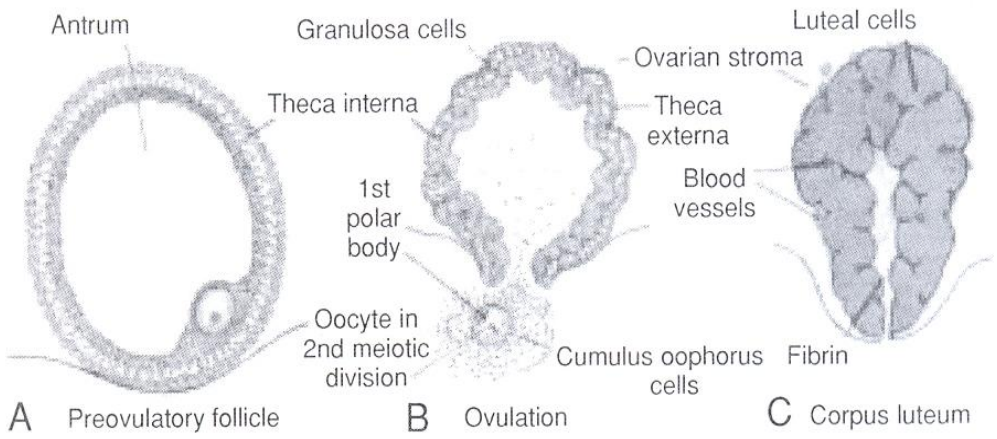


Figure 2.2 A. Preovulatory follicle bulging at the ovarian surface. **B.** Ovulation. The oocyte, in metaphase of meiosis II, is discharged from the ovary together with a large number of cumulus oophorus cells. Follicular cells remaining inside the collapsed follicle differentiate into lutean cells. **C.** Corpus luteum. Note the large size of the corpus luteum, caused by hypertrophy and accumulation of lipid in granulosa and theca interna cells. The remaining cavity of the follicle is filled with fibrin.

During ovulation, some women feel a slight pain, known as **middle pain** because it normally occurs near the middle of the menstrual cycle. Ovulation is also generally accompanied by a rise in basal temperature.

CORPUS LUTEUM

After ovulation, granulosa cells remaining in the wall of the ruptured follicle, together with cells from the theca interna, are vascularized by surrounding vessels. Under the influence of LH, these cells develop a yellowish pigment and change into **lutean cells,** which form the **corpus luteum** and secrete the hormone **progesterone** (Fig. 2.2*C*). Progesterone, together with

estrogenic hormones, causes the uterine mucosa to enter the **progestational** or **secretory stage** in preparation for implantation of the embryo.

CORPUS ALBICANS

If fertilization does not occur, the corpus luteum reaches maximum development approximately 9 days after ovulation. It can easily be recognized as a yellowish projection on the surface of the ovary. Subsequently, the corpus luteum shrinks because of degeneration of lutean cells and forms a mass of fibrotic scar tissue, the **corpus albicans.** Simultaneously, progesterone production decreases,precipitating menstrual bleeding.

If the oocyte is fertilized, degeneration of the corpus luteum is prevented by **human chorionic gonadotropin** (hCG), a hormone secreted by the developing embryo. The corpus luteum continues to grow and forms the **corpus luteum of pregnancy (corpus luteum graviditatis).**

By the end of the third month, this structure may be one-third

to one-half of the total size of the ovary. Yellowish luteal cells continue to secrete progesterone until the end of the fourth month; thereafter, they regress slowly as secretion of progesterone by the trophoblastic component of the placenta becomes adequate for maintenance of pregnancy. Removal of the corpus luteum of pregnancy before the fourth month usually leads to abortion.

The timing of meiosis differs in females and males

In males, the spermatogonia enter meiosis and produce sperm from puberty until death. The process of sperm production takes only a few weeks. In females, this process is more complex. The first meiotic division starts before birth but fails to proceed. It is eventually completed about one month before ovulation in humans. In humans, the second meiotic division occurs just before the actual process of fertilization occurs. Thus, in females, the completion of meiosis can be delayed for over 50 years. This is not always good. In addition, all meiosis is ended in females at menopause.

Egg Polarity

Animal – vegetal polarity: In eggs that have a lot of yolk, the yolk is concentrated in the vegetal pole. The animal pole contains the nucleus and relatively little yolk. The yolk in the vegetal pole interferes with cytokinesis during the process of cleavage leading to incomplete cleavage**.**

On the basis of presence or absence and amount of yolk particles, the eggs classified as follows:

A- According to the amount of yolk , the egg arranged into the following

1-Alecithal type

When the yolk particles are entirely lacking as in placentals (Mammals).

2- Oligolecithal type

Here the yolk particles are present but in little amount as in

Echinodermata Amphioxus, hydra.

3-Mesolecithal type: The yolk particles are present in Moderate amount as in toad.

4-Macrolecithal type

In birds and repticles and egg laying mammals, the yolk particles are in good amount. Here, in such cases the size of nucleus and cytoplasm reduce and lie in the form of a small germinal disc.

B- According to distribution of yolk

1- **Isolecithal type** when yolk particles are distributed evenly throughout the cytoplasm as in fishes.

2- **Centrolecithal type**

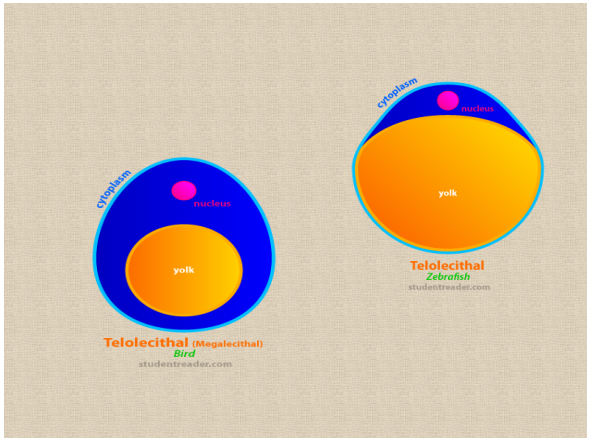
In insects and other Arthropods the yolk particles are restricted to the center of the egg.

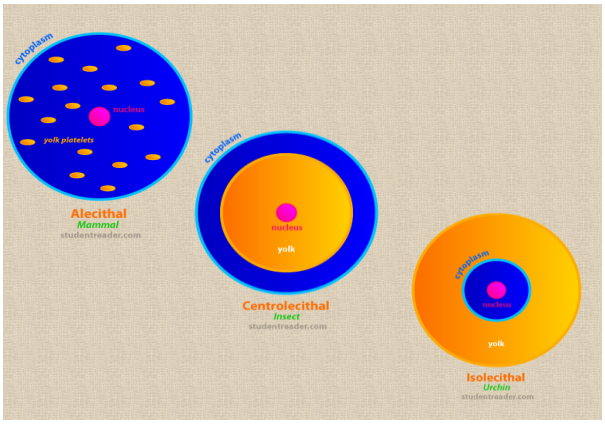
3- **Telolecithal type**

When the yolk increases in quantity and becomes accumulated at one end or pole of the egg as in frog, toad and some fishes.

When little yolk is present, young develop into larval stages that can feed (Indirect development). When lots of nourishing yolk is present, embryos develop into a miniature adult (Direct

development). Mammals have little yolk, but nourish the embryo via the placenta.





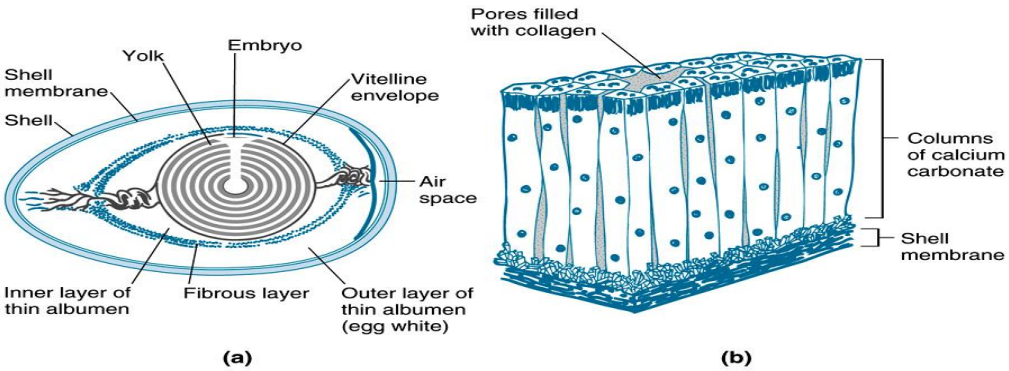
Eggs are protected by elaborate envelopes

After ovulation, eggs are released into a variety of environments both within and outside the body. Most fishes and amphibians shed their eggs into either fresh water or salt water, where the eggs must be protected from predators, disease,and environmental factors such as extremes of osmotic pressure and pH.

Animals that lay their eggs on land (reptiles, birds, primitive mammals) must prevent dessication as well as support and protect the ova. Some egg coverings are secreted by the ova themselves, others by the surrounding follicular cells, and still others by the female reproductive tract after the egg has left the ovary.

Vitelline envelope: a glycoprotein layer covers the plasma membrane of all eggs. This acts to protect the egg. Eggs that are deposited in water have a jelly-like coating that surrounds the egg (frogs eggs)

The eggs of birds have a vitelline envelope, a fibrous layer, an outer layer of albumin (egg white), and a shell composed of calcium carbonate. The outer envelopes are synthesized in the oviduct after the egg has been fertilized.



2- Fertilization

Definition: The process by which the male and female gametes (sperm and ovum) unite to give rise to zygote.

Types of Fertilization

1- External fertilization: When the fertilization occurs in the aquatic medium outside the bodies of male and female parents.

2- Internal fretilization: In terrestrial forms, particularly where eggs are completely enclosed in impermeable envolpes before being laid (birds), or where they are retained within the maternal body throughout development (mammals), thus the fertilization occurs inside the body of the female

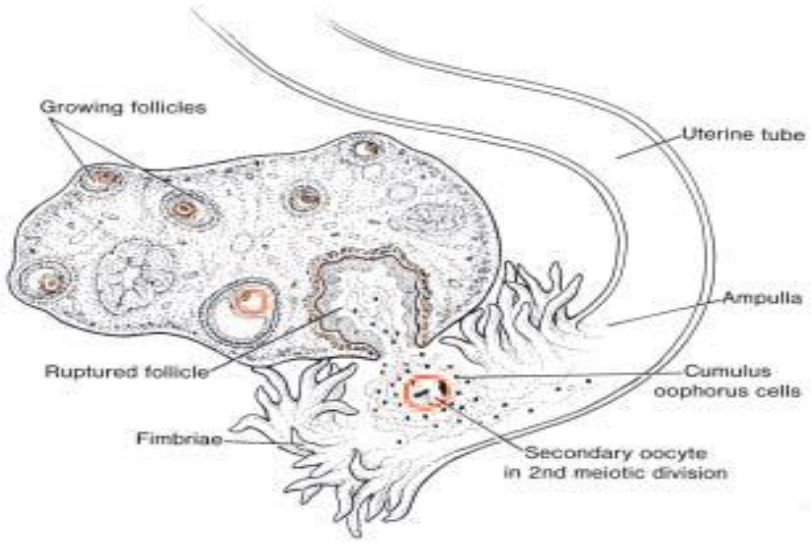
Fertilization in mammals

Fertilization, the process by which male and female gametes fuse, occurs in the **ampullary region of the uterine tube.** This is the widest part of the tube and is close to the ovary. Spermatozoa may remain viable in the female reproductive tract for several days. Only 1% of sperm deposited in the vagina enter the cervix, where they may survive for many hours. The trip from cervix to oviduct requires a minimum of 2 to 7 hours, and after reaching the isthmus, sperm become less motile and cease their migration. Spermatozoa are not able to fertilize the oocyte immediately upon arrival in the female genital tract but must undergo (*a*) **capacitation** and (*b*) the **acrosome reaction**

to acquire this capability.

(a)-Capacitation is a period of conditioning in the female reproductive tract that in the human lasts approximately 7 hours. Much of this conditioning, which occurs in the uterine tube, entails epithelial interactions between the sperm and mucosal surface of the tube. During this time a glycoprotein coat and seminal plasma proteins are removed from the plasma membrane that overlies the acrosomal region of the spermatozoa. Only capacitated sperm can pass through the corona cells and undergo the acrosome reaction.

(b) The **acrosome reaction,** which occurs after binding to the zona pellucida, is induced by zona proteins. This reaction culminates in the release of enzymes needed to penetrate the zona pellucida, including acrosin and trypsin-like substances.



Fimbriae collect the oocyte and sweep it into the uterine tube.

The phases of fertilization

Phase 1, penetration of the corona radiate.

phase 2, penetration of the zona pellucida.

phase 3, fusion of the oocyte and sperm cell membranes.

PHASE 1: PENETRATION OF THE CORONA RADIATA

Of the 200 to 300 million spermatozoa deposited in the female genital tract, only 300 to 500 reach the site of fertilization. Only one of these fertilizes the egg. It is thought that the others aid the fertilizing sperm in penetrating the barriers protecting the female gamete. Capacitated sperm pass freely through corona cells.

PHASE 2: PENETRATION OF THE ZONA PELLUCIDA

The zona is a glycoprotein shell surrounding the egg that facilitates and maintains sperm binding and induces the acrosome reaction. Both binding and the acrosome reaction are mediated by the ligand ZP3, a zona protein. Release of

acrosomal enzymes (acrosin) allows sperm to penetrate the zona, thereby coming in contact with the plasma membrane of the oocyte.

Permeability of the zona pellucida changes when the head of the sperm comes in contact with the oocyte surface. This contact results in release of lysosomal enzymes from cortical granules lining the plasma membrane of the oocyte. In turn, these enzymes alter properties of the zona pellucida **(zona reaction)** to prevent sperm penetration and inactivate species-specific receptor sites for spermatozoa on the zona surface. Other spermatozoa have been found embedded in the zona pellucida, but only one seems to be able to penetrate the oocyte. These reactions prevent polyspermy (penetration of more than one spermatozoon into the oocyte).

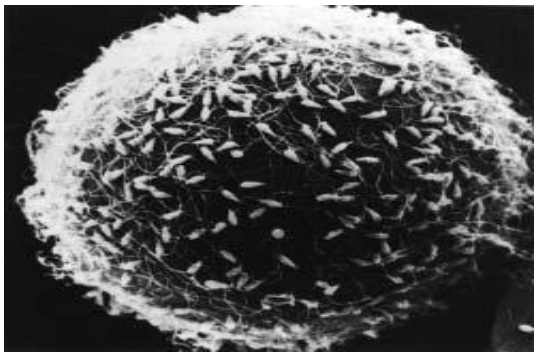
PHASE 3: FUSION OF THE OOCYTE AND SPERM CELL MEMBRANES

After adhesion, the plasma membranes of the sperm and egg fuse. Because the plasma membrane covering the acrosomal head cap disappears during the acrosome reaction, actual fusion is accomplished between the oocyte membrane and the

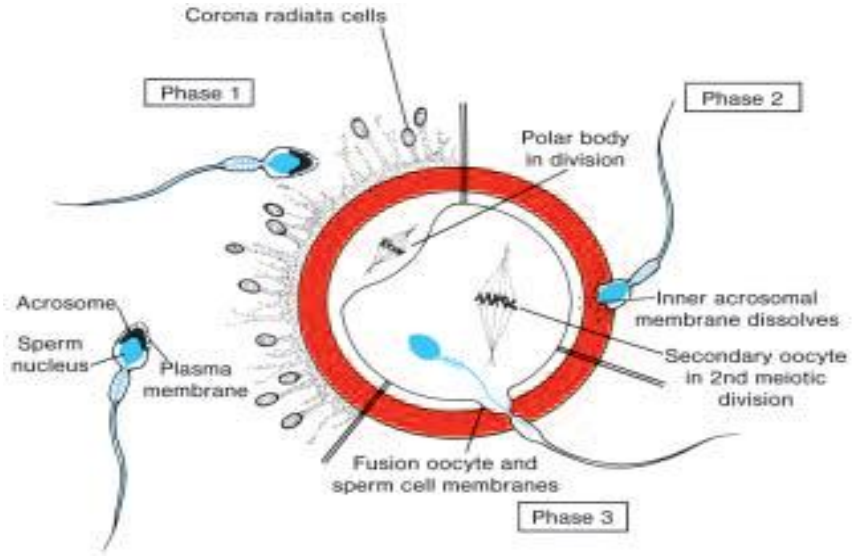
membrane that covers the posterior region of the sperm head. In the human, both the head and tail of the spermatozoon enter the cytoplasm of the oocyte, but the plasma membrane is left behind on the oocyte surface. As soon as the spermatozoon has entered the oocyte, the egg responds in three ways.

The oocyte finishes its second meiotic division immediately after entry of the spermatozoon. One of the daughter cells, which receives hardly any cytoplasm, is known as the **second polar body;** the other daughter cell is the **definitive oocyte.** Its chromosomes (22+X) arrange themselves in a vesicular nucleus known as the **female pronucleus**.

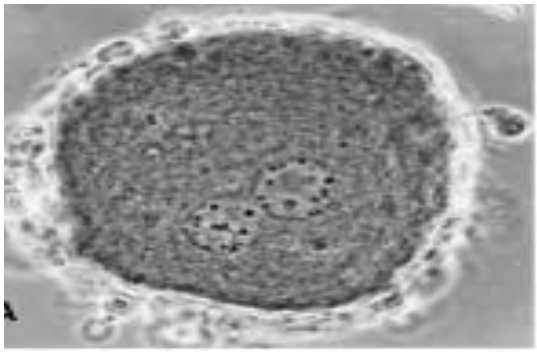
The spermatozoon, meanwhile, moves forward until it lies close to the female pronucleus. Its nucleus becomes swollen and forms the **male pronucleus** ; the tail detaches and degenerates. Morphologically, the male and female pronuclei are indistinguishable, and eventually, they come into close contact and lose their nuclear envelopes. Each pronucleus must replicate its DNA.



Scanning electron micrograph of sperm binding to the zona pellucida.



The three phases of oocyte penetration. In phase 1, spermatozoa pass through the corona radiata barrier; in phase 2, one or more spermatozoa penetrate the zona pellucida; in phase 3, one spermatozoon penetrates the oocyte membrane while losing its own plasma membrane. Inset. Normal spermatocyte with acrosomal head cap.



Phase contrast view of the pronuclear stage of a fertilized human oocyte with male and female pronuclei.

The main results of fertilization are as follows:

1- **Restoration of the diploid number of chromosomes,** half from the father and half from the mother. Hence, the zygote contains a new combination of chromosomes different from both parents.

2- **Determination of the sex of the new individual**. An X-carrying sperm produces a female (XX) embryo, and a Y-carrying sperm produces a male (XY) embryo. Hence, the chromosomal sex of the embryo is determined at fertilization.

3- **Initiation of cleavage.** Without fertilization, the oocyte usually degenerates 24 hours after ovulation.

Zygote is a fertilized egg. A fertilized egg becomes an embryo as soon as the first cleavage occurs.The zygot ultimately, produced a diploid multicellular organism by the several repeated and organised mitotic divisions and cellular differentiation.

3- Cleavage

The development of a normal, adult animal from a zygote involves a series of distinct steps in which specific takes are accomplished, *cleavage*, the mitotic division of the egg or , is the first step after fertilization and is a universally occurring phenomenon among metazoan animals, very little growth occurs. The cells that arise from cleavage are known as *blastomeres*. Influence of yolk on cleavage, in two ways:

1- concentrated at the vegetal pole. Therefore at the vegetal pole the cleavage in most ret It affects the rate of cleavage

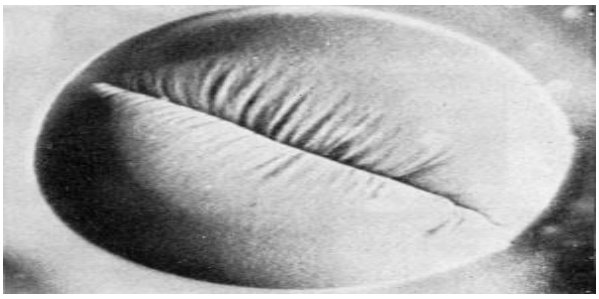
2- It determines the pattern of cleavage

When the yolk is abundant, it tends to retard and even inhibit the process of cleavage. As a result, the blastomeres which are rich in yolk tend to divided at a slower rate, and consequently they remain larger than those which have less yolk. The yolk is more carded, and also where the blastomeres are larger in size.

Planes of cleavage

Cleavage is initiated by the appearance of a groove or constriction called cleavage furrow. The furrow appears first at one point of the egg. For example, the furrow appears at the animal pole. The furrow then deepens and extends downward

on both sides. The two ends meet at the vegetal pole. The furrow then extendeds inwards radially, finally constricting the egg into two blastomeres.



The cleavage furrow divide the egg at different angles or plans. There are four main planes of cleavage. They are as follows:

1- Meridional plane

The cleavage furrow passes through the center of animals-vegetal axis and bisects the both poles of the egg.

2- Vertical plane

The cleavage tend to pass in a direction from the animal pole toward the vegetal pole. It does not pass through the median axis of the egg.

3- Equatoril plane

The cleavage furrow bisects the egg at right angles to the main axis and half way between the animal and vegetal poles.

4- Latitudinal plane

The cleavage furrow is similar to the equatorial but it courses through the cytoplasm on either side of the equatorial plane. It

also called transverse or horizontalplane.

Patterns of cleavage

1- Total or holoblastic cleavage

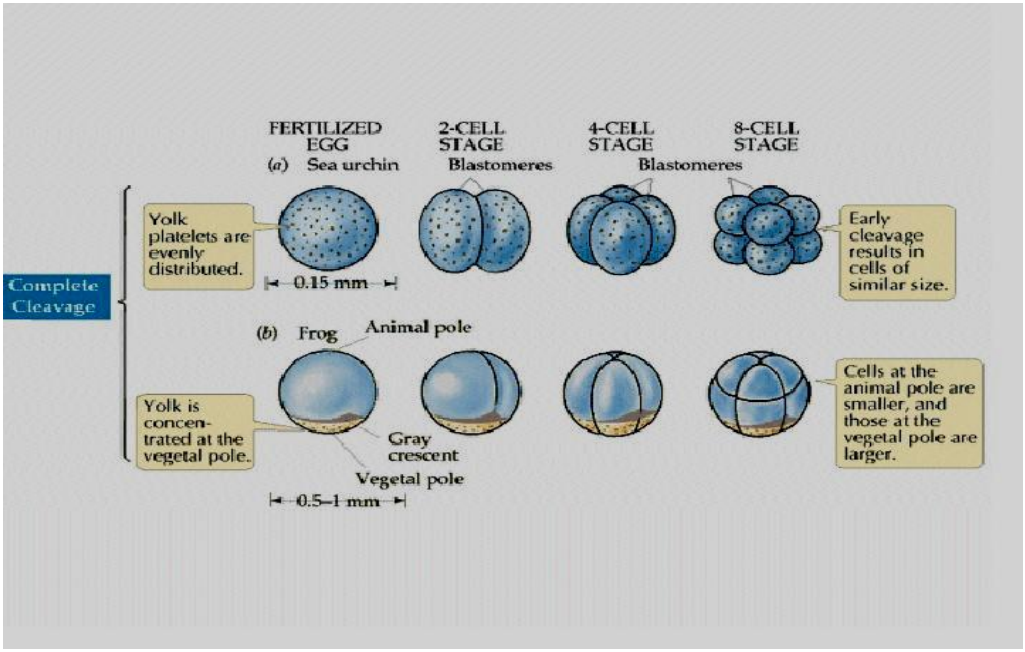
In holoblastic, total or complete cleavage, the entire egg divides by each cleavage furrow. It may be :

a-Equal holoblastic cleavage

In microlecithal or isolecithal egg, it produces blastomeres of approximately equal size e.g., Amphioxus, and placental mammals.

b- Unequal holoblastic cleavage

In telolecithal eggs, the yolk is accumulated at the vegetal pole which retards mitosis and a few but larger blastomeres (macromeres) are formed there. Smaller but large number of micromeres are formed at the animal pole, e.g. lower fishes and amphibians.



2- Meroblastic cleavage

In meroblastic cleavage, only a portion of the egg divides (partial or incomplete cleavage. It is characteristic of telolecithal eggs.

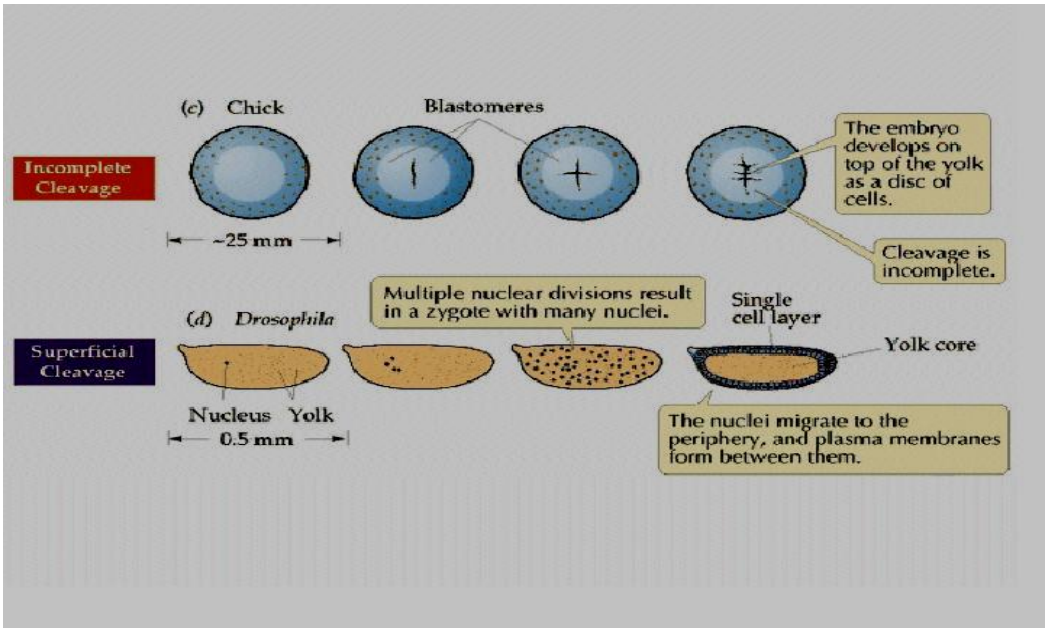
a- Discoidal cleavage

It occurs in fishes, reptiles, and birds. Here the cytoplasm is placed at the animal pole as a disc called blastodisc, and this disc alone divides, the huge mass of yolk does not participate in cleavage.

b- Superficial meroblastic cleavage

In centrolecithal ovum, the cleavage remains restricted to the peripheral cytoplasmic investment. As in insects, where there is a moderate to large amount of yolk, and it is concentrated in the

center of the egg. This means that although there is resistance to cleavage plane development in the center of the egg, it is easier to develop cleavage plane at the periphery of the egg – and thus early zygote.

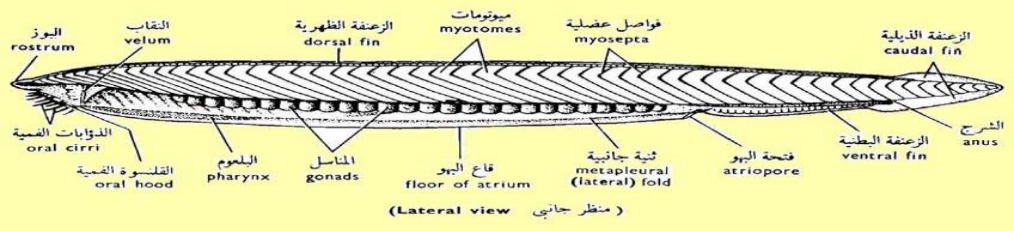


Note: The chromosomes are replicating centrally but there is too much yolk to allow cleavage, so there are multiple nuclei centrally. They then migrate to the periphery, and only there does the meroblastic cleavage (eventually) take place.

Development of Amphioxus

Amphioxus is a diocious animal *i.e.*, sexes are separate but there is no distinction between male and female. The gonads which are in the form of hollow sacs enclosed in coelomic pouches while the genital ducts carrying the gametes from gonads to out

side are lacking. On maturity of gonads the sperms and ova are liberated into the atrium and from where they are discharged out side through the atriopore in breeding season. In fully developed Amphioxus the gonds (testes or ovaries) are cuboidal bodies, twenty six in number on each side between the body wall bordering the atrium and myotomes.



The sperm

The spermatozoa of Amphioxus have typically structure of flagellate spermatozoa (contain head, mid-piece and tail), except that, its head being spherical and mid-piece being very short.

The ovum

An unfertilized ovum of Amphioxus is 0.10 mm to 0.12 mm in diameter. It is microlecithal and isolecithal. Its ooplasm membrane, which in its turn remains surround by a thin membrane of mucopolysaccharides, called vitelline membrane.

The ovum Amphioxus has well determined polarity- the large sized egg nucleus lies at the animal pole, near the egg plasma membrane. The opposite side of the egg from the animal pole ,

the vegtal pole. The yolk granules remain uniformly distributed throughout the ooplasm except near the nucleus at the animal pole .

Cleavage

The pattern of cleavage is holoblastic cleavage. The plane of **first cleavage** is holoblastic and meridional passes through the egg axis from pole to pole. The result of this cleavage is the formation of two identical blastomeres establishing the bilateral symmetry of the adult animals.

The second cleavage plane cleavage in a vertical plane, but at right angles to the first plane, thus forming four cells.

The third cleavage is horizontal (transverse, latitudinal). As the yolk is comparatively more towards the vegetal pole, the four mitotic spindle lie nearer the animal pole. Each of the four blastomeres dividing into a smaller micromeres at the animal pole and a larger macromeres at the vegetal pole. Eight blastomeres are produced.

The fourth cleavage is double plane each one oriented from animal to vegtal pole. This results in eight animal micromeres and eight vegetal macromeres.

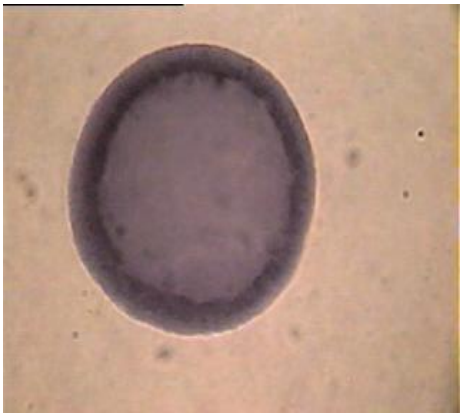
The fifth cleavage is latitudinal and double parallel to plane three – one occurring in the animal, the other in the vegetal hemisphere. They result in 32-cells, arranged in four tiers, with a modest gradation in size from larger, yolk-laden basal macromeres to smaller, less yolky apical micromeres.

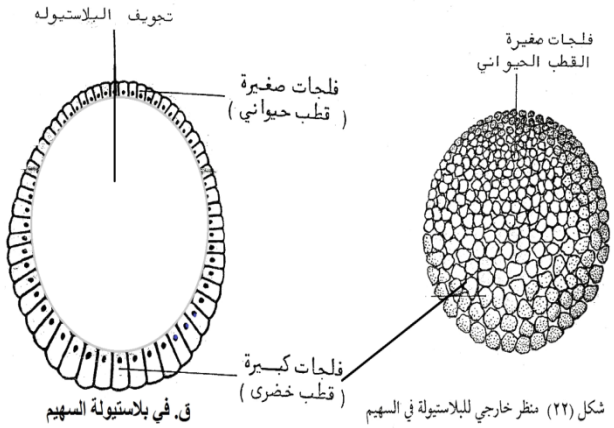
The sixth cleavage are approximately meridional, producing 64-blastomeres. The cleavages pattern beyond this is irregular and difficult to follow.

Blastulation

Up to this stage, the blastomeres remain loosely packed and form the embryonic stage, called morula. Meanwhile, a semifluid materials accumulates in the centre of this mass of cells. This fluid serves to push all the blastomeres outwards, so that they become arranged in single layer called blastoderm, enclosing a central fluid filled cavity, the blastocoel. To form the blastoderm, each blastomre of morula performes some morphogenetic movement and assumes a columnar shape. The resultant hollow, spherical or pear-shaped embryonic stage, in which a single cell thick blastoderm encloses a fluid filled centeric blastocoel, is the blastula. A sagittal section through a

blastula of this stage reveals that, the cells of the vegetal hemisphere (the prospective endoderm) are slightly larger and richer in the yolky material than those of the animal hemisphere (the prospective ectoderm).



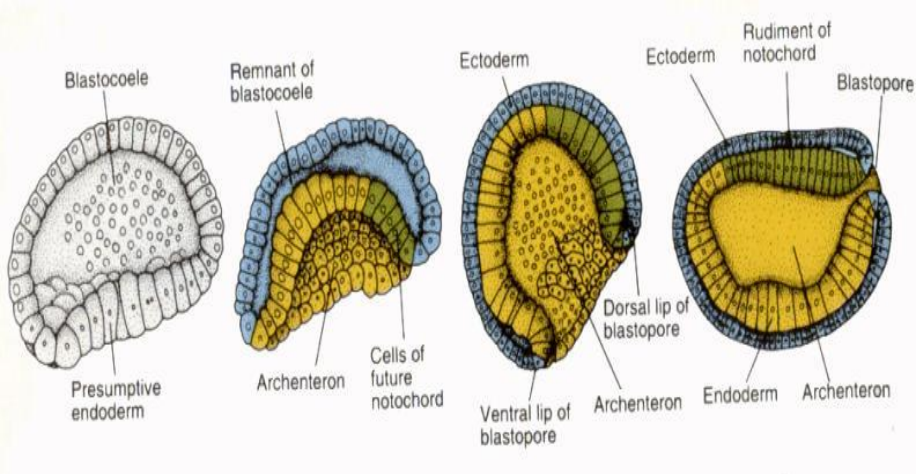


Gastrulation

In Amphioxus, the gastrulation, by which a monoblastic blastula is converted into a diploblastic and stratified gastrula. The onset of gastrulation process is marked by flattening of blastoderm of vegetal pole, i.e., prospective endoderm. This endodermal plate then gradually invaginates, or folds inwardly, into the blastocoel. This invaginating layer of cells gradually eliminates that entire blastocoel and come to lie against the ectodermal micromeres. Thus, whole embryo, instead of being spherical, becomes converted into a cup-shaped structure, having a large cavity, the archenteron (gastrocoel), in open communication

with the exterior by the blastopore. The cup has double walls, an external and internal epitheial layers, both of which remain continuous with each other over the rim of the cup-shaped embryo, the gastrula. The gatrula, at this stage, consists of two layers-an outer epiblast, consisting of neural and epidermal ectoderm, and an inner hypoblast encompassing prospective notochord mesoderm and endoderm. We now have an animal that is a tube within a tube.

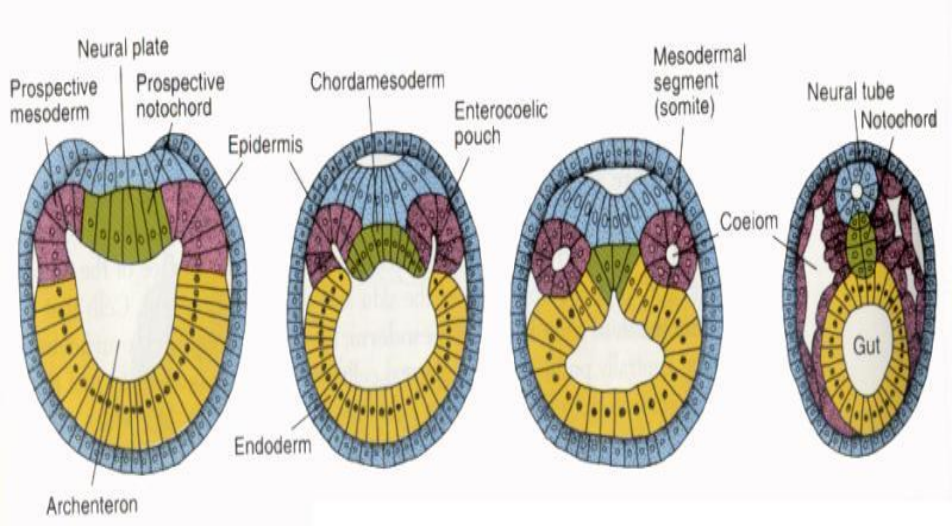
The circular rim of the blastopore is termed the lip, the prospective mesoderm lies in the ventral lip of the blastopore and the prospective notochord lies in the dorsal lip of the blastopore.



Formation of the neural tube

With the completion of gastrulation, a strip of ectodermal cells in the region of midgut dorsal line enlarges to form the neural

plate, which flattens and sinks inwards. The ectodermal on the sides of neural plate now rise up to form the neural folds is gradually extended round the lateral lips of blastopor. Then these folds start growing to meet each other over the neural plate, begining at the posterior end. These folds meet together in the mid-dorsal line. On the other hand, at the same time, the lateral edges of neural plate have grown towards each other, resulting the formation of the neural tube.



Formation of notochord

The chorda cells, in he gastrula are, present along the mid-dorsal wall of archenteron just below the neural plate. These chorda cells becomes in the form of strip due to a median groove. Later on, this groove deepens much resulting in coming together of the lateral sides of the strip of chorda cells. These

sides finally meet each other restricting completely the cavity of the groove. In this way a solid rod-like notochord is formed just below central nervous systems.

Development of mesoderm and coelom

In gastrula the archenteron, is developed by invagination and is bounded by three types of cells namely chorda cells, mesodermal cells on the sides of the chorda cells and manly by endodermal cells. The chorda cells form the notochord. The mesodermal cells separate to form paired pouches or the mesodermal pouches. These pouches are in dorso-lateral position and ultimately develop into initial coelom. Each pouch is disconnected from archenteron and thus encloses a cavity. The remaining cavity with the archenteron becomes the cavity of the gut and persists as alimentary canal in the adult animal.

Formation of gut

The chorda cells and mesodermal cells of archenteron wall separate to form notochord and mesodermal pouches respectively and thus archenteron is left only with endodermal cells. The edges of the endoderm start growing towards each

other (a rolling up process) and finally fuse with each other in mid-dorsal line just below the notochord, forming a tubular structure designated as mesenteron or gut.

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