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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOMEDICAL ENGINEERING

UNIT – I -Cell Biology & Genetics – SBMA1101

CELL STRUCTURE

Cells are the building blocks of life. A cell is chemical system that is able to maintain its structure and reproduce. Cells are the fundamental unit of life. All living things are cells or composed of cells. Although different living things may be as unlike as a violet and an octopus, they are all built in essentially the same way. The most basic similarity is that all living things are composed of one or more cells. This is known as the Cell Theory.

Our knowledge of cells is built on work done with microscopes. English scientist Robert Hooke in 1665 first described cells from his observations of cork slices. Hooke first used the word "cell". Dutch amateur scientist Antonie van Leeuwenhoek discovered microscopic animals in water. German scientists Schleiden and Schwann in 1830's were first to say that all organisms are made of one or more cells. German biologist Virchow in 1858 stated that all cells come from the division of pre-existing cells.

The Cell Theory can be summarized as:

- Cells are the fundamental unit of life - nothing less than a cell is alive.
- All organisms are constructed of and by cells.
- All cells arise from preexisting cells. Cells contain the information necessary for their own reproduction. No new cells are originating spontaneously on earth today.
- Cells are the functional units of life. All biochemical processes are carried out by cells. • Groups of cells can be organized and function as multicellular organisms
- Cells of multicellular organisms can become specialized in form and function to carry out subprocesses of the multicellular organism.

Cells are common to all living beings, and provide information about all forms of life. Because all cells come from existing cells, scientists can study cells to learn about growth, reproduction, and all other functions that living things perform. By learning about cells and how they function, we can learn about all types of living things.

Classification of cells:

All living organisms (bacteria, blue green algae, plants and animals) have cellular organization and may contain one or many cells. The organisms with only one cell in their body are called unicellular organisms (bacteria, blue green algae, some algae, Protozoa, etc.). The organisms having many cells in their body are called multicellular organisms (fungi, most plants and animals). Any living organism may contain only one type of cell either

A. Prokaryotic cells; B. Eukaryotic cells.

The terms prokaryotic and eukaryotic were suggested by Hans Ris in the 1960's. This classification is based on their complexity. Further based on the kingdom into which they may fall i.e the plant or the animal kingdom, plant and animal cells bear many differences. These will be studied in detail in the upcoming sections

PROKARYOTIC CELLS

Prokaryote comes from the Greek words for pre-nucleus. Prokaryotes:

i. One circular chromosome, not contained in a membrane.
ii. No histones or introns are present in Bacteria; both are found in Eukaryotes and Archaea.
iii. No membrane-bound organelles. (Only contain non membrane-bound organelles).
iv. Bacteria contain peptidoglycan in cell walls; Eukaryotes and Archaea do not.
v. Binary fission.

Size, Shape, and Arrangement of Bacterial Cells.

i. Average size of prokaryotic cells: 0.2 -2.0 µm in diameter 1-10 µm (0.001 – 0.01 mm) [book says 2 – 8 µm] in length.

1. Typical eukaryote 10-500 µm in length (0.01 – 0.5 mm).
2. Typical virus 20-1000 nm in length (0.00000002 – 0.000001 m).
3. Thiomargarita is the largest bacterium known. It is about the size of a typed period (0.75 mm).
4. Nanoarchaeum is the smallest cell known. It is at the lower theoretical limit for cell size (0.4 µm).

ii. Basic bacterial shapes:

1. Coccus (sphere/round).
2. Bacillus (staff/rod-shaped).
3. Spirilla (rigid with a spiral/corkscrew shape).
   a. Flagella propel these bacteria.
4. Vibrio (curved rod).
5. Spirochetes (flexible with a spiral shape).
   Axial filaments (endoflagella) propel these bacteria.

iii. Descriptive prefixes:

1. Diplo (two cells).
2. Tetra (four cells).
3. Sarcinae (cube of 8 cells).
4. Staphylo (clusters of cells).
5. Strepto (chains of cells).

iv. Unusual bacterial shapes:

1. Star-shaped Stella.
2. Square/rectangular Haloarcula.

v. Arrangements:

1. Pairs: diplococci, diplobacilli
2. Clusters: staphylococci
3. Chains: streptococci, streptobacilli.

vi. Most bacteria are monomorphic. They do not change shape unless environmental conditions change.

vii. A few are pleomorphic. These species have individuals that can come in a variety of shapes

ULTRA STRUCTURE OF PROKARYOTIC CELLS:

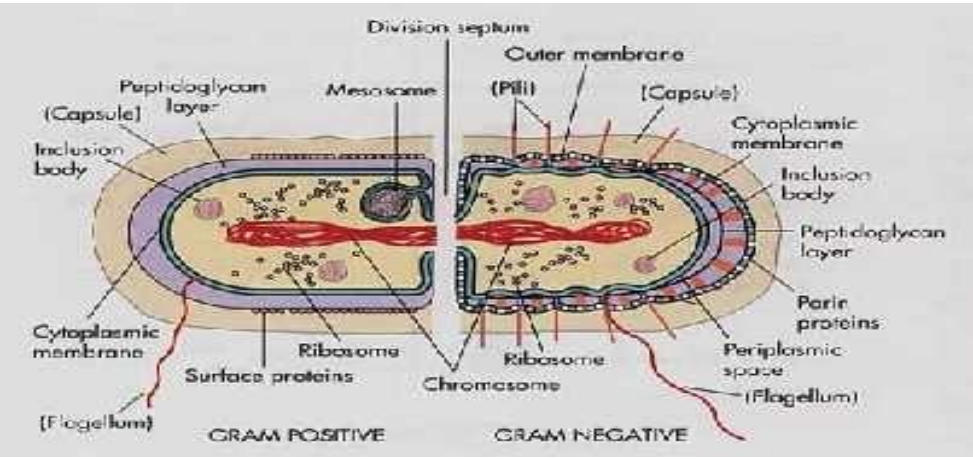


Fig 1.1 Structure of Prokaryotic cell

Structures External to the Prokaryotic Cell Wall.

a. Glycocalyx (sugar coat).

i. Usually very sticky.

ii. Found external to cell wall.

iii. Composed of polysaccharide and/or polypeptide.

iv. It can be broken down and used as an energy source when resources are scarce.

v. It can protect against dehydration.

vi. It helps keep nutrients from moving out of the cell.

1. A capsule is a glycocalyx that is neatly organized and is firmly attached to the cell wall. a. Capsules prevent phagocytosis by the host's immune system.

2. A slime layer is a glycocalyx that is unorganized and is loosely attached to the cell wall.

b. Extracellular polysaccharide(extracellular polymeric substance) is a glycocalyx made of sugars and allows bacterial cells to attach to various surfaces.Prokaryotic Flagella.

i. Long, semi-rigid, helical, cellular appendage used for locomotion.

ii. Made of chains of the protein flagellin.

1. Attached to a protein hook. iii. Anchored to the cell wall and cell membrane by the basal body.

iv. Motile Cells.

1. Rotate flagella to run and tumble.

2. Move toward or away from stimuli (taxis).

a. Chemotaxis. b. Phototaxis.

c. Axial Filaments (Endoflagella).

i. In spirochetes:

1. Anchored at one end of a cell.

2. Covered by an outer sheath.

3. Rotation causes cell to move like a corkscrew through a cork.

d. Fimbriae.

i. Shorter, straighter, thinner than flagella.

ii. Not used for locomotion.

iii. Allow for the attachment of bacteria to surfaces.

iv. Can be found at the poles of the cell, or covering the cell's entire surface.

v. There may be few or many fimbriae on a single bacterium.

e. Pili (sex pili).

i. Longer than fimbriae.

ii. Only one or two per cell.

iii. Are used to transfer DNA from one bacterial cell to another, and in twitching & gliding motility.

IV. The Prokaryotic Cell Wall.

a. Chemically and structurally complex, semi-rigid, gives structure to and protects the cell.

b. Surrounds the underlying plasma membrane.

c. Prevents osmotic lysis.
d. Contributes to the ability to cause disease in some species, and is the site of action for some antibiotics.
e. Made of peptidoglycan (in bacteria).
i. Polymer of a disaccharide. 1. N-acetylglucosamine (NAG) & N-acetylmuramic acid (NAM). ii. Disaccharides linked by polypeptides to form lattice surrounding the cell. Fig.
iii. Penicillin inhibits this lattice formation, and leads to cellular lysis.
f. Gram-positive cell walls. Fig.
i. Many layers of peptidoglycan, resulting in a thick, rigid structure.
ii. Teichoic acids.
1. May regulate movement of cations (+).
2. May be involved in cell growth, preventing extensive wall breakdown and lysis.
3. Contribute to antigenic specificity for each Gram-positive bacterial species.
4. Lipoteichoic acid links to plasma membrane.
5. Wall teichoic acid links to peptidoglycan.
g. Gram-negative cell walls.
i. Contains only one or a few layers of peptidoglycan.
1. Peptidoglycan is found in the periplasm, a fluid-filled space between the outer membrane and plasma membrane.
a. Periplasm contains many digestive enzymes and transport proteins.
ii. No teichoic acids are found in Gram-negative cell walls.
iii. More susceptible to rupture than Gram-positive cells.
iv. Outer membrane:
1. Composed of lipopolysaccharides, lipoproteins, and phospholipids.
2. Protects the cell from phagocytes, complement, antibiotics, lysozyme, detergents, heavy metals, bile salts, and certain dyes.
3. Contains transport proteins called porins.
4. Lipopolysaccharide is composed of:
a. O polysaccharide (antigen) that can be used to ID certain Gram- negative bacterial species.
b. Lipid A (endotoxin) can cause shock, fever, and even death if enough is released into the host's blood.
h. Gram Stain Mechanism.
i. Crystal Violet-Iodine (CV-I) crystals form within the cell.
ii. Gram-positive:
1. Alcohol dehydrates peptidoglycan.
2. CV-I crystals cannot leave.
iii. Gram-negative:
1. Alcohol dissolves outer membrane and leaves holes in peptidoglycan. 2. CV-I washes out. 3. Safranin stains the cell pink.
iv. Table 1, pg. 94, compares Gram-positive and Gram-negative bacteria.
i. Damage to Prokaryotic Cell Walls.
i. Because prokaryotic cell walls contain substances not normally found in animal

cells, drugs or chemicals that disrupt prokaryotic cell wall structures are often used in medicine, or by the host to combat the bacteria.

1. Lysozyme digests the disaccharides in peptidoglycan.
2. Penicillin inhibits the formation of peptide bridges in peptidoglycan.

ii. A protoplast is a Gram-positive cell whose cell wall has been destroyed, but that is still alive and functional. (Lost its peptidoglycan).

iii. A spheroplast is a wall-less Gram-negative cell. (Lost its outer membrane and peptidoglycan).

iv. L forms are wall-less cells that swell into irregular shapes. They can live, divide, and may return to a walled state.

v. Protoplasts and spheroplasts are susceptible to osmotic lysis.

vi. Gram-negative bacteria are not as susceptible to penicillin due to the outer membrane and the small amount of peptidoglycan in their walls.

vii. Gram-negative bacteria are susceptible to antibiotics that can penetrate the outer membrane (Streptomycin, chloramphenicol, tetracycline).

V. Structures Internal to the Cell Wall.

a. Plasma Membrane (Inner Membrane).

a. Phospholipid bilayer lying inside the cell wall.

1. The phospholipid bilayer is the basic framework of the plasma membrane.

2. The bilayer arrangement occurs because the phospholipids are amphipathic molecules. They have both polar (charged) and nonpolar (uncharged) parts with the polar "head" of the phospholipid pointing out and the nonpolar "tails" pointing toward the center of the membrane, forming a nonpolar, hydrophobic region in the membrane's interior.

b. Much of the metabolic machinery is located on the plasma membrane. Photosynthesis, aerobic cellular respiration, and anaerobic cellular respiration reactions occur here. This means that there is a surface area to volume ratio at which bacteria reach a critical size threshold, beyond which bacteria can't survive.

i. Thiomargarita (0.75 mm) is the largest known bacterium and is larger than most eukaryotic cells. It has many invaginations of the plasma membrane, which increases it surface area relative to its volume.

c. Peripheral proteins.

i. Enzymes.
ii. Structural proteins.
iii. Some assist the cell in changing membrane shape.

d. Integral proteins and transmembrane proteins.

i. Provide channels for movement of materials into and out of the cell.

e. Fluid Mosaic Model.

i. Membrane is as viscous as olive oil.
ii. Proteins move to function.
iii. Phospholipids rotate and move laterally.

f. Selective permeability allows the passage of some molecules but not others across the plasma membrane.

i. Large molecules cannot pass through.
ii. Ions pass through very slowly or not at all.
iii. Lipid soluble molecules pass through easily.
iv. Smaller molecules (water, oxygen, carbon dioxide, some simple sugars)

usually pass through easily.

g. The plasma membrane contains enzymes for ATP production.

h. Photosynthetic pigments are found on in-foldings of the plasma membrane called chromatophores or thylakoids. Fig. 15.

i. Damage to the plasma membrane by alcohols, quaternary ammonium compounds (a class of disinfectants) and polymyxin antibiotics causes leakage of cell contents.

j. Movement of Materials Across Membranes.

1. Passive Processes:

a. Simple diffusion: Movement of a solute from an area of high concentration to an area of low concentration (down its concentration gradient) until equilibrium is reached.

b. Facilitated diffusion: Solute combines with a transport protein in the membrane, to pass from one side of the membrane to the other. The molecule is still moving down its concentration gradient. The transport proteins are specific.

c. Osmosis.

i. Movement of water across a selectively permeable membrane from an area of higher water concentration to an area of lower water concentration.

ii. Osmotic pressure.

The pressure needed to stop the movement of water across the membrane.

iii. Isotonic, hypotonic, and hypertonic solutions.

2. Active Processes:

a. Active transport of substances requires a transporter protein and ATP. The solute molecule is pumped against its concentration gradient. Transport proteins are specific. i. In group translocation (a special form of active transport found only in prokaryotes) movement of a substance requires a specific transport protein. 1. The substance is chemically altered during transport, preventing it from escaping the cell after it is transported inside. 2. This process requires high-energy phosphate compounds like phosphoenolpyruvic acid (PEP) to phosphorylate the transported molecule, preventing its movement out of the cell.

b. Cytoplasm.

i. Cytoplasm is the substance inside the plasma membrane.

ii. It is about 80% water.

iii. Contains proteins, enzymes, carbohydrates, lipids, inorganic ions, various compounds, a nuclear area, ribosomes, and inclusions.

c. Nuclear Area (Nucleoid).

i. Contains a single circular chromosome made of DNA.

1. No histones or introns in bacteria.

2. The chromosome is attached to the plasma membrane at a point along its length, where proteins synthesize and partition new DNA for division during binary fission.

ii. Is not surrounded by a nuclear envelope the way eukaryotic chromosomes are.

iii. Also contains small circular DNA molecules called plasmids.

1. Plasmids can be gained or lost without harming the cell.

2. Usually contain less than 100 genes.

3. Can be beneficial if they contain genes for antibiotic resistance, tolerance to toxic metals, production of toxins, or synthesis of enzymes.

4. They can be transferred from one bacterium to another.

5. Plasmids are used in genetic engineering.

d. Ribosomes.

i. Site of protein synthesis.

ii. Composed of a large and small subunit, both made of protein and rRNA. iii. Prokaryotic ribosomes are 70S ribosomes.

1. Made of a small 30S subunit and a larger 50S subunit.

iv. Eukaryotic ribosomes are 80S ribosomes.

1. Made of a small 40S subunit and a larger 60S subunit.

v. Certain antibiotics target only prokaryotic ribosomal subunits without targeting eukaryotic ribosomal subunits.

e. Inclusions.

i. Reserve deposits of nutrients that can be used in times of low resource availability. ii. Include:

1. Metachromatic granules (volutin). Reserve of inorganic phosphate for ATP.

2. Polysaccharide granules. Glycogen and starch.

3. Lipid inclusions.

4. Sulfur granules. Energy reserve for "sulfur bacteria" that derive energy by oxidizing sulfur and sulfur compounds.

5. Carboxysomes. Contain an enzyme necessary for bacteria that use carbon dioxide as their only source of carbon for carbon dioxide fixation.

6. Gas vacuoles. Help bacteria maintain buoyancy.

7. Magnetosomes. Made of iron oxide, they serve as ballast to help some bacteria sink until reaching an appropriate attachment site. They also decompose hydrogen peroxide.

f. Endospores.

i. Resting Gram-positive bacterial cells that form when essential nutrients can no longer be obtained.

ii. Resistant to desiccation, heat, chemicals, radiation.

iii. Bacillus anthracis (anthrax), Clostridium spp. (gangrene, tetanus, botulism, food poisoning).

iv. Sporulation (sporogenesis): the process of endospore formation within the vegetative (functional) cell. This takes several hours.

1. Spore septum (invagination of plasma membrane) begins to isolate the newly replicated DNA and a small portion of cytoplasm. This results in the formation of two separate membrane bound structures.

2. The plasma membrane starts to surround the DNA, cytoplasm, and the new membrane encircling the material isolated in step 1, forming a double-layered membrane-bound structure called a forespore.

3. Thick peptidoglycan layers are laid down between the two membranes of the forespore.

4. Then a thick spore coat of protein forms around the outer membrane of the forespore, which is responsible for the durability of the endospore.

5. When the endospore matures, the vegetative cell wall ruptures, killing the cell, and freeing the endospore.

a. The endospore is metabolically inert, and contains the chromosome,

some RNA, ribosomes, enzymes, other molecules, and very little water.

b. Endospores can remain dormant for millions of years.

v. Germination: the return to the vegetative state.

1. Triggered by damage to the endospore coat. The enzymes activate, breaking down the protective layers. Water then can enter, and metabolism resumes.

vi. Endospores can survive conditions that vegetative cells cannot: boiling, freezing, desiccation, chemical exposure, radiation, etc.

EUKARYOTES:

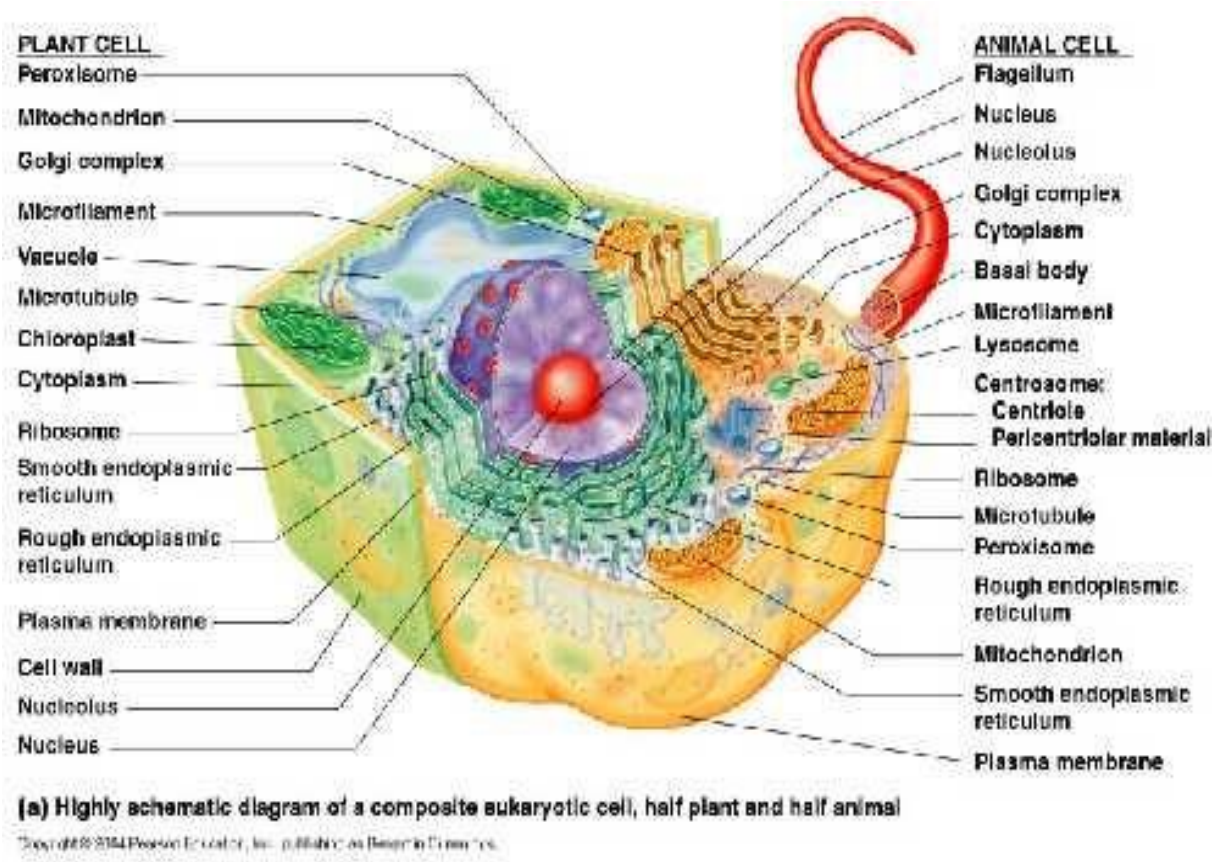


Fig. 1.2 Structure of Eukaryotic cell

a. Make up algae, protozoa, fungi, higher plants, and animals.

Flagella and Cilia. Rotate Cilia are numerous, short, hair-like projections extending from the surface of a cell. They function to move materials across the surface of the cell, or move the cell around in its environment.

i. Flagella are similar to cilia but are much longer, usually moving an entire cell. The only example of a flagellum in the human body is the sperm cell tail.

1. Eukaryotic flagella move in a whip-like manner, while prokaryotic flagella

b. Cell Wall.

i. Simple compared to prokaryotes.

1. No peptidoglycan in eukaryotes.

a. Antibiotics that target peptidoglycan (penicillins and cephalosporins) do not harm us.

ii. Cell walls are found in plants, algae, and fungi.

iii. Made of carbohydrates.

1. Cellulose in algae, plants, and some fungi.

2. Chitin in most fungi. 3. Glucan and mannan in yeasts (unicellular fungi).

c. Glycocalyx.

i. Sticky carbohydrates extending from an animal cell's plasma membrane.

ii. Glycoproteins and glycolipids form a sugary coat around the cell—the glycocalyx—which helps cells recognize one another, adhere to one another in some tissues, and protects the cell from digestion by enzymes in the extracellular fluid.

1. The glycocalyx also attracts a film of fluid to the surface of many cells, such as RBC's, making them slippery so they can pass through narrow vessels.

d. Plasma Membrane.

i. The plasma membrane is a flexible, sturdy barrier that surrounds and contains the cytoplasm of the cell.

1. The fluid mosaic model describes its structure.

2. The membrane consists of proteins in a sea of phospholipids.

a. Some proteins float freely while others are anchored at specific locations.

b. The membrane lipids allow passage of several types of lipid-soluble molecules but act as a barrier to the passage of charged or polar substances.

c. Channel and transport proteins allow movement of polar molecules and ions across the membrane.

ii. Phospholipid bilayer.

1. Has the same basic arrangement as the prokaryotic plasma membrane.

iii. Arrangement of Membrane Proteins.

1. The membrane proteins are divided into integral and peripheral proteins.

a. Integral proteins extend into or across the entire lipid bilayer among the fatty acid tails of the phospholipid molecules, and are firmly anchored in place.

i. Most are transmembrane proteins, which span the entire lipid bilayer and protrude into both the cytosol and extracellular fluid.

b. Peripheral proteins associate loosely with the polar heads of membrane lipids, and are found at the inner or outer surface of the membrane.

2. Many membrane proteins are glycoproteins (proteins with carbohydrate groups attached to the ends that protrude into the extracellular fluid).

iv. Functions of Membrane Proteins.

1. Membrane proteins vary in different cells and function as:

a. Ion channels (pores): Allow ions such as sodium or potassium to cross the cell membrane; (they can't diffuse through the bilayer). Most are selective—they allow only a single type of ion to pass. Some ion channels open and close.

b. Transporters: selectively move a polar substance from one side of the membrane to the other.

c. Receptors: recognize and bind a specific molecule. The chemical binding to the receptor is called a ligand.

d. Enzymes: catalyze specific chemical reactions at the inside or outside surface of the cell.

e. Cell-identity markers (often glycoproteins and glycolipids), such as human leukocyte antigens.

f. Linkers: anchor proteins in the plasma membrane of neighboring cells to each other or to protein filaments inside and outside the cell.

2. The different proteins help to determine many of the functions of the plasma membrane.

v. Selective permeability of the plasma membrane allows passage of some molecules.

1. Transport mechanisms:

a. Simple diffusion. b. Facilitated diffusion. c. Osmosis. d. Active transport. (No group translocation in Eukaryotes). e. Vesicular Transport.

i. A vesicle is a small membranous sac formed by budding off from an existing membrane.

ii. Two types of vesicular transport are endocytosis and exocytosis.

1. Endocytosis.

a. In endocytosis, materials move into a cell in a vesicle formed from the plasma membrane.

b. Viruses can take advantage of this mechanism to enter cells.

c. Phagocytosis is the ingestion of solid particles, such as worn out cells, bacteria, or viruses. Pseudopods extend and engulf particles.

d. Pinocytosis is the ingestion of extracellular fluid. The membrane folds inward bringing in fluid and dissolved substances.

2. In exocytosis, membrane-enclosed structures called secretory vesicles that form inside the cell fuse with the plasma membrane and release their contents into the extracellular fluid.

f. Cytoplasm.

i. Substance inside the plasma membrane and outside nucleus.

ii. Cytosol is the fluid portion of cytoplasm.

iii. Cytoskeleton.

1. The cytoskeleton is a network of several kinds of protein filaments that extend throughout the cytoplasm, and provides a structural framework for the cell.

2. It consists of microfilaments, intermediate filaments, and microtubules.

a. Most microfilaments (the smallest cytoskeletal elements) are composed of actin and function in movement (muscle contraction and cell division) and mechanical support for the cell itself and for microvilli.

b. Intermediate filaments are composed of several different proteins and function in support and to help anchor organelles such as the nucleus.

c. Microtubules (the largest cytoskeletal elements) are composed of a protein called tubulin and help determine cell shape; they function in the intracellular transport of organelles and the migration of chromosome during cell division. They also function in the movement of cilia and flagella.

iv. Cytoplasmic streaming.

1. Movement of cytoplasm and nutrients throughout cells.

2. Moves the cell over surfaces.

g. Organelles.

i. Organelles are specialized structures that have characteristic shapes and perform specific functions in eukaryotic cellular growth, maintenance, reproduction.

1. Nucleus.

The nucleus is usually the most prominent feature of a eukaryotic cell.

b. Most have a single nucleus; some cells (human red blood cells) have none, whereas others (human skeletal muscle fibers) have several in each cell.

c. The parts of the nucleus include the:

i. Nuclear envelope (a double membrane), which is perforated by channels called nuclear pores, that control the movement of substances between the nucleus and the cytoplasm.

1. Small molecules and ions diffuse passively, while movement of most large molecules out of the nucleus involves active transport.

ii. Nucleoli function in producing ribosomes. d. Genetic material (DNA). Within the nucleus are the cell's hereditary units, called genes, which are arranged in single file along chromosomes. Each chromosome is a long molecule of DNA that is coiled together with several proteins (including histones).

2. **RIBOSOMES**.

a. Sites of protein synthesis.

b. 80S in eukaryotes.

i. Membrane-bound ribosomes found on rough ER.

ii. Free ribosomes found in cytoplasm.

c. 70S in prokaryotes.

i. Also found in chloroplasts and mitochondria.

3. **Endoplasmic Reticulum**.

a. The endoplasmic reticulum (ER) is a network of membranes extending from the nuclear membrane that form flattened sacs or tubules.

b. Rough ER is continuous with the nuclear membrane and has its outer surface studded with ribosomes, which synthesize proteins. The proteins then enter the space inside the ER for processing (into glycoproteins or for attachment to phospholipids) and sorting,

and are then either incorporated into organelle membranes, inserted into the plasma membrane, or secreted via exocytosis.
c. Smooth ER extends from the rough ER to form a network of membrane tubules, but it does not contain ribosomes on its membrane surface. In humans, it synthesizes fatty acids and steroids, detoxifies drugs, removes phosphate from glucose 6-phosphate (allowing free glucose to enter the blood), and stores and releases calcium ions involved in muscle contraction.

4. Golgi Complex.

The Golgi complex consists of four to six stacked, flattened membranous sacs (cisterns). The cis (entry) face faces the rough ER, and trans (exit) face faces the cell's plasma membrane. Between the cis and trans faces are the medial cisternae.

b. The cis, medial, and trans cisternae each contain different enzymes that permit each to modify, sort, and package proteins received from the rough ER for transport to different destinations (such as the plasma membrane, to other organelles, or for export out of the cell).

5. Lysosomes.

a. Lysosomes are membrane-enclosed vesicles that form from the Golgi complex and contain powerful digestive enzymes.
b. Lysosomes function in digestion of substances that enter the cell by endocytosis, and transport the final products of digestion into the cytosol.
c. They digest worn-out organelles (autophagy).
d. They digest their own cellular contents (autolysis).
e. They carry out extracellular digestion (as happens when sperm release lysosomal enzymes to aid in penetrating an oocyte).

6. Vacuoles.

a. Space in the cytoplasm enclosed by a membrane called a tonoplast.
b. Derived from the Golgi complex.
c. They serve in the following ways:
i. Temporary storage for biological molecules and ions.
ii. Bring food into cells.
iii. Provide structural support.
iv. Store metabolic wastes.

7. Peroxisomes.

a. Peroxisomes are similar in structure to lysosomes, but are smaller.
b. They contain enzymes (oxidases) that use molecular oxygen to oxidize (remove hydrogen atoms from) various organic substances.

c. They take part in normal metabolic reactions such as the oxidation of amino and fatty acids.
d. New peroxisomes form by budding off from preexisting ones.
e. They produce and then destroy H_2O_2 (hydrogen peroxide) in the process of their metabolic activities.

8. Centrosomes.

a. Centrosomes are dense areas of cytoplasm containing the centrioles, which are paired cylinders arranged at right angles to one another, and serve as centers for organizing microtubules and the mitotic spindle during mitosis.

9. Mitochondria.

a. Found in nearly all eukaryotic cells.
b. A mitochondrion is bound by a double membrane, with a fluid-filled space between called the intermembranous space. The outer membrane is smooth, while the inner membrane is arranged in folds called cristae. The mitochondrial matrix is found inside the inner mitochondrial membrane.
c. The folds of the cristae provide a large surface area for the chemical reactions that are part of the aerobic phase of cellular respiration. These reactions produce most of a eukaryotic cell's ATP, and the enzymes that catalyze them are located on the cristae and in the matrix.
d. Mitochondria self-replicate using their own DNA and contain 70S ribosomes. They grow and reproduce on their own in a way that is similar to binary fission. Mitochondrial DNA (genes) is inherited only from the mother, since sperm normally lack most organelles such as mitochondria, ribosomes, ER, and the Golgi complex. Any sperm mitochondria that do enter the oocyte are soon destroyed.

10. Chloroplasts.

a. Found only in algae and green plants.
b. Contain the pigment chlorophyll and enzymes necessary for photosynthesis.
c. Chloroplasts self-replicate using their own DNA and contain 70S ribosomes. They grow and reproduce on their own in a way that is similar to binary fission.

VII. Endosymbiotic Theory.
a. Large bacterial cells lost their cell walls and engulfed smaller bacteria.

b. A symbiotic (mutualistic) relationship developed.
i. The host cell supplied the nutrients.
ii. The engulfed cell produced excess energy that the host could use.
iii. The relationship evolved.
c. Evidence:

i. Mitochondria and chloroplasts resemble bacteria in size and shape.

1. They divide on their own—independently of the host, and contain their own DNA (single circular chromosome). This process is nearly identical to binary fission seen in bacteria.
2. They contain 70S ribosomes.
3. Their method of protein synthesis is more like that of prokaryotes (no RNA processing).
4. Antibiotics that inhibit protein synthesis on ribosomes in bacteria also inhibit protein

Difference among eukaryotic cells

There are many different types of eukaryotic cells, though animals and plants are the most familiar eukaryotes, and thus provide an excellent starting point for understanding eukaryotic structure. Fungi and many protists have some substantial differences, however.

Animal cell

An **animal cell** is a form of eukaryotic cell that makes up many tissues in animals. Animal cells are distinct from other eukaryotes, most notably plant cells, as they lack cell walls and chloroplasts. They also have smaller vacuoles. Due to the lack of a cell wall, animal cells can adopt a variety of shapes. A phagocytic cell can even engulf other structures.

There are many different types of cell. For instance, there are approximately 210 distinct cell types in the adult human body.

Plant cell

Plant cells are quite different from the cells of the other eukaryotic organisms. Their distinctive features are:

- A large central vacuole (enclosed by a membrane, the tonoplast), which maintains the cell's turgor and controls movement ofmolecules between the cytosol and sap
- A primary cell wall containing cellulose, hemicellulose and pectin, deposited by the protoplast on the outside of the cell membrane; this contrasts with the cell walls of fungi, which contain chitin, and the cell envelopes of prokaryotes, in which peptidoglycans are the main structural molecules
- The plasmodesmata, linking pores in the cell wall that allow each plant cell to communicate with other adjacent cells; this is different from the functionally analogous system of gap junctions between animal cells.

- Plastids, especially chloroplasts that contain chlorophyll, the pigment that gives plants their green color and allows them to perform photosynthesis
- Bryophytes and seedless vascular plants lack flagellae and centrioles except in the sperm cells.[16] Sperm of cycads and *Ginkgo*are large, complex cells that swim with hundreds to thousands of flagellae.
- Conifers (Pinophyta) and flowering plants (Angiospermae) lack the flagellae and centrioles that are present in animal cells.

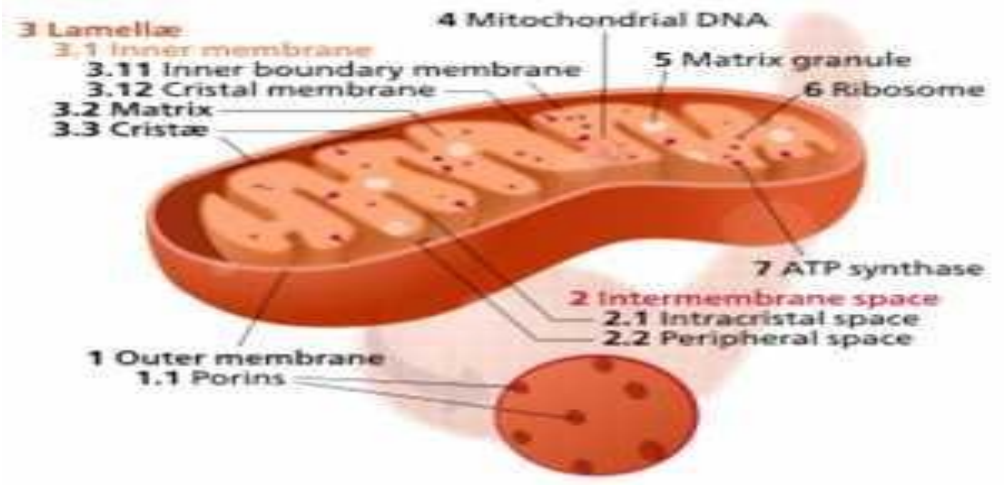
Structure and functions of cell organelles

Mitochondria:

The **mitochondrion** (plural **mitochondria**) is a double membrane-bound organelle found in most eukaryotic cells. The word mitochondrion comes from the Greek *μίτος*, *mitos*, i.e. "thread", and *χονδρίον*, *chondrion*, i.e. "granule" or "grain-like".

Mitochondria range from 0.5 to 1.0 μm in diameter. A considerable variation can be seen in the structure and size of the organelle though. These, unless specifically stained, are not visible. These structures are described as "the powerhouse of the cell" because they generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy.[3]In addition to supplying cellular energy, mitochondria are involved in other tasks, such as signaling, cellular differentiation, cell death, as well as maintaining the control of the cell cycle and cell growth. Mitochondria have been implicated in several human diseases, including mitochondrial disorders, cardiac dysfunction, and heart failure. A recent University of California study including ten children diagnosed with severe autism suggests that autism may be correlated with mitochondrial defects as well.

Several characteristics make mitochondria unique. The number of mitochondria in a cell can vary widely by organism, tissue, and cell type. For instance, red blood cells have no mitochondria, whereas liver cells can have more than 2000. The organelle is composed of compartments that carry out specialized functions. These compartments or regions include the outer membrane, the



intermembrane space, the inner membrane, and the cristae and matrix. Mitochondrial proteins vary depending on the tissue and the species. In humans, 615 distinct types of protein have been identified from cardiacmitochondria, whereas in rats, 940 proteins have been reported. The mitochondrial proteome is thought to be dynamically regulated. Although most of a cell's DNA is contained in the cell nucleus, the mitochondrion has its own independent genome. Further, its DNA shows substantial similarity to bacterial genomes.

Components of Mitochondria

The first observations of intracellular structures that probably represent mitochondria were published in the 1840s. Richard Altmann, in 1894, established them as cell organelles and called them "bioblasts". The term "mitochondria" itself was coined by Carl Benda in 1898. Leonor Michaelis discovered that Janus green can be used as a supravital stain for mitochondria in 1900. Friedrich Meves, in 1904, made the first recorded observation of mitochondria in plants (*Nymphaeaalba*) and in 1908, along with Claudius Regaud, suggested that they contain proteins and lipids. Benjamin F. Kingsbury, in 1912, first related them with cell respiration, but almost exclusively based on morphological observations.In 1913, particles from extracts of guinea-pig liver were linked to respiration by Otto Heinrich Warburg, which he called "grana". Warburg and Heinrich Otto Wieland, who had also postulated a similar particle mechanism, disagreed on the chemical nature of the respiration. It was not until 1925, when David Keilin discovered cytochromes, that the respiratory chain was described.

In 1939, experiments using minced muscle cells demonstrated that cellular respiration using one oxygen atom can form twoadenosine triphosphate (ATP) molecules, and, in 1941, the concept of the phosphate bonds of ATP being a form of energy in cellular metabolism was developed by Fritz Albert Lipmann. In the following years, the mechanism behind cellular respiration was further elaborated, although its link to the mitochondria was not known.[14] The introduction of tissue fractionation by Albert Claude allowed mitochondria to be isolated from other cell fractions and biochemical analysis to be conducted on them alone. In 1946, he concluded that cytochrome oxidase and other enzymes responsible for the respiratory chain were isolated to the mitchondria. Over time, the fractionation method was tweaked, improving the quality of the mitochondria isolated, and other elements of cell respiration were determined to occur in the mitochondria.

The first high-resolution micrographs appeared in 1952, replacing the Janus Green stains as the preferred way of visualising the mitochondria. This led to a more detailed analysis of the structure of the mitochondria, including confirmation that they were surrounded by a membrane. It also showed a second membrane inside the mitochondria that folded up in ridges dividing up the inner chamber and that the size and shape of the mitochondria varied from cell to cell.

The popular term "powerhouse of the cell" was coined by Philip Siekevitz in 1957.

In 1967, it was discovered that mitochondria contained ribosomes. In 1968, methods were developed for mapping the mitochondrial genes, with the genetic and physical map of yeast mitochondria being completed in 1976

Mitochondrion ultrastructure

A mitochondrion has a double membrane; the inner one contains its chemiosmotic apparatus and has deep grooves which increase its surface area. While commonly depicted as an "orange sausage with a blob inside of it" (like it is here), mitochondria can take many shapes and their intermembrane space is quite thin.

A mitochondrion contains outer and inner membranes composed of phospholipid bilayers andproteins.[8] The two membranes have different properties. Because of this double- membraned organization, there are five distinct parts to a mitochondrion. They are:

1. the outer mitochondrial membrane,
2. the intermembrane space (the space between the outer and inner membranes),
3. the inner mitochondrial membrane,
4. the cristae space (formed by infoldings of the inner membrane), and
5. the matrix (space within the inner membrane).

Mitochondria stripped of their outer membrane are called mitoplasts.

Outer membrane

The **outer mitochondrial membrane**, which encloses the entire organelle, is 60 to 75angstroms (Å) thick. It has a protein-to-phospholipid ratio similar to that of the eukaryotic plasma membrane (about 1:1 by weight). It contains large numbers of integral membrane proteinscalled porins. These porins form channels that allow molecules of 5000 daltons or less in molecular weight to freely diffuse from one side of the membrane to the other.[8]Larger proteins can enter the mitochondrion if a signaling sequence at their N-terminus binds to a large multisubunit protein called translocase of the outer membrane, which then actively moves them across the membrane.[18]Mitochondrial pro-proteins are imported through specialised translocate complexes. The outer membrane also contains enzymes involved in such diverse activities as the elongation of fatty acids, oxidation of epinephrine, and the degradation of tryptophan. These enzymes include monoamine oxidase, rotenone-insensitive NADH-cytochrome c- reductase, kynurenine hydroxylase and fatty acid Co-A ligase. Disruption of the outer membrane permits proteins in the intermembrane space to leak into the cytosol, leading to certain cell death.[19] The mitochondrial outer membrane can associate with the endoplasmic reticulum (ER) membrane, in a structure called MAM (mitochondria-associated ER-membrane). This is important in the ER-mitochondria calcium signaling and is involved in the transfer of lipids between the ER and mitochondria.[20]Outside the outer membrane there are small (diameter: 60Å) particles named sub-units of Parson.

Intermembrane space

The intermembrane space is the space between the outer membrane and the inner membrane. It is also known as perimitochondrial space. Because the outer membrane is freely permeable to small molecules, the concentrations of small molecules, such as ions and sugars, in the intermembrane space is the same as the cytosol. However, large proteins must have a specific signaling sequence to be transported across the outer membrane, so the protein composition of this space is different from the protein composition of the cytosol. One protein that is localized to the intermembrane space in this way is cytochrome c.

Inner membrane

The inner mitochondrial membrane contains proteins with five types of functions:[8]

1. Those that perform the redox reactions of oxidative phosphorylation
2. ATP synthase, which generates ATP in the matrix
3. Specific transport proteins that regulate metabolite passage into and out of the matrix
4. Protein import machinery
5. Mitochondrial fusion and fission protein

It contains more than 151 different polypeptides, and has a very high protein-to-phospholipid ratio (more than 3:1 by weight, which is about 1 protein for 15 phospholipids). The inner membrane is home to around 1/5 of the total protein in a mitochondrion. In addition, the inner membrane is rich in an unusual phospholipid, cardiolipin. This phospholipid was originally discovered in cow hearts in 1942, and is usually characteristic of mitochondrial and bacterial plasma membranes.[21] Cardiolipin contains four fatty acids rather than two, and may help to make the inner membrane impermeable. Unlike the outer membrane, the inner membrane doesn't contain porins, and is highly impermeable to all molecules. Almost all ions and molecules require special membrane transporters to enter or exit the matrix. Proteins are ferried into the matrix via the translocase of the inner membrane (TIM) complex or via Oxa1. In addition, there is a membrane potential across the inner membrane, formed by the action of the enzymes of the electron transport chain.

Cristae

The inner mitochondrial membrane is compartmentalized into numerous cristae, which expand the surface area of the inner mitochondrial membrane, enhancing its ability to produce ATP. For typical liver mitochondria, the area of the inner membrane is about five times as large as the outer membrane. This ratio is variable and mitochondria from cells that have a greater demand for ATP, such as muscle cells, contain even more cristae. These folds are studded with small round bodies known as F_1 particles or oxysomes. These are not simple random folds but rather invaginations of the inner membrane, which can affect overall chemiosmotic function.

One recent mathematical modeling study has suggested that the optical properties of the cristae in filamentous mitochondria may affect the generation and propagation of light within the tissue.

Matrix

The matrix is the space enclosed by the inner membrane. It contains about 2/3 of the total protein in a mitochondrion.[8] The matrix is important in the production of ATP with the aid of the ATP synthase contained in the inner membrane. The matrix contains a highly concentrated mixture of hundreds of enzymes, special mitochondrial ribosomes, tRNA, and several copies of the mitochondrial DNA genome. Of the enzymes, the major functions include oxidation of pyruvate and fatty acids, and the citric acid cycle.[8]

Mitochondria have their own genetic material, and the machinery to manufacture their own RNAs and proteins (*see: protein biosynthesis*). A published human mitochondrial DNA sequence revealed 16,569 base pairs encoding 37 genes: 22 tRNA, 2 rRNA, and 13 peptide genes.[24] The 13 mitochondrial peptides in humans are integrated into the inner mitochondrial membrane, along with proteins encoded by genes that reside in the host cell's nucleus.

Mitochondria-associated ER membrane (MAM)

The mitochondria-associated ER membrane (MAM) is another structural element that is increasingly recognized for its critical role in cellular physiology and homeostasis. Once considered a technical snag in cell fractionation techniques, the alleged ER vesicle contaminants that invariably appeared in the mitochondrial fraction have been re-identified as membranous structures derived from the MAM—the interface between mitochondria and the ER. Physical coupling between these two organelles had previously been observed in electron micrographs and has more recently been probed with fluorescence microscopy. Such studies estimate that at the MAM, which may comprise up to 20% of the mitochondrial outer membrane, the ER and mitochondria are separated by a mere 10–25 nm and held together by protein tethering complexes

Purified MAM from subcellular fractionation has be shown to be enriched in enzymes involved in phospholipid exchange, in addition to channels associated with Ca^{2+}signaling.[25][27] These hints of a prominent role for the MAM in the regulation of cellular lipid stores and signal transduction have been borne out, with significant implications for mitochondrial-associated cellular phenomena, as discussed below. Not only has the MAM provided insight into the mechanistic basis underlying such physiological processes as intrinsic apoptosis and the propagation of calcium signaling, but it also favors a more refined view of the mitochondria. Though often seen as static, isolated 'powerhouses' hijacked for cellular metabolism through an ancient endosymbiotic event, the evolution of the MAM underscores the extent to which mitochondria have been integrated into overall cellular physiology, with intimate physical and functional coupling to the endomembrane system.

Phospholipid transfer

The MAM is enriched in enzymes involved in lipid biosynthesis, such as phosphatidylserine synthase on the ER face and phosphatidylserine decarboxylase on the mitochondrial face. Because mitochondria are dynamic organelles constantly undergoing fission and fusion events, they require a constant and well-regulated supply of phospholipids for membrane integrity.[30][31] But mitochondria are not only a destination for the phospholipids they finish synthesis of; rather, this organelle also plays a role in inter-organelle trafficking of the intermediates and products of phospholipid biosynthetic pathways, ceramide and cholesterol metabolism, and glycosphingolipid anabolism.

Such trafficking capacity depends on the MAM, which has been shown to facilitate transfer of lipid intermediates between organelles.[28] In contrast to the standard vesicular mechanism of lipid transfer, evidence indicates that the physical proximity of the ER and mitochondrial membranes at the MAM allows for lipid flipping between opposed bilayers. Despite this unusual and seemingly energetically unfavorable mechanism, such transport does not require ATP. Instead, in yeast, it has been shown to be dependent on a multiprotein tethering structure termed the ER-mitochondria encounter structure, or ERMES, although it remains unclear whether this structure directly mediates lipid transfer or is required to keep the membranes in sufficiently close proximity to lower the energy barrier for lipid flipping.

The MAM may also be part of the secretory pathway, in addition to its role in intracellular lipid trafficking. In particular, the MAM appears to be an intermediate destination between the rough ER and the Golgi in the pathway that leads to very-low-density lipoprotein, or VLDL, assembly and secretion. The MAM thus serves as a critical metabolic and trafficking hub in lipid metabolism.

Calcium signaling

A critical role for the ER in calcium signaling was acknowledged before such a role for the mitochondria was widely accepted, in part because the low affinity of Ca^{2+} channels localized to the outer mitochondrial membrane seemed to fly in the face of this organelle's purported responsiveness to changes in intracellular Ca^{2+} flux.[25] But the presence of the MAM resolves this apparent contradiction: the close physical association between the two organelles results in Ca^{2+} microdomains at contact points that facilitate efficient Ca^{2+} transmission from the ER to the mitochondria.[25] Transmission occurs in response to so-called "Ca^{2+} puffs" generated by spontaneous clustering and activation of IP3R, a canonical ER membrane Ca^{2+} channel.[25][26]

The fate of these puffs—in particular, whether they remain restricted to isolated locales or integrated into Ca^{2+} waves for propagation throughout the cell—is determined in large part by MAM dynamics. Although reuptake of Ca^{2+} by the ER (concomitant with its release) modulates the intensity of the puffs, thus insulating mitochondria to a certain degree from high

Ca^{2+} exposure, the MAM often serves as a firewall that essentially buffers Ca^{2+} puffs by acting as a sink into which free ions released into the cytosol can be funneled. This Ca^{2+} tunneling occurs through the low-affinity Ca^{2+} receptor VDAC1, which recently has been shown to be physically tethered to the IP3R clusters on the ER membrane and enriched at the MAM. The ability of mitochondria to serve as a Ca^{2+} sink is a result of the electrochemical gradient generated during oxidative phosphorylation, which makes tunneling of the cation an exergonic process.[36]

But transmission of Ca^{2+} is not unidirectional; rather, it is a two-way street. The properties of the Ca^{2+} pump SERCA and the channel IP3R present on the ER membrane facilitate feedback regulation coordinated by MAM function. In particular, theclearance of Ca^{2+} by the MAM allows for spatio-temporal patterning of Ca^{2+} signaling because Ca^{2+} alters IP3R activity in a biphasic manner. SERCA is likewise affected by mitochondrial feedback: uptake of Ca^{2+} by the MAM stimulates ATP production, thus providing energy that enables SERCA to reload the ER with Ca^{2+} for continued Ca^{2+} efflux at the MAM.[34][36] Thus, the MAM is not a passive buffer for Ca^{2+} puffs; rather it helps modulate further Ca^{2+}signaling through feedback loops that affect ER dynamics.

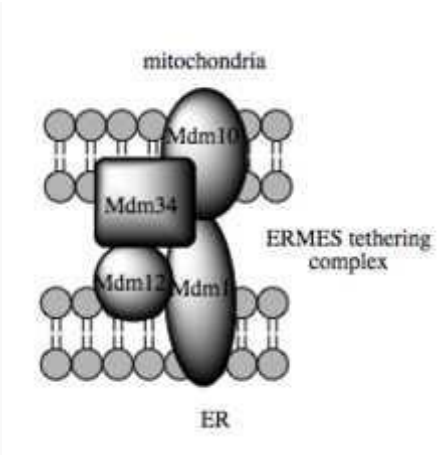
Regulating ER release of Ca^{2+} at the MAM is especially critical because only a certain window of Ca^{2+} uptake sustains the mitochondria, and consequently the cell, at homeostasis. Sufficient intraorganelle Ca^{2+} signaling is required to stimulate metabolism by activating dehydrogenase enzymes critical to flux through the citric acid cycle.[37]However, once Ca^{2+} signaling in the mitochondria passes a certain threshold, it stimulates the intrinsic pathway of apoptosis in part by collapsing the mitochondrial membrane potential required for metabolism. Studies examining the role of pro- and anti-apoptotic factors support this model; for example, the anti-apoptotic factor Bcl-2 has been shown to interact with IP3Rs to reduce Ca^{2+} filling of the ER, leading to reduced efflux at the MAM and preventing collapse of the mitochondrial membrane potential post-apoptotic stimuli. Given the need for such fine regulation of Ca^{2+} signaling, it is perhaps unsurprising that dysregulated mitochondrial Ca^{2+} has been implicated in several neurodegenerative diseases, while the catalogue of tumor suppressors includes a few that are enriched at the MAM.

Molecular basis for tethering

Recent advances in the identification of the tethers between the mitochondrial and ER membranes suggest that the scaffolding function of the molecular elements involved is secondary to other, non-structural functions. In yeast, ERMES, a multiprotein complex of interacting ER- and mitochondrial-resident membrane proteins, is required for lipid transfer at the MAM and exemplifies this principle. One of its components, for example, is also a constituent of the protein complex required for insertion of transmembrane beta-barrel proteins into the lipid bilayer.[31] However, a homologue of the ERMES complex has not yet been identified in

mammalian cells. Other proteins implicated in scaffolding likewise have functions independent of structural tethering at the MAM; for example, ER-resident and mitochondrial-resident mitofusins form heterocomplexes that regulate the number of inter-organelle contact sites, although mitofusins were first identified for their role in fission and fusion events between individual mitochondria.[25] Glucose-related protein 75 (grp75) is another dual-function protein. In addition to the matrix pool of grp75, a portion serves as a chaperone that physically links the mitochondrial and ER Ca^{2+}channels VDAC and IP3R for efficient Ca^{2+} transmission at the MAM. Another potential tether is Sigma-1R, a non-opioid receptor whose stabilization of ER- resident IP3R may preserve communication at the MAM during the metabolic stress response.[38][39]

Perspective



The MAM is a critical signaling, metabolic, and trafficking hub in the cell that allows for the integration of ER and mitochondrial physiology. Coupling between these organelles is not simply structural but functional as well and critical for overall cellular physiology and homeostasis. The MAM thus offers a perspective on mitochondria that diverges from the traditional view of this organelle as a static, isolated unit appropriated for its metabolic capacity by the cell. Instead, this mitochondrial-ER interface emphasizes the integration of the mitochondria, the product of an endosymbiotic event, into diverse cellular processes.

Organization and distribution

Mitochondria are found in nearly all eukaryotes.[40] They vary in number and location according to cell type. A single mitochondrion is often found in unicellular organisms. Conversely, numerous mitochondria are found in human liver cells, with about 1000–2000 mitochondria per cell, making up 1/5 of the cell volume.[8] The mitochondrial content of otherwise similar cells can vary substantially in size and membrane potential,[41] with differences arising from sources including uneven partitioning at cell divisions, leading to extrinsic differences in ATP levels and

downstream cellular processes.[42] The mitochondria can be found nestled between myofibrils of muscle or wrapped around the sperm flagellum. Often, they form a complex 3D branching network inside the cell with the cytoskeleton. The association with the cytoskeleton determines mitochondrial shape, which can affect the function as well.[43] Mitochondria in cells are always distributed along microtubules and the distribution of these organelles is also correlated with the endoplasmic reticulum.[44] Recent evidence suggests that vimentin, one of the components of the cytoskeleton, is also critical to the association with the cytoskeleton.

Function

The most prominent roles of mitochondria are to produce the energy currency of the cell, ATP (i.e., phosphorylation of ADP), through respiration, and to regulate cellularmetabolism.[9] The central set of reactions involved in ATP production are collectively known as the citric acid cycle, or the Krebs cycle. However, the mitochondrion has many other functions in addition to the production of ATP.

Energy conversion

A dominant role for the mitochondria is the production of ATP, as reflected by the large number of proteins in the inner membrane for this task. This is done by oxidizing the major products of glucose: pyruvate, and NADH, which are produced in the cytosol.[9] This process of cellular respiration, also known as aerobic respiration, is dependent on the presence of oxygen. When oxygen is limited, the glycolytic products will be metabolized by anaerobic fermentation, a process that is independent of the mitochondria.[9] The production of ATP from glucose has an approximately 13-times higher yield during aerobic respiration compared to fermentation.[46] Recently it has been shown that plant mitochondria can produce a limited amount of ATP without oxygen by using the alternate substrate nitrite.[47] ATP crosses out through the inner membrane with the help of aspecific protein, and across the outer membrane via porins. ADP returns via the same route.

*Pyruvate and the citric acid cycle**[edit]***

Main articles: Pyruvate dehydrogenase, Pyruvate carboxylase and Citric acid cycle

Pyruvate molecules produced by glycolysis are actively transported across the inner mitochondrial membrane, and into the matrix where they can either be oxidized and combined with coenzyme A to form CO_2, acetyl-CoA and NADH, or they can be carboxylated (by pyruvate carboxylase) to form oxaloacetate. This latter reaction" fills up" the amount of oxaloacetate in the citric acid cycle, and is therefore an anaplerotic reaction, increasing the cycle's capacity to metabolize acetyl-CoA when the tissue's energy needs (e.g. in muscle) are suddenly increased by activity.[48]

In the citric acid cycle, all the intermediates (e.g. citrate, iso-citrate, alpha-ketoglutarate, succinate, fumarate, malate and oxaloacetate) are regenerated during each turn of the cycle. Adding more of any of these intermediates to the mitochondrion therefore means that the additional amount is retained within the cycle, increasing all the other intermediates as one is converted into the other. Hence, the addition of any one of them to the cycle has an anaplerotic effect, and its removal has a cataplerotic effect. These anaplerotic and cataplerotic reactions will, during the course of the cycle, increase or decrease the amount of oxaloacetate available to combine with acetyl-CoA to form citric acid. This in turn increases or decreases the rate of ATP production by the mitochondrion, and thus the availability of ATP to the cell.[48]

Acetyl-CoA, on the other hand, derived from pyruvate oxidation, or from the beta- oxidation of fatty acids, is the only fuel to enter the citric acid cycle. With each turn of the cycle one molecule of acetyl-CoA is consumed for every molecule of oxaloacetate present in the mitochondrial matrix, and is never regenerated. It is the oxidation of the acetate portion of acetyl-CoA that produces CO_2 and water, with the energy thus released captured in the form of ATP

In the liver, the carboxylation of cytosolic pyruvate into intra-mitochondrial oxaloacetate is an early step in the gluconeogenic pathway, which converts lactate and de-aminatedalanine into glucose,[9][48] under the influence of high levels of glucagon and/or epinephrine in the blood.[48] Here, the addition of oxaloacetate to the mitochondrion does not have a net anaplerotic effect, as another citric acid cycle intermediate (malate) is immediately removed from the mitochondrion to be converted into cytosolic oxaloacetate, which is ultimately converted into glucose, in a process that is almost the reverse of glycolysis.

The enzymes of the citric acid cycle are located in the mitochondrial matrix, with the exception of succinate dehydrogenase, which is bound to the inner mitochondrial membrane as part of Complex II. The citric acid cycle oxidizes the acetyl-CoA to carbon dioxide, and, in the process, produces reduced cofactors (three molecules of NADH and one molecule of $FADH_2$) that are a source of electrons for the *electron transport chain*, and a molecule of GTP (that is readily converted to an ATP).[9]

NADH and $FADH_2$: the electron transport chain

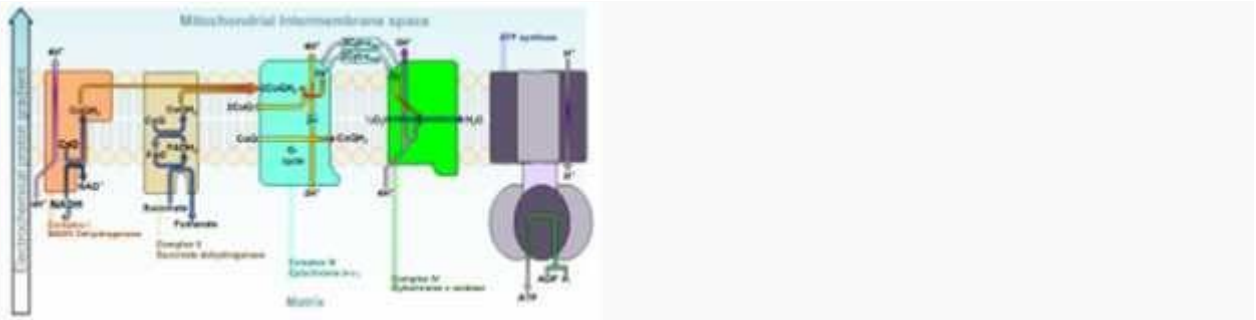


Diagram of the electron transport chain in the mitonchondrialintermembrane space

The redox energy from NADH and $FADH_2$ is transferred to oxygen (O_2) in several steps via the electron transport chain. These energy-rich molecules are produced within the matrix via the citric acid cycle but are also produced in the cytoplasm byglycolysis. Reducing equivalents from the cytoplasm can be imported via the malate-aspartate shuttle system of antiporterproteins or feed into the electron transport chain using a glycerol phosphate shuttle. Protein complexes in the inner membrane (NADH dehydrogenase (ubiquinone), cytochrome c reductase, and cytochrome c oxidase) perform the transfer and the incremental release of energy is used to pump protons (H^+) into the intermembrane space. This process is efficient, but a small percentage of electrons may prematurely reduce oxygen, forming reactive oxygen species such as superoxide. This can cause oxidative stress in the mitochondria and may contribute to the decline in mitochondrial function associated with the aging process.

As the proton concentration increases in the intermembrane space, a strong electrochemical gradient is established across the inner membrane. The protons can return to the matrix through the ATP synthase complex, and their potential energy is used to synthesize ATP from ADP and inorganic phosphate (P_i). This process is called chemiosmosis, and was first described by Peter Mitchell who was awarded the 1978 Nobel Prize in Chemistry for his work. Later, part of the 1997 Nobel Prize in Chemistry was awarded to Paul D. Boyer and John E. Walker for their clarification of the working mechanism of ATP synthase.

Heat production

Under certain conditions, protons can re-enter the mitochondrial matrix without contributing to ATP synthesis. This process is known as *proton leak* or *mitochondrial uncoupling*and is due to the facilitated diffusion of protons into the matrix. The process results in the unharnessed potential energy of the proton electrochemical gradient being released as heat.[9] The process is mediated by a proton channel called thermogenin, or UCP1. Thermogenin is a 33 kDa protein first discovered in 1973. Thermogenin is primarily found in brown adipose tissue, or brown fat, and is responsible for non-shivering thermogenesis. Brown adipose tissue is found in mammals, and is at its highest levels in early life and in hibernating animals. In humans, brown adipose tissue is present at birth and decreases with age

Storage of calcium ions

The concentrations of free calcium in the cell can regulate an array of reactions and is important for signal transduction in the cell. Mitochondria can transiently store calcium, a contributing process for the cell's homeostasis of calcium. In fact, their ability to rapidly take in calcium for later release makes them very good "cytosolic buffers" for calcium. The endoplasmic reticulum (ER) is the most significant storage site of calcium, and there is a significant interplay between the mitochondrion and ER with regard to calcium. The calcium is taken up into the matrix by a calcium

uniporter on the inner mitochondrial membrane. It is primarily driven by the mitochondrial membrane potential. Release of this calcium back into the cell's interior can occur via a sodium-calcium exchange protein or via "calcium-induced-calcium-release" pathways. This can initiate calcium spikes or calcium waves with large changes in the membrane potential. These can activate a series of second messenger system proteins that can coordinate processes such as neurotransmitter release in nerve cells and release of hormones in endocrine cells.

Ca^{2+} influx to the mitochondrial matrix has recently been implicated as a mechanism to regulate respiratorybioenergetics by allowing the electrochemical potential across the membrane to transiently "pulse" from ΔΨ-dominated to pH-dominated, facilitating a reduction of oxidative stress.[62] In neurons, concominant increases in cytosolic and mitochondrial calcium act to synchronize neuronal activity with mitochondrial energy metabolism. Mitochondrial matrix calcium levels can reach the tens of micromolar levels, which is necessary for the activation of isocitrate dehydrogenase, one of the key regulatory enzymes of the Kreb's cycle.

Additional functions

Mitochondria play a central role in many other metabolic tasks, such as:

- Signaling through mitochondrial reactive oxygen species
- Regulation of the membrane potential
- Apoptosis-programmed cell death
- Calcium signaling (including calcium-evoked apoptosis
- Regulation of cellular metabolism
- Certain heme synthesis reactions
- Steroid synthesis.
- Hormonal signaling Mitochondria are sensitive and responsive to hormones, in part by the action of mitochondrial estrogen receptors (mtERs). These receptors have been found in various tissues and cell types, including brain and heart

Some mitochondrial functions are performed only in specific types of cells. For example, mitochondria in liver cells contain enzymes that allow them to detoxify ammonia, a waste product of protein metabolism. A mutation in the genes regulating any of these functions can result in mitochondrial diseases.

Endoplasmic reticulum:

Endoplasmic reticulum is a network of interconnected internal membranes generally, the largest membrane in a eukaryotic cell—an extensive network of closed, flattened membrane-bounded sacs called cisternae(Figure 3). The name “endoplasmic reticulum” was coined in 1953 by Porter, who had observed it in electron micrographs of liver cells. The endoplasmic reticulum has a number of functions in the cell but is particularly important in the synthesis of lipids, membrane proteins, and secreted proteins.

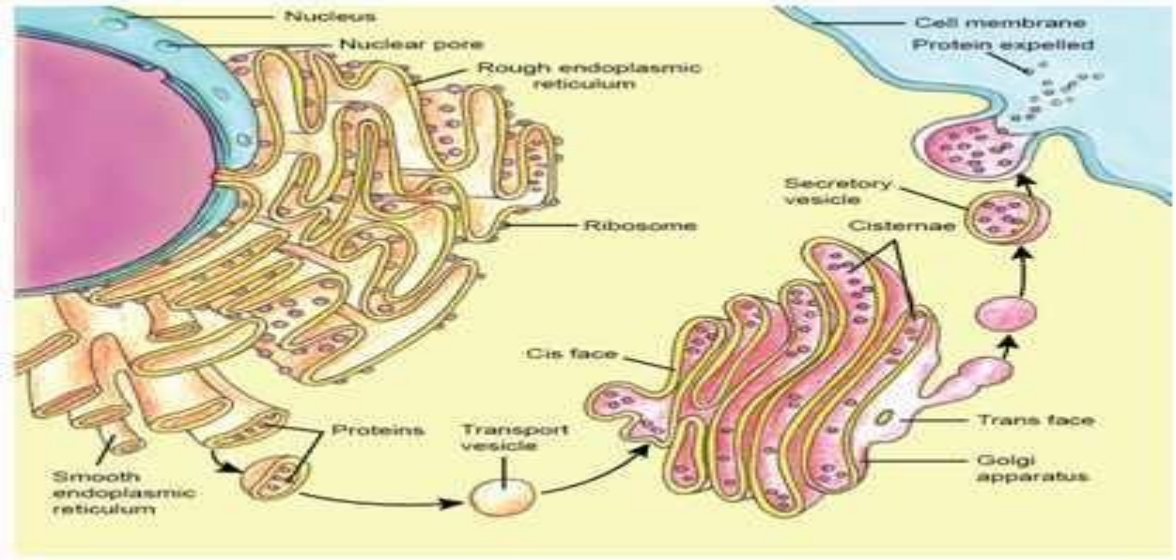


Figure 3: The Endoplasmic reticulum.

Occurrence:

The occurrence of the endoplasmic reticulum is in eukaryotic cells with variation in its position from cell to cell. The erythrocytes (RBC), egg and embryonic cells lack in endoplasmic reticulum. ER is poorly developed in certain cells as the RBC which produces only proteins to be retained in the cytoplasmic matrix (haemoglobin), although the cell may contain many ribosomes). The spermatocytes also have poorly developed endoplasmic reticulum.

Morphology:

The endoplasmic reticulum occurs in three forms:

1. Lamellar form or cisternae which is a closed, fluid-filled sac, vesicle or cavity is called cisternae;
2. Vesicular form or vesicle and
3.Tubularform

1. Cisternae:

The cisternae are long, flattened, sac-like, unbranched tubules having diameter of 40 to 50 µm. They remain arranged parallely in bundles or stakes. RER mostly exists as cisternae which occur in those cells which have synthetic roles as the cells of pancreas, notochord and brain.

2. Vesicles:

The vesicles are oval, membrane-bound vacuolar structures having diameter of 25 to 500 µm. They often remain isolated in the cytoplasm and occur in most cells but especially abundantintheSER.

3. Tubules: The tubules are branched structures forming the reticular system along with the cisternae and vesicles. They usually have the diameter from 50 to 190 µm and occur almost in all the cells. Tubular form of ER is often found in SER and is dynamic in nature, *i.e.*, it is associated with membrane movements, fission and fusion between membranes of cytocavity network.

Ultrastructure:

The cavities of cisternae, vesicles and tubules of the endoplasmic reticulum are bounded by a thin membrane of 50 to 60 Aº thickness. The membrane of endoplasmic reticulum is fluid- mosaic like the unit membrane of the plasma membrane, nucleus, Golgi apparatus. The membrane of

endoplasmic reticulum remains continuous with the membranes of plasma membrane, nuclear membrane and Golgi apparatus. The cavity of the endoplasmic reticulum is well developed and acts as a passage for the secretory products. Palade in the year 1956 has observed secretory granules in the cavity of endoplasmic reticulum amking it rough in appearance. Sometimes, the cavity of RER is very narrow with two membranes closely apposed and is much distended in certain cells which are actively engaged in protein synthesis (acinar cells, plasma cells and goblet cells). The membranes of the endoplasmic reticulum contains many kinds of enzymes which are needed for various important synthetic activities. Some of the most common enzymes are found to have different transverse distribution in the ER membranes. The most important enzymes are the stearases, NADH-cytochrome C reductase, NADH diaphorase, glucose-6-phosphotase and Mg^{++} activated ATPase. Certain enzymes of the endoplasmic reticulum such as nucleotide diphosphate are involved in the biosynthesis of phospholipid, ascorbic acid, glucuronide, steroids and hexose metabolism.

Types of endoplasmic reticulum:

Agranular or smooth endoplasmic reticulum:

ER with no studded ribosomes makes it smooth in appearance. The adipose tissues, brown fat cells and adrenocortical cells, interstitial cells of testes and cells of corpus luteum of ovaries, sebaceous cells and retinal pigment cells contain only smooth endoplasmic reticulum (SER). The synthesis of fatty acids and phospholipids takes place in the smooth ER. It is abundant in hepatocytes. Enzymes in the smooth ER of the liver modify or detoxify hydrophobic chemicals such as pesticides and carcinogens by chemically converting them into more water-soluble, conjugated products that can be excreted from the body. High doses of such compounds result in a large proliferation of the smooth ER in liver cells.

Granular or rough endoplasmic reticulum:

Ribosomesbound to the endoplasmic reticulum make it appear rough. The rough ER synthesizes certain membrane and organelle proteins and virtually all proteins to be secreted from the cell. A ribosome that fabricates such a protein is bound to the rough ER by the nascent polypeptide chain of the protein. As the growing polypeptide emerges from the ribosome, it passes through the rough ER membrane, with the help of specific proteins in the membrane. Newly made membrane proteins remain associated with the rough ER membrane, and proteins to be secreted accumulate in the lumen of the organelle. All eukaryotic cells contain a discernible amount of rough ER because it is needed for the synthesis of plasma membrane proteins and proteins of the extracellular matrix. Rough ER is particularly abundant in specialized cells that produce an abundance of specific proteins to be secreted. The cells of those organs which are actively engaged in the synthesis of proteins such as acinar cells of pancreas, plasma cells, goblet cells and cells of some endocrine glands are found to contain rough endoplasmic reticulum (RER) which is highly developed.

Rough endoplasmic reticulum and protein secretion:

George Palade and his colleagues in the 1960s were the first to demonstrate the role of endoplasmic reticulum in protein secretion. The defined pathway taken by secreted protein is: Rough ER - Golgi - secretory vesicles- cell exterior. The entrance of proteins into the ER represents a major branch point for the traffic of proteins within eukaryotic cells. In mammalian cells most proteins are transferred into the ER while they are being translated on membrane bound ribosomes. Proteins that are destined for secretion are then targeted to the endoplasmic reticulum by a signal sequence (short stretch of hydrophobic amino acid residues) at the amino terminus of the growing polypeptide chain. The signal sequence is K/HDEL which is Lys/His- Asp-Glu-Leu. This signal peptide is recognized by a signal recognition particle consisting of six polypeptides and srpRNA.

The SRP binds the ribosome as well as the signal sequence, inhibiting further translation and targeting the entire complex (the SRP, ribosome, and growing polypeptide chain) to the rough ER by binding to the SRP receptor on the ER membrane. Binding to the receptor releases the SRP from both the ribosome and the signal sequence of the growing polypeptide chain. The ribosome then binds to a protein translocation complex in the ER membrane, and the signal sequence is inserted into a membrane channel or translocon with the aid of GTP. Transfer of the ribosome mRNA complex from the SRP to the translocon opens the gate on the translocon and allows translation to resume, and the growing polypeptide chain is transferred directly into the translocon channel and across the ER membrane as translation proceeds. As translocation proceeds, the signal sequence is cleaved by signal peptidase and the polypeptide is released into the lumen of the ER.

Smooth endoplasmic reticulum and lipid synthesis:

Phospholipids are synthesized in the cytosolic side of the ER membrane from water-soluble cytosolic precursors. Other lipids that are synthesized in the ER are cholesterol and ceramide which is further converted to either glycolipids or sphingomyelin in the golgi apparatus. Smooth ER are also the site for the synthesis of the steroid hormones from cholesterol. Thus steroid producing cells in the testis and ovaries are abundant in smooth ER.

Common functions of SER and RER:

1. The endoplasmic reticulum provides an ultrastructural skeletal framework to the cell and gives mechanical support to the colloidal cytoplasmic martix.
2. The exchange of molecules by the process of osmosis, diffusion and active transport occurs through the membranes of endoplasmic reticulum. The ER membrane has permeases and carriers.
3. The endoplasmic membranes contain many enzymes which perform various synthetic and metabolic activities and provides increased surface for various enzymatic reactions.
4. The endoplasmic reticulum acts as an intracellular circulatory or transporting system. Various secretory products of granular endoplasmic reticulum are transported to various organelles as follows: Granular ER- agranular ER - Golgi membrane-lysosomes, transport vesicles or secretory granules. Membrane flow may also be an important mechanism for carrying particles, molecules and ions into and out of the cells. Export of RNA and nucleoproteins from nucleus to cytoplasm may also occur by this type of flow.
5. The ER membranes are found to conduct intra-cellular impulses. For example, the sarcoplasmic reticulum transmits impulses from the surface membrane into the deep region of the muscle fibres.
6. The sarcoplasmic reticulum plays a role in releasing calcium when the muscle is stimulated and actively transporting calcium back into the sarcoplasmic reticulum when the stimulation stops and the muscle must be relaxed.

The Golgi Complex: Processes and Sorts Secreted and Membrane Proteins

The golgi complex was discovered by Camillo Golgi during an investigation of the nervous system and he named it the “internal reticular apparatus”.Functionally it is also known as the post office of the cell.Certain important cellular functions such as biosynthesis of polysaccharides, packaging (compartmentalizing) of cellular synthetic products (proteins), production of

exocytotic (secretory) vesicles and differentiation of cellular membranes, occurs in the Golgi complex or Golgi apparatus located in the cytoplasm of animal and plant cells.

Occurrence:

The Golgi apparatus occurs in all eukaryotic cells. The exceptions are the prokaryotic cells (mycoplasmas, bacteria and blue green algae) and eukaryotic cells of certain fungi, sperm cells of bryophytes and pteridiophytes, cells of mature sieve tubes of plants and mature sperm and red blood cells of animals. Their number per plant cell can vary from several hundred as in tissues of corn root and algal rhizoids (*i.e.*, more than 25,000 in algal rhizoids, Sievers,1965), to a single organelle in some algae. In higher plants, Golgi apparatuses are particularly common in secretory cells and in young rapidly growing cells. In animal cells, there usually occurs a single Golgi apparatus, however, its number may vary from animal to animal and from cell to cell. *Paramoeba* species has two golgi apparatuses and nerve cells, liver cells and chordate oocytes have multiple golgi apparatuses, there being about 50 of them in the liver cells.

Morphology

The Golgi apparatus is morphologically very similar in both plant and animal cells. However, it is extremely pleomorphic: in some cell types it appears compact and limited, in others spread out and reticular (net-like). Its shape and form may vary depending on cell type. It appears as a complex array of interconnecting tubules, vesicles and cisternae. There has been much debate concerning the terminology of the Golgi's parts. The simplest unit of the Golgi apparatus is the cisterna. This is a membrane bound space in which various materials and secretions may accumulate. Numerous cisternae are associated with each other and appear in a stack-like (lamellar) aggregation. A group of these cisternae is called the dictyosome, and a group of dictyosomes makes up the cell's Golgi apparatus. All dictyosomes of a cell have a common function. The detailed structure of three basic components of the Golgi apparatus are as follows:

1. Flattened Sac or Cisternae

Cisternae of the golgi apparatus are about 1 μm in diameter, flattened, plate-like or saucer-like closed compartments which are held in parallel bundles or stacks one above the other. In each stack, cisternae are separated by a space of 20 to 30 nm which may contain rod- like elements or fibres. Each stack of cisternae forms a dictyosome which may contain 5 to 6 Golgi cisternae in animal cells or 20 or more cisternae in plant cells. Each cisterna is bounded by a smooth unit membrane (7.5 nm thick), having a lumen varying in width from about 500 to 1000 nm. Polarity. The margins of each cisterna are gently curved so that the entire dictyosome of Golgi apparatus takes on a bow-like appearance. The cisternae at the convex end of the dictyosome comprise proximal, forming or cis-face and the cisternae at the concave end of the dictyosome comprise the distal, maturing or trans-face. The forming or cis face of Golgi is located next to either the nucleus or a specialized portion of rough ER that lacks bound ribosomes and is called "transitional" ER. Trans face of Golgi is located near the plasma membrane. This polarization is called cis-trans axis of the Golgi apparatus.

2. Tubules

A complex array of associated vesicles and tubules (30 to 50 nm diameter) surround the

dictyosome and radiate from it. The peripheral area of dictyosome is fenestrated or lace-like in structure.

3. Vesicles

The vesicles are 60 nm in diameter and are of three types:

(i) Transitional vesicles are small membrane limited vesicles which are form as blebs from the transitional ER to migrate and converge to cis face of Golgi, where they coalasce to form new cisternae.

(ii) Secretory vesicles are varied-sized membrane-limited vesicles which discharge from margins of cisternae of Golgi. They, often, occur between the maturing face of Golgi and the plasma membrane.

(iii) Clathrin-coated vesicles are spherical protuberances, about 50 μm in diameter and with a rough surface. They are found at the periphery of the organelle, usually at the ends of single tubules, and are morphologically quite distinct from the secretory vesicles. The clathrin-coated vesicles are known to play a role in intra- cellular traffic of membranes and of secretory products.

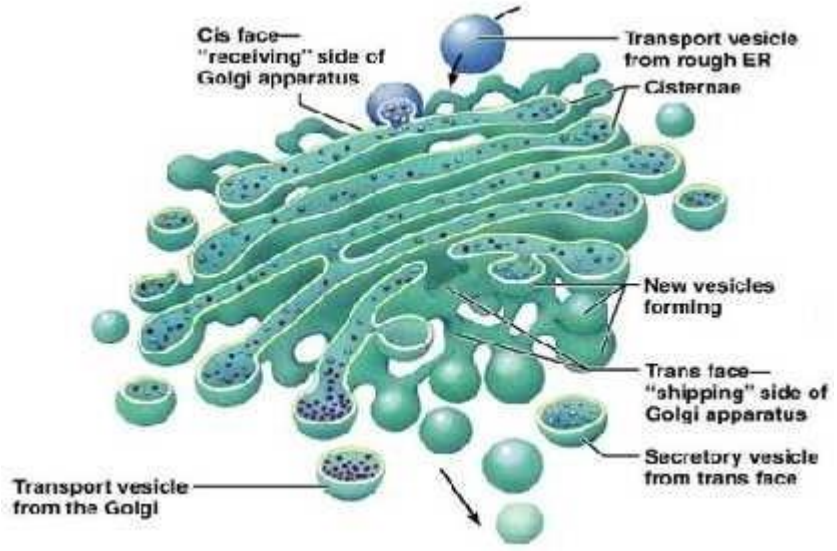


Figure : The Golgi complex.

Functions:

1. Modifying, sorting, and packaging of macromolecules for cell secretion: The golgi complex is involved in the transport of lipids around the cell, and the creation of lysosomes.Proteins are modifiedby enzymes in cisternae by glycosylation and phosphorylation by identifying the signal sequence of the protein in question. For example, the Golgi apparatus adds a mannose-6-phosphate label to proteins destined for lysosomes. One molecule that is phosphorylated in the Golgi is Apolipoprotein, which forms a molecule known as VLDL that is a constituent of blood serum. The phosphorylation of these molecules is important to help aid in their sorting for secretion into the blood serum.

2. Proteoglycans and carbohydrate synthesis: This include the production of glycosaminoglycans (GAGs), long unbranched polysaccharides which the Golgi then attaches to a protein synthesised in the endoplasmic reticulum to form proteoglycans.

3. Golgi Functions in Animals:

In animals, Golgi apparatus is involved in the packaging and exocytosis of the following: Zymogen of exocrine pancreatic cells; Mucus (a glycoprotein) secretion by goblet cells of intestine; Lactoprotein (casein) secretion by mammary gland cells (Merocrine secretion); Secretion of compounds (thyroglobulins) of thyroxine hormone by thyroid cells; Secretion of tropocollagen and collagen; Formation of melanin granules and other pigments; and Formation of yolk and vitelline membrane of growing primary oocytes. It is also involved in the formation of certain cellular organelles such as plasma membrane, lysosomes, acrosome of spermatozoa and cortical granules of a variety of oocytes.

4. Golgi Functions in Plants:

In plants, Golgi apparatus is mainly involved in the secretion of materials of primary and secondary cell walls (formation and export of glycoproteins, lipids, pectins and monomers for hemicellulose, cellulose, lignin). During cytokinesis of mitosis or meiosis, the vesicles originating from the periphery of Golgi apparatus, coalesce in the phragmoplast area to form a semisolid layer, called cell plate. The unit membrane of Golgi vesicles fuses during cell plate formation and becomes part of plasma membrane of daughter

Ribosome

Ribosomes are the protein synthesis units of a cell described by G.E. Palade in 1952. They are complex of ribosomal RNA and various proteins. Their presence in both free and endoplasmis reticulum membrane attached form (rough endoplasmic reticulum) was confirmed by Palade and Siekevitz by the electron microscopy. We will have discussion about endoplasmic reticulum in this lecture after discussion about ribosome. Ribosomes are small, dense, rounded and granular particles of the ribonucleoprotein. As mentioned, they occur either freely in the matrix of mitochondria, chloroplast and cytoplasm or remain attached with the membranes of the endoplasmic reticulum. They occur in most prokaryotic and eukaryotic cells and provide a scaffold for the ordered interaction of all the molecules involved in protein synthesis. They are the most abundant RNA-protein complex in the cell, which directs elongation of a polypeptide at a rate of three to five amino acids added per second. Small proteins of 100–200 amino acids are therefore made in a minute or less. On the other hand, it takes 2–3 hours to make the largest known protein, titin, which is found in muscle and contains about 30,000 amino acid residues.

Occurrence and distribution:

The ribosomes occur in both prokaryotic and eukaryotic cells. In prokaryotic cells the ribosomes often occur freely in the cytoplasm or sometimes as polyribosome. In eukaryotic cells the ribosomes either occur freely in the cytoplasm or remain attached to the outer surface of the membrane of endoplasmic reticulum. The yeast cells, reticulocytes or lymphocytes, meristamatic plant tissues, embryonic nerve cells and cancerous cells contain large number of ribosomes which

often occur freely in the cytoplasmic matrix. Cells like the erythroblasts, developing muscle cells, skin and hair which synthesize specific proteins for the intracellular utilization and storage contain also contain large number of free ribosomes. In cells with active protein synthesis, the ribosomes remain attached with the membranes of the endoplasmic reticulum. Examples are the pancreatic cells, plasma cells, hepatic parenchymal cells, Nissls bodies, osteoblasts, serous cells, or the submaxillary gland, thyroid cells and mammary gland cells.

Types of ribosomes:

Ribosomes are classified into two types based on their sedimentation coefficient, 70S and 80S. S stands for Svedberg unit and related to sedimentation rate (sedimentation depends on mass and size). Thus, the value before S indicates size of ribosome.

70S Ribosomes: Prokaryotes have 70S ribosemes. The 70S ribosomes are comparatively smaller in size and have sedimentation coefficient 70S with molecular weight 2.7×10^6daltons. Electron microscopy measures the dimension of the 70S ribosomes as$170 \times 170 \times 200$ A^o. They occur in the prokaryotic cells of the blue green algae and bacteria and also in mitochondria and chloroplasts of eukaryotic cells.

80S Ribosomes: Eukaryotes have 80S ribosomes. The 80S ribosomes have sedimentation coefficient of 80S and molecular weight 40×10^6daltons. The 80S ribosomes occur in eukaryotic cells of the plants and animals. The ribosomes of mitochondria and chloroplasts are always smaller than 80S cytoplasmic ribosomes and are comparable to prokaryotic ribosomes in both size and sensitivity to antibiotics. However their sedimentation values vary in different phyla, 77S in mitochondria of fungi, 60S in mitochondria of mammals and 60S in mitochondria of animals.

Number of ribosomes:

An *E. coli* cell contains 10,000 ribosomes, forming 25 per cent of the total mass of the bacterial cell. Whereas, mammalian cultured cells contain 10 million ribosomes per cell.

Chemical composition:

The ribosomes are chemically composed of RNA and proteins as their major constituents; both occurring approximately in equal proportions in smaller as well as larger subunit. The 70S ribosomes contain more RNA (60 to 40%) than the proteins (36 to 37%). The ribosomes of *E. coli* contain 63% rRNA and 37% protein. While the 80S ribosomes contain less RNA (40 to 44%) than the proteins (60 to 56%), yeast ribosomes have 40 to 44% RNA and 60 to 56% proteins; ribosomes of pea seedling contain 40% RNA and 60% proteins. There is no lipid content in ribosomes.

Ribosomal RNAs:

RNA constitutes about 60 percent of the mass of a ribosome. The 70S ribosomes contain three types of rRNA, *viz.*, 23S rRNA, 16S rRNA, 5S rRNA. The 23S and 5S rRNA occur in the larger 50S ribosomal subunit, while the 16S rRNA occurs in the smaller 30S ribosomal subunit. Assuming an average molecular weight for one nucleotide to be 330 daltons, one can calculate the total number of each type of rRNA. Thus, the 23S rRNA consists of 3300 nucleotides, 16S rRNA contains 1650 nucleotides and 5S rRNA includes 120 nucleotides in it. The 80S ribosomes contain four types of rRNA, 28S rRNA (or 25-26 rRNA in plants, fungi and protozoa), 18S rRNA, 5S rRNA and 5.8S rRNA. The 28S, 5S and 5.8S rRNAs occur in the larger 60S ribosomal subnit, while the 18S rRNA occurs in the smaller 40S ribosomal subunit. About 60 per cent of the rRNA

is helical (*i.e*., double stranded) and contains paired bases. These double stranded regions are due to hairpin loops between complimentary regions of the linear molecule.

The 28S rRNA has the molecular weight 1.6×10^6daltons and its molecule is double stranded and having nitrogen bases in pairs. The 18S rRNA has the molecular weight $0.6x10^6$daltons and consists of 2100 nucleotides. The 18S and 28S ribosomal RNA contain a characteristic number of methyl groups, mostly as 2'-O-methyl ribose. The molecule of 5S rRNA has a clover leaf shape and a length equal to 120 nucleotides. The 5.8S rRNA is intimately associated with the 28S rRNA molecule and has, therefore, been referred to as 28S-associated ribosomal RNA (28S- ArRNA). The 55S ribosomes of mammalian mitochondria lack 5S rRNA but contain 21S and 12S rRNAs. The 21S rRNA occurs in larger or 35S ribosomal subunits, while 12S rRNAoccur in smaller or 25S ribosomal subunit. It is thought that each ribosomal subunit contains a highly folded ribonucleic acid filament to which the various proteins adhere. But as the ribosomes easily bind the basic dyes so it is concluded that RNA is exposed at the surface of the ribosomal subunits, and the protein is assumed to be in the interior in relation to non-helical part of the RNA.

Ribosomal Proteins:

A ribosome is composed of three (in bacteria) or four (in eukaryotes) different rRNA molecules and as many as 83 proteins, organized into a large subunit and a small subunit. The primary structure of several of these proteins has been elucidated. Most of the recent knowledge about the structure of ribosomal proteins has been achieved by dissociation of ribosomal subunits into their component rRNA and protein molecules. When both 50S and 30S ribosomal subunits are dissociated by centrifuging both of them in a gradient of 5 M cesium chloride, then there are two inactive core particles (40S and 23S, respectively) which contain the RNA and some proteins called core proteins (CP) at the same time several other proteins—the so-called split proteins (SP) are released from each particle.There are SP50 and SP30 proteins which may reconstitute the functional ribosomal subunit when added to their corresponding core. Some of the split proteins are apparently specific for each ribosomal subunit. The split proteins have been further fractionated and divided into acidic (A) and basic (B) proteins. According to Nomura (1968, 1973) and Garett and Wittmann (1973) each 70S ribosome of *E. coli* is composed of about 55 ribosomal proteins. Out of these 55 proteins, about 21 different molecules have been isolated from the 30S ribosomal subunit, and some 32 to 34 proteins from the 50S ribosomal subunit. Similar organization of ribosomal proteins and RNA is found in 80S Ribosomes. Different rRNA molecules evidently play a central role in the catalytic activities of ribosomes in the process of protein synthesis.

Structure

The ribosomes are oblate spheroid structures of 150 to 250 A^o in diameter. Each ribosome is porous, hydrated and composed of two subunits. One ribosomal subunit is large in size and has a domelike shape, while the other ribosomal subunit is smaller in size, occurring above the larger subunit and forming a cap-like structure. The small ribosomal subunit contains a single rRNA molecule, referred to as small *rRNA*. The large subunit contains a molecule of large *rRNA* and one molecule of 5S rRNA, plus an additional molecule of 5.8S rRNA in vertebrates. The lengths of the rRNA molecules, the quantity of proteins in each subunit, and consequently the sizes of the subunits differ in bacterial and eukaryotic cells. The assembled ribosome is 70S in bacteria and

80S in vertebrates. There are great structural and functional similarities between ribosomes from all species which is another reflection of the common evolutionary origin of the most basic constituents of living cells.

The 70S ribosome consists of two subunits, 50S and 30S. The 50S ribosomal subunit is larger in size and has the size of 160 A^o to 180 A^o. The 30S ribosomal subunit is smaller in size and occurs above the 50S subunit like a cap. The 80S ribosome also consists of two subunits, 60S and 40S. The 60S ribosomal subunit is dome-shaped and larger in size. In the ribosomes which remain attached with the membranes of endoplasmic reticulum and nucleus, the 60S subunit remains attached with the membranes. The 40S ribosomal subunit is smaller in size and occurs above the 60s subunit forming a cap-like structure. Both the subunits remain separated by a narrow cleft. The two ribosomal subunits remain united with each other due to high concentration of the Mg^{++}(.001M) ions.

The actual three-dimensional structures of bacterial rRNAs from *Thermusthermopolis* recently have been determined by x-ray crystallography of the 70S ribosome. The multiple, much smaller ribosomal proteins for the most part are associated with the surface of the rRNAs. During translation, a ribosome moves along an mRNA chain, interacting with various protein factors and tRNAs and very likely undergoing large conformational changes. Despite the complexity of the ribosome, great progress has been made in determining the overall structure of bacterial ribosomes and in identifying various reactive sites. X-ray crystallographic studies on the *T. thermophilus* 70S ribosome, for instance, not only have revealed the dimensions and overall shape of the ribosomal subunits but also have localized the positions of tRNAs bound to the ribosome during elongation of a growing protein chain. In addition, powerful chemical techniques such as footprinting, have been used to identify specific nucleotide sequences in rRNAs that bind to protein or another RNA. Figure 1 illustrates the ribosomes.

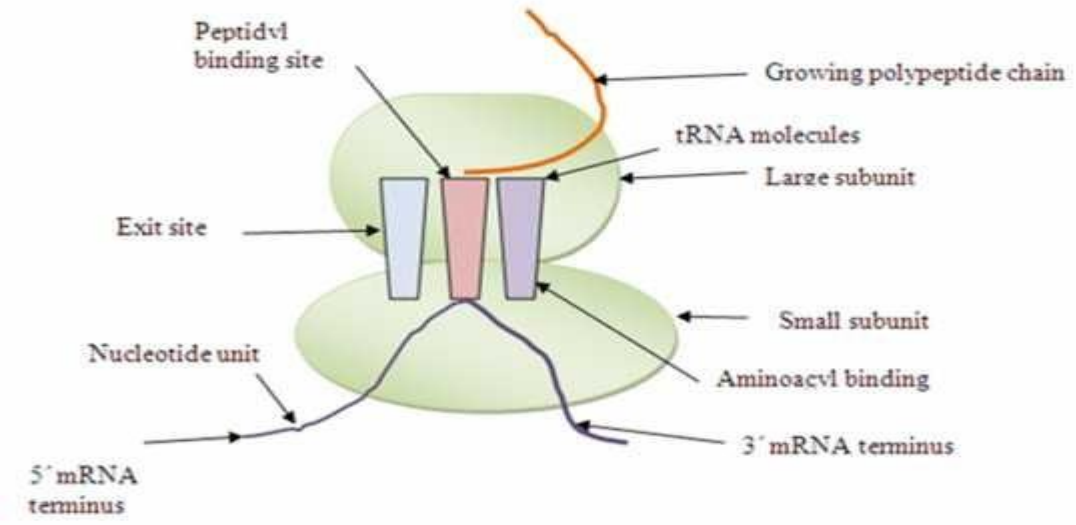


Figure : The detailed structure of a ribosome involved in protein synthesis.

Golgi bodies,

The **Golgi apparatus** (/ˈgoʊldʒiː/), also known as the **Golgi complex**, **Golgi body**, or simply the **Golgi**, is an organelle found in most eukaryotic cells.[1] It was identified in 1897 by the Italian physician Camillo Golgi and named after him in 1898.[2]

Part of the cellular endomembrane system, the Golgi apparatus packages proteins into membrane-bound vesicles inside the cell before the vesicles are sent to their destination. The Golgi apparatus resides at the intersection of the secretory, lysosomal, and endocytic pathways. It is of particular importance in processing proteins for secretion, containing a set of glycosylationenzymes that attach various sugar monomers to proteins as the proteins move through the apparatus.

Discovery

Owing to its large size and distinctive structure, the Golgi apparatus was one of the first organelles to be discovered and observed in detail. It was discovered in 1898 by Italian physician Camillo Golgi during an investigation of the nervous system.[2] After first observing it under his microscope, he termed the structure the *internal reticular apparatus*. Some doubted the discovery at first, arguing that the appearance of the structure was merely an optical illusion created by the observation technique used by Golgi. With the development of modern microscopes in the 20th century, the discovery was confirmed.[3] Early references to the Golgi referred to it by various names including the "Golgi–Holmgren apparatus", "Golgi–Holmgren ducts", and "Golgi–Kopsch apparatus".[2] The term "Golgi apparatus" was used in 1910 and first appeared in scientific literature in 1913.[2]

Subcellular localization

Among eukaryotes, the subcellular localization of the Golgi apparatus differs. In mammals, a single Golgi apparatus complex is usually located near the cell nucleus, close to the centrosome. Tubular connections are responsible for linking the stacks together. Localization and tubular connections of the Golgi apparatus are dependent on microtubules. If microtubules are experimentally depolymerized, then the Golgi apparatus loses connections and becomes individual stacks throughout the cytoplasm.[4] In yeast, multiple Golgi apparatuses are scattered throughout the cytoplasm (as observed in *Saccharomyces cerevisiae*). In plants, Golgi stacks are not concentrated at the centrosomal region and do not form Golgi ribbons.[5] Organization of the plant Golgi depends on actin cables and not microtubules.[5] The common feature among Golgi is that they are adjacent to endoplasmic reticulum (ER) exit sites.[6]

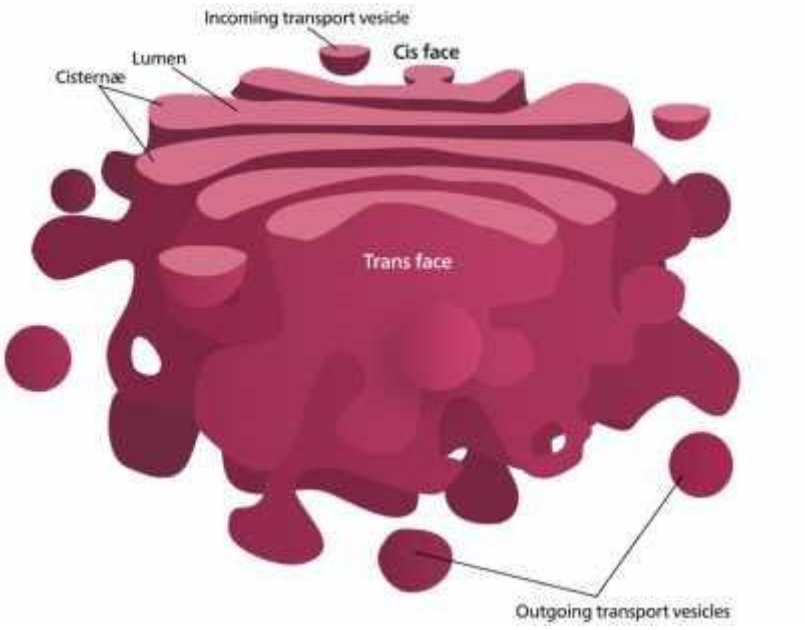
Structure:

In most eukaryotes, the Golgi apparatus is made up of a series of compartments consisting of two main networks: the *cis* Golgi network (CGN) and the *trans* Golgi network (TGN). The CGN is a collection of fused, flattened membrane-enclosed disks known as cisternae (singular: *cisterna*),

originating from vesicular clusters that bud off theendoplasmic reticulum. A mammalian cell typically contains 40 to 100 stacks.[7] Between four and eight cisternae are usually present in a stack; however, in some protists as many as sixty cisternae have been observed.[3] This collection of cisternae is broken down into *cis*, medial, and *trans* compartments. The TGN is the final cisternal structure, from which proteins are packaged into vesicles destined tolysosomes, secretory vesicles, or the cell surface. The TGN is usually positioned adjacent to the stacks of the Golgi apparatus, but can also be separate from the stacks. The TGN may act as an early endosome in yeast and plants.

The Golgi apparatus tends to be larger and more numerous in cells that synthesize and secrete large amounts of substances; for example, the antibody-secreting plasma B cells of the immune system have prominent Golgi complexes.

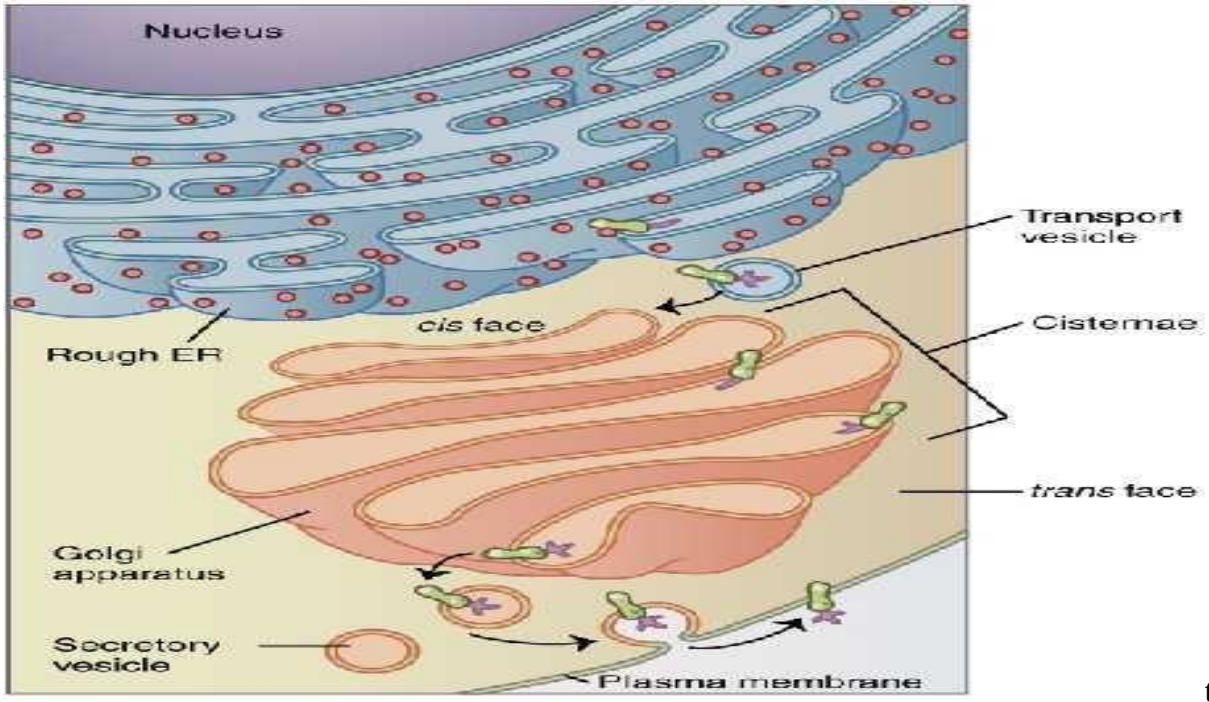
In all eukaryotes, each cisternal stack has a *cis* **entry face** and a *trans* **exit face**. These faces are characterized by unique morphology and biochemistry.[8] Within individual stacks are assortments of enzymes responsible for selectively modifying protein cargo. These modifications influence the fate of the protein. The compartmentalization of the Golgi apparatus is advantageous for separating enzymes, thereby maintaining consecutive and selective processing steps: enzymes catalyzing early modifications are gathered in the *cis* face cisternae, and enzymes catalyzing later modifications are found in *trans* face cisternae of the Golgi stacks.[4][8]



Functions:

The Golgi apparatus is a major collection and dispatch station of protein products received from the endoplasmic reticulum (ER). Proteins synthesized in the ER are packaged into vesicles, which then fuse with the Golgi apparatus. These cargo proteins are modified and destined for secretion

via exocytosis or for use in the cell. In this respect, the Golgi can be thought of as similar to a post office: it packages and labels items which it then sends to different parts of the cell or to the extracellular space. The Golgi apparatus is also involved in lipid transport and lysosome formation. The structure and function of the Golgi apparatus are intimately linked. Individual stacks have different assortments of enzymes, allowing for progressive processing of cargo proteins as they travel from the cis to the trans Golgi face.[4][8] Enzymatic reactions within the Golgi stacks occur exclusively near its membrane surfaces, where enzymes are anchored. This feature is in contrast to the ER, which has soluble proteins and enzymes in its lumen. Much of the enzymatic processing is post-translational modification of proteins. For example, phosphorylation of oligosaccharides on lysosomal proteins occurs in the early CGN. *Cis* cisternaare associated with the removal of mannose residues.[4][8] Removal of mannose residues and addition of N- acetylglucosamine occur in medial cisternae.[4] Addition of galactose and sialicacid occurs in the *trans*cisternae. Sulfation of tyrosines and carbohydrates occurs within the TGN.[4] Other general post-translational modifications of proteins include the addition of carbohydrates (glycosylation) and phosphates (phosphorylation). Protein modifications may form a signal sequence that determines the final destination of the protein. For example, the Golgi apparatus adds a mannose-6-phosphate label to proteins destined forlysosomes. Another important function of the Golgi apparatus is in the formation of proteoglycans. Enzymes in the Golgi append proteins to glycosaminoglycans, thus creating proteoglycans. Glycosaminoglycans are long unbranched polysaccharide molecules present in extracellular matrix of animals.



the

Nucleus

Nucleus means kernel and was the first organelle to be discovered. It was discovered and named by Robert Brown in 1833 in the plant cells and is recognized as a constant feature of all animal and plant cells. Certain eukaryotic cells such as the mature sieve tubes of higher plants and mammalian erythrocytes contain no nucleus. It is the largest cellular organelle in eukaryotes. Prokaryotic cells lack nucleus and is complemented by nucleoid. In mammalian cells, the average diameter of the nucleus is approximately 6 micrometers (μm), occupying about 10% of the total cell volume. The contents of the nucleus are DNA genome, RNA synthetic apparatus, and a fibrous matrix. It is surrounded by two membranes, each one a phospholipid bilayer containing many different types of proteins. The inner nuclear membrane defines the nucleus itself. In most cells, the outer nuclear membrane is continuous with the rough endoplasmic reticulum, and the space between the inner and outer nuclear membranes is continuous with the lumen of the rough endoplasmic reticulum. The two nuclear membranes appear to fuse at nuclear pores, the ringlike complexes composed of specific membrane proteins through which material moves between the nucleus and the cytosol. It contains cell's genetic material, organized as multiple long linear DNA molecules in complex with histones, to form chromosomes. The genes within these chromosomes are the cell's nuclear genome. The function is to maintain the integrity of the genes that controls the activities of the cell by regulating gene expression. The schematic presentation of nucleus is in Figure 1.

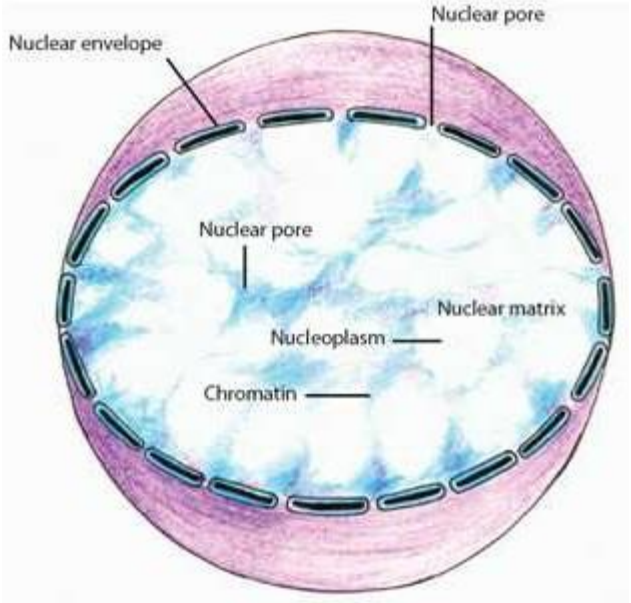


Figure 1: The schematic representation of nucleus.

In a growing or differentiating cell, the nucleus is metabolically active, replicating DNA and synthesizing rRNA, tRNA, and mRNA. Within the nucleus mRNA binds to specific proteins, forming ribonucleoprotein particles. Most of the cell's ribosomal RNA is synthesized in the nucleolus, a subcompartment of the nucleus that is not bounded by a phospholipid membrane. Some ribosomal proteins are added to ribosomal RNAs within the nucleolus as well. The finished or partly finished ribosomal subunits, as well as tRNAs and mRNA-containing particles, pass through a nuclear pore into the cytosol for use in protein synthesis. In a nucleus that is not dividing, the chromosomes are dispersed and not dense enough to be observed in the light microscope. Only during cell division are individual chromosomes visible by light microscopy. In the electron

microscope, the nonnucleolar regions of the nucleus, called the nucleoplasm,can be seen to have dark and light staining areas. The dark areas, which are often closely associated with the nuclear membrane, contain condensed concentrated DNA, called heterochromatin. Fibrous proteins called lamins form a two-dimensional network along the inner surface of the inner membrane, giving it shape and apparently binding DNA to it. The breakdown of this network occurs early in cell division.

Cell Nucleus: Ultrastructure

The structure of a cell nucleus consists of a nuclear membrane (nuclear envelope), nucleoplasm, nucleolus, and chromosomes. Nucleoplasm, also known as karyoplasm, is the matrix present inside the nucleus. Following section discusses in brief about the several parts of a cell nucleus.

a. Nuclear Membrane

It is a double-membrane structure each 5–10 nm thick . Numerous pores occur in the envelope, allowing RNA and other chemicals to pass, but not the DNA. Because the nuclear membrane is impermeable to most molecules, nuclear pores are required to allow movement of molecules across the envelope. These pores cross both of the membranes, providing a channel that allows free movement of small molecules and ions. The movement of larger molecules such as protein requires active transport regulated by carrier proteins. Figure 2 illustrates the nuclear membrane. The nuclear envelope (or perinuclear cisterna) encloses the DNA and defines the nuclear compartment of interphase and prophase nuclei. The spherical inner nuclearmembranecontains specific proteins that act as binding sites for the supporting fibrous sheath ofintermediate filaments (IF), called nuclear lamina. Nuclear lamina has contact with the chromatin (or chromosomes) and nuclear RNAs. The inner nuclear membrane is surrounded by the outer nuclear membrane, which closely resembles the membrane of the endoplasmic reticulum, that is continuous with it. Like the membrane of the rough ER, the outer surface of outer nuclear membrane is generally studded with ribosomes engaged in protein synthesis. The proteins made on these ribosomes are transported into space between the inner and outer nuclear membrane, called perinuclear space. The perinuclear space is a 10 to 50 nm wide fluid-filled compartment which is continuous with the ER lumen and may contain fibres, crystalline deposits, lipid droplets or electrondense material. Nuclear pores and nucleocytoplasmic traffic. The nuclear envelope in all eukaryotic forms, from yeasts to humans, is perforated by nuclear pores which have the following structure and function:

Structure of nuclear pores: Nuclear pores appear circular in surface view and have a diameter between 10nm to 100 nm. Previously it was believed that a diaphragm made of amorphous to fibrillar material extends across each pore limiting free transfer of material. Such a diaphragm called annulus has been observed in animal cells, but lack in plant cells. Recent electron microscopic studies have revealed that a nuclear pore has far more complex structure, so it is called nuclear pore complex with an estimated molecular weight of 50 to 100 million daltons. Negative staining techniques have demonstrated that pore complexes have an eight-fold or octagonal symmetry.

Nuclear Pore density: In nuclei of mammals it has been calculated that nuclear pores account for 5 to 15 per cent of the surface area of the nuclear membrane. In amphibian oocytes, certain plant cells and protozoa, the surface occupied by the nuclear pores may be as high as 20 to 36 per cent.

Arrangement of nuclear pores on nuclear envelope: In somatic cells, the nuclear pores are evenly or randomly distributed over the surface of nuclear envelope. However, pore arrangement in other cell types is not random but rather range from rows (spores of *Eqisetum*) to Clusters (oocytes of *Xenopuslaevis*) to hexagonal (Malpighian tubules of leaf hoppers) packing order.

Nucleo-cytoplasmic traffic: Quite evidently there is considerable trafficking across the nuclear envelope during interphase. Ions, nucleotides and structural, catalytic and regulatory proteins are imported from the cytosol (cytoplasmic matrix); mRNA, tRNA are exported to the cytosol (cytoplasmic matrix). However, one of the main functions of the nuclear envelope is to prevent the entrance of active ribosomes into the nucleus.

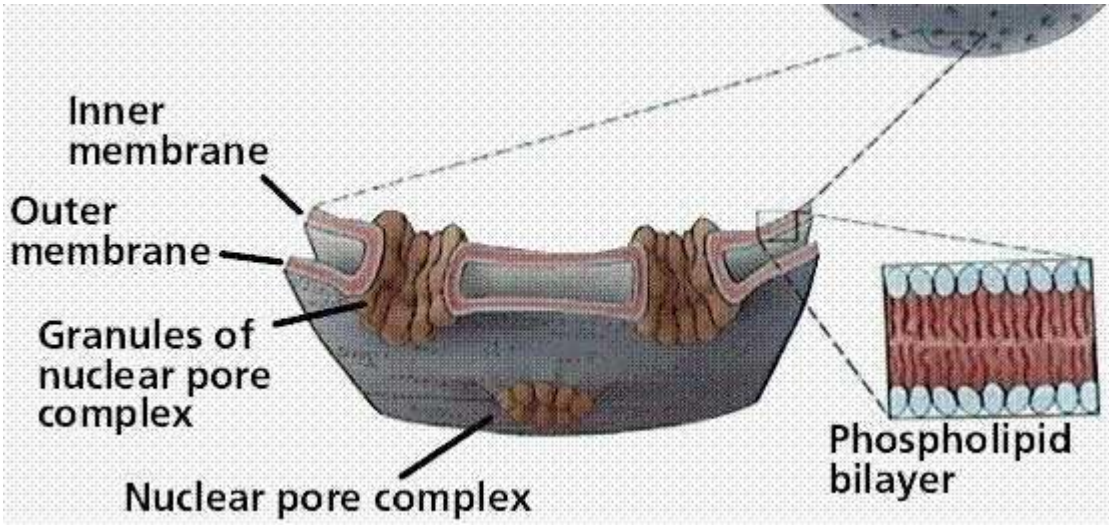


Figure 2: An illustration of the nuclear membrane

Nucleoplasm:
The space between the nuclear envelope and the nucleolus is filled by a transparent, semi-solid, granular and slightly acidophilic ground substance or the matrix known as the nuclear sap or nucleoplasm or karyolymph. The nuclear components such as the chromatin threads and the nucleolus remain suspended in the nucleoplasm which is composed mainly of nucleoproteins but it also contains other inorganic and organic substances, namely nucleic acids, proteins, enzymes and minerals. The most common nucleic acids of the nucleoplasm are the DNA and RNA. The nucleoplasm contains many types of complex proteins categorized into:

(i) Basic proteins. The proteins which take basic stain are known as the basic proteins. The most important basic proteins of the nucleus are nucleoprotamines and the nucleohistones.

(ii) (ii) Non-histone or Acidic proteins. The acidic proteins either occur in the nucleoplasm or in the chromatin. The most abundant acidic proteins of the euchromatin (a type of chromatin) are the phosphoproteins. The nucleoplasm contains many enzymes which are necessary for the synthesis of the DNA and RNA. Most of the nuclear enzymes are composed of non-histone (acidic) proteins. The most important nuclear enzymes are the DNA polymerase, RNA polymerase, NAD synthetase.

The nucleoplasm contains many thread-like, coiled and much elongated structures which take readily the basic stains such as the basic fuchsin. These thread-like structures are known as the chromatin (*chrome*=colour) substance or chromatin fibres.

Nucleolus:
Most cells contain in their nuclei one or more prominent spherical colloidal acidophilic bodies, called nucleoli. However, cells of bacteria and yeast lack nucleolus. The nucleolus is mainly involved in the assembly of ribosomes. After being produced in the nucleolus, ribosomes are

exported to the cytoplasm where they translate mRNA. Some of the eukaryotic organisms have nucleus that contains up to four nucleoli. The nucleolus plays an indirect role in protein synthesis by producing ribosomes. Nucleolus disappears when a cell undergoes division and is reformed after the completion of cell-division. The size of the nucleolus is found to be related with the synthetic activity of the cell. Therefore, the cells with little or no synthetic activities, sperm cells, blastomeres, muscle cell, etc., are found to contain smaller or no nucleoli, while the oocytes, neurons and secretory cells which synthesize the proteins or other substances contain comparatively large-sized nucleoli. The number of the nucleoli in the nucleus depends on the species and the number of the chromosomes. The number of the nucleoli in the cells may be one, two or four. A nucleolus is often associated with the nucleolar organizer (NO) which represents the secondary constriction of the nucleolar organizing chromosomes, and are 10 in number in human beings. Nucleolar organizer consists of the genes for 18S, 5.8S and 28S rRNAs. The genes for fourth type of rRNA, *i.e.*, 5S rRNA occur outside the nucleolar organizer. Nucleolus is not bounded by any limiting membrane; calcium ions are supposed to maintain its intact organization. Nucleolus also contains some enzymes such as acid phosphatase, nucleoside phosphorylase and NAD^+ synthesizing enzymes for the synthesis of some coenzymes, nucleotides and ribosomal RNA. RNA methylase enzyme which transfers methyl groups to the nitrogen bases occurs in the nucleolus of some cells. Functionally nucleolus is the site where biogenesis of ribosomal subunits (40S and 60S) takes place. In it three types of rRNAs, namely 18S, 5.8S and 28S rRNAs, are transcribed as parts of a much longer precursor molecule (45S transcript) which undergoes processing (RNA splicing) by the help of two types of proteins such as nucleolin and U3 sn RNP (U3 is a 250 nucleotide containing RNA, sn RNP represents small nuclear ribonucleoprotein). The 5S r RNA is transcribed on the chromosome existing outside the nucleolus and the 70S types of ribosomal proteins are synthesized in the cytoplasm. All of these components of the ribosomes migrate to the nucleolus, where they are assembled into two types of ribosomal subunits which are transported back to the cytoplasm. The smaller (40S) ribosomal subunits are formed and migrate to the cytoplasm much earlier than larger (60S) ribosomal subunits; therefore, nucleolus contains many more incomplete 60S ribosomal subunits than the 40S ribosomal subunits. Such a time lag in the migration of 60S and 40S ribosomal subunits, prevents functional ribosomes from gaining access to the incompletely processed heterogeneous RNA (hn RNA; the precursor of m RNA) molecule inside the nucleus.

FUNCTION OF THE NUCLEUS

Speaking about the functions of a cell nucleus, it controls the hereditary characteristics of an organism. This organelle is also responsible for the protein synthesis, cell division, growth, and differentiation. Some important functions carried out by a cell nucleus are:

1. Storage of hereditary material, the genes in the form of long and thin DNA (deoxyribonucleic acid) strands, referred to as chromatins.
2. Storage of proteins and RNA (ribonucleic acid) in the nucleolus.
3. Nucleus is a site for transcription in which messenger RNA (mRNA) are produced for the protein synthesis.
4. Exchange of hereditary molecules (DNA and RNA) between the nucleus and rest of the cell.
5. During the cell division, chromatins are arranged into chromosomes in the nucleus.
6. Production of ribosomes (protein factories) in the nucleolus.
7. Selective transportation of regulatory factors and energy molecules through nuclear pores.

As the nucleus regulates the integrity of genes and gene expression, it is also referred to as the control center of a cell. Overall, the cell nucleus stores all the chromosomal DNA of an organism.

Structure and organization of Plasma membrane

All living cells possess a cell membrane. These membranes serve to contain and protect cell components from the surroundings as well as regulate the transport of material into and out of the cell. Cell membranes are the selectively permeable lipid bilayers inclusive of membrane proteins which delimits all prokaryotic and eukaryotic cells. In prokaryotes and plants, the plasma membrane is an inner layer of protection bounded to the inner side of a rigid cell wall. Eukaryotes lack this external layer of protection or the cell wall. In eukaryotes the membrane also forms boundary of cell organelles. The cell membrane has been given different specific names based on their lipid and protein composition such as "sarcolemma" in myocytes and "oolemma" in oocytes. The plasma membrane is just 5-10nm wide thus cannot be detected under the light microscope. It can only be observed under the Transmission electron microscope as a trilaminar structure which is a layer of hydrophobic tails of phospholipids sandwiched between two layers of hydrophilic heads. The plasma membrane contains lipids (32%), proteins (42%), carbohydrates (6%) and water (20%) although variations are always there.

Carbohydrates

The carbohydrates occur only at the outer surface of the membrane. Their molecules are covalently linked to (*i*) the polar heads of some lipid molecules (forming **glycolipids**) and (*ii*) most of the proteins exposed at outer surface (forming **glycoproteins**). The carbohydrates so bound to membrane components constitute the **glycocalyx** of cell surface.

The sugar portions of glycolipids and glycoproteins are involved in recognition mechanisms:-

(a) Sugar recognition sites of two neighbouring cells may bind each other causing cell-to-cell adhesion. This enables cells to orient themselves and to form tissues.

(b) Through glycoproteins, bacteria recognise each other (female bacteria are recognized by male bacteria; *Paramoecia*of different mating types recognize each other).

(c) These provide the basic of immune response and various control systems, where glycoproteins act as antigens.

Lipids

Lipids and integral proteins are **amphipathic** in nature (i.e. have both hydrophobic and hydrophilic groups). The hydrophobic ends are situated inside the bilayer while the hydrophilic groups are directed outwards. Thus, the membrane is held together primarily by hydrophobic attraction. However, the lipids have links in their fatty acid tails. These links prevent close packing of molecules and make the membrane structure more fluid. The fluidity increases with decreasing length of fatty acid tails.

The cell membrane lipids are highly complex comprising of

- Phospholipids,
- Glycolipids,
- Cholesterols.

The major membrane phospholipids and glycolipids are phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns) and phosphatidylserine

(PtdSer) Eukaryotic membrane lipids are glycerophospholipids, sphingolipids, and sterols. Sphingolipids (SPs) and sterols enable eukaryotic cellular membranes with the property of vesicular trafficking important for the establishment and maintenance of distinct organelles.

Proteins

Thousands of different types of proteins can occur in cell membranes. These may be purely structural (provide elasticity and mechanical support) or have additional functions as

(a) Carriers, for transporting specific molecules into or out of the cell.

(b) Receptors, for immediate flow of information into the cells.

Endocytosis occurs by an infolding or extension of plasma membrane to form a vacuole or a vesicle (small vacuole). It is of two types:

(a) **Phagocytosis** (i.e. cell eating): Material is taken up in solid form. The cells involved in phagocytosis are called **phagocytes** or **phagocytic** cells (e.g. white blood cells). The vesicle/vacuole formed is called **phagocytic vacuole**.

(b) **Pinocytosis** (i.e. cell drinking): Material is taken up in liquid (solution/colloid/ suspension) form. If the vesicle formed is extremely small, the process is known as **micropinocytosis**and the vesicle is **micropinocytotic**.

Functions

Functionally membranes take part in several cellular activities covering motility, energy transduction in lower unicellular organisms to immunorecognition in higher eukaryotes. The most valuable function is segregation of the cell into compartments. This functional diversity is due to the variability in lipid and protein composition of the membranes. The various functions can be summarized as given below.

1. Diffusion: Diffusion of small molecules such as carbon dioxide, oxygen (O2), and water happens by passive transport.
2. 2. Osmosis: Cell membrane is semipermeable thus it sets up an osmotic flow for solvent such as water, which can be transported across the membrane by osmosis.
3. 3. Mediated Transport: Nutrients are moved across the membrane by special proteins called transport proteins or permeases which are quite specific, recognizing and transporting only a limited group of chemical substances, often even only a single substance.
4. Endocytosis: Endocytosis is the process in which cells absorb molecules by engulfing them small molecules and ions and macromolecules through active transport which requires ATP.
5. Exocytosis: The plasma membrane can extrude its contents to the surrounding medium to remove undigested residues of substances brought in by endocytosis, to secrete substances such as hormones and enzymes, and to transport a substance completely across a cellular barrier.
6. Cell adhesion.
7. Cell signalling.

Models of plasma membrane

Quincke first perceived the lipid nature of the cell membranes and proposed it to be less than 100 nm thick. With time many researchers have proposed models for cell membrane. In 1935, Danielli and Davson, proposed a model, called sandwich model, for membrane structure in which a lipid bilayer was coated on its either side with hydrated proteins (globular proteins). Mutual attraction between the hydrocarbonchains of the lipids and electrostatic forces between the protein and the "head" of the lipid molecules, were thought to maintain the stability of the membrane. From the speed at which various molecules penetrate the membrane, they predicted the lipid bilayer to be about 6.0 nm in thickness, and each of the protein layer of about 1.0 nm thickness, giving a total thickness of about 8.0 nm. The Danielli-Davson model got support from electron microscopy. Electron micrographs of the plasma membrane showed that it consists of two dark layers (electron dense granular protein layers), both separated by a lighter area in between (the central clear area of lipid bilayer). The total thickness of the membranes too turned out to be about 7.5 nm.

Currently, the most accepted model for cell membrane is fluid mosaic model proposed by S.J.Singer and G.L.Nicolson (1972). According to this model, the plasma membrane contains a bimolecular lipid layer, both surfaces of which are interrupted by protein molecules. Proteins occur in the form of globular molecules and they are dotted about here and there in a mosaic pattern **(see Figure 1)** . Some proteins are attached at the polar surface of the lipid (i.e., the extrinsic proteins); while others (i.e., integral proteins) either partially penetrate the bilayer or span the membrane entirely to stick out on both sides (called transmembrane proteins). Further, the peripheral proteins and those parts of the integral proteins that stick on the outer surface (i.e., ectoproteins) frequently contain chains of sugar or oligosaccharides (i.e., they are glycoproteins). Likewise, some lipids of outer surface are glycolipids. The fluid-mosaic membrane is thought to be a far less rigid than was originally supposed.

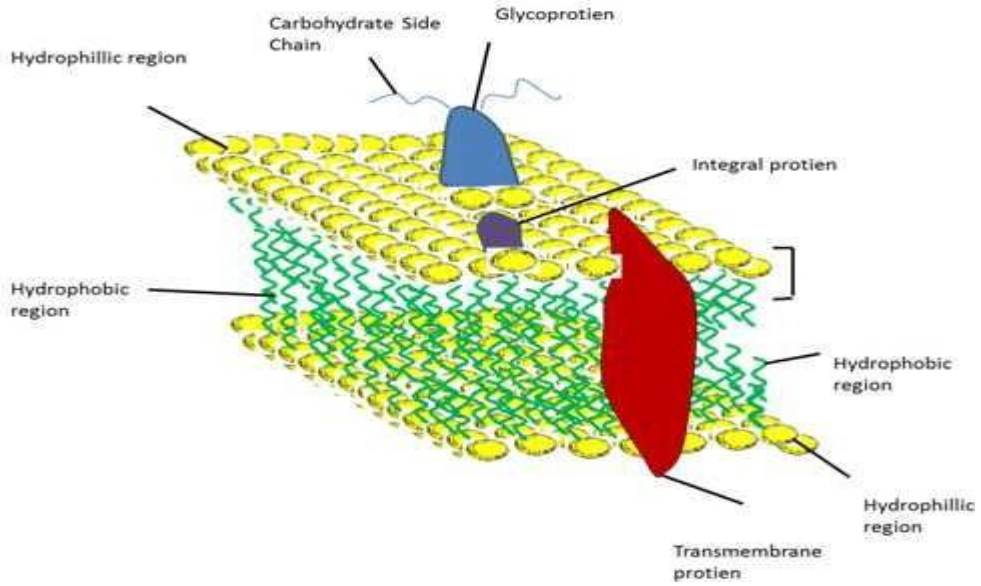


Figure 1: The architecture of the cell membrane

MEMBRANE TRANSPORT

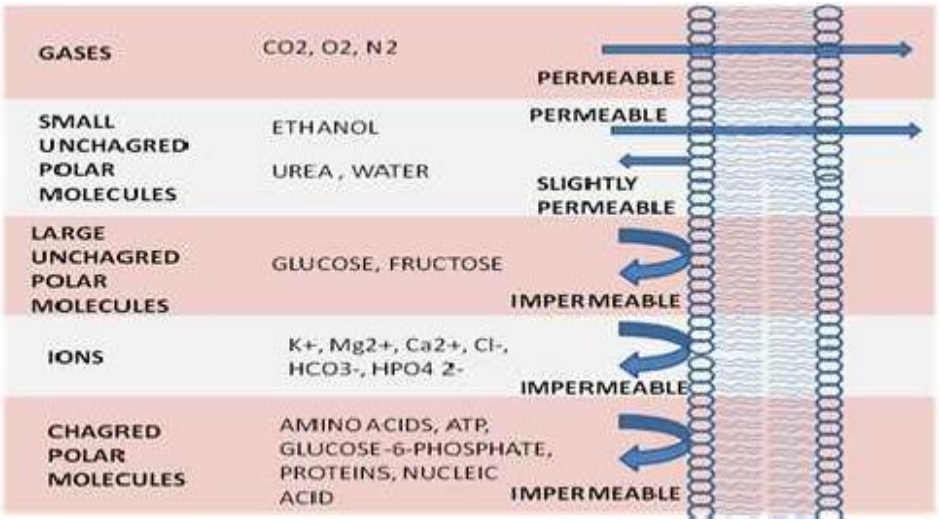
In cellular biology, **membrane transport** refers to the collection of mechanisms that regulate the passage of solutes such as ions and small molecules through biological membranes, which are lipid bilayers that contain proteins embedded in them. The regulation of passage through the membrane is due to selective membrane permeability - a characteristic of biological membranes which allows them to separate substances of distinct chemical nature. In other words, they can be permeable to certain substances but not to others. The movements of most solutes through the membrane are mediated by membrane transport proteins which are specialized to varying degrees in the transport of specific molecules. As the diversity and physiology of the distinct cells is highly related to their capacities to attract different external elements, it is postulated that there is a group of specific transport proteins for each cell type and for every specific physiological stage. This differential expression is regulated through the differential transcription of the genescoding for these proteins and its translation, for instance, through genetic-molecular mechanisms, but also at the cell biology level: the production of these proteins can be activated by cellular signaling pathways, at the biochemical level, or even by being situated in cytoplasmic vesicles.

Background

Thermodynamically the flow of substances from one compartment to another can occur in the direction of a concentration or electrochemical gradient or against it. If the exchange of substances occurs in the direction of the gradient, that is, in the direction of decreasing potential, there is no requirement for an input of energy from outside the system; if, however, the transport is against the gradient, it will require the input of energy, metabolic energy in this case. For example, a classic chemical mechanism for separation that does not require the addition of external energy is dialysis. In this system a semipermeable membrane separates two solutions of different concentration of the same solute. If the membrane allows the passage of water but not the solute the water will move into the compartment with the greatest solute concentration in order to establish an equilibrium in which the energy of the system is at a minimum. This takes place because the water moves from a high solvent concentration to a low one (in terms of the solute, the opposite occurs) and because the water is moving along a gradient there is no need for an external input of energy.

1.phospholipid 2. cholesterol 3. glycolipid 4. sugar 5. polytopic protein (transmembrane protein) 6. monotopic protein (here, a glycoprotein) 7. monotopic protein anchored by a phospholipid 8. peripheralmonotopic protein (here, a glycoprotein.)

The nature of biological membranes, especially that of its lipids, is amphiphilic, as they form bilayers that contain an internal hydrophobic layer and an external hydrophilic layer. This structure makes transport possible by simple or passive diffusion, which consists of the diffusion of substances through the membrane without expending metabolic energy and without the aid of transport proteins. If the transported substance has a net electrical charge, it will move not only in response to a concentration gradient, but also to a gradient due to the membrane potential. electrochemical



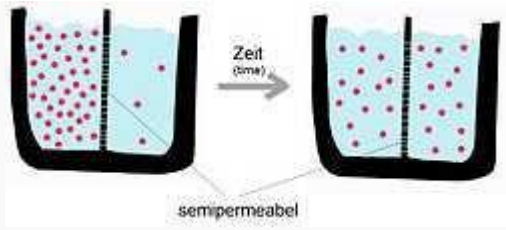
Relative permeability of a phospholipid bilayer to various substances

As few molecules are able to diffuse through a lipid membrane the majority of the transport processes involve transport proteins. These transmembrane proteins possess a large number of alpha helices immersed in the lipid matrix. In bacteria these proteins are present in the beta lamina form.[4] This structure probably involves a conduit through hydrophilic protein environments that cause a disruption in the highly hydrophobic medium formed by the lipids.[1] These proteins can be involved in transport in a number of ways: they act as pumps driven by ATP, that is, by metabolic energy, or as channels of facilitated diffusion.

Transport types

Passive diffusion

Main article: Passive transport



As mentioned above, passive diffusion is a spontaneous phenomenon that increases the entropy of a system and decreases the free energy.[5] The transport process is influenced by the characteristics of the transport substance and the nature of the bilayer. Membrane proteins (with the exception of channels - facilitated diffusion) are not involved in passive diffusion. The diffusion velocity of a pure phospholipid membrane will depend on:

- concentration gradient,

- hydrophobicity,
- size,
- charge, if the molecule has a net charge.

Active and co-transport[ ]

Active transport

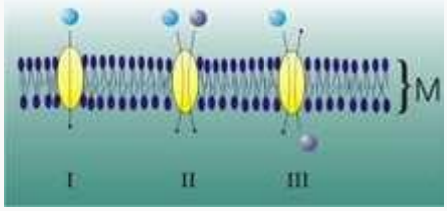
In active transport a solute is moved against a concentration or electrochemical gradient, in doing so the transport proteins involved consume metabolic energy, usually ATP. Inprimary active transport the hydrolysis of the energy provider (e.g. ATP) takes place directly in order to transport the solute in question, for instance, when the transport proteins are ATPase enzymes. Where the hydrolysis of the energy provider is indirect as is the case in secondary active transport, use is made of the energy stored in an electrochemical gradient. For example, in co-transport use is made of the gradients of certain solutes to transport a target compound against its gradient, causing the dissipation of the solute gradient. It may appear that, in this example, there is no energy use, but hydrolysis of the energy provider is required to establish the gradient of the solute transported along with the target compound. The gradient of the co-transported solute will be generated through the use of certain types of proteins called biochemical pumps.

The discovery of the existence of this type of transporter protein came from the study of the kinetics of cross-membrane molecule transport. For certain solutes it was noted that the transport velocity reached a plateau at a particular concentration above which there was no significant increase in uptake rate, indicating a log curve type response. This was interpreted as showing that transport was mediated by the formation of a substrate-transporter complex, which is conceptually the same as the enzyme-substrate complex ofenzyme kinetics. Therefore, each transport protein has an affinity constant for a solute that is equal to the concentration the solute when the transport velocity is half its maximum value. This is equivalent in the case of an enzyme to the Michaelis-Menten constant.

Some important features of active transport in addition to its ability to intervene even against a gradient, its kinetics and the use of ATP, are its high selectivity and ease of

selective pharmacol gical inhibition

*Secondary active transporter proteins**[ ]***



Secondary active transporter proteins move two molecules at the same time: one
against a gradient and the other with its gradient. They are distinguished according to the directionality of the two molecules:

A protein involved in moving only one molecule across a membrane is called a uniport. If two molecules are moved in opposite directions across the bilayer, the protein is called an antiport. If two molecules are moved in same directions across the bilayer, the protein is called symport Proteins involved in moving ions are called ionophores.

Na+/K+ pump

A pump is a protein that hydrolyses ATP in order to transport a particular solute through a membrane in order to generate an electrochemical gradient to confer certain membrane potential characteristics on it. This gradient is of interest as an indicator of the stat of the cell through parameters such as the Nernst potential. Interms of membrane transport the gradient is of interest as it contributes to increased system entropy in the co-transport of substances against their gradient. One of the most important pumps in animal cells is thesodium potassium pump, that operates through the following mechanism:

1. binding of three Na^+ ions to their active sites on the pump which are bound to ATP.
2. ATP is hydrolyzed leading to phosphorylation of the cytoplasmic side of the pump, this induces a structure change in the protein. The phosphorylation is caused by the transfer of the terminal group of ATP to a residue of aspartate in the transport protein and the subsequent release of ADP.
3. the structure change in the pump exposes the Na^+ to the exterior. The phosphorylated form of the pump has a low affinity for Na^+ions so they are released.
4. once the Na^+ ions are liberated, the pump binds two molecules of K^+ to their respective bonding sites on the extracellular face of the transport protein.This causes the dephosphorylation of the pump, reverting it to its previous conformational state, transporting the K^+ ions into the cell.
5. The unphosphorylated form of the pump has a higher affinity for Na^+ ions than K^+ ions, so the two bound K^+ ions are released into the cytosol. ATP binds, and the process starts again.

Membrane selectivity

As the main characteristic of transport through a biological membrane is its selectivity and its subsequent behavior as a barrier for certain substances, the underlying physiology of the phenomenon has been studied extensively. Investigation into membrane selectivity have classically been divided into those relating to electrolytes and non-electrolytes.

Electrolyte selectivity

The ionic channels define an internal diameter that permits the passage of small ions that is related to various characteristics of the ions that could potentially be transported. As the size of the ion is related to its chemical species, it could be assumed *a priori* that a channel whose pore diameter was sufficient to allow the passage of one ion would also allow the transfer of others of smaller size, however, this does not occur in the majority of cases. There are two characteristics alongside size that are important in the determination of the selectivity of the membrane pores: the facility for dehydration and the interaction of the ion with the internal charges of the pore.
In order for an ion to pass through a pore it must dissociate itself from the water molecules that cover it in successive layers of solvation. The tendency to dehydrate, or the facility to do this, is related to the size of the ion: larger ions can do it more easily that the smaller ions, so that a pore with weak polar centres will preferentially allow passage of larger ions over the smaller ones. When the interior of the channel is composed of polar groups from the side chains of the component amino acids, the interaction of a dehydrated ion with these centres can be more important than the facility for dehydration in conferring the specificity of the channel. For example, a channel made up of histidines and arginines, with positively charged groups, will selectively repel ions of the same polarity, but will facilitate the passage of negatively charged ions. Also, in this case, the smallest ions will be able to interact more closely due to the spatial arrangement of the molecule (stericity), which greatly increases the charge-charge interactions and therefore exaggerates the effect.

Non-electrolyte selectivity

Non-electrolytes, substances that generally are hydrophobic and lipophylic, usually pass through the membrane by dissolution in the lipid bilayer, and therefore, by passive diffusion. For those non-electrolytes whose transport through the membrane is mediated by a transport protein the ability to diffuse is, generally, dependent on the partition coefficient K. Partially charged non-electrolytes, that are more or less polar, such as ethanol, methanol or urea, are able to pass through the membrane through aqueous channels immersed in the membrane. It is interesting to note that there is no effective regulation mechanism that limits this transport, which indicates an intrinsic vulnerability of the cells to the penetration of these molecules.

Membrane transport facilitators

Membrane transport is assisted by various facilitators to ease their job. We will study a few of them in detail.

Permeases

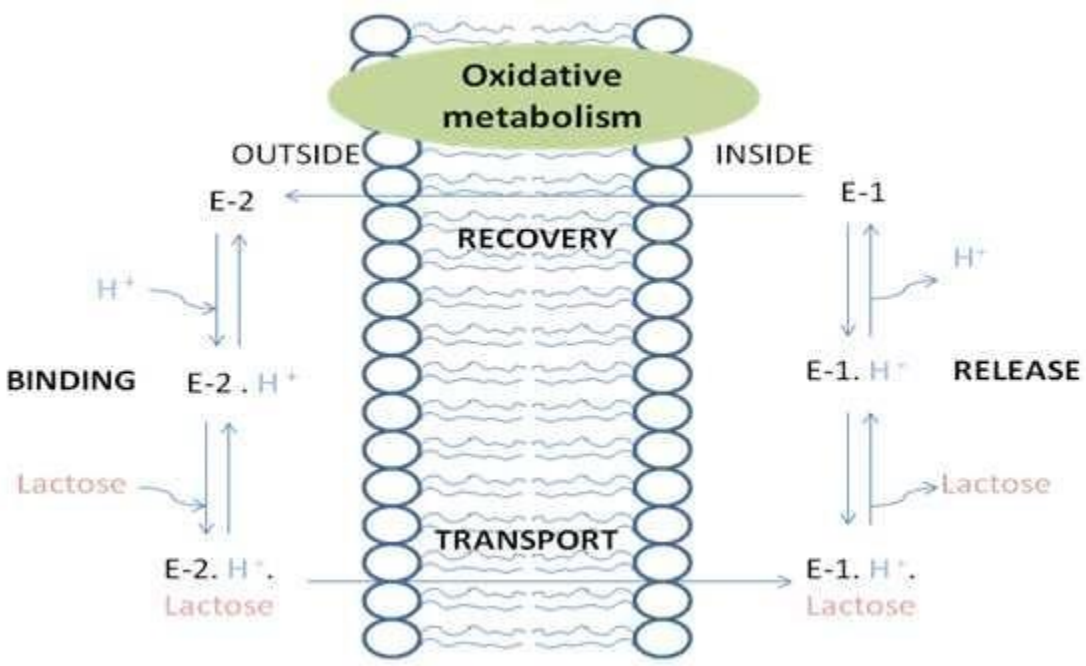
Permeases are a class of membrane transport proteins which facilitate the diffusion of a specific molecule by passive mediated transport.

These are divided into following types:

Lactose permease: It is a transmembrane protein that consists of N- and C- terminal domains, each consisting of six membrane-spanning alpha helices in a symmetrical fashion. These two domains are well separated and are joined by a single stretch of polypeptide. There are six side chains amino acids that play an important role in the active transport of lactose through the protein. Some of the examples are: Glutamic Acid 126, Arginine 144, and Glutamic Acid 269 plays role in substrate binding activities whereas Arginine 302, Histidine 322, and Glutamic Acid 325 plays a significant role in proton translocation throughout the transport process. These side chains, make up the active

site of the protein and found within the large internal hydrophilic cavity of the lactose permease where the substrate is received for transport and it is the location from which it is sent into the cell. It is an active co-transport that facilitates the passage of lactose across the phospholipid bi-layer of the cell membrane by using the inwardly directed H+ electrochemical gradient as its driving force. The proton gradient is metabolically generated through oxidative metabolism. The electrochemical potential gradient created by both these systems is used mainly to drive the synthesis of ATP. As a result, the lactose is accompanied from the periplasam to the cytoplasm of the cell by an H+ proton.

Lactose permease has two major conformational states: 1. E-1, which has a low-affinity lactose-binding site facing the interior of the cell. 2. E-2, which has a high-affinity lactose-binding site facing the exterior of the cell.



Schematic diagram for the cotransport of H+ and lactose by lactose permease in E.Coli. H+ binds first to E-2 outside the cell, followed by lactose. They are sequentially released from E-1 inside the cell. E-2 must bind to lactose and H+ in order to change the conformation to E-1, thereby cotransporting these substances in the cell. E-1 changes the conformation to E-2 when neither lactose nor H+ is bound, thus completing the transport cycle.

2. β-galactosidepermease is a membrane-bound transport protein that facilitates the uptake of β-galactosides across the cell. The common example is melibiose carrier protein from Klebsiella pneumonia, which is capable of using hydrogen and lithium cations as coupling cations for cotransport, depending on the particular sugar transported (H+ -melibiose, Li+ -lactose).

3. Amino acid permeases are integral membrane proteins involved in the transport of amino acids into the cell. One of the examples of amino acid permease is histidinepermease which is a bacterial ABC protein in E.coli and located in the periplasmic space of cell.

Histidine binding protein binds histidine tightly and directs it to T sub-units of permease, through which hisitidine crosses the plasma membrane along with ATP hydrolysis.

ATP dependent proton pumps

ATP dependent proton pumps or transport ATPase are the pumps that transport H^+ ions against their concentration gradients. These pumps are transmembrane proteins with one or more binding sites for ATP located on the cytosolic face of the membrane and these proteins are called ATPases. They normally do not hydrolyze ATP into ADP and Pi unless H^+ ions are simultaneously transported. Because of this tight coupling between ATP hydrolysis and transport, the energy stored in the phosphoanhydride bond is not dissipated but rather used to move ions or other molecules uphill against an electrochemical gradient.

ATP dependent proton pumps can be categorized into different classes. Generally, ATP dependent proton pumps are divided into 4 classes:

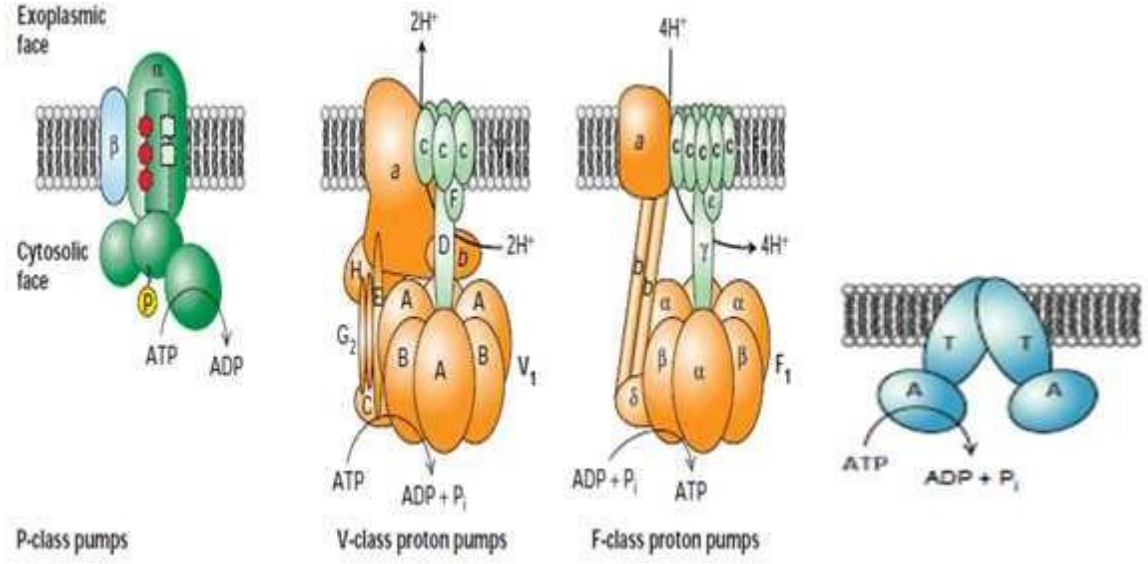


Figure 1: Different types of ATP dependent proton pumps

1. P-class ion pumps

These are multipasstransmembrane proteins having two identical catalytic α-subunits that contain an ATP binding site. Some have two smaller β-subunits that usually have regulatory functions. During the transport process or pumping cycle at least one of the α-subunit must be phosphorylated and the H^+ ions are thought to move through the phosphorylated subunit. This class includes many ion pumps that are responsible for setting up and maintaining gradients of Na^+, K^+, H^+ and Ca^{2+} across the cell membrane.

a) The common P-type pump is mostly found in parietal cells of the mammalian stomach which transport protons (H^+ ions) out of cell and K^+ ions into the cell and is mainly responsible for the acidification of the stomach contents. The pump is known as H^+/K^+ ATPase. It is a heterodimeric protein. The H^+/K^+ ATPase transports one H^+ from the cytoplasm of the parietal cell in exchange for one K^+ retrieved from the gastric lumen. As an ion pump the H^+/K^+ ATPase is able to transport ions against a concentration gradient using energy derived from the hydrolysis of ATP. Like all P-type ATPases, a phosphate group is transferred from ATP to the H^+/K^+ ATPase during the transport cycle.

b) Another example of P-type pump is Na^+/K^+ ATPase in the plasma membrane, whichgenerates low cytosolic Na^+ and high cytosolic K^+ concentration which is typical of animal cells.

c) Certain Ca^{2+} ATPase pump Ca^{2+} ions out of the cytosol into the external medium while others pump $Ca^{2+}$from the cytosol into the endoplasmic recticulum or into the specialized sarcoplasmic reticulum, which is more common in muscle cells.

2. F-class ion pumps:

The F class ion pumps contain different transmembrane and cytosolic subunits. They are known for only transport of protons, in a process that does not involve phosphoprotein intermediate. They generally behave as reverse proton pump by synthesizing ATP from ADP and Pi by movement of protons from the exoplasmic to the cytosolic face of the membrane down the proton electrochemical gradient. Therefore, these pumps are also known as ATP synthases or F_0F_1 complex. F-class ion pump is most common in bacteria, yeast and animal mitochondria and also in chloroplast.

The F_0F_1 complex is a multi-protein having two components $F_0$and F_1. Both are multimeric proteins. The F_0 component contains three integral membrane proteins named a, b and c. The a and two b subunits are linked tightly but not to the donut- shaped ring of c subunits. And the F_1 component is water soluble complex of five distinct polypeptides with the composition $\alpha 3\beta 3\gamma\delta\varepsilon$. The lower part of the $F_1\gamma$ subunit is a coil which fits into the centre of the c-subunit ring of F_0 and appears rigidly attached to it. The F_1 ε subunit is rigidly attached to γ and also forms rigid contacts with c subunits. The F_1 and subunits associate in alternating order to form a hexamer αβαβαβ. The $F_1\delta$ subunit is permanently linked to one of the F_1 subunits and also to the b subunit of F_0. Thus the F_0 a and b subunits and the δ subunit and (αβ)3hexamer of the F_1 complex form a rigid structure anchored in the membrane. The rodlike b subunits form a stator that prevents the (αβ)3hexamer from moving while it rests on the γ subunit.

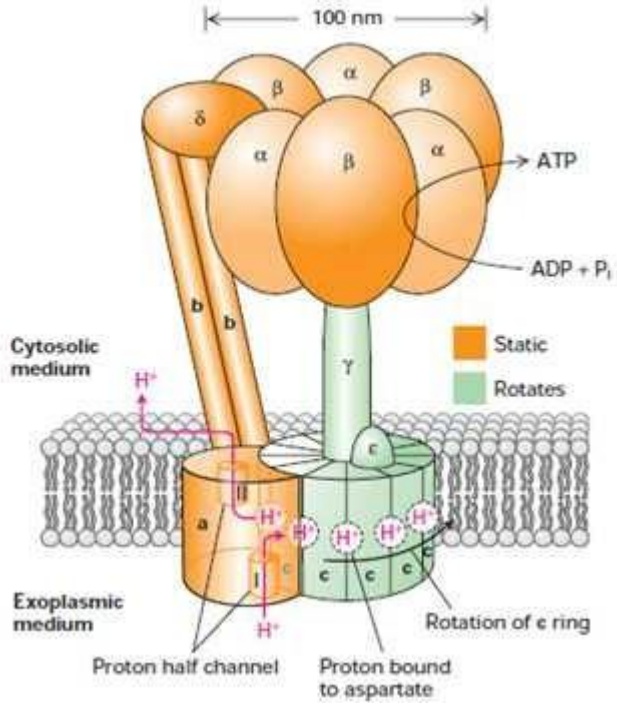


Figure 2: Model of the structure and function of ATP synthase (the F_0F_1 complex) in the bacterial plasma membrane. The F_0 portion is built of three integral membrane proteins: one copy of a, two copies of b, and on average 10 copies of c arranged in a ring in the plane of the membrane. Two proton half-channels lie at the interface between the a subunit and the c ring. Half-channel I allows protons to move one at a time from the exoplasmic medium and bind to

aspartate-61 in the center of a c subunit near the middle of the membrane. Half-channel II (after rotation of the c ring) permits protons to dissociate from the aspartate and move into the cytosolic medium

3. V-class ion pumps:

It is almost similar to F-class ion pumps in structure and function. But none of their subunits are related to each other. F-class pumps operate in reverse direction to F-class. These pumps generally function to maintain low pH of plant vacuoles and lysosome and other acidic vesicles in animal cells by pumping protons from cytosolic to exoplasmic face (inside) of membrane against the proton electrochemical gradient. The acidification between the lysosomal lumen and cytosol lumen can be maintained by production of ATP by cells.

These V-class proton pumps contain two domains: a cytosolic hydrophilic domain (V_1) and a transmembrane domain (V_0) with multiple subunits in each domain. Binding and hydrolysis of ATP by the B subunits in V_1 provide the energy for pumping of H^+ ions through the proton-conducting channel formed by the c and a subunits in V_0. These V-class proton pumps are not phosphorylated and dephosphorylated during proton transport.

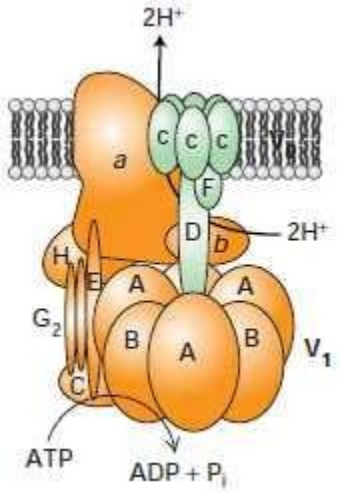


Figure 3: V-class proton pump

These protons cannot acidify by themselves because a net movement of electric charge occurs. Only a few protons build up positive H^+ ions on exoplasmic face (inside) and for each H^+ pumped across, a negative ion will be left behind on cytosolic face, building negatively charged ions. These oppositely charged ions attract each other on opposite faces of the membrane, generating a charge separation, or electric potential, across the membrane. If more protons pumped, the excess positive ions on exoplasmic face repels other H^+ ions and prevents pumping of extra proton long before a significant transmembrane H^+ concentration gradient had been established. If the organelle lumen or the extracellular space has to be acidified, the net movements of protons must be accompanied either by movement of equal number of anion egCl^- in same direction or my movement of different cation in the opposite direction. The first process occurs in lysosomes and plant vacuoles whose membrane contains V-class H^+ ATPase and anion channels for Cl^- movement. And the second process is observed in the lining of the stomach which contains a H^+ /K^+ ATPase and pumps one H^+ outward and one K^+ inward.

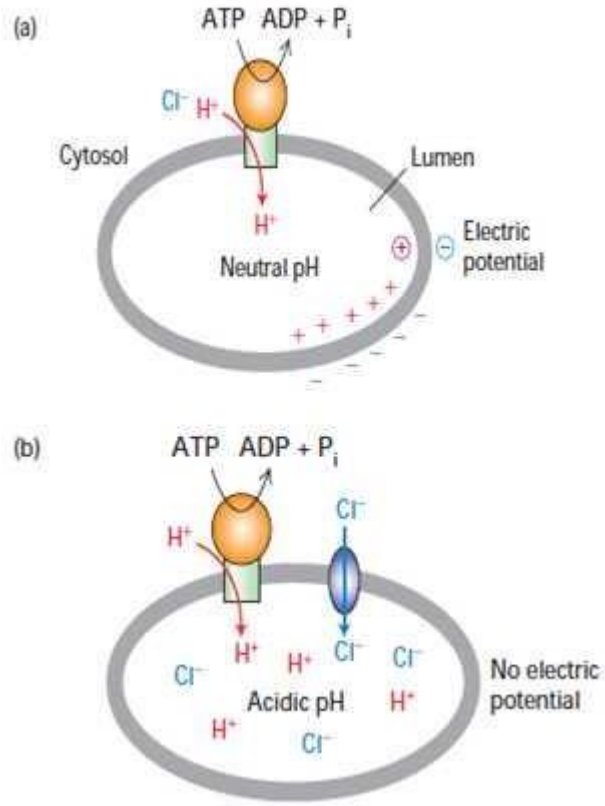


Figure 4: Effect of proton pumping by V-class ion pumps on H^+ concentration gradients and electric potential gradients across cellular membranes. (a) If an intracellular organelle contains only V-class pumps, proton pumping generates an electric potential across the membrane, luminal-side positive, but no significant change in the intraluminal pH. (b) If the organelle membrane also contains Cl^-channels, anions passively follow the pumped protons, resulting in an accumulation of H^+ ions (low luminal pH) but no electric potential across the membrane.

4. ABC (ATP binding cassettes) superfamily:

The final class of ATP-powered pumps is a large family of multiple membranes. This class includes several hundred different transport proteins found in all organisms ranging from bacteria to mammals. Each ABC protein is specific for single substrate or group of related substrate, which may be ions, sugars, amino acids, phospholipids, cholesterol, peptides, polysaccharides or proteins. All ABC transport protein share a structural organization consisting of four core domains: two transmembarne (T) domains, forming the passageway through which transported molecules cross the membrane and two cytosolic ATP-binding (A) domains. The core domains are generally present in separate polypeptides which are more common in bacterial cell. In others, the core domains are fused into one or two multidomain polypeptides. ATP binding leads to dimerization of two ATP-binding domains and ATP hydrolysis leads to their dissociation. Theses structural changes in the cytosolic domains are thought to be transmitted to the transmembrane segments, driving cycles of conformational changes that alternately expose substrate-binding sites on one side of the membrane and then on the other. In this way, ABC transporters use ABC binding and hydrolysis to transport small molecules across the bilayer. Some common example of ABC transporters are found in bacterial plasma membranes which contain amino acid, sugar and peptide transporters. These cells use H+ gradient across the membrane to pump variety of nutrients into the cell. It is also present is mammalian plasma

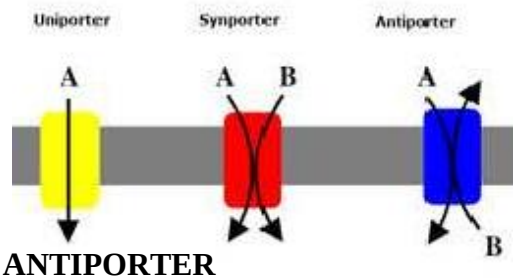
membrane that contains transporters of phospholipids, small lipophillic drugs, cholesterol and other small molecules. One example of eukaryotic ABC transporters is multidrug resistance (MDR) protein which has the ability to pump hydrophobic drugs out of the cytosol. Overexpression of these MDR protein in human cancer cells, make the cells resistant to variety of chemically unrelated cytototoxic drugs.

Interesting facts

- Valinomycin is a carrier for potassium.
- Lactose permease has been crystallized with thiodigalactoside (TDG), an analog of lactose.
- Adenine nucleotide translocase (ADP/ATP exchanger), which catalyzes 1:1 exchange of ADP for ATP across the inner mitochondrial membrane.
- The reaction mechanism for a P-class ion pump involves transient covalent modification of the enzyme.
- Gramicidin is an example of a channel. It is an unusual peptide, with alternating D and L amino acids. In lipid bilayer membranes, gramicidin dimerizes and folds as a right handed β-helix. The dimer just spans the bilayer.

CO-TRANSPORTERS

Cotransporters are a subcategory of transporters that couple the favorable movement of one molecule with its concentration gradient and unfavorable movement of another molecule against its concentration gradient and include antiporters and symporters. In general, cotransporters consist of two out of the three classes of integral membrane proteins known as transporters that move molecules and ions across biomembranes. Uniporters are also transporters but move only one type of molecule down its c ncentration gradient and are not classified as cotransporters.



ANTIPORTER

An **antiporter** (also called **exc ł anger** or **counter-transporter**) is a cotransporter and integral

membrane protein involved insecondary active transport of two or more different molecules or ions (i.e., solutes) across a phospholipid membrane such as theplasma membrane in opposite directions.

In secondary active transport, one species of solute moves along its electroc emical gradient, allowing a different species to ove against its own electrochemical gradient. his movement is

in contrast to primary active transport, in which all solutes are moved against their concentration gradients, fueled by ATP.

Transport may involve one or more of each type of solute. For example, the **Na^+/Ca^{2+} exchanger**, used by many cells to remove cytoplasmic calcium, exchanges one

calcium ion for three sodium ions.

SYMPORTER

A symporter is an integral membrane protein that is involved in movement of two or more

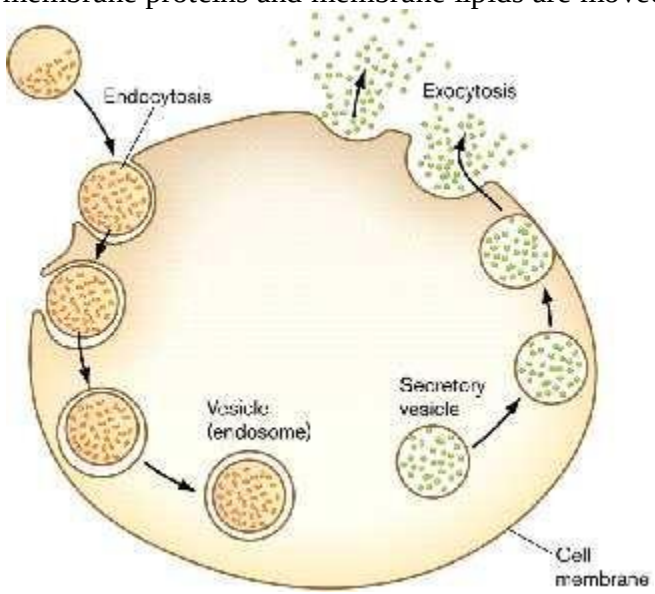
different molecules or ions across a phospholipid membrane such as the plasma membrane in the same direction, and is, therefore, a type of cotransporter. Typically, the ion(s) will move down the electrochemical gradient, allowing the other molecule(s) to move against the concentration gradient. The movement of the ion(s) across the membrane is facilitated diffusion, and is coupled with the active transport of the molecule(s). Although two or more types of molecule are transported, there may be several molecules transported of each type.

ENDOCYTOSIS AND EXOCYTOSIS

Endocytosis is the process of capturing a substance or particle from outside the cell by engulfing it with the cell membrane. The membrane folds over the substance and it becomes completely enclosed by the membrane. At this point a membrane-bound sac, or vesicle, pinches off and moves the substance into the cytosol. There are two main kinds of endocytosis:

- **Phagocytosis**, or *cellular eating*, occurs when the dissolved materials enter the cell. The plasma membrane engulfs the solid material, forming a phagocytic vesicle.
- **Pinocytosis**, or *cellular drinking*, occurs when the plasma membrane folds inward to form a channel allowing dissolved substances to enter the cell. When the channel is closed, the liquid is encircled within a pinocytic vesicle.

Exocytosis describes the process of vesicles fusing with the plasma membrane and releasing their contents to the outside of the cell. Exocytosis occurs when a cell produces substances for export, such as a protein, or when the cell is getting rid of a waste product or a toxin. Newly made membrane proteins and membrane lipids are moved on top the plasma membrane by exocytosis.



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DEPARTMENT OF BIOMEDICAL ENGINEERING

UNIT – II -Cell Biology & Genetics – SBMA1101

MEMBRANE RECEPTORS

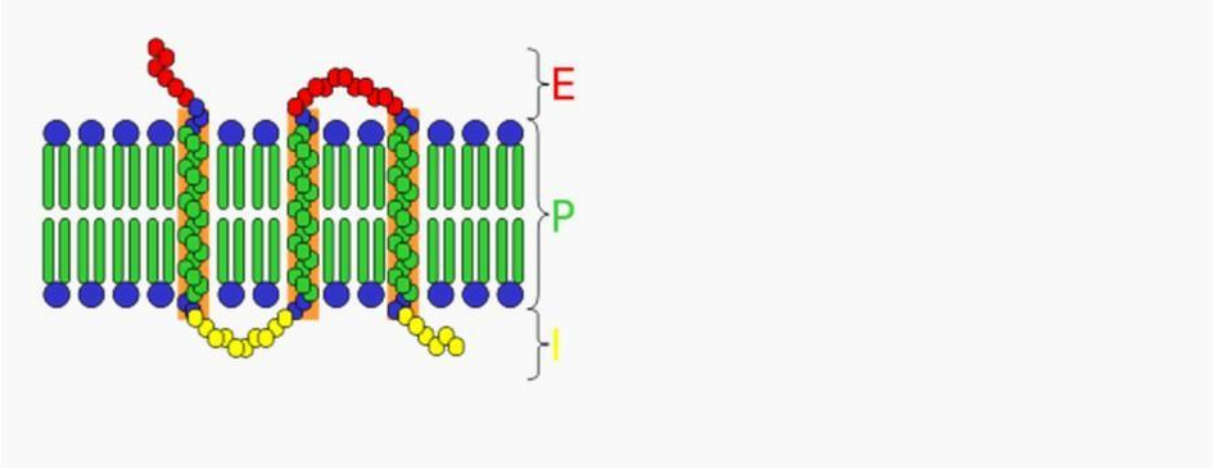
Cell surface receptors (**membrane receptors**, transmembrane **receptors**) are specialized integral membrane proteins which communicate signals between the cell and the outside world. Extracellular molecules such as: hormones; neurotransmitters; cytokines; growth factors; cell recognition molecules; nutrients; or waste products; all react with the archetypical receptor to induce changes in the metabolism and activity of a cell. The process is referred to as signal transduction: in which Ligand Binding affects a cascading chemical change through the plasma membrane. In this way, surface receptors act as triggers of cellular communication and signal transduction.

Structure and mechanism

Transmembrane receptors are composed of two or more peptides which cooperate and may dissociate when ligands bind, or fall off during another stage of their signaling cycles. They are typically classified based on their tertiary structure. But, if the three-dimensional structure is yet undiscovered, then they can be classified based on experimentally verifiable membrane topology. The simplest polypeptide chains are found to cross the lipid bilayer once; while others, such as the G-protein coupled receptors, cross as many as seven times.

There are various kinds, such as glycoprotein and lipoprotein. Hundreds of different receptors are known and many more have yet to be studied. Many membrane receptors include transmembrane proteins. Each cell membrane can have several kinds of membrane receptor, in varying surface distribution. A specific receptor may also be differently distributed on different membrane surfaces, depending on the membrane sort and cell function. Since receptors usually cluster on the membrane surface, the placement of every receptor on each membrane surface is heterogeneous.

Domains



Like any integral membrane protein, a transmembrane receptor may be divided into three domains.

Extracellular domain

The extracellular domain juts externally from the cell or organelle. If the polypeptide chain crosses the bilayer several times, the external domain comprises loops entwined through the membrane. By definition, a receptor's main function is to recognize and respond to a type of ligand. For example, a neurotransmitter, hormone, or atomic ions may each bind to the extracellular domain as a ligand coupled to receptor. Klotho is an enzyme which effects a receptor to recognize the ligand (FGF23).

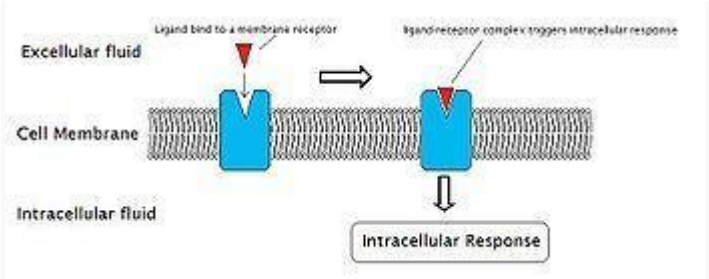
Transmembrane domain

In the majority of receptors with known structures, transmembrane alpha helices constitute most of the transmembrane component. In certain receptors, such as the nicotinic acetylcholine receptor, the transmembrane domain forms a protein pore through the membrane, or around the ion channel. Upon activation of an extracellular domain by binding of the appropriate ligand, the pore becomes accessible to ions, which then diffuse. In other receptors, the transmembrane domains undergo a conformational change upon binding, which effects intracellular conditions. In some receptors, such as members of the 7TM superfamily, the transmembrane domain includes a ligand binding pocket.Bacteriorhodopsin is an example, the detailed structure of which has been determined by crystallography.

Intracellular domain

The intracellular (or cytoplasmic) domain of the receptor interacts with the interior of the cell or organelle, relaying the signal. There are two fundamental paths for this interaction:

- The intracellular domain communicates via protein-protein interactions against *effector proteins*, which in turn pass a signal to the destination.
- With enzyme-linked receptors, the intracellular domain has *enzymatic activity*. Often, this is tyrosine kinase activity. The enzymatic activity can also be due to an enzyme associated with the intracellular domain.



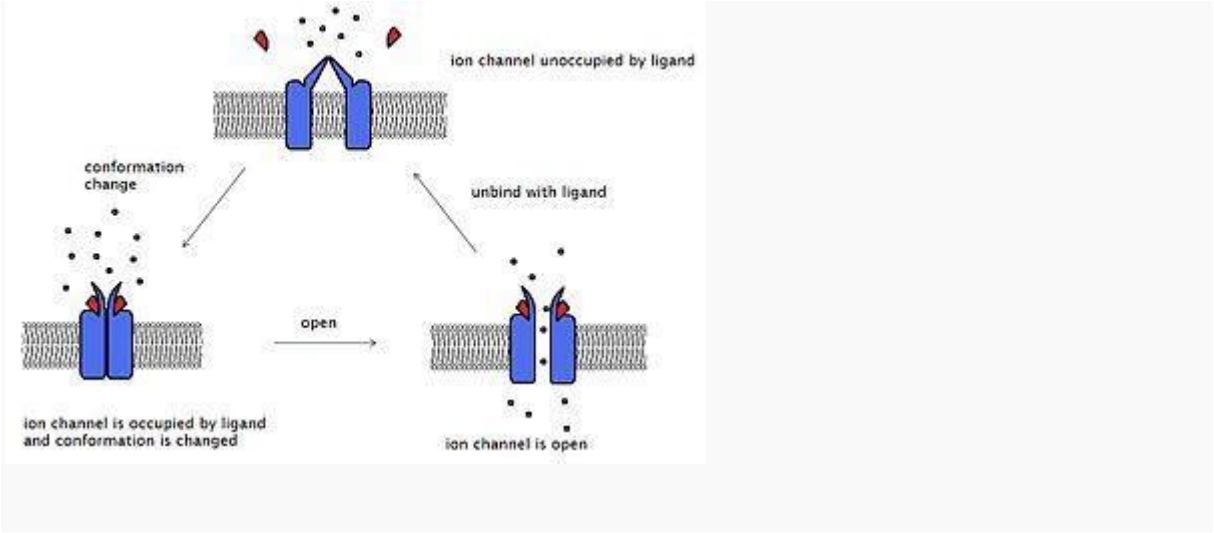
Signal transduction

External reactions and internal reactions for signal transduction

Signal transduction processes through membrane receptors involve the external reactions, in which the ligand binds to a membrane receptor, and the internal reactions, in which intracellular response is triggered.

Signal transduction through membrane receptors requires four parts:

- Extracellular signaling molecule: an extracellular signaling molecule is produced by one cell and is at least capable of traveling to neighboring cells.
- Receptor protein: cells must have cell surface receptor proteins which bind to the signaling molecule and communicate inward into the cell.
- Intracellular signaling proteins: these pass the signal to the organelles of the cell. Binding of the signal molecule to the receptor protein will activate intracellular signaling proteins that initiate a signaling cascade.
- Target proteins: the conformations or other properties of the target proteins are altered when a signaling pathway is active and changes the behavior of the cell.



Three conformation states of acetylcholine receptor

Membrane receptors are mainly divided by structure and function into 3 classes: The ion channel-linked receptor; The enzyme-linked receptor; and The G protein-coupled receptor.

- **Ion channel linked receptors** have ion channels for anions and cations, and constitute a large family of multipass transmembrane proteins. They participate in rapid signaling events usually found in electrically active cells such as neurons. They are also called ligand-gated ion channels. Opening and closing of ion channels is controlled by neurotransmitters.

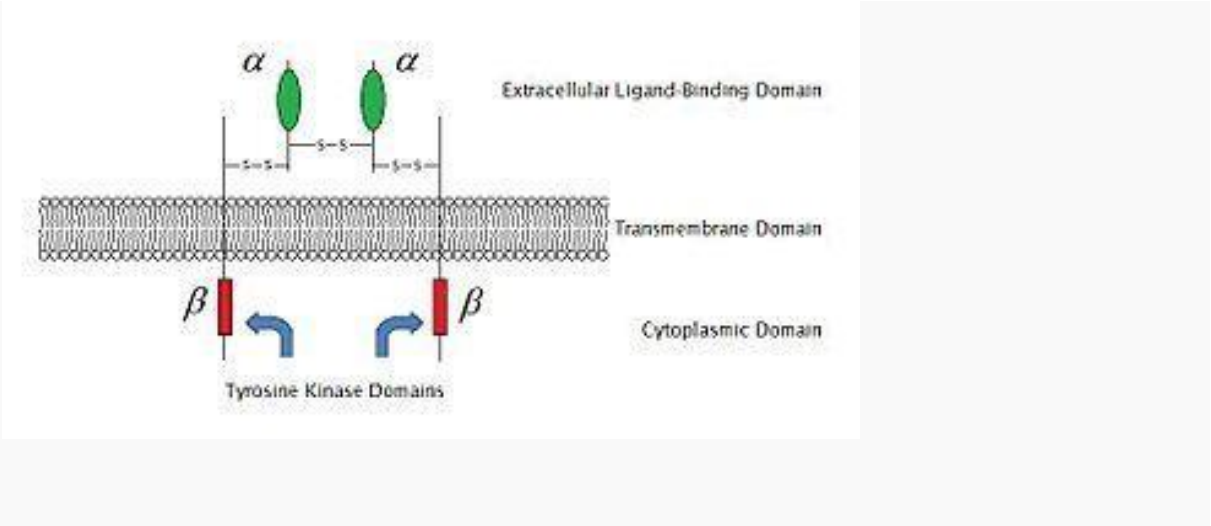
- **Enzyme-linked receptors** are either enzymes themselves, or are directly activate associated enzymes. These are typically single-pass transmembrane receptors, with the enzymatic component of the receptor kept intracellular. The majority of enzyme-linked receptors are, or associate with, protein kinases.
- **G protein-coupled receptors** are integral membrane proteins that possess seven transmembrane helices. These receptors activate a G protein with ligand binding. G-protein is a trimeric protein. The 3 subunits are called α 、 β and γ. The α subunit can bind with guanosine diphosphate, GDP. This causes phosphorylation of the GDP toguanosine triphosphate, GTP, and activates the α subunit to then dissociate from the β and γ subunits. The activated α subunit can further effect intracellular signaling proteins or target functional proteins directly.

Ion channel-linked receptor

During the signal transduction event in a neuron, the neurotransmitter binds to the receptor and alters the conformation of the protein. This opens the ion channel, allowing extracellular ions into the cell. Ion permeability of the plasma membrane is altered, and this transforms the extracellular chemical signal into an intracellular electric signal which alters the cell excitability.

Acetylcholine receptor is a receptor linked to a cation channel. The protein consists of 4 subunits: α, β, γ, and δ subunits. There are two α subunits, with one acetylcholine binding site each. This receptor can exist in three conformations. The closed and unoccupied state is the native protein conformation. As two molecules of acetylcholine both bind to the binding sites on α subunits, the conformation of the receptor is altered and the gate is opened, allowing for the entry of many ions and small molecules. However, this open and occupied state only lasts for a minor duration and then the gate is closed, becoming the closed and occupied state. The two molecules of acetylcholine will soon dissociate from the receptor, returning it to the native closed and unoccupied state.

Enzyme-linked receptors



As of 2009, there are 6 known types of enzyme-linked receptors: Receptor tyrosine kinases; Tyrosine kinase associated receptors; Receptor-like tyrosine phosphatases; Receptor

serine/threonine kinases; Receptor Guanylyl cyclases and Histidine kinase associated receptors. Receptor tyrosine kinases have the largest population and widest application. The majority of these molecules are receptors for growth factors and hormones like epidermal growth factor (EGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), insulin, nerve growth factor (NGF) etc.

Most of these receptors will dimerize after binding with their ligands, in order to activate further signal transductions. For example, after the epidermal growth factor (EGF) receptor binds with its ligand EGF, the two receptors dimerize and then undergo phosphorylation of the tyrosine residues in the enzyme portion of each receptor molecule. This will activate the tyrosine protein kinase and catalyze further intracellular reactions.

G protein-coupled receptors

G protein-coupled receptors comprise a large protein family of transmembrane receptors. They are found only in eukaryotes.[11] The ligands which bind and activate these receptors include: photosensitive compounds, odors, pheromones, hormones, and neurotransmitters. These vary in size from small molecules to peptides and large proteins. G protein-coupled receptors are involved in many diseases, and thus are the targets of many modern medicinal drugs.

There are two principal signal transduction pathways involving the G-protein coupled receptors: cAMP signal pathway and Phosphatidylinositol signal pathway. Both activate aG protein ligand binding. G-protein is a trimeric protein. The 3 subunits are called α 、 β and γ. The α subunit can bind with guanosine diphosphate, GDP. This causesphosphorylation of the GDP to guanosine triphosphate, GTP, and activates the α subunit, which then dissociates from the β and γ subunits. The activated α subunit can further affect intracellular signaling proteins or target functional proteins directly.

NUCLEAR RECEPTORS

In the field of molecular biology, **nuclear receptors** are a class of proteins found within cells that are responsible for sensing steroid and thyroid hormones and certain other molecules. In response, these receptors work with other proteins to regulate the expression of specific genes, thereby controlling the development, homeostasis, and metabolism of the organism.

Nuclear receptors have the ability to directly bind to DNA and regulate the expression of adjacent genes, hence these receptors are classified as transcription factors. The regulation of gene expression by nuclear receptors generally only happens when a ligand — a molecule that affects the receptor's behavior — is present. More specifically, ligand binding to a nuclear receptor results in a conformational change in the receptor, which, in turn, activates the receptor, resulting in up- or down-regulation of gene expression.

A unique property of nuclear receptors that differentiates them from other classes of receptors is their ability to directly interact with and control the expression of genomic DNA. As a consequence, nuclear receptors play key roles in both embryonic development and adult homeostasis. As discussed below, nuclear receptors may be classified according to either mechanism or homology.

- Group A: Estrogen receptor (Sex hormones: Estrogen)
  - 1: Estrogen receptor-α (ERα; NR3A1, *ESR1*)
  - 2: Estrogen receptor-β (ERβ; NR3A2, *ESR2*)
- Group B: Estrogen related receptor
  - 1: Estrogen-related receptor-α (ERRα; NR3B1, *ESRRA*)
  - 2: Estrogen-related receptor-β (ERRβ; NR3B2, *ESRRB*)
  - 3: Estrogen-related receptor-γ (ERRγ; NR3B3, *ESRRG*)
- Group C: 3-Ketosteroid receptors
  - 1: Glucocorticoid receptor (GR; *NR3C1*) (Cortisol)
  - 2: Mineralocorticoid receptor (MR; *NR3C2*) (Aldosterone)
  - 3: Progesterone receptor (PR; NR3C3, *PGR*) (Sex hormones: Progesterone)
  - 4: Androgen receptor (AR; NR3C4, *AR*) (Sex hormones: Testosterone)

Structure

Intracellular steroid hormone receptors share a common structure of four units that are functionally homologous, so-called "domains":

1. *Variable domain*: It begins at the N-terminal and is the most variable domain between the different receptors.
2. *DNA binding domain*: This centrally located highly conserved DNA binding domain (DBD) consists of two non-repetitive globular motifs[3] where zinc is coordinated with four cysteine and no histidine residues. Their secondary and tertiary structure is distinct from that of classic zinc fingers.[4] This region controls which gene will be activated. On DNA it interacts with the hormone response element (HRE).
3. *Hinge region*: This area controls the movement of the receptor to the nucleus.
4. *Hormone binding domain*: The moderately conserved ligand-binding domain (LBD) can include a nuclear localization signal, amino-acid sequences capable of binding chaperones and parts of dimerization interfaces. Such receptors are closely related to chaperones (namely heat shock proteins hsp90 and hsp56), which are required to maintain their inactive (but receptive) cytoplasmic conformation. At the end of this domain is the C-terminal. The terminal connects the molecule to its pair in the homodimer or heterodimer. It may affect the magnitude of the response.

MECHANISM OF ACTION

Mechanism of action and subcellular distribution, nuclear receptors may be classified into at least two classes. Nuclear receptors that bind steroid hormones are all classified as type I receptors. Only type I receptors have a heat shock protein (HSP) associated with the inactive receptor that will be released when the receptor interacts with the ligand. Type I receptors may be found in homodimer or heterodimer forms. Type II nuclear receptors have no HSP, and in contrast to the classical type I receptor are located in the cell nucleus.Free (that is, unbound)

steroids enter the cell cytoplasm and interact with their receptor. In this process heat shock protein is dissociated, and the activated receptor-ligand complex is translocated into the nucleus.

After binding to the ligand (steroid hormone), steroid receptors often form dimers. In the nucleus, the complex acts as a transcription factor, augmenting or suppressingtranscription particular genes by its action on DNA.

Type II receptors are located in the nucleus. Thus, their ligands pass through the cell membrane and cytoplasm and enter the nucleus where they activate the receptor without release of HSP. The activated receptor interacts with the hormone response element and the transcription process is initiated as with type I receptors.

Non-genomic

The cell membrane aldosterone receptor has shown to increase the activity of the basolateral Na/K ATPase, ENaC sodium channels and ROMK potassium channels of theprincipal cell in the distal tubule and cortical collecting duct of nephrons (as well as in the large bowel and possibly in sweat glands).

There is some evidence that certain steroid hormone receptors can extend through lipid bilayer membranes at the surface of cells and might be able to interact with hormones that remain outside of cells.

Steroid hormone receptors can also function outside of the nucleus and couple to cytoplasmic signal transduction proteins such as PI3k and Akt kinase.

Other

A new class of steroid hormone receptors has recently been elucidated and these new receptors are found on the cell membrane. New studies suggest that along with the well documented intracellular receptors that cell membrane receptors are present for several steroid hormones and that their cellular responses are much quicker than the intracellular receptors.[9]

G protein-coupled receptors

At least one G protein-coupled receptor, GPR30 has been found to function as a steroid receptor. GPR30 binds to and is activated by estrogen.

Ion channels

Neuroactive steroids bind to and modulate the activity of several ion channels including the GABAA NMDA, and sigma receptors.

The steroid progesterone has been found to modulate the activity of CatSper (cation channels of sperm) voltage-gated Ca^{2+} channels. Since eggs release progesterone, sperm may use progesterone as a homing signal to swim toward eggs (chemotaxis).

SHBG/SHBG-R complex

Sex hormone-binding globulin (SHBG) is thought to mainly function as a transporter and reservoir for the estradiol and testosterone sex hormones. However it has also been demonstrated that SHBG can bind to a cell surface receptor (SHBG-R). The SHBG-R has not been completely characterized. A subset of steroids are able to bind to the SHBG/SHBG-R complex resulting in an activation of adenylyl cyclase and synthesis of the cAMP second messenger.[19] Hence the SHBG/SHBG-R complex appears to act as atransmembrane steroid receptor that is capable of transmitting signals to the interior of cells.

Cell signaling is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity as well as normal tissue homeostasis. Errors in cellular information processing are responsible for diseases such as cancer, autoimmunity, and diabetes. By understanding cell signaling, diseases may be treated effectively and, theoretically, artificial tissues may be created.

Traditional work in biology has focused on studying individual parts of cell signaling pathways. Systems biology research helps us to understand the underlying structure of cell signaling networks and how changes in these networks may affect the transmission and flow of information. Such networks are complex systems in their organization and may exhibit a number of emergent properties including bistability and ultrasensitivity. Analysis of cell signaling networks requires a combination of experimental and theoretical approaches including the development and analysis of simulations and modeling. Long-range allostery is often a significant component of cell signaling events

Signaling within, between, and among cells is subdivided into the following classifications:

- *Intracrine* signals are produced by the target cell that stay within the target cell.
- *Autocrine* signals are produced by the target cell, are secreted, and affect the target cell itself via receptors. Sometimes autocrine cells can target cells close by if they are the same type of cell as the emitting cell. An example of this are immune cells.
- *Juxtacrine* signals target adjacent (touching) cells. These signals are transmitted along cell membranes via protein or lipid components integral to the membrane and are capable of affecting either the emitting cell or cells immediately adjacent.
- *Paracrine* signals target cells in the vicinity of the emitting cell. Neurotransmitters represent an example.
- *Endocrine* signals target distant cells. Endocrine cells produce hormones that travel through the blood to reach all parts of the body.

Types of Signals

There are four categories of chemical signaling found in multicellular organisms: paracrine signaling, endocrine signaling, autocrine signaling, and direct signaling across gap junctions (Figure 1). The main difference between the different categories of signaling is the distance that the signal travels through the organism to reach the target cell. Not all cells are affected by the same signals.

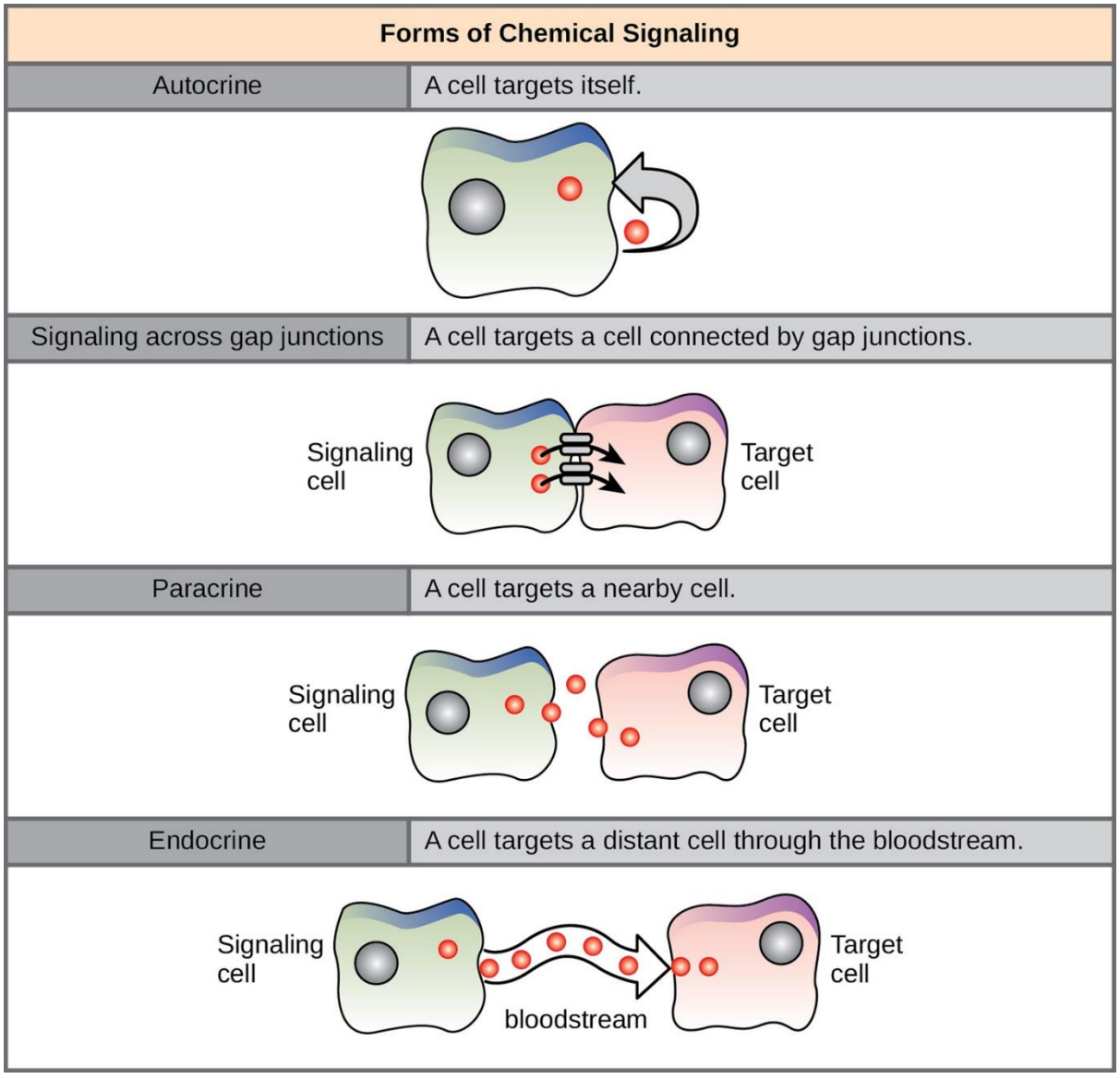


Fig. Cell signaling

Paracrine Signaling

Signals that act locally between cells that are close together are called **paracrine signals**. Paracrine signals move by diffusion through the extracellular matrix. These types of signals usually elicit quick responses that last only a short amount of time. In order to keep the response localized, paracrine ligand molecules are normally quickly degraded by enzymes or removed by neighboring cells. Removing the signals will reestablish the concentration gradient for the signal, allowing them to quickly diffuse through the intracellular space if released again.

One example of paracrine signaling is the transfer of signals across synapses between nerve cells. A nerve cell consists of a cell body, several short, branched extensions called dendrites that receive stimuli, and a long extension called an axon, which transmits signals to other nerve cells or muscle cells. The junction between nerve cells where signal transmission occurs is called a synapse. A **synaptic signal** is a chemical signal that travels between nerve cells. Signals within the nerve cells are propagated by fast-moving electrical impulses. When these impulses reach the end of the axon, the signal continues on to a dendrite of the next cell by the release of chemical ligands called **neurotransmitters** by the presynaptic cell (the cell emitting the signal). The neurotransmitters are transported across the very small distances between nerve cells, which are called **chemical synapses** (Figure 2). The small distance between nerve cells allows the signal to travel quickly; this enables an immediate response, such as, Take your hand off the stove!

When the neurotransmitter binds the receptor on the surface of the postsynaptic cell, the electrochemical potential of the target cell changes, and the next electrical impulse is launched. The neurotransmitters that are released into the chemical synapse are degraded quickly or get reabsorbed by the presynaptic cell so that the recipient nerve cell can recover quickly and be prepared to respond rapidly to the next synaptic signal.

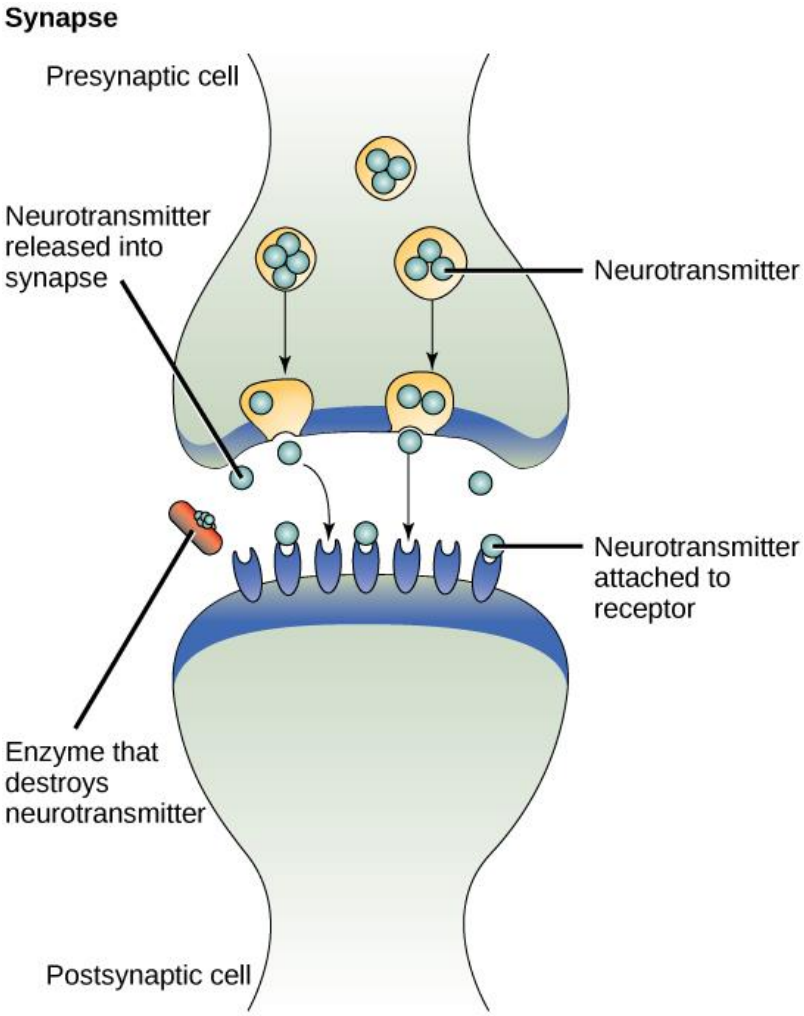


Figure 2. The distance between the presynaptic cell and the postsynaptic cell—called the synaptic gap—is very small and allows for rapid diffusion of the neurotransmitter. Enzymes in the synaptic cleft degrade some types of neurotransmitters to terminate the signal.

Endocrine Signaling

Signals from distant cells are called **endocrine signals**, and they originate from **endocrine cells**. (In the body, many endocrine cells are located in endocrine glands, such as the thyroid gland, the hypothalamus, and the pituitary gland.) These types of signals usually produce a slower response but have a longer-lasting effect. The ligands released in endocrine signaling are called hormones, signaling molecules that are produced in one part of the body but affect other body regions some distance away.

Hormones travel the large distances between endocrine cells and their target cells via the bloodstream, which is a relatively slow way to move throughout the body. Because of their form of transport, hormones get diluted and are present in low concentrations when they act on

their target cells. This is different from paracrine signaling, in which local concentrations of ligands can be very high.

Autocrine Signalling

Autocrine signals are produced by signaling cells that can also bind to the ligand that is released. This means the signaling cell and the target cell can be the same or a similar cell (the prefix *auto*- means self, a reminder that the signaling cell sends a signal to itself). This type of signaling often occurs during the early development of an organism to ensure that cells develop into the correct tissues and take on the proper function. Autocrine signaling also regulates pain sensation and inflammatory responses. Further, if a cell is infected with a virus, the cell can signal itself to undergo programmed cell death, killing the virus in the process. In some cases, neighboring cells of the same type are also influenced by the released ligand. In embryological development, this process of stimulating a group of neighboring cells may help to direct the differentiation of identical cells into the same cell type, thus ensuring the proper developmental outcome.

Direct Signalling Across Gap Junctions

Gap junctions in animals and plasmodesmata in plants are connections between the plasma membranes of neighboring cells. These water-filled channels allow small signaling molecules, called **intracellular mediators**, to diffuse between the two cells. Small molecules, such as calcium ions (Ca^{2+}), are able to move between cells, but large molecules like proteins and DNA cannot fit through the channels. The specificity of the channels ensures that the cells remain independent but can quickly and easily transmit signals. The transfer of signaling molecules communicates the current state of the cell that is directly next to the target cell; this allows a group of cells to coordinate their response to a signal that only one of them may have received. In plants, plasmodesmata are ubiquitous, making the entire plant into a giant, communication network.

Cell signaling in multicellular organisms

A particular molecule is generally used in diverse modes of signaling, and therefore a classification by mode of signaling is not possible. At least three important classes of signaling molecules are widely recognized, although non-exhaustive and with imprecise boundaries, as such membership is non-exclusive and depends on the context:

- Hormones are the major signaling molecules of the endocrine system, though they often regulate each other's secretion via local signaling (e.g. islet of Langerhans cells), and most are also expressed in tissues for local purposes (e.g. angiotensin) or

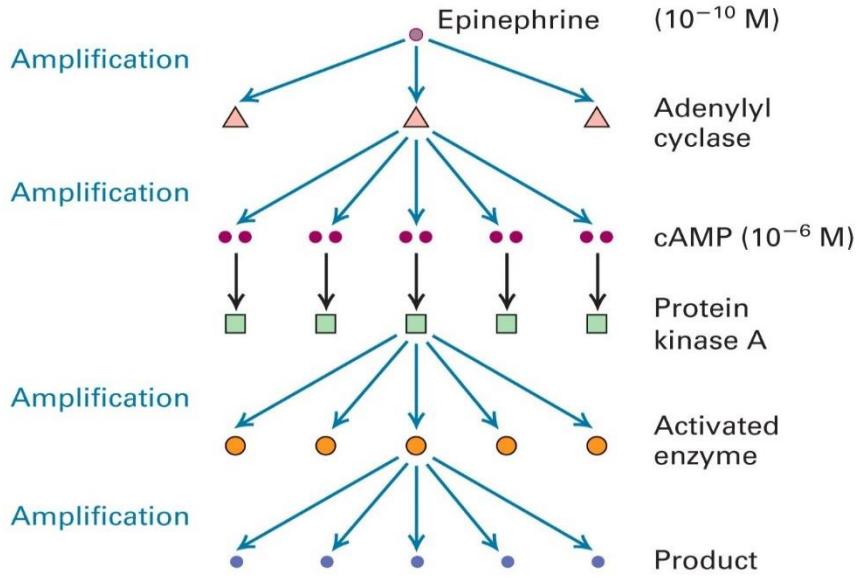
failing that, structurally related molecules are (e.g. PTHrP).

- Neurotransmitters are signaling molecules of the nervous system, also including neuropeptides and neuromodulators. Neurotransmitters like the catecholamines are also secreted by the endocrine system into the systemic circulation.
- Cytokines are signaling molecules of the immune system, with a primary paracrine or juxtacrine role, though they can during significant immune responses have a strong presence in the circulation, with systemic effect. Growth factors can be considered as cytokines or a different class.

Signaling molecules can belong to several chemical classes: lipids, phospholipids, amino acids, monoamines, proteins, glycoproteins, or gases. Signaling molecules binding surface receptors are generally large and hydrophilic (e.g. TRH, Vasopressin, Acetylcholine), while those entering the cell are generally small and hydrophobic (e.g.glucocorticoids, thyroid hormones, cholecalciferol, retinoic acid), but important exceptions to both are numerous, and a same molecule can act both via surface receptor or in an intracrine manner to different effects.[10] In intracrine signaling, once inside the cell, a signaling molecule can bind to intracellular receptors, other elements, or stimulate enzymeactivity (e.g. gasses). The intracrine action of peptide hormones remains a subject of debate.

Signal Amplification in Signalling Pathways

At each step of many signal transduction pathways, the number of activated participants in the pathway increases. This is referred to as signal amplification, and hormone signaling pathways are often referred to as amplification cascades. For example, one epinephrine-activated GPCR activates 100s of G_{as}-GTP complexes, which in turn activate 100s of adenylyl cyclase molecules, that each produce hundreds of cAMP molecules, and so on. The overall amplification associated with epinephrine signaling is estimated to be ~10^8-fold.



cAMP-dependent pathway

In the field of molecular biology, the cAMP-dependent pathway**, also known as the** adenylyl cyclase pathway**, is a G protein-coupled receptor-triggered signaling cascadeused in cell communication.**

Mechanism

G protein-coupled receptors (GPCRs) are a large family of integral membrane proteins that respond to a variety of extracellular stimuli. Each GPCR binds to and is activated by a specific ligand stimulus that ranges in size from small molecule catecholamines, lipids, or neurotransmitters to large protein hormones. When a GPCR is activated by its extracellular ligand, a conformational change is induced in the receptor that is transmitted to an attached intracellular heterotrimeric G protein complex. The G_S alpha subunit of the stimulated G protein complex exchanges GDP for GTP and is released from the complex.

In a cAMP-dependent pathway, the activated G_S alpha subunit binds to and activates an enzyme called adenylyl cyclase, which, in turn, catalyzes the conversion of ATP intocyclic adenosine monophosphate (cAMP). Increases in concentration of the second messenger cAMP may lead to the activation of

- cyclic nucleotide-gated ion channels
- exchange proteins activated by cAMP (EPAC) such as RAPGEF3
- popeye domain containing proteins (Popdc)
- an enzyme called protein kinase A (PKA).
- The PKA enzyme is also known as cAMP-dependent enzyme because it gets activated only if cAMP is present. Once PKA is activated, it phosphorylates a number of other proteins including:
- enzymes that convert glycogen into glucose
- enzymes that promote muscle contraction in the heart leading to an increase in heart rate
- transcription factors, which regulate gene expression

Specificity of signaling between a GPCR and its ultimate molecular target through a cAMP- dependent pathway may be achieved through formation of a multiprotein complex that includes the GPCR, adenylyl cyclase, and the effector protein

MECHANISM

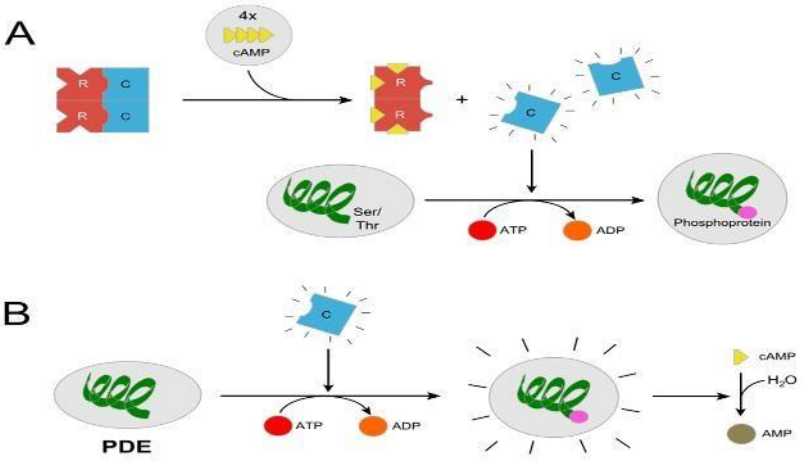
In humans, cAMP works by activating protein kinase A (PKA, cAMP-dependent protein kinase), and, thus, further effects mainly depend on cAMP-dependent protein kinase, which vary based on the type of cell.

cAMP-dependent pathway is necessary for many living organisms and life processes.

Many different cell responses are mediated by cAMP. These include increase in heart rate, cortisol secretion, and breakdown of glycogen and fat.

This pathway can activate enzymes and regulate gene expression. The activation of preexisting enzymes is a much faster process, whereas regulation of gene expression is much longer and can take up to hours. The cAMP pathway is studied through loss of function (inhibition) and gain of function (increase) of cAMP.

If cAMP-dependent pathway is not controlled, it can ultimately lead to hyper-proliferation, which may contribute to the development and/or progression of cancer.



The PKA enzyme is also known as cAMP-dependent enzyme because it gets activated only if cAMP is present. Below is a list of the steps involved in PKA activation:

1. Cytosolic cAMP increases

2. Two cAMP molecules bind to each PKA regulatory subunit

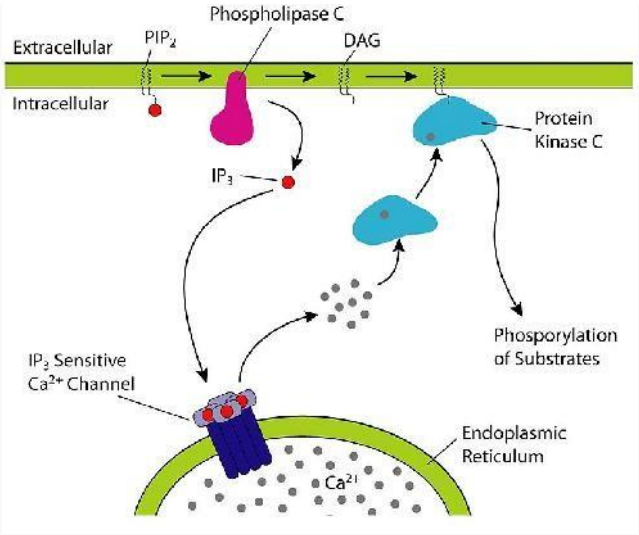
3. The regulatory subunits move out of the active sites of the catalytic subunits and the R2C2 complex dissociates

4. The free catalytic subunits interact with proteins to phosphorylate Ser or Thr residues.

INOSITOL TRISPHOSPHATE

Inositol trisphosphate or **inositol 1,4,5-trisphosphate** (also commonly known as **triphosphoinositol**; abbreviated **InsP3** or**Ins3P** or **IP3**), together with diacylglycerol (DAG), is a secondary messenger molecule used in signal transduction and lipid signalingin biological cells. While DAG stays inside the membrane, IP3 is soluble and diffuses through the cell. It is made by hydrolysis ofphosphatidylinositol 4,5-bisphosphate (PIP2), a phospholipid that is located in the plasma membrane, by phospholipase C (PLC).

SIGNALING



PATHWAY

Increases in the intracellular Ca^{2+} concentrations are often a result of IP3 activation. When a ligand binds to a G protein-coupled receptor(GPCR) that is coupled to a Gq heterotrimeric G protein, the α-subunit of Gq can bind to and induce activity in the PLC isozyme PLC-β, which results in the cleavage of PIP2 into IP3 and DAG.

If a receptor tyrosine kinase (RTK) is involved in activating the pathway, the isozyme PLC-γ has tyrosine residues that can become phosphorylated upon activation of an RTK, and this will activate PLC-γ and allow it to cleave PIP2 into DAG and IP3. This occurs in cells that are capable of responding to growth factors such as insulin, because the growth factors are the ligands responsible for activating the RTK.

IP3 (also abbreviated Ins3P) is a soluble molecule and is capable of diffusing through the cytoplasm to the ER, or the sarcoplasmic reticulum (SR) in the case of muscle cells, once it has been produced by the action of PLC. Once at the ER, IP3 is able to bind to the Ins3P receptor (Ins3PR) on a ligand-gated Ca^{2+} channel that is found on the surface of the ER. The binding of IP3 (the ligand in this case) to InsP3R triggers the opening of the Ca^{2+} channel, and thus release of Ca^{2+} into the cytoplasm. In heart muscle cells this increase in Ca^{2+} activates the ryanodine receptor-operated channel on the SR, results in further increases in Ca^{2+} through a process known as calcium-induced calcium release. IP3 may also activate Ca^{2+} channels on the cell membrane indirectly, by increasing the intracellular Ca^{2+} concentration.

G-PROTEIN-DEPENDENT SIGNALING

There are three main G-protein-mediated signaling pathways, mediated by four sub-classes of G-proteins distinguished from each other by sequence homology (Gαs, Gαi/o, Gαq/11, and Gα12/13). Each sub-class of G-protein consists of multiple proteins, each the product of multiple genes and/or splice variations that may imbue them with differences ranging from subtle to distinct with regard to signaling properties, but in general they appear reasonably grouped into four classes. Because the signal transducing properties of the various possible βγ combinations do not appear to radically differ from one another, these classes are defined according to the isoform of their α-subunit.

While most GPCRs are capable of activating more than one Gα-subtype, they also show a preference for one subtype over another. When the subtype activated depends on the ligand that is bound to the GPCR, this is called functional selectivity(also known as agonist-directed trafficking, or conformation-specific agonism). However, the binding of any single particularagonist may also initiate activation of multiple different G-proteins, as it may be capable of stabilizing more than one conformation of the GPCR's GEF domain, even over the course of a single interaction. In addition, a conformation that preferably activates one isoform of Gα may activate another if the preferred is less available. Furthermore, feedback pathways may result in receptor modifications (e.g., phosphorylation) that alter the G-protein preference. Regardless of these various nuances, the GPCR's preferred coupling partner is usually defined according to the G-protein most obviously activated by the endogenous ligand under most physiological and/or experimental conditions.

Gα signaling

1. The effector of both the Gαs and Gαi/o pathways is the cyclic-adenosine monophosphate (cAMP)-generating enzyme adenylate cyclase, or AC. While there are ten different AC gene products in mammals, each with subtle differences in tissue distribution and/or function, all catalyze the conversion of cytosolic adenosine triphosphate (ATP) to cAMP, and all are directly stimulated by G-proteins of the Gαs class. In contrast, however, interaction with Gα subunits of the Gαi/o type inhibits AC from generating cAMP. Thus, a GPCR coupled to Gαs counteracts the actions of a GPCR coupled to Gαi/o, and vice versa. The level of cytosolic cAMP may then determine the activity of variousion channels as well as members of the ser/thr- specific protein kinase A (PKA) family. Thus cAMP is considered a second messenger and PKA a secondary effector.
2. The effector of the Gαq/11 pathway is phospholipase C-β (PLCβ), which catalyzes the cleavage of membrane-bound phosphatidylinositol 4,5-biphosphate (PIP2) into the second messengers inositol (1,4,5) trisphosphate

(IP3) and diacylglycerol (DAG). IP3 acts on IP3 receptors found in the membrane of the endoplasmic reticulum (ER) to elicit Ca^{2+} release from the ER, while DAG diffuses along the plasma membrane where it may activate any membrane localized forms of a second ser/thr kinase calledprotein kinase C (PKC). Since many isoforms of PKC are also activated by increases in intracellular Ca^{2+}, both these pathways can also converge on each other to signal through the same secondary effector. Elevated intracellular Ca^{2+} also binds and allosterically activates proteins called calmodulins, which in turn go on to bind and allosterically activate enzymes such as Ca^{2+}/calmodulin-dependent kinases (CAMKs).

3. The effectors of the Gα12/13 pathway are three RhoGEFs (p115-RhoGEF, PDZ-RhoGEF, and LARG), which, when bound to Gα12/13 allosterically activate the cytosolicsmall GTPase, Rho. Once bound to GTP, Rho can then go on to activate various proteins responsible for cytoskeleton regulation such as Rho-kinase (ROCK). Most GPCRs that couple to Gα12/13 also couple to other sub-classes, often Gαq/11.

Gβγ signalling

The above descriptions ignore the effects of Gβγ–signalling, which can also be important, in particular in the case of activated Gαi/o-coupled GPCRs. The primary effectors of Gβγ are various ion channels, such as G-protein-regulated inwardly rectifying

K^+ channels (GIRKs), P/Q- and N-type voltage-gated Ca^{2+} channels, as well as some isoforms of AC and PLC, along with some phosphoinositide-3-kinase (PI3K) isoforms.

CALCIUM ION FLUX

Calcium ions are also important intracellular messengers. In fact, calcium ions are probably the most widely used intracellular messengers. Calcium (Ca^{2+}) plays an essential role in the physiology and biochemistry of organisms and the cell. It plays role in common signalling mechanism because once it enters the cytoplasm it exerts allosteric regulatory affects on many enzymes and proteins. Calcium is a second messenger produced by indirect signal transduction pathways such as G-protein coupled receptors. Calcium ions (Ca^{2+}) impact nearly every aspect of cellular life. The principles of Ca^{2+}signaling, from changes in protein conformations driven by Ca^{2+} to the mechanisms that control Ca^{2+} levels in the cytoplasm and organelles. The highly localized nature of Ca^{2+} -mediated signal transduction and its specific roles in excitability, exocytosis, motility, apoptosis, and transcription. Normally, cytosolic calcium [Ca^{2+}] ions is kept very low (10^{-7} M) by the action of Ca^{2+} pumps in the ER, mitochondria and plasma membrane. Hormonal, neural, or other stimuli cause either an influx of Ca^{2+} into the cell through specific Ca^{2+} channels in the plasma membrane or the release of sequestered Ca^{2+} from the ER or mitochondria, in either case raising the

cytosolic [Ca^{2+}] and triggering a cellular response. This phenomenon is called Calcium ion flux.

Role of Calcium in cell signalling:

In response to many different signals, a rise in the concentration of Ca^{2+} in the cytosol triggers many types of events such as:

- Muscle contraction
- Exocytosis

a) Release of neurotransmitters at synapses (and essential for the long-term synaptic changes that produce Long-Term Potentiation (LTP) and Long-Term Depression (LTD)

b) Secretion of hormones like insulin

- Activation of T cells and B cells when they bind antigen with their antigen receptors (TCRs and BCRs respectively)
- Adhesion of cells to the extracellular matrix (ECM)
- Apoptosis
- A variety of biochemical changes mediated by P rotein K inase C (PKC)

The concentration of calcium ions in a particular cellular compartment is guarded by the regulated activity of Ca^{2+} pumps, Ca^{2+} exchangers, and/or Ca^{2+} ion channels located within the membranes that surrounds the compartment. There are two major types of signaling receptors - G-protein coupled receptor (GPCRs) and receptor protein-tyrosine kinases (RTKs). One of the most important pathways of intracellular signalling is based on the use of second messengers derived from the membrane phospholipids phosphatidylinositol 4, 5-bisphosphate (PIP2). PIP2 is a minor component of the plasma membrane, localized to the inner leaflet of the phospholipids bilayer. Hydrolysis of PIP2by phospholipase C-β is stimulated by variety of hormones and neurotransmitters. After hydrolysis PIP2 is cleaved into two components- Diacyl glycerol (DAG) and inositol l,4,5- triphosphate (IP3), both are also the secondary messengers of cell. Diacylglycerol and IP3 stimulate distinct down-stream signalling pathways (protein kinase C and Ca^{2+} mobilization, respectively), so PIP2 hydrolysis triggers a two-armed cascade of intracellular signalling.

The first secondary messenger diacylglycerol produced by hydrolysis of PIP2 remains associated with the plasma membrane and activates protein-serine/threonine kinases belonging to the protein kinase C family, many of which play important roles in the control of cell growth and differentiation.

The other second messenger produced by PIP2 cleavage, IP3 , is a small polar molecule that is released into the cytosol, where it acts to signal the release of Ca^{2+} from intracellular stores.

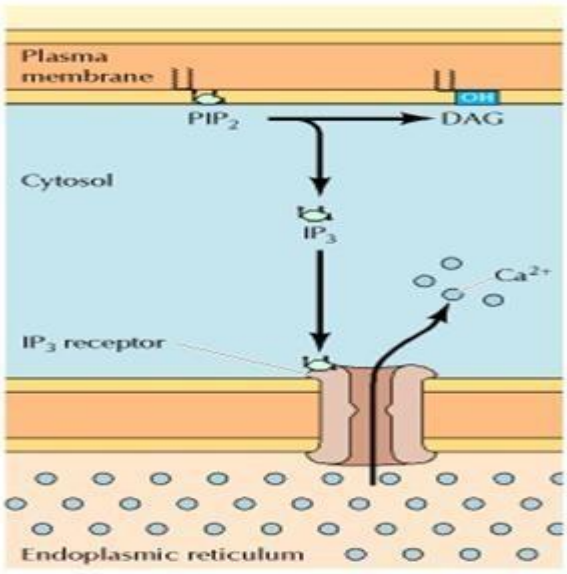


Figure 1: Ca^{2+} mobilization by IP3

Whereas the other important pathway in which extracellular messengers that signal through RTKs can trigger a similar response as in GPCR signal pathway. The major difference is that RTKs activate members of the phospholipase C-γ subfamily, which possess an SH2 domain that allows them to bind to the activated, phosphorylated RTK.

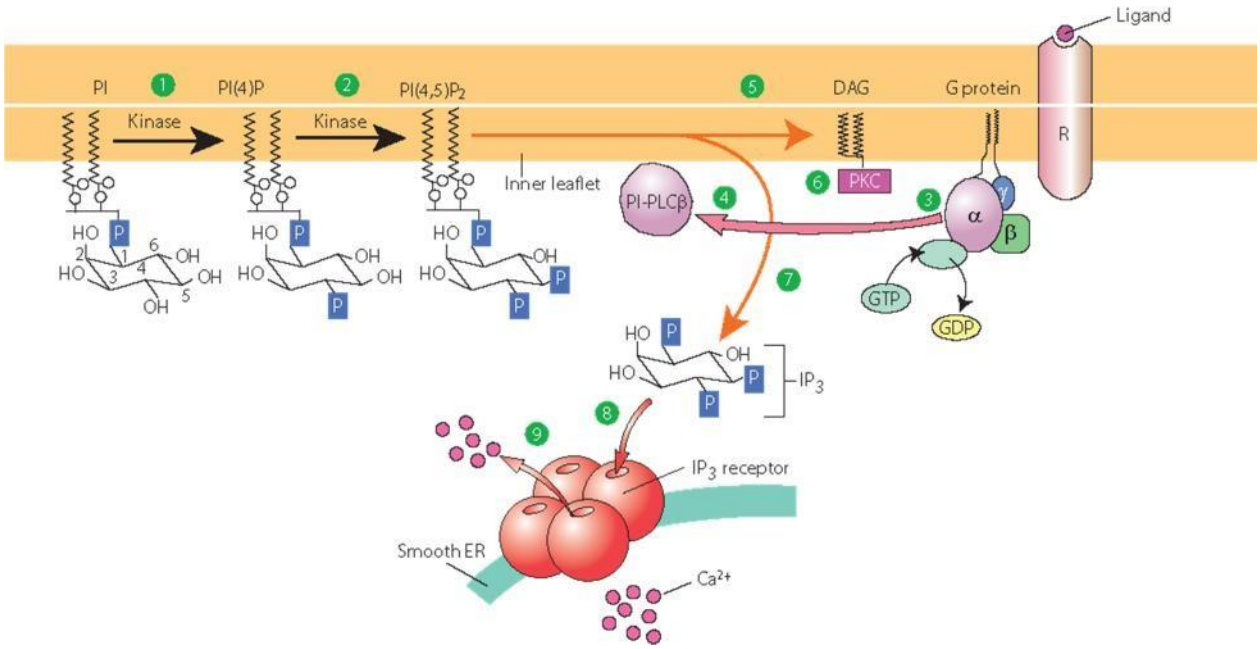


Figure 2: Comparative pathway of GPCR and RTKs for release of calcium from ER

There are numerous other PLC isoforms. For example, PLC δ is activated by Ca^{2+} ions, and PLCε is activated by Ras-GTP. All PLC isoforms carry out the same reaction, producing IP 3 and linking a multitude of cell surface receptors to an increase in

cytoplasmic Ca^{2+} . There is another major route leading to elevation of cytosolic [Ca^{2+}] which involve in synaptic transmission. In this case, a nerve impulse leads to a depolarization of the plasma membrane, which triggers the opening of voltage-gated calcium channels in the plasma membrane, allowing the influx of Ca^{2+} ions from the extracellular medium.

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DEPARTMENT OF BIOMEDICAL ENGINEERING

UNIT – III - Cellbiology & Genetics – SBMA1101

CELL DIVISION

IMPORTANT TERMINOLOGIES

CHROMATID: Each pair of identical DNA molecules after DNA replication, joined at the centromere

CHROMATIN: Protein/DNA complex making the chromosome

CHROMOSOMES: Molecules of DNA complexed with specific proteins responsible in eukaryotes for storage and transmission of genetic information

HISTONES: Five kinds of protein forming complexes with eukaryotic DNA

KINETOCHORE: Structure forming at centromere during mitosis for binding microtubules

NUCLEOSOMES: Basic structural unit of eukaryotic chromosome forming "beads on a string".

Cell division

Cell division generally takes place asexually by mitosis, a process that allows each daughter nucleus to receive one copy of each chromosome. In most eukaryotes, there is also a process of sexual reproduction, typically involving an alternation between haploid generations, wherein only one copy of each chromosome is present, and diploid generations, wherein two copies of each chromosome are present, occurring through meiosis. There is considerable variation in this pattern.

Eukaryotes have a smaller surface area to volume ratio than prokaryotes, and thus have lower metabolic rates and longer generation times. In some multicellular organisms, cells specialized for metabolism will have enlarged surface areas, such as intestinal vili.

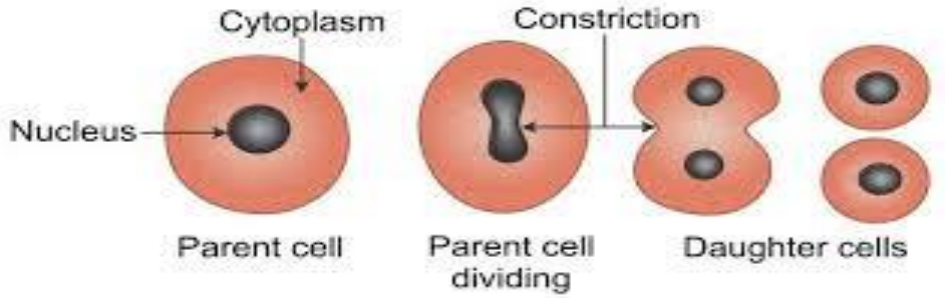
The evolution of sexual reproduction may be a primordial and fundamental characteristic of eukaryotes. Based on a phylogenetic analysis, Dacks and Roger proposed that facultative sex was present in the common ancestor of all eukaryotes. A core set of genes that function in meiosis is present in both *Trichomonasvaginalis* and *Giardia intestinalis*, two organisms previously thought to be asexual. Since these two species are descendants of lineages that diverged early from the eukaryotic evolutionary tree, it was inferred that core meiotic genes, and hence sex, were likely present in a common ancestor of all eukaryotes. Other studies on eukaryotic species once thought to be asexual have revealed evidence for a sexual cycle. For instance, parasitic protozoa of the genus Leishmania have recently been shown to have a sexual cycle. Also, evidence now indicates that amoeba, that were previously regarded as asexual, are anciently sexual and that the majority of present day asexual groups likely arose recently and independently.

The division of cells into daughter cells is called cell division. It is of two types:-

- Amitosis – It is a direct division
- Mitosis – Equational division (in somatic cells)
- Meiosis – Reduction division (in reproductive cells)

AMITOSIS

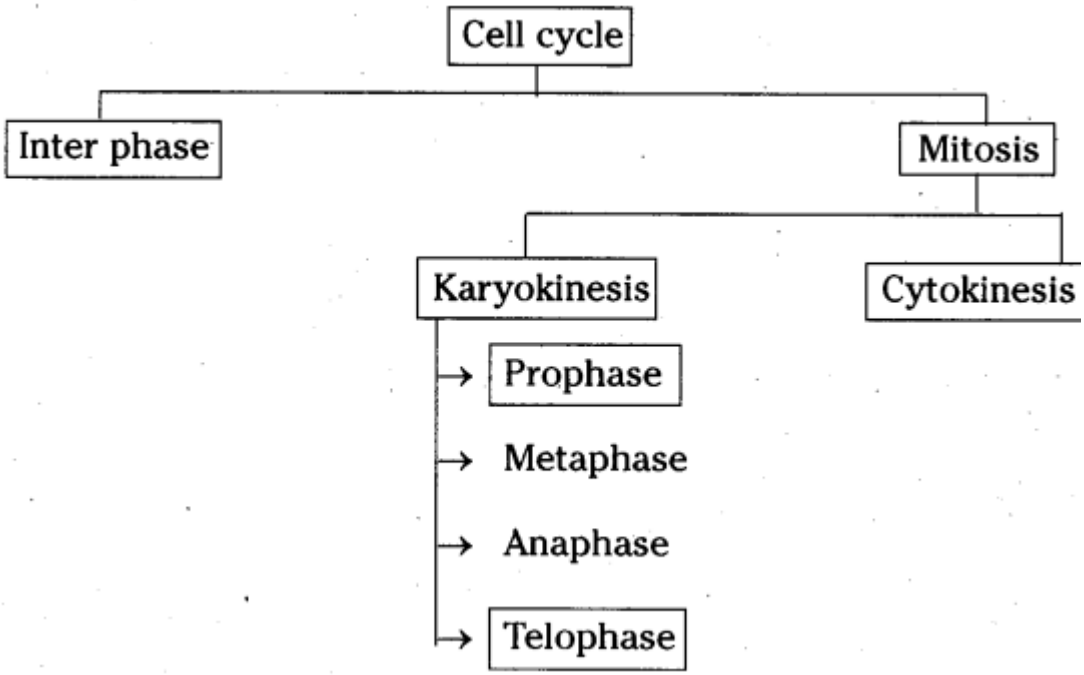
- It is the simplest mode of cell division
- First described by Lemark-1841
- During amitosis the nucleus elongates first and then constriction occurs. This constriction deepens and divides the nucleus into two.
- This is followed by the division of cytoplasm which results in the formation of two daughter cells. The division occurs in unicellular organisms



Stages in amitosis

MITOSIS

- Cells undergo a period of growth called interphase before entering mitosis.
- During the interphase, the genetic material replicates and the organelles prepare for division.
- In the process of mitosis, the parent's cell genome is transferred into the two daughter cells.
- The daughter cells are similar to each other and to their parent cell.
- Mitosis consists of four basic phases: prophase, metaphase, anaphase, and telophase.



INTERPHASE

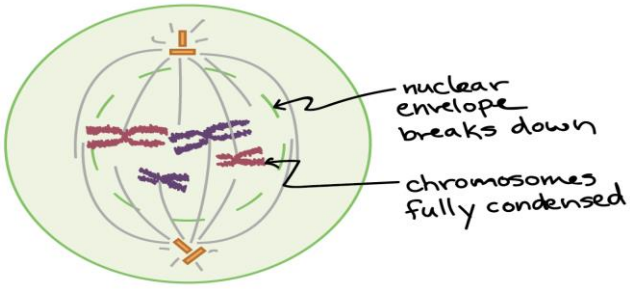
- Genetic material replicates
- Organelles ready for division

KARYOKINESIS

- The division of nucleus into two nuclei
- It has four phases : PMAT (Prophase , Metaphase, Anaphase, Telophase)

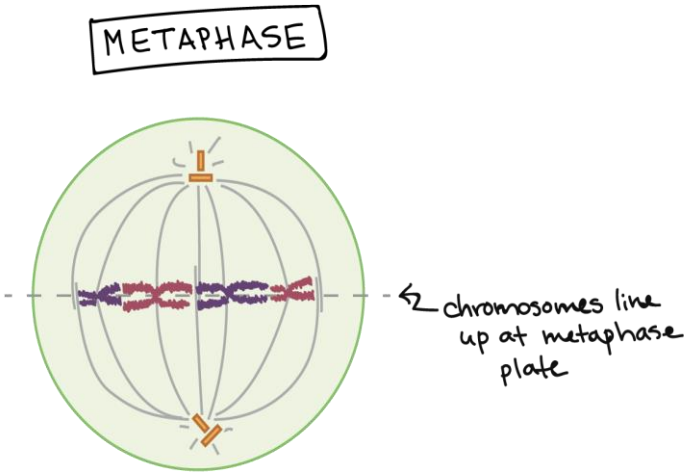
PROPHASE

- Condensation of chromatin material into chromosomes starts taking place
- Nuclear membrane starts disappearing
- Nucleolus disappears



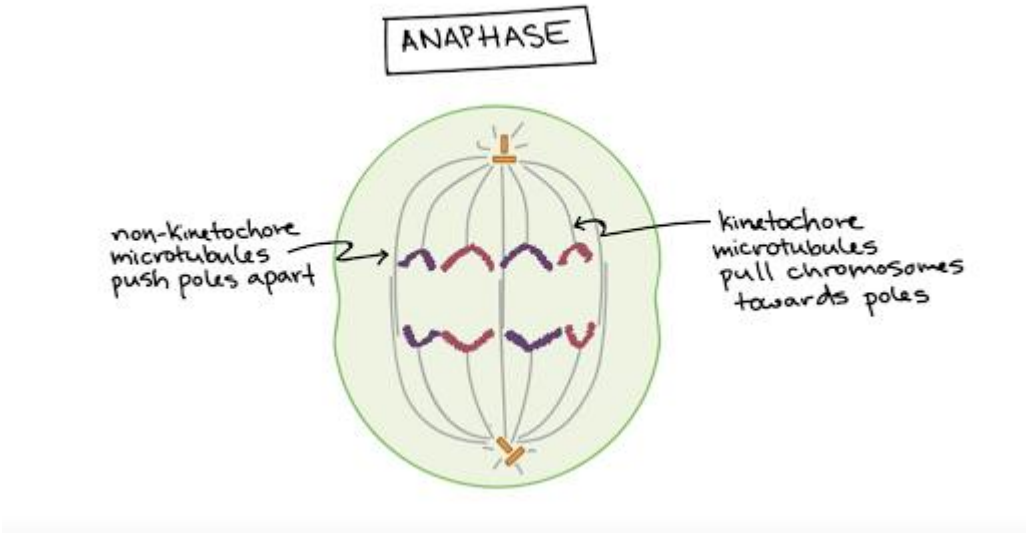
METAPHASE

- The completely condensed chromosomes align themselves on the equatorial plane
- Centromere splits, spindle fibres get attached to each chromatid



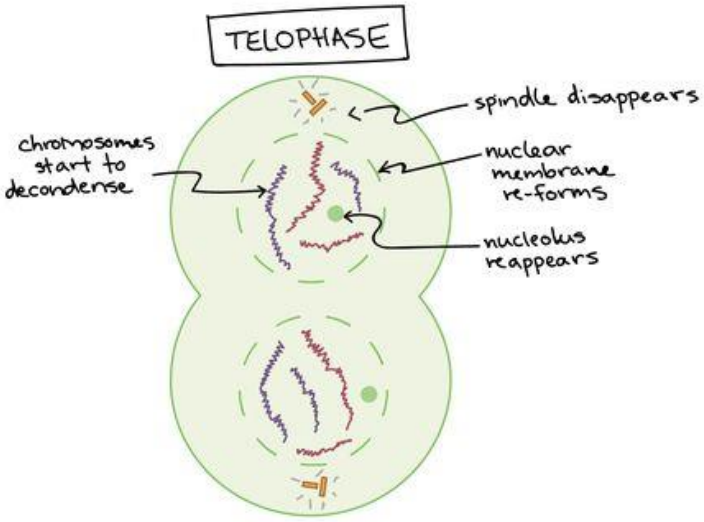
ANAPHASE

- Spindle fibres start contracting, pulling the chromatids toeards the opposite poles



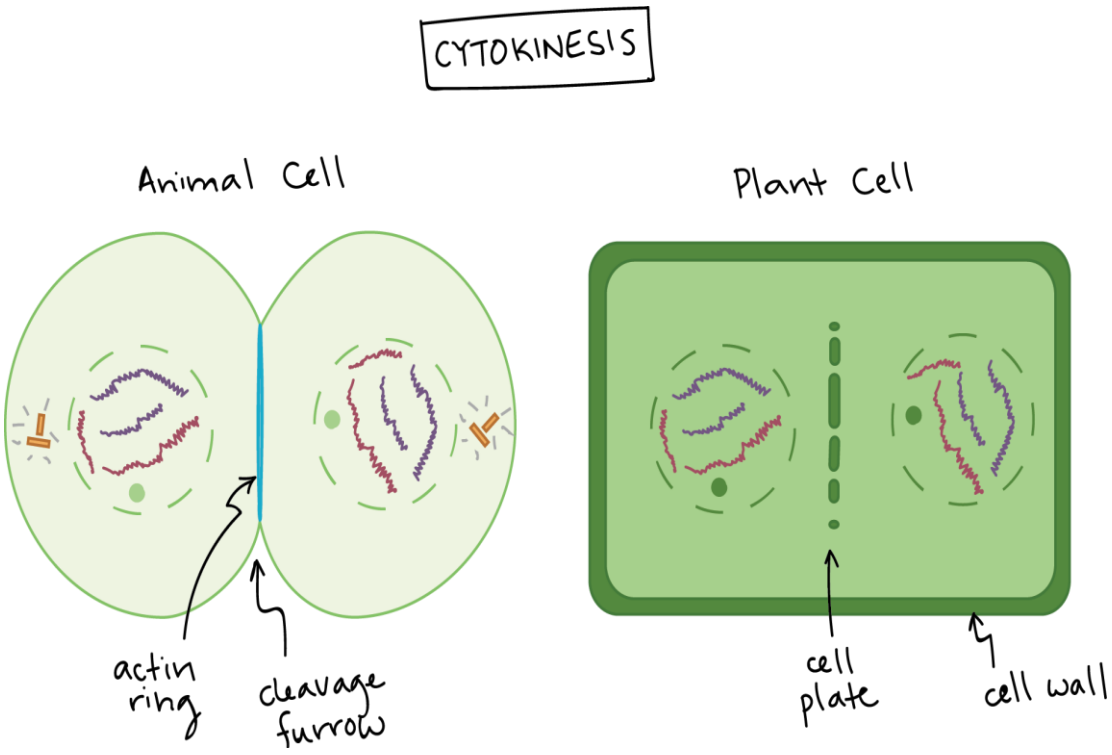
TELOPHASE

- Chromosomes convert back to chromatin
- Nuclear membrane starts appearing around the separated chromatin
- Nucleolus starts reappearing



CYTOKINESIS

- The division of cytoplasm into two daughter cells is called cytokinesis
- In animal cells, the area of cell membrane pinches inwards to form two daughter cells , the imaginary line is called the cleavage furrow which separates the developing nuclei
- In plant cells, the new dividing cell wall is constructed in between the daughter cells. The parent cell thus splits in half and gives rise to two daughter cells



SIGNIFICANCE OF MITOSIS

- It is an equational division
- The chromosomal number is maintained constant
- Mitosis helps in the growth and development of organelles
- Mitosis helps in the asexual reproduction of organisms and helps in repair of tissues

MEIOSIS

- Meiosis takes place only in the reproductive cells during the formation of gametes
- It is called as reduction division because the chromosome number is reduced to haploid from diploid [2n -> n]
- Meiosis produces four daughter cells from a parent cell
- It consists of two divisions:-
  - Heterotypic / Meiosis - I
  - Homotypic / Meioisis – II
- Meiosis – I , has different phases:-
  - Prophase – I
  - Metaphase – I
  - Anaphse – I
  - Telophase – I
  - Cytokinesis – I

MEIOSIS - I

- PROPHASE – I
  - Prophase I has been further subdivided into
    - Leptotene

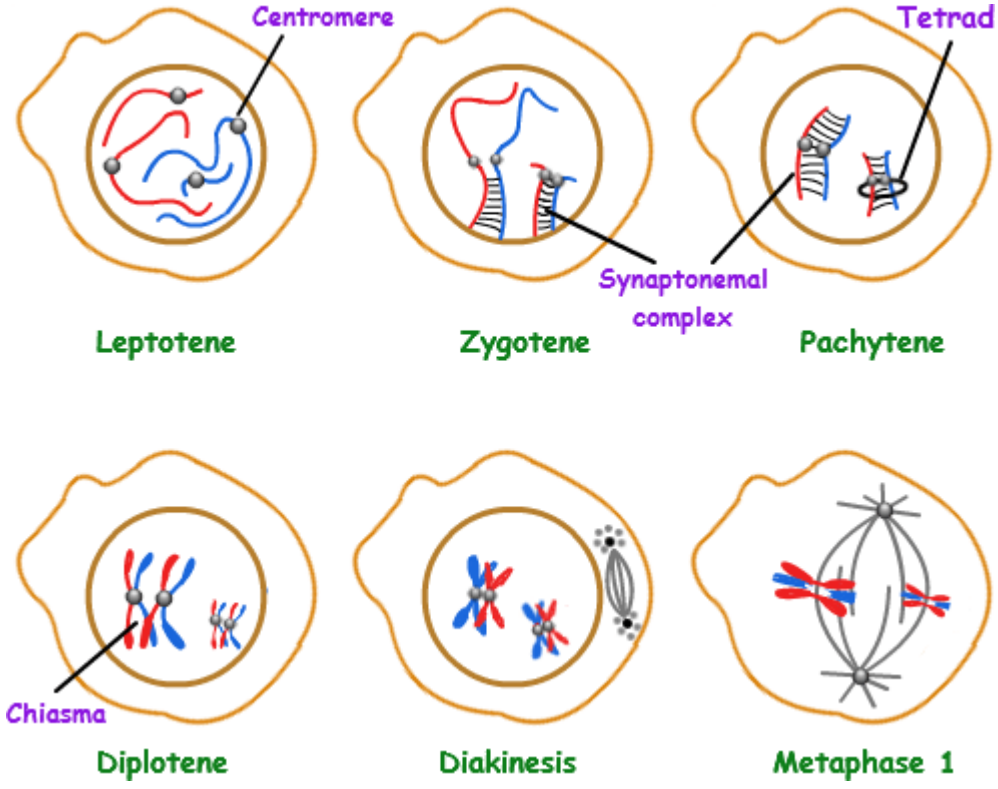
- Zygotene
- Pachytene
- Diplotene
- Diakinesis

- Leptotene

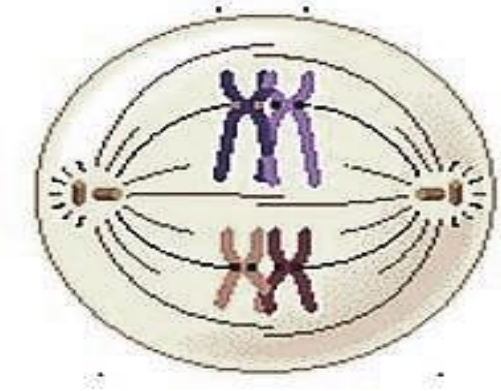
In leptotene stage, the chromosomes condense and become visible.

- Zygotene
  In zygotene, chromosomes start pairing together to form homologous chromosomes by the process of association called **synapsis**, which is accompanied by the formation of a complex structure called **synaptonemal complex** and the complex formed by a pair of synapsed homologous chromosomes called a **bivalent** or a **tetrad**.
- Pachytene
  In pachytene, exchange of genetic material between two homologous chromosomes known as **crossing over** takes place between non-sister chromatids of the homologous chromosomes.
- Diplotene
  Diplotene stage is characterized by the dissolution of the synaptonemal complex, and X-shaped structures called **chiasmata** form by the tendency of the recombined homologous chromosomes of the bivalents to separate from each other except at the sites of crossovers.
- Diakinesis

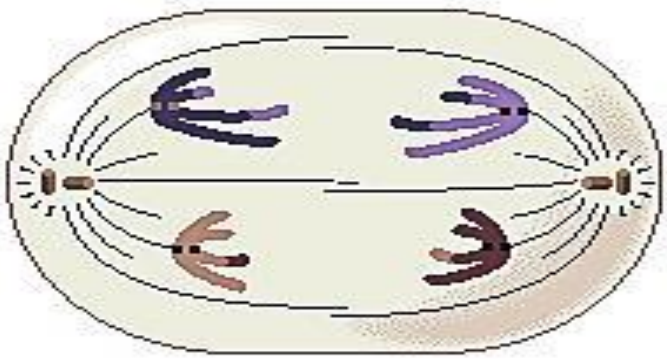
In diakinesis, the chromosomes are fully condensed and the meiotic spindle is assembled to prepare the homologous chromosomes for separation and by the end of diakinesis, the nucleolus disappears and the nuclear envelope also breaks down.



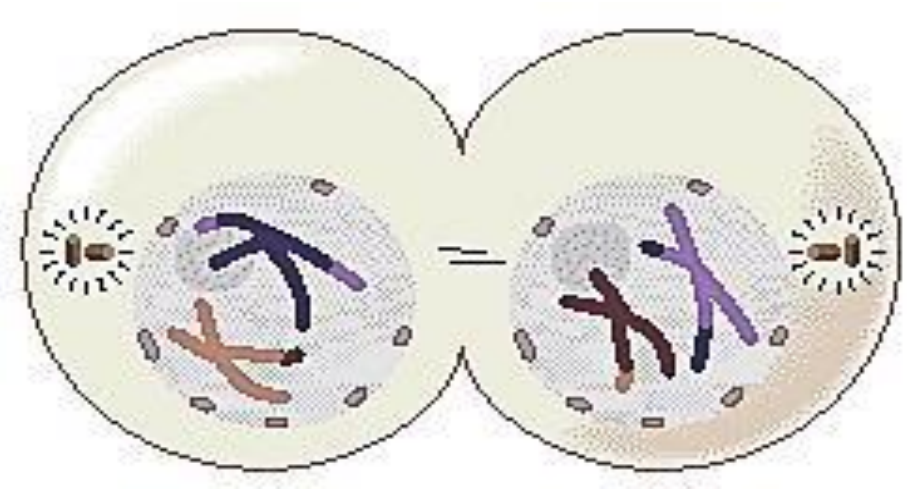
- METAPHASE – I
  - The bivalent chromosomes align on the equatorial plate.
  - The microtubules from the opposite poles of the spindle attach to the pair of homologous chromosomes.



- ANAPHASE – I
  - The homologous chromosomes separate, while sister chromatids remain associated at their centromeres.

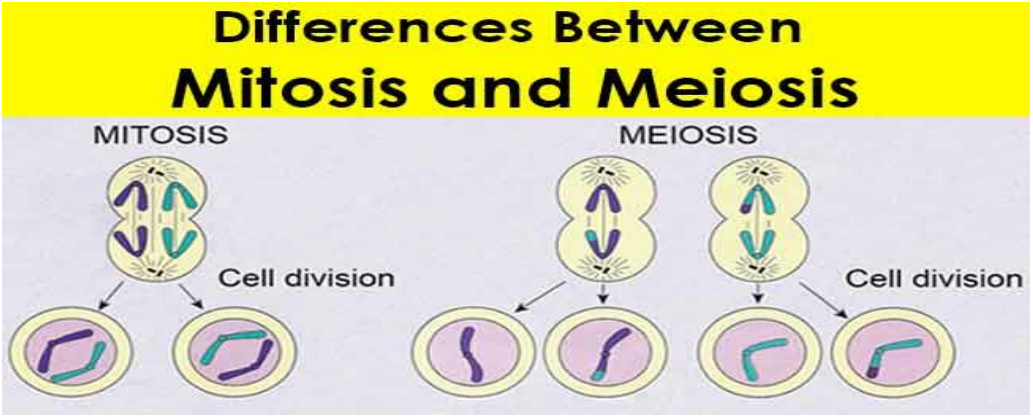


- TELOPHASE – I
  - The nuclear membrane and nucleolus reappear, cytokinesis follows and this is called as **diad** of cells.
  - The stage between the two meiotic divisions is called **interkinesis**, which is followed by prophase II.



MEIOSIS – II

- PROPHASE – II
  - In contrast to meiosis I, meiosis II resembles a normal mitosis.
  - The nuclear membrane disappears by the end of prophase II and the chromosomes again become compact.
- METAPHASE – II
  - The chromosomes align at the equator and the microtubules from opposite poles of the spindle get attached to the kinetochores of sister chromatids.
- ANAPHASE – II
  - The centromere of each chromosome splits, allowing them to move toward opposite poles of the cell.
- TELOPHASE – II
  - The two groups of chromosomes get enclosed by a nuclear envelope.
  - Cytokinesis follows resulting in the formation of tetrad of cells i.e., four haploid daughter cells.



Structural Organization of Chromosome

Chromosomes are thread-like structures present in the nucleus, which carries genetic information from one generation to another. They play a vital role in cell division, heredity, variation, mutation, repair and regeneration.

In Eukaryotic cells, genetic material is present in the nucleus in chromosomes, which is made up of highly organized DNA molecules with histone proteins supporting its structure.

Chromosome means 'coloured body', that refers to its staining ability by certain dyes.

Karl Nägeli in 1842, first observed the rod-like structure present in the nucleus of the plant cell.

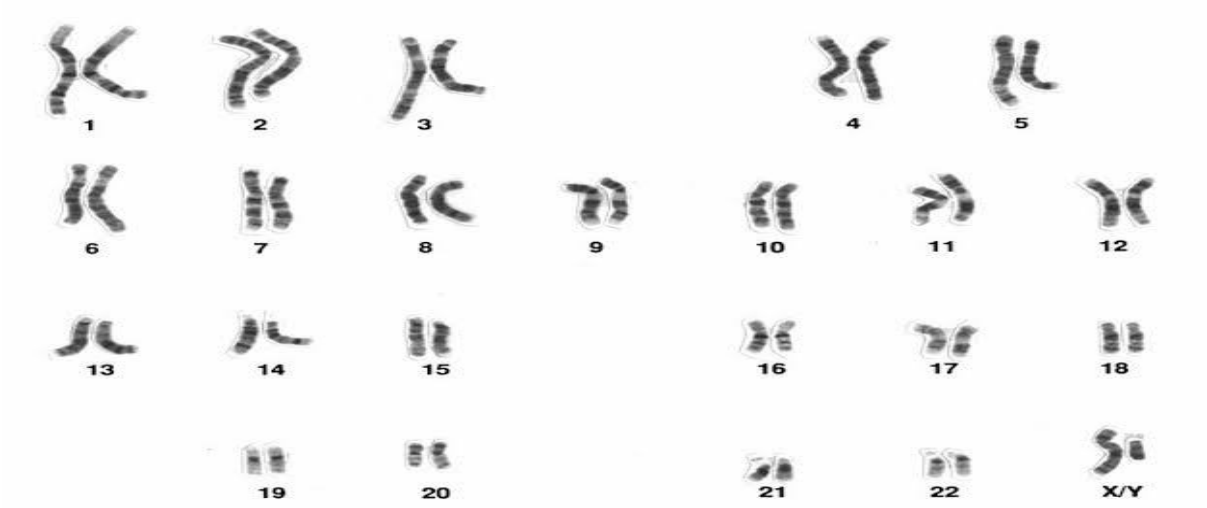
W. Waldeyer in 1888 coined the term 'chromosome'.

Walter Sutton and Theodor Boveri in 1902 suggested that chromosomes are the physical carrier of genes in the eukaryotic cells.

The number of chromosomes in any species is constant for all the cells. The number of chromosomes in gametes (e.g. sperms, egg) is half of the somatic cell and known as a haploid set of chromosomes, which is the result of meiosis during sexual reproduction. Chromosome number is preserved in the mitotic division of somatic cells, which is required for an organism to grow, repair and regenerate.

Chromosome number varies in different species. A nematode species contains only 2 chromosomes in a cell, whereas a protozoan species contains as much as 1600 chromosomes in the cell. Most of the plant and animal species contain 8 to 50 number of chromosomes in its somatic cell. The number of chromosomes does not reflect the complexity of a species. A human cell contains total 23 pair of chromosomes (2n, total 23×2=46), of which 22 are autosomes and 1 sex chromosome.

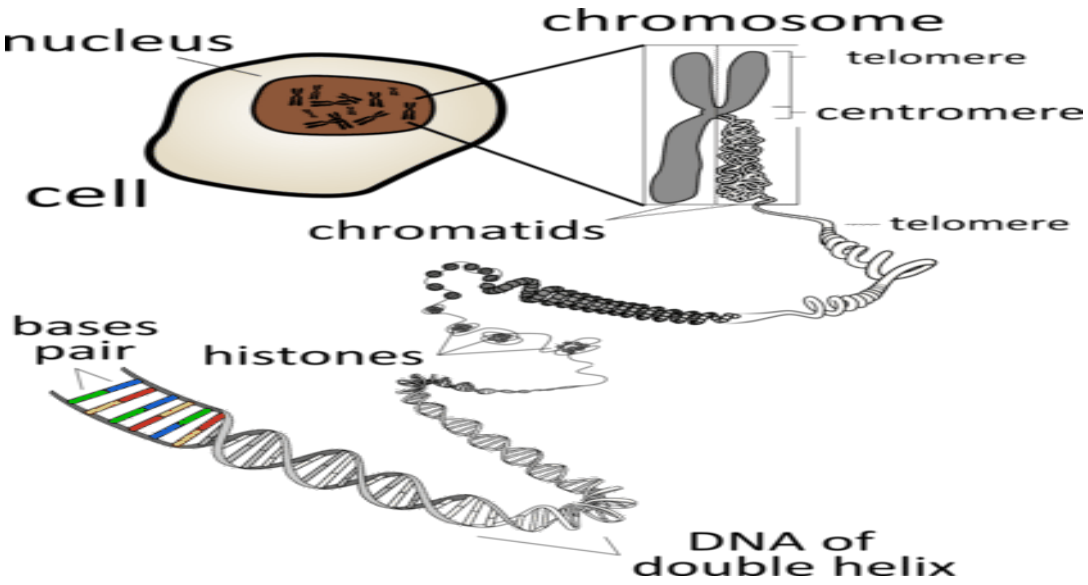
Karyotyping is a technique to study the structure of chromosomes present in a species. Chromosomes are isolated, stained and photographed. This technique is useful in finding out any chromosomal abnormalities.



Karyotype of a human cell (male)

Chromosome Structure

Each cell has a pair of each kind of chromosome known as a homologous chromosome. Chromosomes are made up of chromatin, which contains a single molecule of DNA and associated proteins. Each chromosome contains hundreds and thousands of genes that can precisely code for several proteins in the cell. Structure of a chromosome can be best seen during cell division.



Main parts of chromosomes are:

- **Chromatid:** Each chromosome has two symmetrical structures called chromatids or sister chromatids which is visible in mitotic metaphase.
    - Each chromatid contains a single DNA molecule
    - At the anaphase of mitotic cell division, sister chromatids separate and migrate to opposite poles
- **Centromere and kinetochore:** Sister chromatids are joined by the centromere.
    - Spindle fibres during cell division are attached at the centromere
    - The number and position of the centromere differs in different chromosomes
    - The centromere is called **primary constriction**
    - Centromere divides the chromosome into two parts, the shorter arm is known as **'p' arm** and the longer arm is known as **'q' arm.**
    - The centromere contains a disc-shaped **kinetochore**, which has specific DNA sequence with special proteins bound to them
    - The kinetochore provides the centre for polymerisation of tubulin proteins and assembly of microtubules
- **Secondary constriction and nucleolar organisers:** Other than centromere, chromosomes possess secondary constrictions.
    - Secondary constrictions can be identified from centromere at anaphase because there is bending only at the centromere (primary constriction)
    - Secondary constrictions, which contain genes to form nucleoli are known as the **nucleolar organiser**

- **Telomere:** Terminal part of a chromosome is known as a telomere.
    - Telomeres are polar, which prevents the fusion of chromosomal segments
- **Satellite:** It is an elongated segment that is sometimes present on a chromosome at the secondary constriction.
    - The chromosomes with satellite are known as **sat-chromosome**
- **Chromatin:** Chromosome is made up of **chromatin.** Chromatin is made up of DNA, RNA and proteins. At interphase, chromosomes are visible as thin chromatin fibres present in the nucleoplasm. During cell division, the chromatin fibres condense and chromosomes are visible with distinct features.
    - The darkly stained, condensed region of chromatin is known as **heterochromatin.** It contains tightly packed DNA, which is genetically inactive
    - The light stained, diffused region of chromatin is known as **euchromatin.** It contains genetically active and loosely packed DNA
    - At prophase, the chromosomal material is visible as thin filaments known as **chromonemata**
    - At interphase, bead-like structures are visible, which are an accumulation of chromatin material called **chromomere.** Chromatin with chromomere looks like a necklace with beads

Structural Organisation of Chromatin

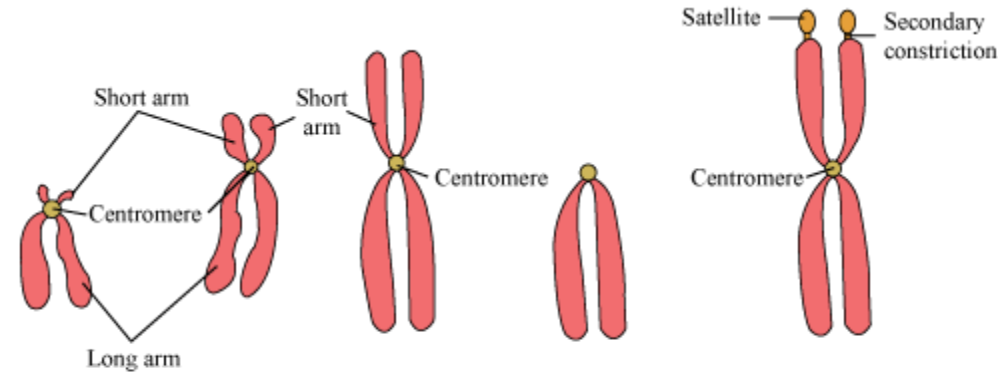
- Chromatin consists of DNA and associated proteins. DNA is packaged in a highly organised manner in chromosomes

1. **Nucleosomes** are the basic unit of chromatin. It is 10 nm in the diameter
    - DNA packing is facilitated by proteins called **histones.** DNA is wound around histone proteins to form a nucleosome
    - There are 5 types of histone proteins in the eukaryotic chromosomes, namely H1, H2A, H2B, H3 and H4
    - Histones are positively charged due to the presence of amino acids with basic side chains and it associates with negatively charged DNA due to the presence of phosphate groups
    - Histone proteins play an important role in gene regulation
    - Nucleosomes are made up of 146 base pair of DNA coiled around a core of eight histone molecules (2 molecules of 4 histone proteins)
    - Nucleosomes prevent DNA from getting tangled
2. Linker DNA and the fifth histone (H1) pack adjacent nucleosomes to a 30 nm **compact chromatin fibre**
3. These fibres form a large coiled loop held together by non-histone proteins (actin, **α** and **β** tubulin, myosin) called **scaffolding proteins** to form **extended chromatin** which is 300 nm in diameter
4. Chromatin further condenses with the help of protein known as **condensin,** it binds to DNA and wraps it into coiled loops and we get the compacted chromosome

Types of Chromosome
Based on the number of centromeres present

i. **Monocentric:** having only one centromere
ii. **Holocentric:** having diffused centromere and microtubules are attached along the length of a chromosome
iii. **Acentric:** chromosome may break and fuse together to form a chromosome without a centromere. It cannot attach to the mitotic spindle
iv. **Dicentric:** chromosomal aberration where chromosomes break and fuse together with two centromeres. They are also unstable as two centromeres tend to migrate to opposite poles resulting in fragmentation

Based on the position of the centromere



Types of chromosomes

i. **Telocentric:** rod-like chromosome with centromere present on the proximal end. No 'p' arm (short arm) present. Telomeric chromosomes are not found in humans
ii. **Acrocentric:** rod-like, centromere present at one end giving rise to one very short arm and an exceptionally long arm
iii. **Submetacentric:** L-shaped or J-shaped, with centromere near the centre of the chromosome giving rise to two unequal arms
iv. **Metacentric:** V-shaped chromosomes with centromere in the middle giving rise to two equal arms

Giant Chromosomes

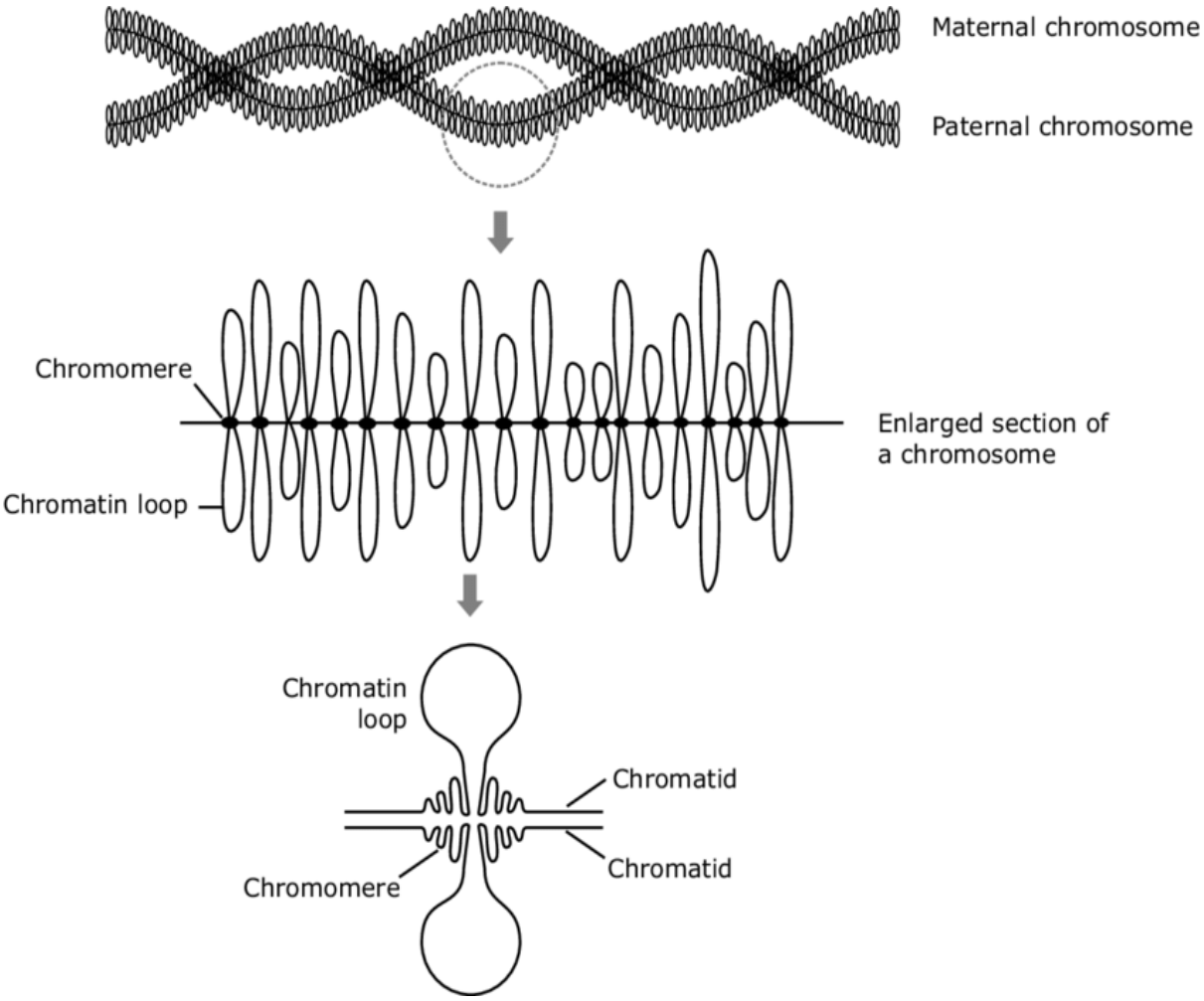
i. **Polytene chromosome**

a. **Balbiani** first discovered a structure in the nuclei of secretory glands of midges
b. Painter, Heitz and Bauer, rediscovered them in the salivary gland of Drosophila and recognised them as a chromosome
c. Also known as **Salivary gland chromosome**
d. These are called polytene by Kollar due to the presence of many chromonemata in them
e. These are present in some cells of the larvae of Dipteran insects

f. These are very large due to the presence of high DNA content
g. The polytene chromosome of Drosophila's salivary gland has 1000 DNA molecules Chironomus has 1600 DNA molecules in its each polytene chromosome
h. There is a series of alternating dark and clear bands called interband
i. Chromosome puffs or **Balbiani rings** are present, which are the swelling of bands due to DNA unfolding into open loops. These are the region of the intense transcription or mRNA formation

ii. **Lampbrush chromosome**

a) First discovered in the oocytes of salamander
b) The name is given due to its resemblance with a brush that is used for cleaning lamp, glass chimneys, etc.
c) They occur at the diplotene stage of oocytes in vertebrates and invertebrates
d) Lampbrush chromosomes are also found in the spermatocytes of many animals and also found in the giant nucleus of an algae **Acetabularia**
e) They are present as a bivalent with 4 chromatids
f) Chromosomal axis is formed from highly condensed chromatin and **lateral loops** extend from the row of chromomeres
g) Lateral loops of DNA are always symmetrical and formed due to intense RNA synthesis
h) In the oocytes of salamander, there are 10,000 loops present per haploid set of chromosomes
i) The centromere doesn't bear any loops



Functions of Chromosomes

- The main function of chromosomes is to carry the genetic material from one generation to another
- Chromosomes play an important role and act as a guiding force in the growth, reproduction, repair and regeneration process, that is important for their survival
- Chromosomes protect the DNA from getting tangled and damaged
- Histone and non-histone proteins help in the regulation of gene expression
- Spindle fibres attached to centromere help in the movement of the chromosome during cell division
- Each chromosome contains thousands of genes that precisely code for multiple proteins present in the body

Variation in Chromosome Number and Structure

- The karyotype can change due to rare events.
- Changes are inherited.
- The karyotype can vary somewhat within a species.
- The karyotype evolves.
- Different species, and less often different individuals within a species, have

different numbers of chromosomes and different arrangements of genes on the chromosomes.

VARIATIONS IN CHROMOSOME NUMBER

Variations in chromosome number include euploidy (varying numbers of complete chromosome sets) and aneuploidy (partial chromosome sets).

Euploids have varying numbers of complete chromosome sets; varieties include diploid, haploid, auto- and allopolyploid, polytene. Aneuploids have partial chromosome sets.

Ploidy of organism = number of chromosome sets

Euploid: multiples of complete sets

haploid	N	A B C
diploid	2N	A A B B C C
polyploid	> 2N	
triploid	3N	A AA B B B C C C
tetraploid	4N	A A A A B B B B C C C C

Aneuploids: diploid ± partial set

monosomic	2N - 1	A A B B C monosomic C
trisomic	2N + 1	A A A B B C C trisomic A

Table 1: Number of chromosomes in different organisms

Organism	No. of chromosomes
Arabidopsis thaliana (diploid)	10
Maize (diploid)	20
Wheat (hexaploid)	42
Common fruit fly (diploid)	8
Earthworm (diploid)	36
Mouse (diploid)	40
Human (diploid)	46
Elephants (diploid)	56
Donkey (diploid)	62
Dog (diploid)	78
Gold Fish (diploid)	100-104
Tobacco(tetraloid)	48
Oat (hexaploid)	42

Aneuoploidy important because:

- genetic tool
- results in genetic defects

Polyploidy important because:

- plant evolutionpolyploidization -> new species, especially in grasses
- plant breeding polyploidization -> new useful variety

Breadwheat(Triticumaestivum)isallotetraploidwithdiploidchromosomesets from 3 different parent species, each of which had 2N = 14, so total in wheat is 42.

Variations in chromosome number are usually caused by errors in mitosis, meiosis, or fertilization.

Errors in mitosis, meiosis, or fertilization cause polyploidy. E.g.:

(a) 2 sperm + 1 egg -->3N

(b) Failure of anaphase separation in mitosis in germ line --> 4N gametocyte --> 2N gametes. Can be induced with agents that block spindle formation or dissolve spindle.

Aneuploids result from nondisjunction.

Changes in chromosome number often cause abnormalities in gene expression (and hence the phenotype) and in meiosis.

Gene dosage effects: aneuploidy results in unbalanced genomes and abnormal development; hence aneuploidy is usually lethal or detrimental.

Embryo with one extra or one too few chromosome has **unbalanced genome --> abnormal development**. Genome evolved to work with two functional copies of each gene. If have too few or too many of some genes, those make too much or too little gene product, and may upset metabolic pathways; e.g. if some enzymes are too abundant, may make too much product.

Aneuploidy in humans is common medical problem. Occurs in ca. 3.5% of all embryos.

Aneuploidy for large chromosome in humans usually causes unbalance for one or many important genes, usually lethal --> stillbirth. Accounts for ca. 20% of all stillbirths.

Aneuploidy for small chromosomes maybe viable. E.g. Down's syndrome = trisomy 21. (Or part of 21, attached to another chromosome.)

Causes of trisomy 21:

≈ 75% female MI or MII nondisjunction

≈ 25% male MI or MII "

Female eggs arrested in prophase of MI at birth. Eggs age --> increasing frequency of nondisjunction with increasing maternal age, especially > 45 years.

Meiosis in aneuploids and autopolyploids may be abnormal because of problems with synapsis, resulting in sterility. Don’t have time to cover in course, can omit corresponding sections of reading.

VARIATIONS IN CHROMOSOME STRUCTURE

Also called chromosomal mutations (OK when they first occur), chromosomal abnormalities or aberrations (not strictly applicable because sometimes ≥ 2 different structures are present in population in high frequency; can't say that either one is aberrant orabnormal).

Changes in chromosome structure occur in both prokaryotes and eukaryotes. We will focus on eukaryotes.

1. **Transposable elements = TEs**"jumping genes" Tranposition:
   - TE moves to new location
   - rare event; frequency is on order of mutation rate or somewhat higher, but not so frequent that they interfere with mapping
   - duplicative transposition puts a copy of the TE in the new position, leaving the old one behind; nonduplicative transposition moves the TE.

1 2 3 4 5 6 7 8 9 10 --duplicative transposition--> 1 2 3 4 5 6 7 8 9 2 10

--non-duplicative transposition--> 1 3 4 5 67 8 9 2 10

   - Duplicative transposable elements can spread in genome even if they are of no selective advantage to the organism, and even if they are mildly detrimental. Sometimes called **selfishDNA**.

Transposable elements were first discovered by Barbara McClintock (first woman elected to National Academy of Science).in maize; Nobel Prize.

Mechanisms:

Some have transposase gene -> transposase protein which cuts TE out and inserts in target sequence.

Retrotransposons transcribed to make RNA copy, then reverse transcriptase makes DNA copy of the RNA, and DNA is integrated into target sequence. The act of transposition itself may cause rearrangements of adjacent sequences. TE inserted in gene or in controlling sequences can make gene inactive, just like a mutation.

If transposon excises cleanly, gene activity can be restored.

The copies of a transposable element are sites of homology at which crossing- over can occur within a chromosome and between homologous or nonhomologous chromosomes --> changes in chromosome structure. Crossing- over probably in interphase.

1. Repeats = repeatedsequences

Repeats are segments of DNA that are present two or more times in the genome of an

organism.

Many short repeats arise in the course of evolution due to mutations, just by chance:

Short (simple sequence) tandem repeats arise by replication slippage: ATTTCG – replication--> ATTTTCG ATGATGATG ATGATGATG. Some human hereditary defects are due to increases in number of tandem repeats; e.g. Myotonic muscular dystrophy. Some regions of chromosomes are rich in short tandem repeats, notably centromeres. These are the bane of DNA sequencers; this is why a large part of the human genome still hasn't been sequenced. Longer repeated sequences arise by duplicative transposition.

Polytene Chromosomes
Some insects, including Drosophila:

During differentiation of some tissues (salivary glands, Malpighian tubules, etc.) cells go through repeated S phases (e.g. 10) without mitosis --> polyploid or polytene nuclei.

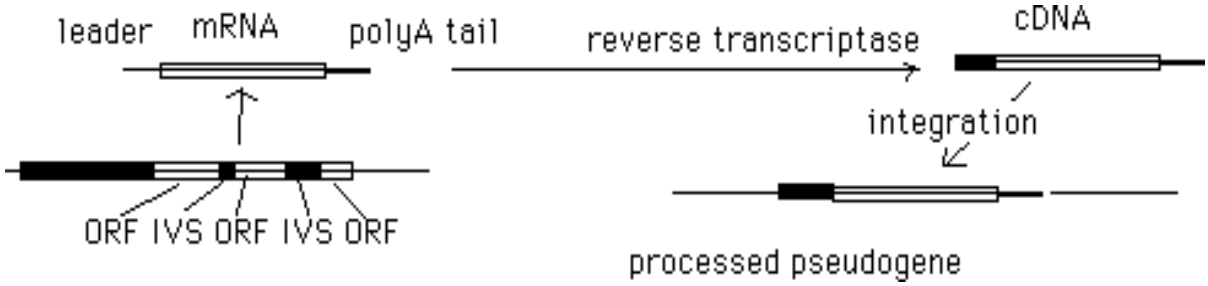
Polytene chromosomes: all copies held tightly together and in alignment.

Duplication = process of duplicating a segment (usually a whole gene or genes); also used to denote the repeated copies

1 2 3 4 5 6 --duplication--> 1 2 3 4 5 4 5 6

Might be called dup45

(1) Duplications can arise by duplicative transposition.
(2) Duplications can occur via processed pseudogenes:



Duplication can occur via unequal crossing-over between existing repeats, usually in sister chromatids produced by replication of a chromosome.

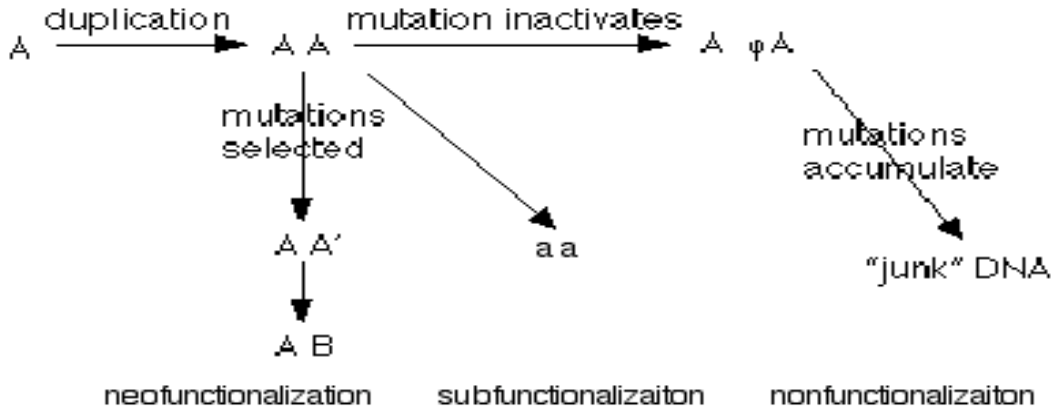
Classic case: Bar eye mutation is actually a duplication of several bands. Sturtevant & Bridges. Unequal crossing-over between sister chromatids or homologous X chromosomes (in females)

Ribosomal RNA genes in eukaryotes are present in long tandem arrays which vary in number.

Duplications are important in the evolution of new genes.

Duplication 2 copies of a gene in a genome.
Diploids have two genomes, so diploid initially heterozygous for duplication and has 3 copies.

Sexual reproduction can produce individuals with 4 copies. We will just track the fate of two copies.



Most often, pseudogene diverges further so eventually not recognizable as related to gene. Contributes to "junk" DNA, unique or repeated sequences with no detectable function. Eventually deleted.

Duplicate can also acquire mutations that turn it into a gene with simnilar function, e.g. binding protein duplicates, one copy --> hemoglobin (blood), other copy --> myoglobin (e.g. human globins: example of clustered multigene family.

□	alpha	major adult
□	beta	major adult
□	gamma	fetal
□	delta	infant, minor adult
□	epsilon	embryonic
□	zeta	embryonic
□	psi	pseudogene

When we discuss molecular evolution, will see how we can trace the evolution of all of these genes in detail from a single ancestral gene. That gene duplicated to give myoglobin and globin genes; the latter duplicated to form □ and □ globin genes; and each of those duplicated further.

1. **Deletion = loss of a segment of DNA, and the result of that loss.**

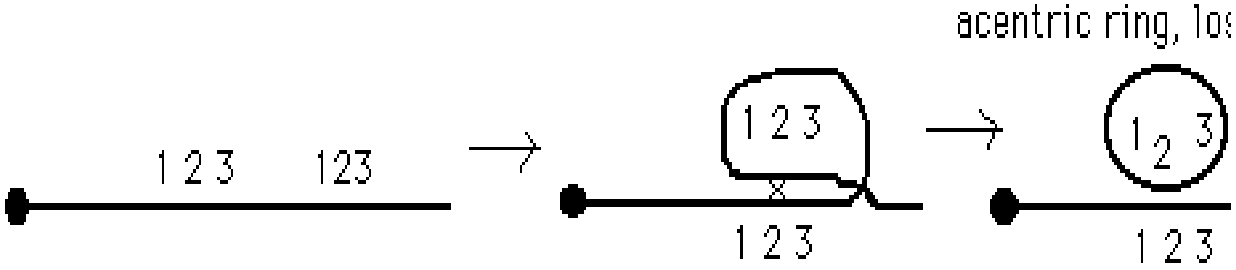
(1) Deletions arise by unequal crossing-over (together with duplication).

(2) Deletions arise by intrachromosomal crossing-over between direct repeats. deletion = deficiency

1 2 3 4 5 6 --deletion--> 1 2 3 6 Might be called def45.

Origins of **deletions**:

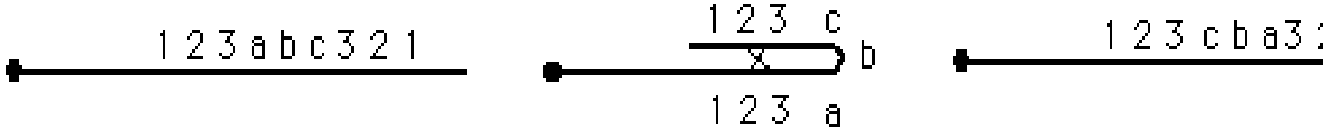
Intrachromosomal crossing-over between **direct** repeats:



2. **Inversions arise by intrachromosomal crossing-over between inverted repeats.**

Inversions arise by intrachromosomal crossing-over between **inverted** repeats.

Inversions act as cross-over "suppressors" in inversion heterozygotes because cross-



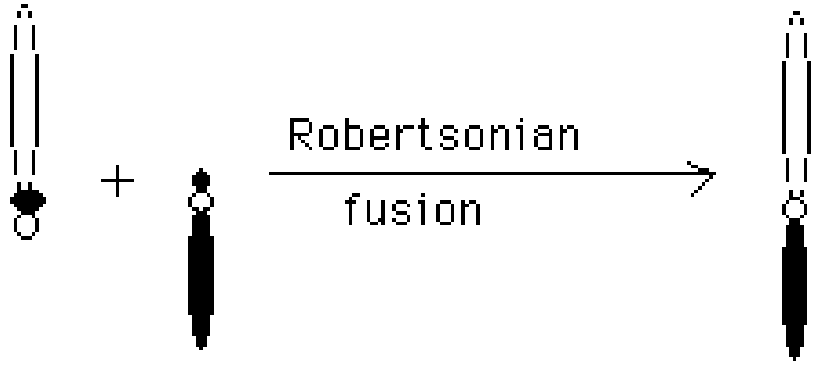
overs within the inverted region --> unbalanced genomes with duplications and deletions, hence inviable gametes and/or progeny.

1. Reciprocal translocations arise by crossing-over between homologous regions on nonhomologous chromosomes.

Reciprocal translocations arise by crossing-over between homologous regions on **non**homologous chromosomes.

1 2 3 4 r 5 6 7 8 r 9 10 --reciprocal translocation--> 1 2 3 4 r 9 10 7 8 r 5 6

2. Robertsonian fusions change N.



Small arms are lost.

Extreme example of change in chromosome number is seen in deer. Reeve's muntjac 2N = 46, same order of magnitude as most other deer species. Indian muntjac 2N = 6.

Note that most rearrangements may take place by crossing-over between repeats or other homologous DNA segments (segments with similar sequence).

Duplicative transposition is a major source of such duplicated regions.

If a transposable element invades a genome, it can spread and facilitate rearrangements. Thus it can increase variation in chromosome structure and eventually lead to evolution of new gene arrangements and new phenotypes.

Transpsable elements can contribute to, or even give rise to, promoters and other regulatory sequences.

Transposable elements can spread from one organism to another. e.g. P element was transferred from Drosophila willistoni (or a close relative) to Drosophila melanogaster, probably by a parasitic mite that sucks cytoplasm from fly eggs.

The evolutionary origin(s) of transposable elements are unknown (last I heard).

Rearrangements can affect meiosis and mitosis

What happens when rearranged chromosomes synapse in meiosis I?

synapsis and recombination is always between homologous chromosome regions. Centromeres segregate normally, but chromosome segments may not. Will have example(s) in practice problems.

Visible in polytene chromosomes in heterozygotes for rearrangements.

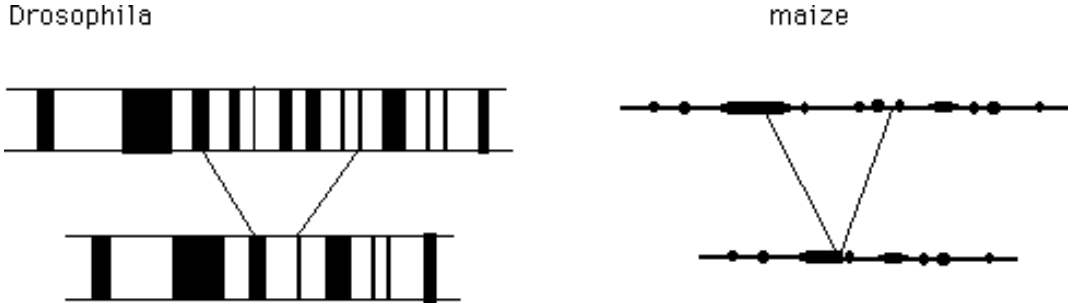
Position effect = phenotype of a gene or region of a chromosome depends on its neighbors. One obvious rationale: move gene to different position, may put under control of different set of upstream controlling sequences.

Deletions (and other structural changes) are important tools for mapping genes on chromosomes (tying linkage maps to physical maps).

Relating recombination maps and physical maps of landmarks. Can use deletions (and other structural changes) to map genes.

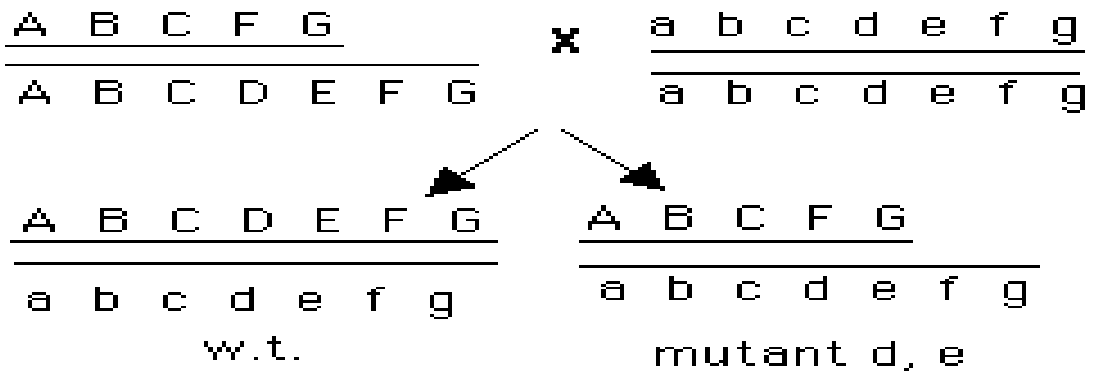
Can use deletions (and other structural changes) to tie linkage map to physical map of chromosome.

(1) Map deletion on physical map. See what landmarks are missing.



Map deletions on genetic map: determine which recessive mutations are "uncovered" by deletions. An alternative way of looking at this is to see what mutations complement a deletion. If a mutation and a deletion don't complement each other, they involve the same gene.

Note this is a slightly different way of using the term “complement”. Testcross a deletion heterozygote:



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DEPARTMENT OF BIOMEDICAL ENGINEERING

UNIT – IV - Cellbiology & Genetics – SBMA1101

GENETIC MATERIAL AND MUTATION

TOPIC CHECKLIST:

- LINKAGE
- CROSSING OVER
- GENETIC MATERIAL
- MUTATION
- C-VALUE PARADOX

I. LINKAGE;

DEFINITION Linkage is the tendency of genes to stay together during inheritance through generations without anychange or separation due to their being present on the same chromosome. The genes located in the same chromosome are called linked genes and those present in different chromosomes are termed as unlinked genes.

DEMONSTRATION OF LINKAGE

a. linked genes:

These genes occur on the same chromosome and do not show independent assortment but remain but remaintogether and are inherited en block due to their being present on the same chromosome. They show a dihybrid ratio of 3:1 (like monohbrid ratio) and test cross ratio of 1:1(like monohybrid test cross).Progeny consists of only parental types. Recombinants are absent.

b. Unlinked Genes:

These genes occur on different chromosomes and show independent assortment. Dihybrid ratio 9:3:3:1 while the dihybrid test cross ratio 1:1:1:1. In both the crosses, 50% are parental types while 50% are recombinants.

Linked genes	Unlinked genes
1. these genes are placed on yhe same chromosome and do not show independent assortment at the time of gamete formation . 2. They show dihybrid ratio of 3:1. 3. In dihybrid they show test cross ratio of 1:1.	1. these genes are located on different chroosomes an undergo independent assortment(segregation) 2. They show dihybrid ratio of 9:3:3:1. 3.In dihybrid,they show a test cross ratio of 1:1:1:1.

CHROMOSOME THEORY OF LINKAGE :

In 1911 Morgan and Castle proposed chromosomal theory of linkage. It states that,

• Linked Genes occur in the same chromosome

• They lie in a linear sequence in the chromosome

• There is a tendency to maintain the parental combination of genes except for occasional cross overs

• Strength of the linkage between two genes is inversely proportional to the distance between the two, I.e,two linked genes show their distance between them is higher and lower frequency if the distance is small.

LINKAGE GROUPS:

- Genes that are present on the same chromosome make one linkage group. They inherit together except for crossing over.
- It corresponds to a chromosome which bears a linear sequence of genes linked and inherited together.
- Because the two homologous chromosomes posess either similar or allelic genes on the same loci ,they constitute the same linkage group.
- Therefore the number of linkage groups present in an individual corresponds to the number of chromosomes in its one genome.
- It is known as principle of limitation of linkage groups.

EXAMPLE: fruit fly *Drosophila melanogaster* has 4 linkage groups(4 pairs of chromosomes.),humans have 23 linkage groups(23 pairs of chromosomes),maize has 10 linkage groups(10 pairs of chromosome)

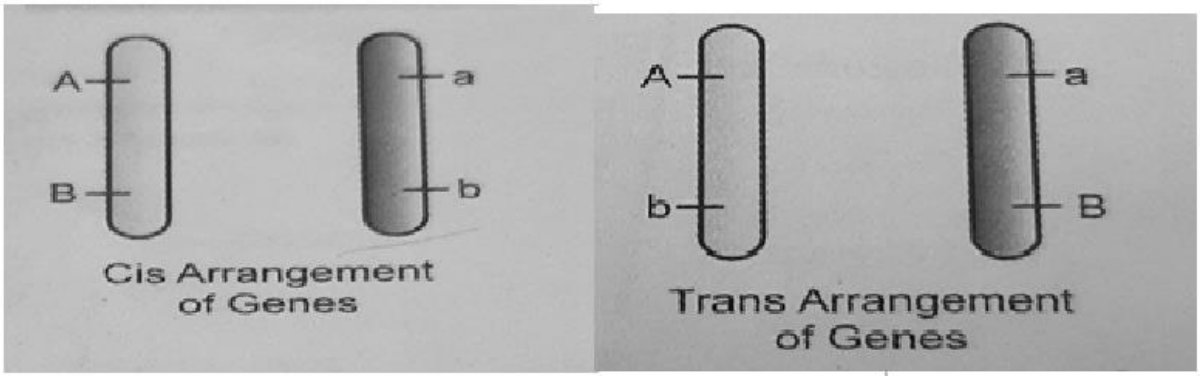
IMPORTANCE:

1. The number of linkage groups is equivalent to number of chromosomes present in a genome. It proves that genes are present on the chromosomes.
2. Linkage prevents or reduces the incidence of recombination so that specific varietal or racial characters are retained over the generations.
3. It is highly useful for maintaining the good characters of the newly developed variety.
4. Linkage is the headache for breeders because it does not allow them to freely bring all the desirable traits in one variety
5. It dilutes the use of desirable traits if undesirable ones are also present on the same linkage group.
6. Marker genes or genes which express their effect in early growth can indicate the effect of a linked gene which is to express late.

ARRANGEMENT OF LINKED GENES:

Linked genes in heterozygous organisms can how the following two types of arrangements;

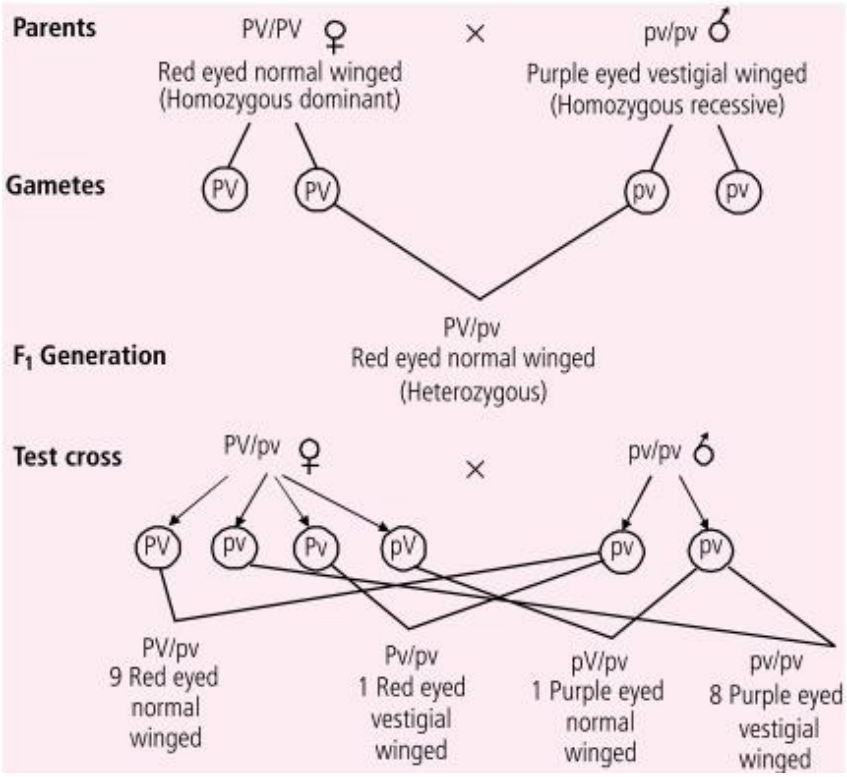
1. Cis-arrangement: if dominant alleles of both the linked genes are present on one chromosome and their recessive alleles on its homologous chromosome(AB|ab).this is known as cis-arrangement and genes are said to be in coupling state.
2. Tras-arrangement: If dominant allele of one pair and recessive allele of the second pair are present on one chromosome and recessive and dominant alleles on the other homologous chromosome of a pair (Ab|aB),this arrangement is known as trans-arrangement and genes are said to be in repulsive state.



TYPES OF LINKAGE:

Linkage is of two types: complete and incomplete

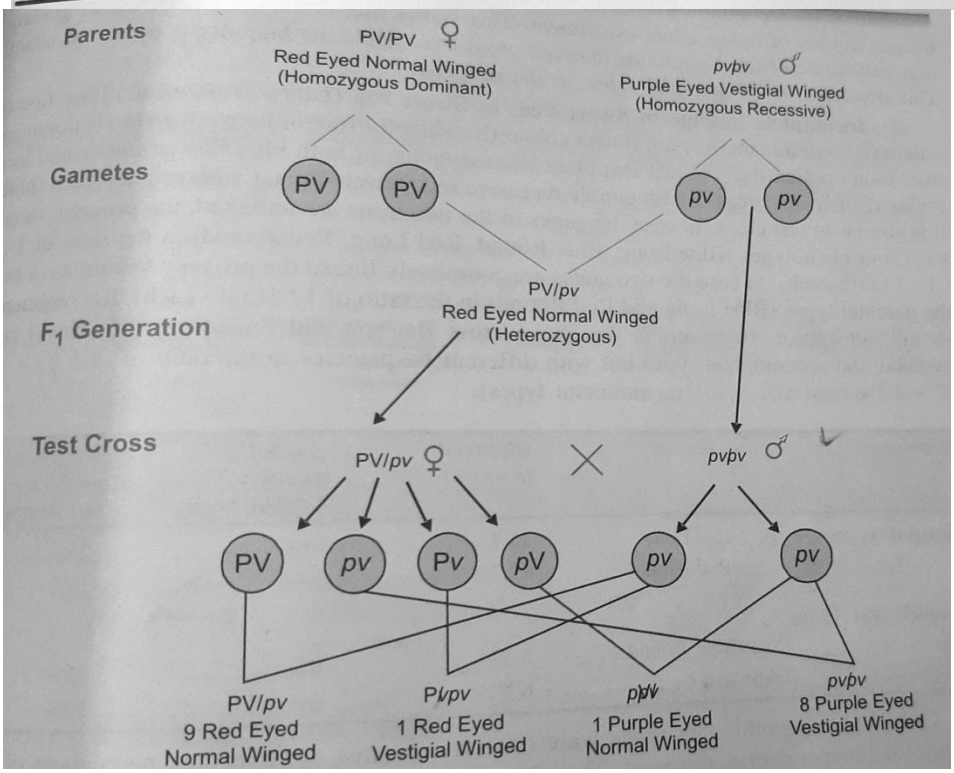
1. **Complete Linkage:** the genes located in the same chromosome do not separate and are inherited together over the generations due to the absence of crossing over. Complete linkage allows the combination of parental traits to be inherited as such.
   Example: Complete linkage in male drosophila



Result : when F1 males are testcrossed to homozygous recessive females only two types of indviduals are produced – red eyed normal winged and purple eyed vestigial winged in the ratio of 1:1(parental phenotypes).similarly during inbreeding of F1 indviduals ,recombinant types arec absent.there was no crossing over which indicated that the linkage in male Drosophila was complete.

2. **Incomplete Linkage:** linkage is incomplete when new or non-parental combinations of linked genes are also formed. It is due to crossing over and hence produce recombinant progeny besides the parental type. The number of recombinant individuals is usually less than the number expected in independent assortment. In independent assortment all the four types are each 25%. In case of linkage each of the two parental types is more than 25% while each of the recombinant types is less than 25%
   Example: incomplete linkage in female drosophila.
   When F1 female flies are test crossed with homozygous recessive males, it does not yield the ratio of 1:1:1:1,instead it comes to be 9:1:1:8.this shoes that the two genes did not segregate independently of each other. the data obtained is:

Phenotype	Progeny	Observed	Expected if Complete Linkage	Expected if Independent Assortment
Parental Types				
	(a) Red eyed, normal winged	1339	1420	710
	(b) Purple eyed vestigial winged	1195	1420	710
Recombinant Types				
	(a) Red eyed, vestigial winged	152	zero	710
	(b) Purple eyed, normal winged	152	zero	710



FACTORS AFFECTING LINKAGE:

1. Age: with advancing age the chances of crossing over are reduced and hence strength of linkage increases.
2. Temperature: rise in temperature increases the chances of crossing over therefore decreases the strength of linkage
3. Radiation : radiations like UV and X-ray decreases the linkage,and causes recombination and crossing over.
4. Distance : the increase in distance between two genes decreases the percentage of linkage.

SIGNIFICANCE OF LINKAGE:

- Linkage reduces the chances of the formation of new combinations of genes in the gametes. Thus it helps to keep the parental racial and important traits together.
- It helps to maintain the important traits of a newly developed variety.
- Linkage does not allow the plant and animal breeders to combine all valuable traits in a single variety.
- Linkage groups give important information about the location of genes in the chromosomes.

CROSSING OVER:

DEFINITION: Crossing over is the mutual exchange of segments between two non-sister chromatids of homologous chromosomes in pachytene stage of meiosis-1,producing new combinations of alleles of linked genes

The non-sister chromatids in which exchange of segments had taken place are called as re-combinants or cross overs, while the other chromatids in which crossing over has not taken place are known as parental chromatids or non-cross overs.

MECHANISM:

SYNAPSIS

- Homologous chromosomes pair up. This pairing is done in such a way that the genes of the same character present on the two chromosomes lie exactly opposite to each other.
- This process of association is known as synapsis

TETRAD FORMATION

- Soon after the completion of synapsis, the cell enters the pachytene stage. Each of the homologous chromosome in bivalent splits longitudinally into two sister chromatids.
- Thus, the bivalent now consists of four chromatidsand is known as tetrad.

CROSSING OVER & CHIASMA FORMATION

- The non sister chromatids remain in contact at one or more points.These points of contact are known as chiasmata(sigular:chiasma).
- there is a brakage of chromatid segment of twonon sister chromatids.after the completion of crossing over, the homologius chromosomes move apart.

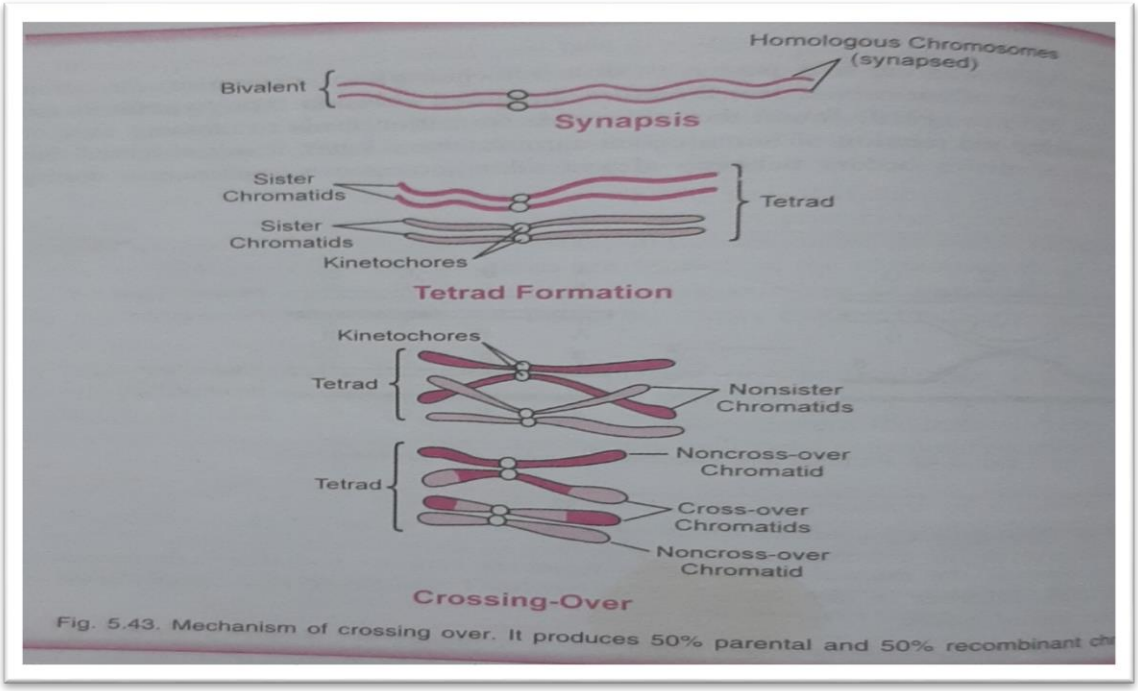


Fig. 5.43. Mechanism of crossing over. It produces 50% parental and 50% recombinant ch

TYPES OF CROSSING OVER:

Crossing over is of the following types:

1. Single crossing over: Crossing over occurs at one point between two non-sister chromatids of a homologous chromosome pair. There are two parental types and two recombinants.
2. Double Crossing over: crossing over occurs at two points in a homologous pair of chromosomes.
   (a) Reciprocal Double Crossing over : two in of crossing over occur between the same non-sister chromatids.
   (b) Complementary Double Crossing: the two crossing overs involve three or all the four chromatids so that the number of cross overs is three of four with occurrence of one or no parental type.

3. Multiple Crossing over: three or more points of crossing over occur in the same homologous chromosome. Double cross-overs and parental types may or may not occur.

FACTORS INFLUENCING CROSSING OVER:

1. Distance: the physical distance between two genes determines the frequency of the crossing over two genes
2. Age and sex
3. X-rays
4. Temperature
5. Heterochromatin: presence of centromere and heterochromatic areas decrease the rate of crossing over.
6. Chemicals
7. Interference: one cross over reduces the occurrence of another cross over in vicinity. This phenomenon is called interference. Coincidence is the ratio of observed double cross over in relation to expected double cross over on the basis of non-interference or independent occurrence. Coincidence is small when interference is high**.**

IMPORTANCE OF CROSSING OVER:

1. CROSSING OVER is a means of introducing new combinations of genes ad hence traits.
2. It increases variability which is useful for natural selection and under changed environment.
3. Since the frequency of crossing over depends upon the distance between the two genes ,the phenomenon is used for preparing linkage chromosome maps.
4. It has proved that genes lie in a linear fashion in a chromosome.
5. Breeders have to select small or large population for obtaining the required cross-overs. For obtaining cross overs between closely linked genes a very large population is required .
6. Useful recombinations produced by crossing over are picked up by breeders to develop useful new varieties of crop plants and animals. Green revolution has been achieved in India due to this selective picking up of useful recombinations. Operation flood or white revolution is also being carried out on similar lines.

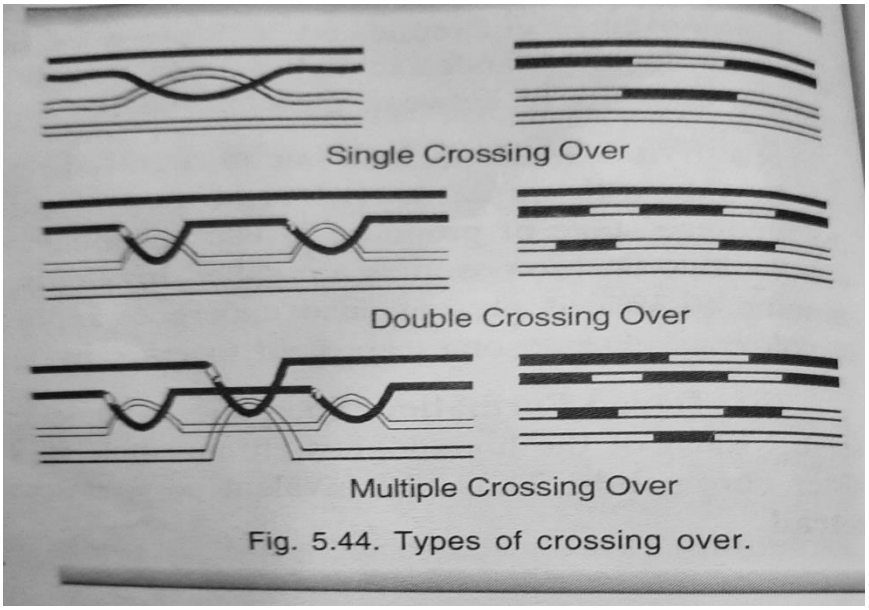


Fig. 5.44. Types of crossing over.

GENETIC MATERIAL:

The search of Genetic material started during the mid-nineteenth century. The principle of *inheritance* was discovered by Mendel. Based on his investigation, Mendel concluded that some 'factors' are transferred from one generation to another. Mendel's Law of Inheritance was the basis for the researchers on genetic material. Keeping his conclusions in mind, scientists who came after him, focused on chromosomes in search of genetic material. Even though the chromosomal components were identified, the material which is responsible for inheritance remained unanswered. It took a long time for the acceptance of DNA as the genetic transformation. Let's go through a brief account of the discovery of genetic material and Griffith experiment.

Griffith Experiment & Transforming Principle

Griffith experiment was a stepping stone for the discovery of genetic material. Frederick Griffith experiments were conducted with Streptococcus pneumoniae.

During the experiment, Griffith cultured Streptococcus pneumoniae bacteria which showed two patterns of growth. One culture plate consisted of smooth shiny colonies (S) while other consisted of rough colonies (R). The difference was due to the presence of mucous coat in S strain bacteria, whereas the R strain bacteria lacked them.

Experiment: Griffith injected both S and R strains to mice. The one which was infected with the S strain developed pneumonia and died while that infected with the R strain stayed alive.

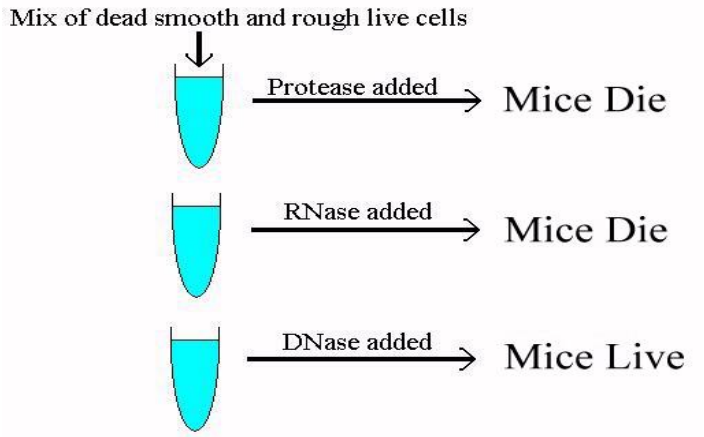
In the second stage, Griffith heat-killed the S strain bacteria and injected into mice, but the mice stayed alive. Then, he mixed the heat-killed S and live R strains. This mixture was injected into mice and they died. In addition, he found living S strain bacteria in dead mice.

Injected form of Streptococcus	Effect
Live non-capsulated	mice live
Live capsulated	mice die
Heat-killed capsulated	mice live
Heat-killed capsulated + live non-capsulated	mice die

Conclusion: Based on the observation, Griffith concluded that R strain bacteria had been transformed by S strain bacteria. The R strain inherited some 'transforming principle' from the heat-killed S strain bacteria which made them virulent. And he assumed this transforming principle as genetic material.

Biochemical Characterisation of Transforming Principle:

- Oswald Avery, Colin MacLeod and Maclyn McCarty (1933-44) worked to determine the biochemical nature of 'transforming principle' in Griffith's experiment in an in vitro system.
- From the heat-killed S-cells, they purified biochemicals (proteins, DNA, RNA, etc.) to observe, that which biochemicals could transform live R-cells into S-cells.
- Therefore, they discovered that DNA alone from heat-killed S-type bacteria caused the transformation of non-virulent R-type bacteria into S-type virulent bacteria.
- Protein-digesting enzymes (proteases) and RNA-digesting enzymes (RNases) did not cause this transformation. This proved that the 'transforming substance' was neither the protein no RNA.
- DNA-digesting enzyme (DNase) caused inhibition of transformation, which suggests that the DNA caused the transformation. Thus, these scientists came to the conclusion that DNA is the hereditary material.

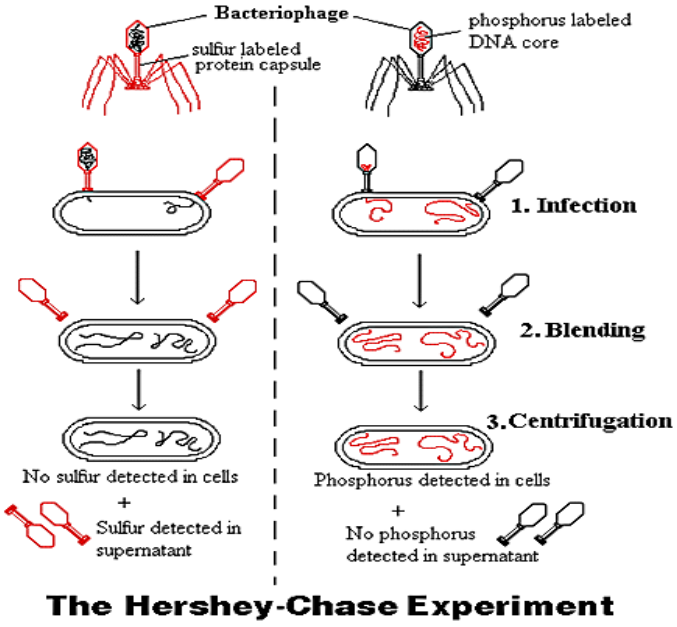


THE GENETIC MATERIAL IS THE DNA!

In 1952, Alfred Hershey and Martha Chase took an effort to find the genetic material in and centrifugation.

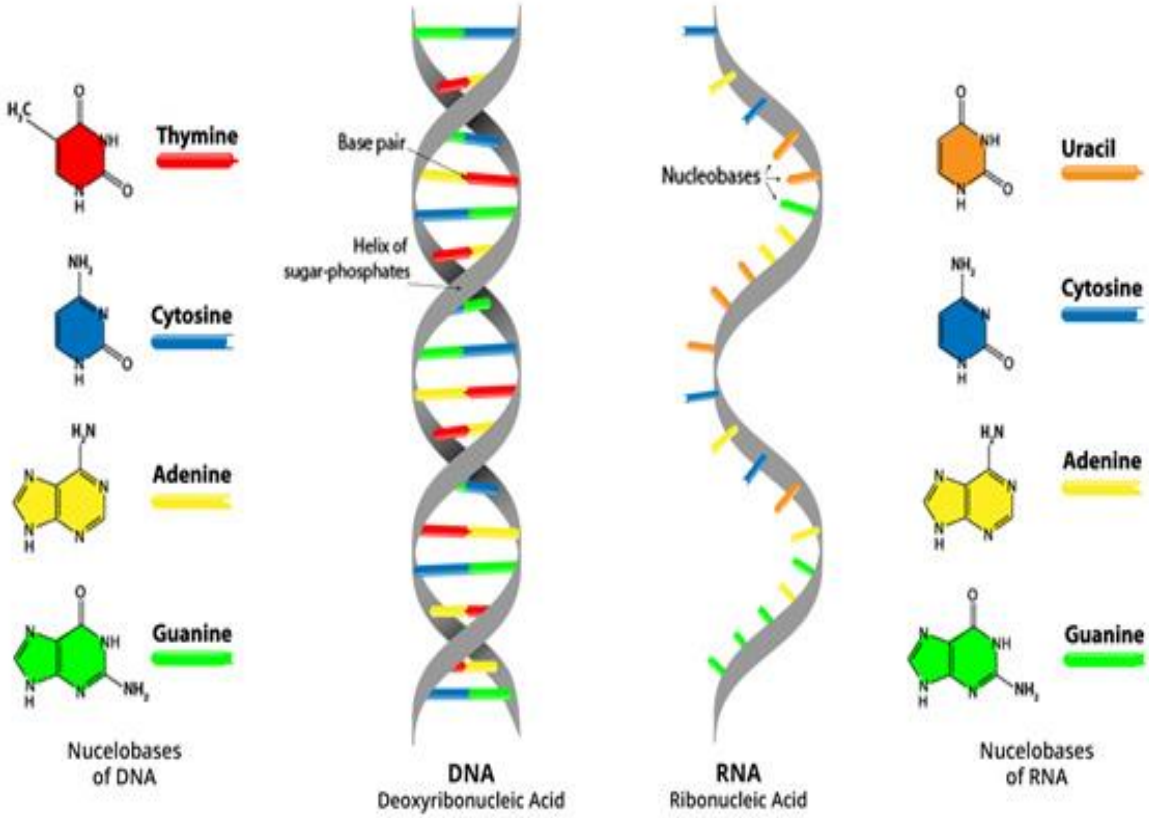
- **Observation:** E.coli bacteria which were infected by radioactive DNA viruses (A) were radioactive but the ones that were infected by radioactive protein viruses (B) were non-radioactive. organisms. Their experiments led to an unequivocal proof to DNA as genetic material. Bacteriophages (viruses that affect bacteria) were the key element for Hershey and Chase experiment.
- The virus doesn't have their own mechanism of reproduction but they depend on a host for the same. Once they attach to the host cell, their genetic material is transferred to the host. Here in case of bacteriophages, bacteria are their host. The infected bacteria are manipulated by the bacteriophages such that bacterial cells start to replicate the viral genetic material. Hershey and Chase conducted an experiment to discover whether it was protein or DNA that acted as the genetic material that entered the bacteria.
- **Experiment:** The experiment began with the culturing of viruses in two types of medium. One set of viruses (A) was cultured in a medium of radioactive phosphorus whereas another set (B) was cultured in a medium of radioactive sulfur. They observed that the first set of viruses (A) consisted of radioactive DNA but not radioactive proteins. This is because DNA is a phosphorus-based compound while protein is not. The latter set of viruses (B) consisted of radioactive protein but not radioactive DNA.
- The host for infection was E.coli bacteria. The viruses were allowed to infect bacteria by removing the viral coats through a number of blending

- **Conclusion:** Resultant radioactive and non-radioactive bacteria infer that the viruses that had radioactive DNA transferred their DNA to the bacteria but viruses that had radioactive protein didn't get transferred to the bacteria. Hence, DNA is the genetic material and not the protein.



The Hershey-Chase Experiment

DNA VERSUS RNA:



Comparison	DNA	RNA
Full Name	Deoxyribonucleic Acid	Ribonucleic Acid
Function	DNA replicates and stores genetic information. It is a blueprint for all genetic information contained within an organism	RNA converts the genetic information contained within DNA to a format used to build proteins, and then moves it to ribosomal protein factories.
Structure	DNA consists of two strands, arranged in a double helix. These strands are made up of subunits called nucleotides. Each nucleotide contains a phosphate, a 5-carbon sugar molecule and a nitrogenous base.	RNA only has one strand, but like DNA, is made up of nucleotides. RNA strands are shorter than DNA strands. RNA sometimes forms a secondary double helix structure, but only intermittently.
Length	DNA is a much longer polymer than RNA. A chromosome, for example, is a single, long DNA molecule, which would be several centimetres in length when unravelled.	RNA molecules are variable in length, but much shorter than long DNA polymers. A large RNA molecule might only be a few thousand base pairs long.
Sugar	The sugar in DNA is deoxyribose, which contains one less hydroxyl group than RNA's ribose.	RNA contains ribose sugar molecules, without the hydroxyl modifications of deoxyribose.
Bases	The bases in DNA are Adenine ('A'), Thymine ('T'), Guanine ('G') and Cytosine ('C').	RNA shares Adenine ('A'), Guanine ('G') and Cytosine ('C') with DNA, but contains Uracil ('U') rather than Thymine.
Base Pairs	Adenine and Thymine pair (A-T) Cytosine and Guanine pair (C-G)	Adenine and Uracil pair (A-U) Cytosine and Guanine pair (C-G)
Location	DNA is found in the nucleus, with a small amount of DNA also present in mitochondria.	RNA forms in the nucleolus, and then moves to specialised regions of the cytoplasm depending on the type of RNA formed.
Reactivity	Due to its deoxyribose sugar, which contains one less oxygen-containing hydroxyl group, DNA is a more stable molecule than RNA, which is useful for a molecule which has the task of keeping genetic information safe.	RNA, containing a ribose sugar, is more reactive than DNA and is not stable in alkaline conditions. RNA's larger helical grooves mean it is more easily subject to attack by enzymes.

Ultraviolet (UV) Sensitivity	DNA is vulnerable to damage by ultraviolet light.	RNA is more resistant to damage from UV light than DNA.

Criteria for a molecule to be a genetic material:

1. It should be able to generate its replica
2. It should chemically and structurally be stable
3. It should provide the scope for slow changes that are required for evolution.
4. It should be able to express itself in Mendelian characters.

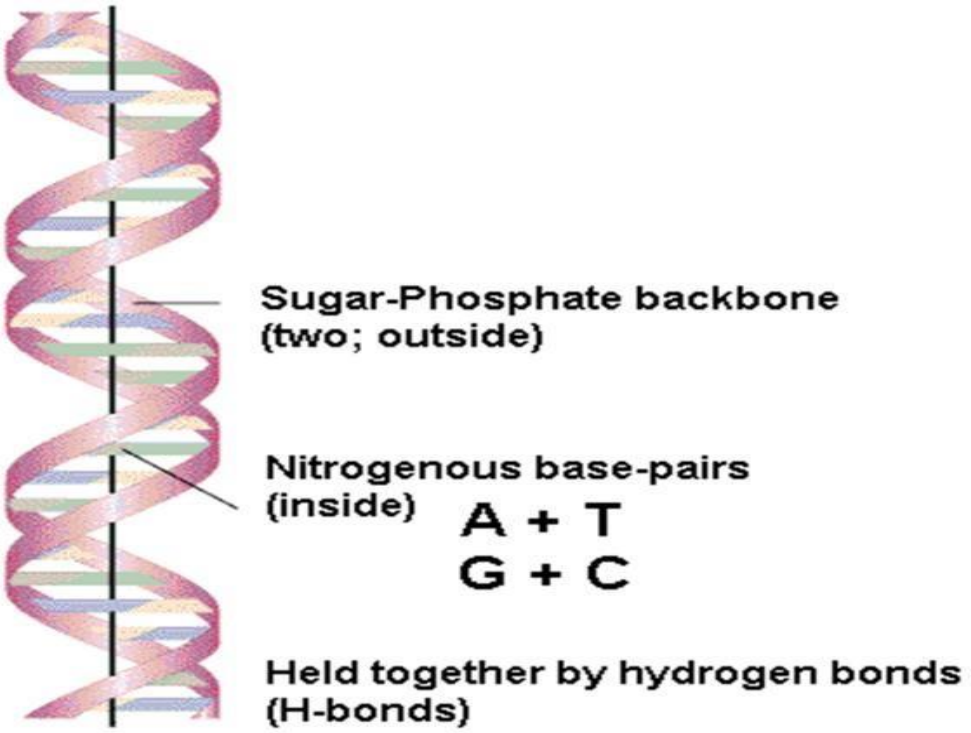
STRUCTURE OF DNA:

The genetic material in most organisms is DNA or Deoxyribonucleic acid; whereas in some viruses, it is RNA or Ribonucleic acid. A DNA molecule consists of two polynucleotide chains i.e. chains with multiple nucleotides. Let's understand the structure of this chain in detail.

The Watson-Crick Model of DNA (1953)

Deoxyribonucleic Acid (**DNA**) is a **double-stranded, helical molecule**. It consists of two **sugar-phosphate backbones** on the outside, held together by **hydrogen bonds** between pairs of **nitrogenous bases** on the inside. The bases are of four types (**A**, **C**, **G**, & **T**): pairing always occurs between **A** & **T**, and **C** & **G. James Watson** (1928 -) and **Francis Crick** (1916- 2004)realized that these pairing rules meant that either strand contained all the **information** necessary to make a new copy of the entire molecule, and that the order of bases might provide a**"genetic code"**.

Watson and Crick shared the **Nobel Prize in 1962** for their discovery, along with **Maurice Wilkins** (1916-2004), who had produced a large body of crystallographic data supporting the mode. Working in the same lab, **Rosalind Franklin** (1920-1958) had earlier produced the first clear crystallographic evidence for a helical structure. Crick went on to do fundamental work in molecular biology and neurobiology. Watson become Director of the Cold Spring Harbor Laboratory, and headed up the **Human Genome Project** in the 1990s.



A nucleotide is made of the following components:

- **Pentose sugar** – A pentose sugar is a 5-carbon sugar. In case of DNA, this sugar is deoxyribose whereas, in RNA, it is ribose.
- **Phosphate group**
- **Nitrogenous base** – These can be of two types – Purines and Pyrimidines. Purines include Adenine and Guanine whereas pyrimidines include Cytosine and Thymine. In RNA, thymine is replaced by Uracil.

Nitrogenous base + pentose sugar (via N-glycosidic linkage) = Nucleoside.
Nucleoside + phosphate group (via phosphoester linkage) = Nucleotide.
Nucleotide + Nucleotide (via 3′-5′ phosphodiester linkage) = Dinucleotide.
Many nucleotides linked together = Polynucleotide.
A polynucleotide has a free phosphate group at the 5′ end of the sugar and this is called the 5′ end. Similarly, the sugar also has a free 3′-OH group at the other end of the polynucleotide which is called the 3′ end. The backbone of a polynucleotide chain consists of pentose sugars and phosphate groups; whereas the nitrogenous bases project out of this backbone.
DNA is a long polymer and therefore, difficult to isolate from cells in an intact form. This is why it is difficult to study its structure. However, in 1953, James Watson and Francis revealed the 'double helix' model of the structure of DNA, based on X-ray diffraction data from Maurice Wilkins and Rosalind Franklin.
This model also reveals a unique property of polynucleotide chains – Base pairing. It refers to the hydrogen bonds that connect the nitrogen bases on opposite DNA strands. This pairing gives rise to complementary strands i.e. if you know the sequence of bases on one strand, you can predict the bases on the other strand. Additionally, if each DNA strand acts as a template for synthesis (parent) of a new strand, then the new double-stranded DNA (daughters) produced are identical to the parental DNA strand.
Salient Features of DNA Double-Helix

- It consists of two polynucleotide chains where the sugar and phosphate group form the backbone and the nitrogenous bases project inside the helix.

- The two polynucleotide chains have anti-parallel polarity i.e. if one strand has 5′ → 3′ polarity, the other strand has 3′ → 5′ polarity.
- The bases on the opposite strands are connected through hydrogen bonds forming base pairs (bp). Adenine always forms two hydrogen bonds with thymine from the opposite strand and vice-versa. Guanine forms three hydrogen bonds with cytosine from the opposite strand and vice-versa. Therefore, a purine always pairs with a pyrimidine on the other strand, giving rise to a uniform distance between the two strands of the helix.
- The two strands coil in a right-handed fashion. Each turn of the helix is 3.4nm (or 34 Angstrom units) consisting of 10 nucleotides. These nucleotides are at a distance of 0.34nm (or 3.4 Angstrom units).
- The helix is stable because of the base pairs that stack over one another and hydrogen bonds that hold the bases together.

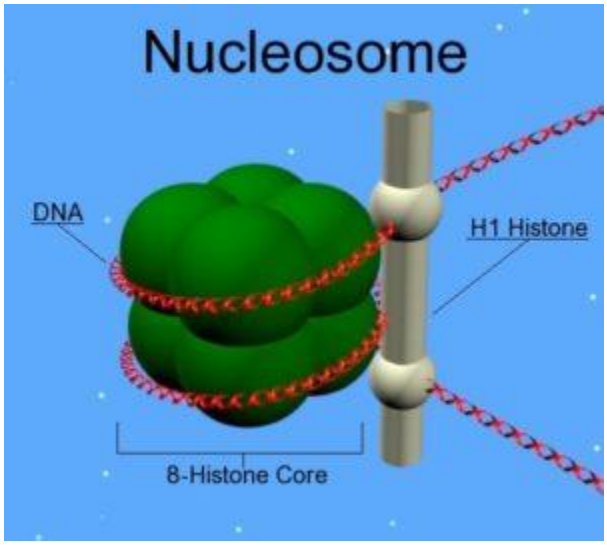
Packaging of DNA Helix

If you calculate the length of DNA in a typical mammalian cell, it is approximately 2.2 meters. The dimension of a typical nucleus is only about 10^{-6} meters! Then, how does such a long polymer fit in the nucleus of a cell?

Prokaryotes like *E. coli*, do not have a defined nucleus. Here, the negatively-charged DNA is held together in large loops by positively-charged proteins in a structure called 'nucleoid'. In Eukaryotes, however, the organization of DNA in the nucleus is much more complex and is as follows:

- The negatively-charged DNA is wrapped around a positively-charged **histone octamer** i.e. a unit of 8 histone molecules. This forms a '**Nucleosome**'. Histones are positively-charged proteins that are rich in basic amino acids – arginines and lysines. A typical nucleosome has 200bp of DNA helix.
- Many nucleosomes join together to form a thread-like structure – **Chromatin** in the nucleus. The nucleosomes in chromatin appear as 'beads-on-string' under the electron microscope.
- The chromatin is packaged to form **chromatin fibres** which are further coiled and condensed to form **chromosomes**. The higher level packaging of chromatin requires another set of proteins – **Non-histone Chromosomal (NHC) proteins**.

Note: **Euchromatin** is the region of chromatin that is loosely packed and therefore stains lightly; whereas **Heterochromatin** is the densely packed region and therefore stains dark.



MUTATION

Definition: mutations are new sudden inheritable discontinuous variations which appear in the organisms due to permanent change in their genotypes. The term mutation is derived from the Latin word 'mutare'meaning to change.

TYPES OF MUTATION: Mutations appearing in germinal cell are called germinal mutations.they are passed on to the offspring. Mutations appearing in the body cells(other than germinal are known as somatic mutations. They generally die with the death of the body.

CHROMOSOMAL MUTATIONS:

- Mutations that occur in chromosome.
- They are of two types genomic and chromosomal aberrations.

I. Genomic mutations:

- Changes in chromosomal number
- They are of two types polyploidy and aneuploidy.
- Polyploidy :it is the phenomenon of having more than two sets of chromosomes or genomes.polyploidy occurs in nature due to the failure of chromosome to separate at the time of anaphase either due to non-disjunction or non-formation of spindle. An organism or its karyotype having more than two genomes is called a polyploid. Depending upon the number of genomes present in a polyploid it is known as triploid /tetraploid/pentaploid/hexaploidetc.triploids and pentaploids have odd chromosomes and do not form synapsis hence are sexually sterile. polyploidy is of 3 types-autoploidy ,alloploidy and autoalloploidy
- Aneuploidy :it is a condition of having fewer or extra chromosomes than the normal genome number of the species.it arises due to nondisjunction of the two chromosomes of homologous pair so that one gamete comes to have an extra chromosome while the other becomes deficient in one chromosome. Aneuploidy is of two types hypoploidy or loss of chromosomes,(which is further classified into monosomic,nullisomic,and mixed aneuploids) and hyperploidy or addition of chromosomes.(which is further classified into trisomic and tetrasomic)

Chromosomal aberrations:

- Changes in chromosomal structure
- They are changes in the number and arrangement of genes in the chromosomes
- Chromosome aberrations may involve changes in single chromosomes (intrachromosomal aberrations) or two chromosomes (interchromosomal aberrations)
- Intra chromosomal aberrations are of types-deficiency, deletion, and inversion.
- Inter chromosomal aberrations are of types- duplication, translocation

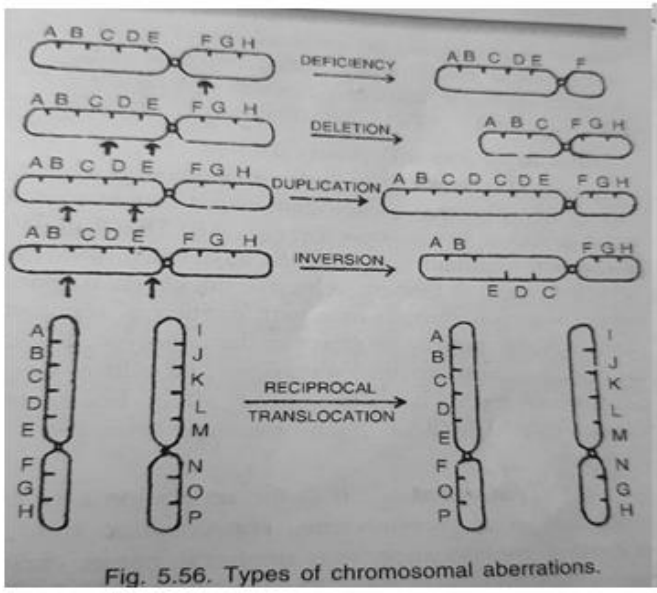


Fig. 5.56. Types of chromosomal aberrations.

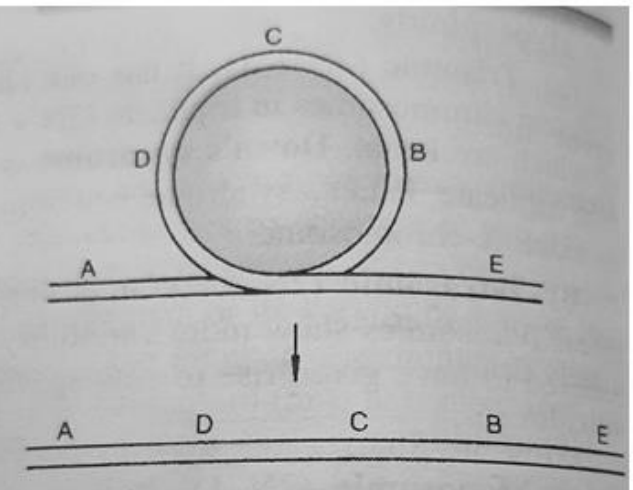


Fig. 5.57. Inversion through loop formation.

GENE MUTATIONS:

Genes are segments of DNA located on chromosomes. A gene mutation is defined as an alteration in the sequence of nucleotides in DNA. This change can affect a single nucleotide pair or larger gene segments of a chromosome. DNA consists of a polymer of nucleotides joined together. During protein synthesis, DNA is transcribed into RNA and then translated to produce proteins. Altering nucleotide sequences most often results in nonfunctioning proteins. Mutations cause changes in the genetic code that lead to genetic variation and the potential to develop the disease. Gene mutations can be generally categorized into two types: point mutations and base-pair insertions or deletions.

Point Mutations

Point mutations are the most common type of gene mutation. Also called a base-pair substitution, this type of mutation changes a single nucleotide base pair. Point mutations can be categorized into three types:

- **Silent Mutation:** Although a change in the DNA sequence occurs, this type of mutation does not change the protein that is to be produced. This is because multiple genetic codons can encode for the same amino acid. Amino acids are coded for by three-nucleotide sets called codons. For example, the amino acid arginine is coded for by several DNA codons including CGT, CGC, CGA, and CGG (A = adenine, T = thymine, G = guanine, and C = cytosine). If the DNA sequence CGC is changed to CGA, the amino acid arginine will still be produced.
- **Missense Mutation:** This type of mutation alters the nucleotide sequence so that different amino acid is produced. This change alters the resulting protein. The change may not have much effect on the protein, may be beneficial to protein function, or may be dangerous. Using our previous example, if the codon for arginine CGC is changed to GGC, the amino acid glycine will be produced instead of arginine.
- **Nonsense Mutation:** This type of mutation alters the nucleotide sequence so that a stop codon is coded for in place of amino acid. A stop codon signals the end of the translation process and stops protein production. If this process is ended too soon, the amino acid sequence is cut short and the resulting protein is most always nonfunctional.

Base-Pair Insertions and Deletions

Mutations can also occur in which nucleotide base pairs are inserted into or deleted from the original gene sequence. This type of gene mutation is dangerous because it alters the template from which amino acids are read. Insertions and deletions can cause frame-shift mutations

when base pairs that are not a multiple of three are added to or deleted from the sequence. Since the nucleotide sequences are read in groupings of three, this will cause a shift in the reading frame. For example, if the original, transcribed DNA sequence is CGA CCA ACG GCG..., and two base pairs (GA) are inserted between the second and third groupings, the reading frame will be shifted.

- **Original Sequence:** CGA-CCA-ACG-GCG...
- **Amino Acids Produced:** Arginine/Proline/Threonine/Alanine...
- **Inserted Base Pairs (GA):** CGA-CCA-GAA-CGG-CG...
- **Amino Acids Produced:** Arginine/Proline/Glutamic Acid/Arginine...

The insertion shifts the reading frame by two and changes the amino acids that are produced after the insertion. The insertion can code for a stop codon too soon or too late in the translation process. The resulting proteins will be either too short or too long. These proteins are for the most part defunct.

Causes of Gene Mutation

Gene mutations are most commonly caused as a result of two types of occurrences. Environmental factors such as chemicals, radiation, and ultraviolet light from the sun can cause mutations. These mutagens alter DNA by changing nucleotide bases and can even change the shape of DNA. These changes result in errors in DNA replication and transcription.

Other mutations are caused by errors made during mitosis and meiosis. Common errors that occur during cell division can result in point mutations and frameshift mutations. Mutations during cell division can lead to replication errors which can result in the deletion of genes, translocation of portions of chromosomes, missing chromosomes, and extra copies of chromosomes.

Introduction to DNA Damage and Repair:

DNA is a highly stable and versatile molecule. Though sometimes the damage is caused to it, it is able to maintain the integrity of information contained in it. The perpetuation of genetic material from generation to generation depends upon keeping the rates of mutation at low level. DNA has many elaborate mechanisms to repair any damage or distortion.

The most frequent sources of damage to DNA are the inaccuracy in DNA replication and chemical changes in DNA. Malfunction of the process of replication can lead to incorporation of wrong bases, which are mismatched with the complementary strand. The damage causing chemicals break the backbone of the strand and chemically alter the bases. Alkylation, oxidation and methylation cause damage to bases. X-rays and gamma radiations cause single or double stranded breaks in DNA.

A change in the sequence of bases if replicated and passed on to the next generation becomes permanent and leads to mutation. At the same time mutations are also necessary which provide raw material for evolution. Without evolution, the new species, even human beings would not have arisen. Therefore, a balance between mutation and repair is necessary.

Types of Damage:
Damage to DNA includes any deviation from the usual double helix structure.

1. Simple Mutations:

Simplest mutations are switching of one base for another base. In transition one pyrimidine is substituted by another pyramidine and purine with another purine. Trans-version involves substitution of a pyramidine by a purine and purine by a pyrimidine such as T by G or A and A by C or T. Other simple mutations are detection, insertion of a single nucleotide or a small number of nucleotides. Mutations which change a single nucleotide are called point mutations.

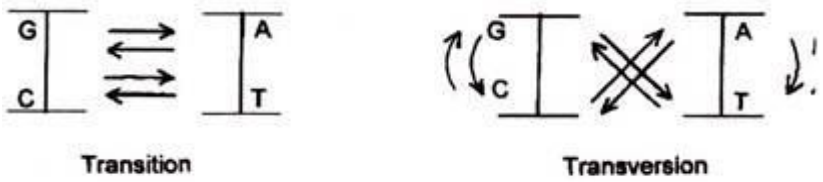


Fig. 5.1.

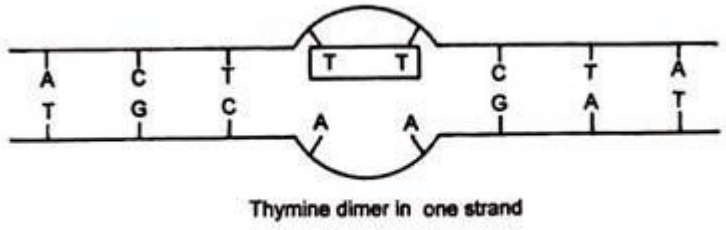
2. Deamination:
The common alteration of form or damage includes deamination of cytosine (C) to form uracil (u) which base pairs with adenine (A) in next replication instead of guanine (G) with which the original cytosine would have paired. As uracil is not present in DNA, adenine base pairs with thymine (T). Therefore C-G pair is replaced by T-A in next replication cycle. Similarly, hypoxanthine results from adenine deamination.

3. Missing Bases:

Cleavage of N-glycosidic bond between purine and sugar causes loss of purine base from DNA. This is called depurination. This apurinic site becomes non-coding lesion.

4. Chemical Modification of Bases:

Chemical modification of any of the four bases of DNA leads to modified bases. Methyl groups are added to various bases. Guanine forms 7- methylguanine, 3-methylguanine. Adenine forms 3-methyladenine. Cytosine forms 5- Methylcytosine. Replacement of amino group by a keto group converts 5-methylcytosine to thymine.



Thymine dimer in one strand

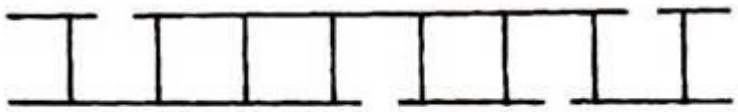
Fig. 5.2.

5. Formation of Pyrimidine Dimers (Thymine Dimers):

Formation of thymine dimers is very common in which a covalent bond (cyclobutyl ring) is formed between adjacent thymine bases. This leads to loss of base pairing with opposite stand. A bacteria may have thousands of dimers immediately after exposure to ultraviolet radiations.

6. Strand Breaks:

Sometimes phosphodiester bonds break in one strand of DNA helix. This is caused by various chemicals like peroxides, radiations and by enzymes like DNases. This leads to breaks in DNA backbone. Single strand breaks are more common than double strand breaks.



Double strand breaks

Fig. 5.3.

Sometimes X-rays, electronic beams and other radiations may cause phosphodiester bonds breaks in both strands which may not be directly opposite to each other. This leads to double strand breaks. Some sites on DNA are more susceptible to damage. These are called hot-stops.

Repair Mechanisms:

Most kinds of damage create impediments to replication or transcription. Altered bases cause mispairing and can cause permanent alteration to DNA sequence after replication.

In order to maintain the integrity of information contained in it, the DNA has various repair mechanisms.

1. Direct Repair:

The damage is reversed by a repair enzyme which is called photoreactivation. This mechanism involves a light dependant enzyme called DNA photolyase. The enzyme is present in almost

all cells from bacteria to animals. It uses energy from the absorbed light to cleave the C-C bond of cyclobutyl ring of the thymine dimers. In this way thymine dimers are monomerized.

2. Excision Repair:

It includes base excision repair and nucleotide excision repair. Base excision repair system involves an enzyme called N-glycosylase which recognizes the abnormal base and hydrolyes glycosidic bond between it and sugar. Another enzyme, an endonuclease cleaves the DNA backbone on the 5′-side of the abnormal base. Then the DNA polymerase by its exonuclease activity removes the abnormal base. DNA polymerase then replaces it with normal base and DNA ligase seals the region.

Nucleotide repair system includes three steps, incision, excision and synthesis. Incision is done by endonuclease enzyme precisely on either side of the damaged patch of the strand. In this way damaged portion of the strand is cleaved.

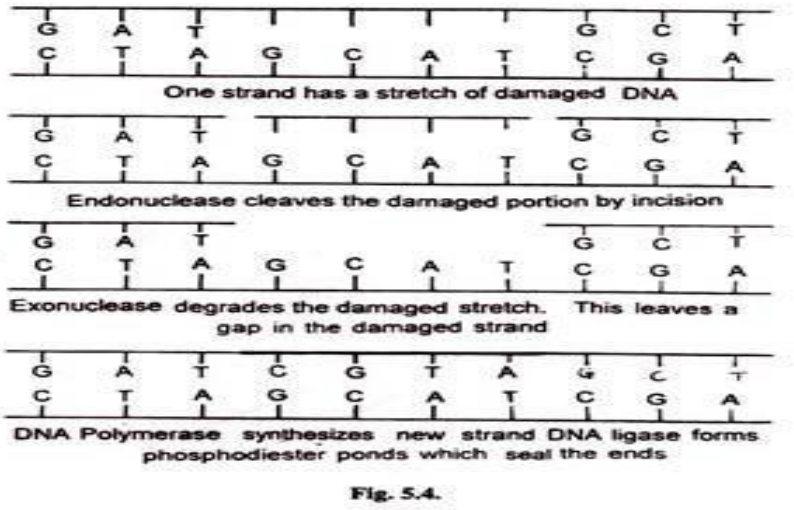


Fig. 5.4.

Endonuclease enzymes involved are UvrA, UvrB which recognize the damaged stretch of the strand. UvrC makes two cuts (incision) on either side. Exonuclease removes the damaged strand. Enzyme involved is UvrD. Later, DNA polymerase synthesizes the new strand by using complementary strand as a template. DNA ligase forms phosphodiester bonds which seal the ends on newly synthesized strand.

3. Mismatch Base Repair:

Sometimes wrong bases are incorporated during replication process, G-T or C-A pairs are formed. The wrong base is always incorporated in the daughter strand only. Therefore in order to distinguish the two strands for the purpose of repair, the adenine bases of the template strand are labelled or tagged by methyl groups. In this way the newly replication DNA helix is hemimethylated. The excision of wrong bases occur in the non-methylated or daughter strand.

4. Recombination Repair or Retrieval System:

In thymine dimer or other type of damage, DNA replication cannot proceed properly. A gap opposite to thymine dimer is left in the newly synthesized daughter strand. The gap is repaired by recombination mechanism or retrieval mechanism called also sister strand exchange.

During replication of DNA two identical copies are produced. Replicating DNA molecule has four strands A, B, C and D. Strands A and C have same DNA , sequence. Strands B and D also have same sequence as they are identical. A thymine dimer is present in strand A. The replication fork passes the dimmer as it cannot form hydrogen bonds with incoming adenine bases, thus creating a gap in the newly synthesized strand B.

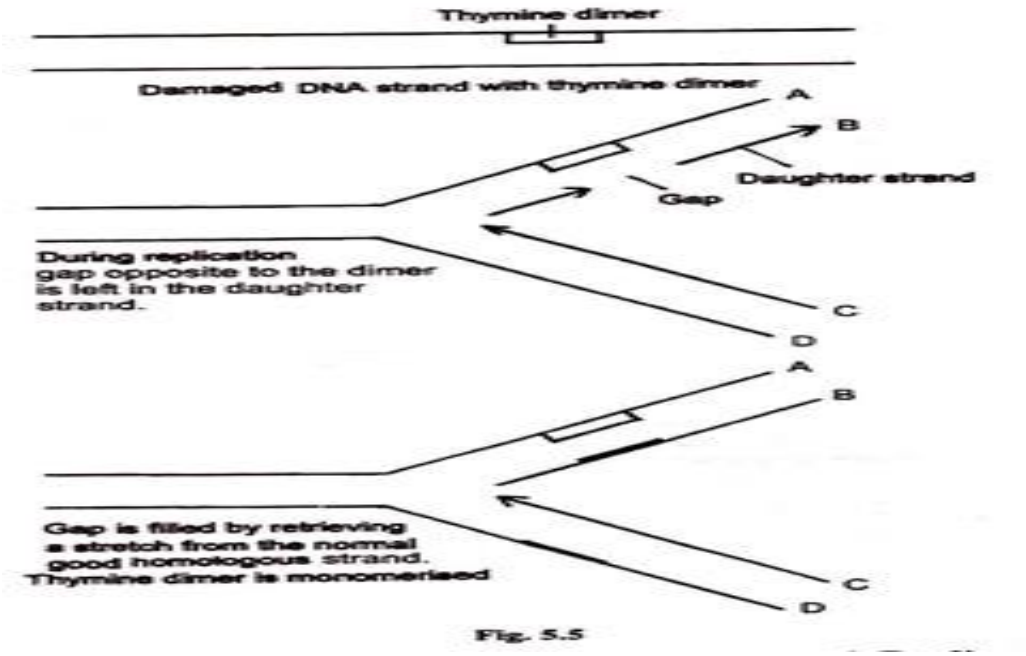


Fig. 5.5

In recombination repair system a short identical segment of DNA is retrieved from strand D and is inserted into the gap of strand B. But this creates a gap in strand D which is easily filled up by DNA polymerase using normal strand C as a template. This event is dependent on the activity of a special protein Rec A. The Rec A protein plays its role in retrieving a portion of the complementary strand from other side of the replication fork to fill the gap. Rec A is a strand exchange protein.After filling both gaps, thymine is monomerised. So in this repair mechanism a portion of DNA strand is retrieved from the normal homologous DNA segment. This is also known as daughter strand gap repair mechanism.

5. SOS Repair Mechanism:

Sometimes the replicating machinery is unable to repair the damaged portion and bypasses the damaged site. This is known as translesion synthesis also called bypass system and is emergency repair system. This mechanism is catalyzed by a special class of DNA polymerases

called Y-family of DNA polymerases which synthesized DNA directly across the damaged portion.

Physicochemical properties of DNA

Denaturation of DNA:

(i) **Denaturation by Temperature:**

If a DNA solution is heated to approximately 90°C or above there will be enough kinetic energy to denature the DNA completely causing it to separate into single strands. This denaturation is very abrupt and is accelerated by chemical reagents like urea and formamide.

The chemicals enhance the aqueous solubility of the purine and pyrimidine groups. This separation of double helix is called melting as it occurs abruptly at a certain characteristic temperature called denaturation temperature or melting temperature (T_m).

It is defined as temperature at which 50% of the DNA is melted. The abruptness of the transition indicates that the DNA double helix is highly cooperative structure, held together by many reinforcing bonds. The melting of DNA can be followed spectrophotometrically by monitoring the absorbance of DNA at 260 nm. T_m is analogous to the melting point of crystal. The T_m value depends on the nature of the DNA.

If several samples of DNA are melted, it is found that the T_m is highest for those DNAs that contain the highest proportion of G—C. Actually the value is used to estimate the percentage of G—C in a DNA sample. In fact, the T_m of DNA from many species varies linearly with G—C content.

This relationship between T_m and G—C content arises due to guanine and cytosine form three hydrogen bonds when base paired, whereas adenine and thymine form only two.

Denaturation involves the following changes of the properties of DNA:

(a) Increase in Absorption of UV-Light:

If denaturation is followed spectrophotometrically by monitoring the absorbance of light at 260 nm, it is observed that the absorbance at 260 nm increases as the DNA become denatured, a phenomenon known as the hyperchromatic effect or hyperchromacity or hyperchromism. This is due to un-stacking of base pairs.

A plot of the absorbance at 260 nm against the temperature of a DNA solution indicates that little denaturation occurs below approximately 70°C, but further increases in temperature result in a marked increase in the extent of denaturation.

(b) Decrease in Specific Optical Rotation:

Double-stranded DNA shows a strong positive rotation which highly decreases with denaturation. This change is analogous to the change in rotation observed when the proteins are denatured.

(c) Decrease in Viscosity:

The solutions of native DNA exhibit high viscosity because of the relatively rigid double helical, long and rod like character of DNA molecule. Denaturation causes a marked decrease in viscosity.

If melted DNA is cooled it is possible to reassociate the separated strands, a process known as renaturation. However, a stable double-stranded molecule may be formed only if the complementary strands collide in such a way that their bases are paired precisely. But renaturation may not be precise if the DNA is very long and complex.

Thus the rate of renaturation (renaturation kinetics) can give information about the complexity of a DNA molecule. Complete denaturation is not a readily reversible process. If a heat-

denatured DNA solution is cooled slowly (anneling) and hold the solution at about 25°C below T_m and above a concentration of 0.4M Na^+ for several hours, some amount of
DNA (50-60%) is renatured. Rapid cooling does not reverse denaturation, but if the cooled solution is again heated and then cooled slowly, renaturation takes place.

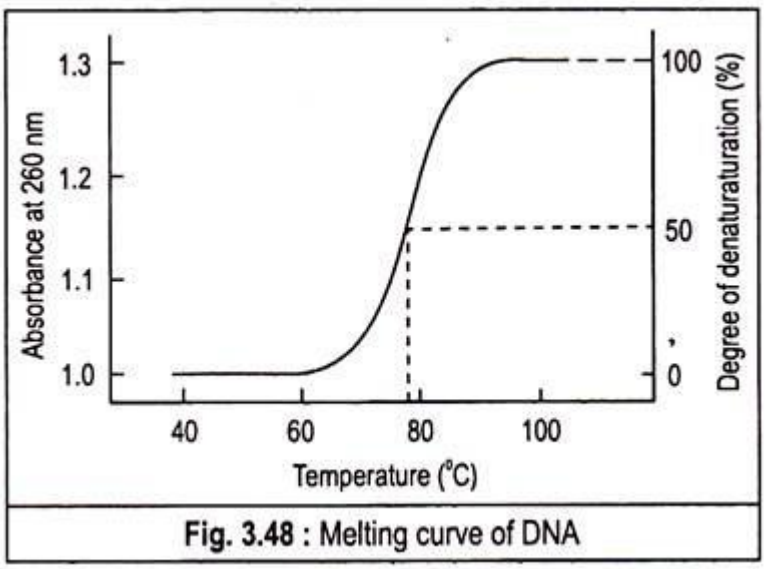


Fig. 3.48 : Melting curve of DNA

(ii) Denaturation by Chemical Agents:
Denaturation of DNA double helix can also be brought about by certain chemical agents such as urea and formamide. These chemical reagents enhance the aqueous solubility of the purine and pyrimidine groups. The T_m value is lowered by the addition of urea. In 8M urea, T_m is decreased by nearly 20°C. DNA can be completely denatured by 95% formamide at room temperature only.

(iii) **Effect of pH on Denaturation:**
Denaturation also occurs at acidic and alkaline solutions in which ionic changes of the purine and pyrimidine bases can occur. In acidic solutions at pH values 2-3 the amino groups bind with protons and the DNA double helix is disrupted. Similarly, in alkaline solutions at pH 12, the enolic hydroxyl groups ionize, thus preventing the keto-amino hydrogen bonding.

Renaturation of DNA:
When preparations of double-stranded DNA are denatured and allowed to renature, the rate of renaturation can give valuable information about the complexity of the DNA if there are repetitive sequences in the DNA, it shows less complexity in comparison to its total length, but the complexity is equal to its total length if all sequences are unique.

The 1kb DNA fragments are denatured by heating above its T_m and then renatured at a temperature 10°C below the T_m and monitored either by decrease in absorbance at 260 nm (hypochromic effect), or by-passing samples at intervals through a column of hydroxylapatite, which retains only double stranded DNAs, and estimating how much of the sample is retained. The degree of renaturation after a given time depends on C_0, the concentration of double stranded DNA prior to denaturation, and t, the duration of the renaturation in seconds. The concentration is measured in nucleotides per unit volume. In order to compare the rates of renaturation of different samples of DNA it is usual to measure C_0 and the time taken for renaturation to proceed half way to completion, $t_{1/2}$, and to multiply these values together to give a $C_0t_{1/2}$ value. The larger the $C_0t_{1/2}$, the greater the complexity of the DNA; hence λ DNA has a far lower $C_0t_{1/2}$ than does human DNA.

if the extent of renaturation is plotted against log C_0t (known as Cot curve), it is observed that part of the DNA is renatured quite rapidly while the rest is very slow to renature. This indicates that some sequences have a higher concentration than others i.e., part of the genome consists of repetitive sequences.
These repetitive sequences can be separated from the single-copy unique DNA by passing the renaturating sample through a hydroxylapatite column early in the renaturation process, at a time which gives a low value of C_0t. At this stage only the rapidly renaturating sequences will be double stranded, and will, therefore, bind to the column.

C VALUE PARADOX:

C-value is the amount, in picograms, of DNA contained within a haploid nucleus (e.g. a gamete) or one half the amount in a diploid somatic cell of a eukaryotic organism.
C-value' means the 'constant' (or 'characteristic') value of haploid DNA content per nucleus, typically measured in picograms (1 picogram is roughly 1 gigabase). Around 1950, the observation that different cell types in the same organism generally have the same C-value was part of the evidence supporting the idea that DNA was responsible for heredity.
This chart shows the range of **C-value** [genome size, measured as number of **Kbp of DNA**] for a variety of organisms. So-called "simple" prokaryotic organisms in general have less **DNA** per genome than do more "complex," eukaryotic organisms, such as plants and animals. The so-called **C-Value Paradox** refers to the observation that genome size does not uniformly increase with respect to perceived complexity of organisms, for example vertebrate with respect to invertebrate animals, or "lower" versus "higher" vertebrate animals (red box). Note for examples that some Amphibians have more than 10-fold more **DNA** than do Mammals, including humans.

There is in fact no "paradox." Evolution does not proceed in a linear manner, nor is there a linear succession of organisms from "lower" to "higher." Despite differences in **DNA** content, the number of genes in any vertebrate genome is roughly similar. Also, plant and amphibian genomes in particular are frequently **polyploid**, in which the chromosome number undergoes doubling to two-, four, or eight-fold. without a radical change to the form of the organisms.

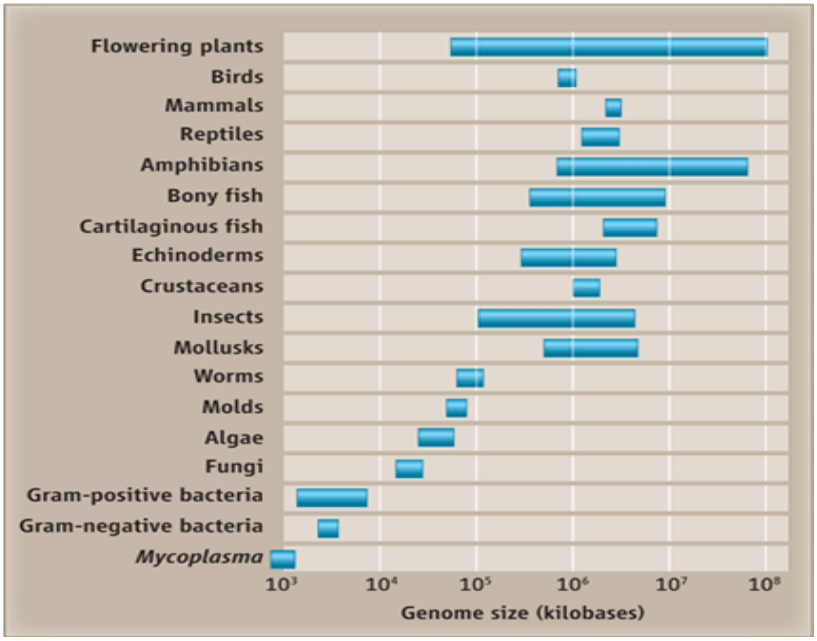


Fig. Genomic size of different organism

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DEPARTMENT OF BIOMEDICAL ENGINEERING

UNIT – V - Cell biology & Genetics – SBMA1101

Human Chromosome:

The human genome is 3 x 10^9 base pairs of DNA and the smallest human chromosome is several times larger than the entire yeast genome; and the extended length of DNA that makes up the human genome is about 1 m long. The human genome is distributed among 24 chromosomes (22 autosomes and the 2 sex chromosomes), each containing between 45 and 280 Mb of DNA **(Figure 1)**. The sex chromosomes are denoted by X and Y and they contain genes which determine the sex of an individual i.e., XX for female and XY for male. The rest are known as autosomes. The haploid human genome contains about 23,000 protein-coding genes, which are far fewer than had been expected before sequencing. In fact, only about 1.5% of the genome codes for proteins, while the rest consists of non-coding genes, regulatory sequences, introns, and noncoding DNA. Chromosomes are stained with A-T (G bands) and G-C (R bands) base pair specific dyes **(Figure 1)**. When they are stained, the mitotic chromosomes have a banded structure that unambiguously identifies each chromosome of a karyotype. Each band contains millions of DNA nucleotide pairs which do not correspond to any functional structure. G-banding is obtained with Giemsa stain yielding a series of lightly and darkly stained bands. The dark regions tend to be heterochromatic and AT rich. The light regions tend to be euchromatic and GC rich. R-banding is the reverse of G- banding where the dark regions are euchromatic and the bright regions are heterochromatic.

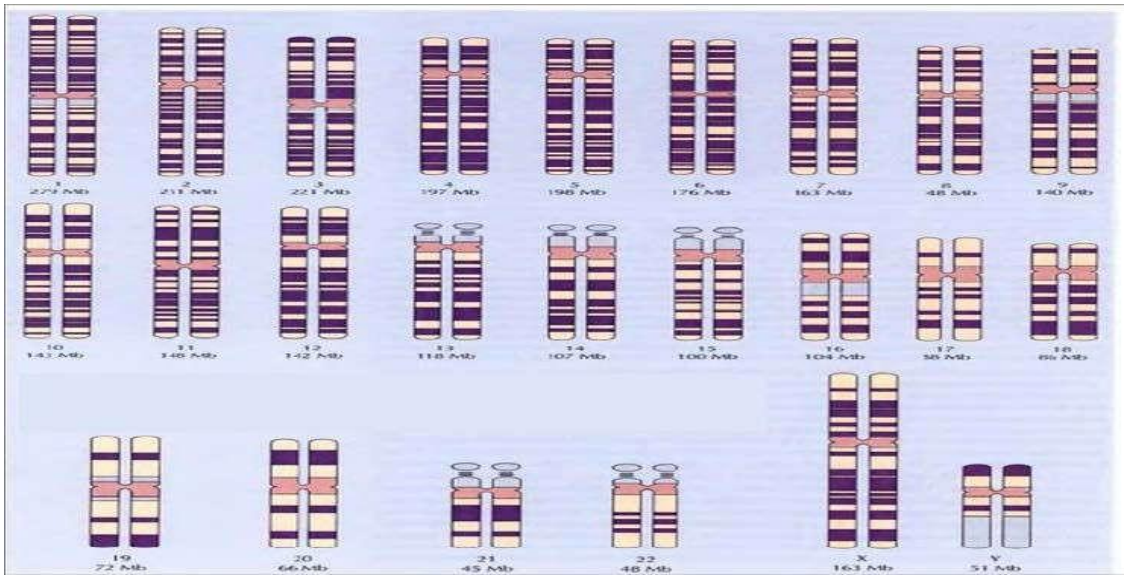


Figure 1: Human metaphase chromosome showing the banding pattern obtained after cytogenetic staining.

Types of human chromosomes

There are four types of chromosomes based upon the position of the centromere in humans **(Figure 2)**.

a. **Metacentric**: In this type of chromosome the centromere occurs in the centre and all the four chromatids are of equal length.

b. **Submetacentric**: In this type of chromosome the centromere is a little away from the centre and therefore chromatids of one side are slightly longer than the other side.

c. **Acrocentric**: In this type of chromosome the centromere is located closer to one end of chromatid therefore the chromatids on opposite side are very long. A small round structure, attached by a very thin thread is observed on the side of shorter chromatid. The small round structure that is a part of the chromatid is termed as satellite. The thin strands at the satellite region are termed as Nucleolar Organiser Region

d. **Telocentric**: In this type of chromosome the centromere is placed at one end of the chromatid and hence only one arm. Such telocentric chromosomes are not seen in human cells.

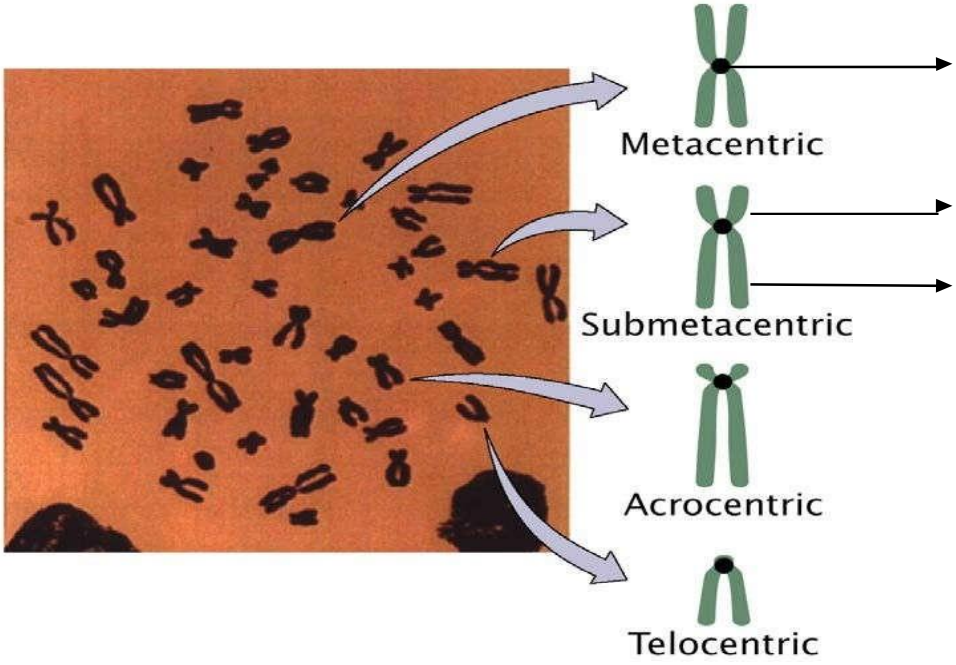


Figure 2: Types of human chromosomes.

Human Chromosome Karyotype

Eukaryotic species have several chromosomes and are detected only during mitosis or meiosis. They are best observed during the metaphase stage of cell division as they are found in the most condensed state. Thus each eukaryotic species is characterized by a **karyotype** which is the numerical description (number and size) of chromosomes in the normal diploid cell. For example, the *Homo sapiens* possess 46 chromosome i,e., 23 pairs **(Figure 3)**. The karyotype is important because genetic research can correlate changes in the karyotype with changes in the phenotype of the individual. For example, Down's syndrome is caused by duplication of the human chromosome number 21. Insertions, deletions and changes in chromosome number can be detected by the skilled cytogeneticist, but correlating these with specific phenotypes is difficult.

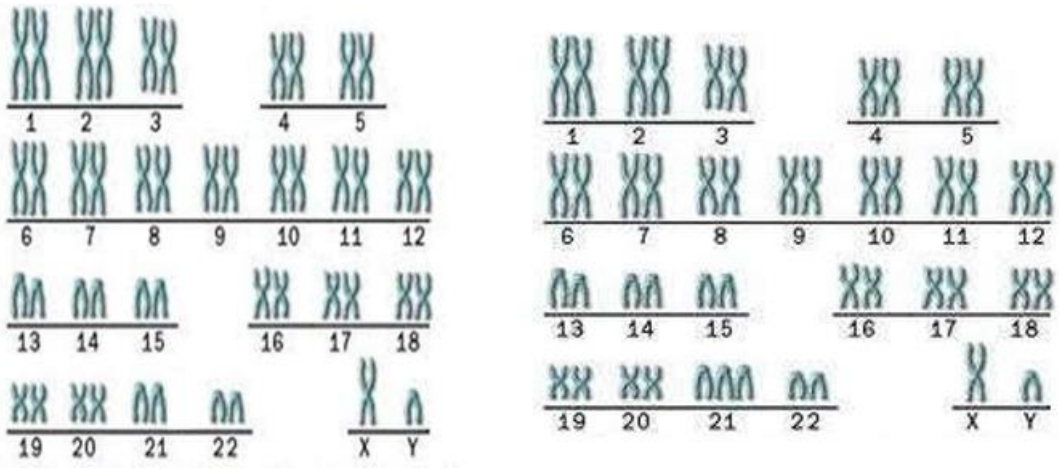


Figure 3: The normal human karyotype (left) and human karyotype in Down's syndrome (Right).

Chromosomal Abnormalities

Normally, humans have 23 pairs of chromosomes - making 46 in total. This includes one pair of chromosomes which are the sex chromosomes. The ova and the sperm each carry 23 chromosomes.

- **Numerical Aberrations**
- **Structural Aberrations**

Chromosomal abnormalities occur when there is a defect in a chromosome, or in the arrangement of the genetic material on the chromosome. Very often, chromosome abnormalities give rise to specific physical symptoms, however, the severity of these can vary from individual to individual.

Abnormalities can be in the form of additional material which may be attached to a chromosome, or where part or a whole chromosome is missing, or even in defective formation of a chromosome. Any increases or decreases in chromosomal material interfere with normal development and function. There are two main types of chromosomal abnormality which can occur during meiosis and fertilization: numerical aberrations and structural aberrations.

Numerical Aberrations

Numerical Aberrations Numerical aberrations are those that cause a change (addition or deletion) in the number of chromosomes. They are further classified as euploidy changes or aneuploidy changes. • Euploidy is the condition when an organism gains or losses one or more complete set of chromosomes, thus causing change in the ploidy number. For example, triploid (3n), tetraploid (4n) etc. • Aneuploidy is the condition when an organism gains or losses one or more chromosomes and not the entire set. For example, trisomy (2n+ 1), monosomy (2n – 1) (Table 2.1). In humans, euploidy conditions do not exist because the extent of abnormality is too large to sustain life. Aneuploidy conditions, however, are more common and are manifested in disorders such as Down syndrome (**Autosomal**), Klinefelter syndrom e and Turner syndrome (**Allosomal Aberrations**)

.

2. **Down syndrome:** This syndrome is a type of trisomy as there is an extra copy of the chromosome 21. It is named so after the person who discovered this chromosomal disorder – Langdon Down. The symptoms in a person include the following:
   - Person is short and has a small and round head
   - Physical and mental development is retarded
   - Furrowed tongue and partially open mouth Broad palm

3. **Klinefelter syndrome**: This genetic disorder arises due to the presence of an additional X chromosome in males. Thus, resulting in a chromosome count of 47 (44 + XXY) instead of 46.
   - Such a person has a masculine physique but has feminine development like the development of breasts
   - Such individuals are sterile, i.e.; they cannot reproduce

4. **Turner syndrome**: Unlike Klinefelter syndrome, in this chromosomal disorder there is the absence of one X chromosome in females. Hence, decreasing the chromosomes count to 45 (44 + X0). The symptoms include the following:
   - Such females are sterile
   - Have rudimentary ovaries and there is the absence of secondary sexual characters.

Structural Aberrations

These occur due to a loss or genetic material, or a rearrangement in the location of the genetic material. They include: deletions, duplications, inversions, ring formations, and translocations.

 Deletions: A portion of the chromosome is missing or deleted. Known disorders

include Wolf-Hirschhorn syndrome, which is caused by partial deletion of the short arm of chromosome 4; and Jacobsen syndrome, also called the terminal 11q deletion disorder.

- Duplications: A portion of the chromosome is duplicated, resulting in extra genetic material. Known disorders include Charcot-Marie-Tooth disease type 1A which may be caused by duplication of the gene encoding peripheral myelin protein 22 (PMP22) on chromosome 17.

- Translocations: When a portion of one chromosome is transferred to another chromosome. There are two main types of translocations. In a reciprocal translocation, segments from two different chromosomes have been exchanged. In a Robertsonian translocation, an entire chromosome has attached to another at the centromere; these only occur with chromosomes 13, 14, 15, 21 and 22.

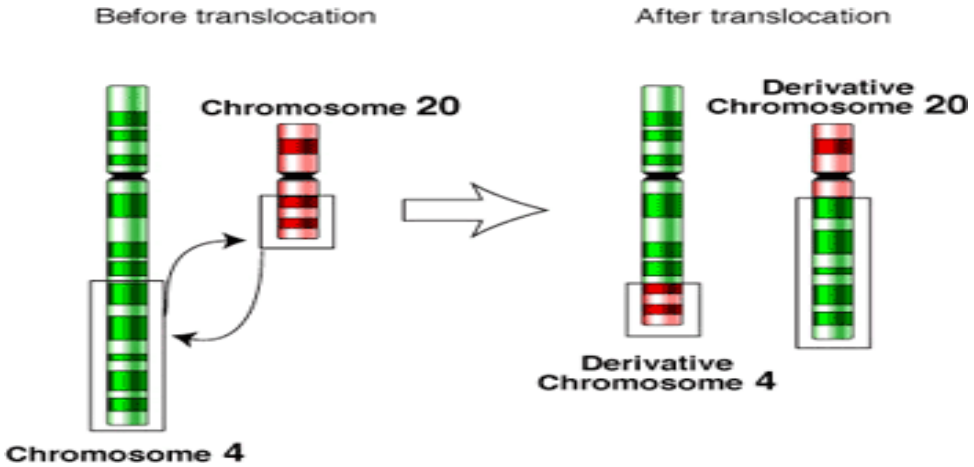
- Inversions: A portion of the chromosome has broken off, turned upside down and reattached, therefore the genetic material is inverted.

- Rings: A portion of a chromosome has broken off and formed a circle or ring. This can happen with or without loss of genetic material.

- Isochromosome: Formed by the mirror image copy of a chromosome segment including the centromere.

Structural aberrations also include some disorders which are characterized by chromosomal instability and breakage. One example, is the creation of a fragile site on the X Chromosome - Fragile X syndrome. Boys are worse affected by this because they only have one X-Chromosome but even in girls, Fragile X syndrome can cause learning difficulties.

Most chromosome anomalies occur as an accident in the egg or sperm, and are therefore not inherited. The anomaly is present in every cell of the body.Some



anomalies, however, can happen after conception, resulting in mosaicism (where some cells have the anomaly and some do not). Chromosome anomalies can be inherited from a parent or be "de novo". This is why chromosome studies are often performed on parents when a child is found to have an anomaly.

Translocation - showing a portion of one chromosome transferred to another chromosome

Inheritance of Autosomal Recessive Genetic Diseases

These genetic diseases are diseases caused by an error in a single DNA gene. Autosomal means the errors occurs on chromosome 1. 22 rather than on the 23rd sex-linked X chromosome. Recessive means that disease only occurs when a person has two copies of the bad gene. Usually this means they must inherit the disease from both parents. Some examples of autosomal recessive diseases are Cystic Fibrosis, Phenylketonuria, Sickle Cell Anemia, Tay Sachs, Albinism, and galactosemia.

Recessive diseases often occur in genes that produce an enzyme. In a carrier, who has only one bad copy, there is often no disease, because the second gene can pull up the slack, and maintain health. In some recessive diseases, a carrier gets a mild form of the disease. Autosomal recessive diseases are relatively rare, because to get the disease a person must inherit a bad gene from each parent, not just one. So both parents must have a bad gene. However, parents can be carriers without the disease, since they typically only have one bad gene themselves.

Inheritance patterns: There are various clear patterns common to any autosomal recessive genetic disease:

Children of an affected person will typically not have the disease (except in the rare case that they too marry someone who is also affected or a carrier of exactly the same disease), but the odds are 100% the child will be a carrier. The affected parent has two bad copies of the gene, so the child gets a bad gene from that parent, but usually a good second copy from the other unaffected parent.

If only one parent is a carrier (and the other unaffected), the child cannot get the disease, but might still be a carrier (typically 50% chance of being a carrier).

If both parents are a carrier, there is a 25% chance that their child will have the disease. There is also a 50% chance the child will be a carrier, and only 25% chance the child will be neither

diseased nor carrier. The situation where both parents are carriers is the most likely way that children with the disease are born.

If one parent has the disease, and the other is a carrier, a child has a 50% chance of getting the disease, and 50% chance of being a carrier. The child definitely gets one bad gene from the diseased parent, and has a 50% chance of getting a second one from the carrier parent.

Other children: If parents have one affected child, the odds of a second are usually 25%. If parents have a child with the disease, this almost always means that they are both carriers. The chances a second child will also have the disease are the same as above for two parent carriers: 25% chance of disease, 50% chance the second child is a carrier, and 25% chance of neither disease nor carrier. Note that genetic testing can often detect the rarer case where a child gets a genetic disease without both parents being carriers (perhaps only one is a carrier).

Gender bias: Male or females get the disease equally, because an autosomal error is unrelated to the sex chromosomes.

Inheritance patterns tend to be "horizontal", which a generation being affected (i.e. many siblings of the same parents), but not their parents nor their own children. Parents and next-generation children will usually be carriers.

Sporadic cases: A genetic disease that occurs when neither parent has any genetic defect is called a sporadic genetic disease. These cases arise via random genetic mutations in the DNA. A sporadic genetic mutation is not likely as a cause of an autosomal recessive disease, because it would require two identical random mutations (one in each copy of the gene) at the same time.

Inheritance of X-Linked Dominant Diseases

These genetic diseases are diseases caused by an error in a single DNA gene. X-linked means the genetic error occurs in a gene on the 23rd sex-linked X chromosome. These diseases are sometimes called "sex-linked" rather than x-linked. This pair of chromosomes is different from all others because male and females are different: males have XY (one X and one Y chromosome), whereas females are XX (two copies of X and no Y).

Rare type of genetic disease: X-linked dominant genetic conditions are rarer than other types of genetic disease. Most X- linked genetic diseases are recessive. The majority of

dominant genetic diseases affect the autosomal chromosomes 1..22. However, X-linked dominant genetic disease can occur.

Inheritance patterns for x-linked dominance: The patterns of genetic inheritance for X-linked dominant genetic diseases have the following features:

No carriers: There is no such thing as a carrier in a dominant disease, because you cannot have the bad gene without having the disease.

Father (XY) to Son (XY): 0% chance of disease (because the father's sperm provides only the Y chromosome, the son's good X comes from the mother's egg).

Father (XY) to Daughter (XX): 100% chance of disease (because the father's sperm gives the daughter an X, and the XY father only has one X chromosome to give, i.e. the bad one).

Mother (XX) to Son (XY): 50% chance of disease because the son gets one X chromosome from the mother, and has a 50% chance of getting the bad one versus the good gene.

Mother (XX) to Daughter (XX): 50% chance of disease, because it depends on whether the child gets the good or bad X from the mother.

Double dominant mother to child: The rare case of a mother with double-dominant disease (i.e. both X chromosomes have bad genes), has a 100% chance of passing disease to a child of either gender. Only females can be double-dominant.

Gender bias: Both males and females can have the disease, since both can have an X chromosome with a bad gene that dominates. However, more females have the disease than men. This is because daughters have 100% inheritance from fathers combined with 50% from mothers, whereas sons only have 50% from mothers. Statistically, there should be 3 times as many women with the disease as men.

Milder female disease: However, the female version of the disease can be milder for some diseases, because females have a second X chromosome, which should have a good gene that may mitigate somewhat against the dominant bad gene in the other X (i.e. perhaps the bad gene is not totally dominant).

Double dominance: A female can have the double dominant form of the disease, with two bad copies of the gene. Double dominance can only occur if both the mother and father have

the disease. This form is usually more severe than a dominant disease. (However it is unclear whether this is usually the same as the male version, who also has no good genes.)

Inheritance of X-Linked Recessive Genetic Diseases

These genetic diseases are diseases caused by an error in a single DNA gene. X-linked means the error occurs on the X chromosome which is the 23rd sex-linked X chromosome. Such diseases are sometimes called "sex-linked" rather than X- linked.

Some examples of X-linked recessive disorders are Hemophilia and Duchenne muscular dystrophy. These occurs only in boys, which is what we expected from an X-linked recessive disorder, as discussed below. For a list of this type of disorders, sex-linked recessive disorders.

Recessive means that disease only occurs when a person has two copies of the bad gene.

For autosomal recessive diseases, this usually means they must inherit the disease from both parents, but this is not the case for X-linked recessive diseases.
Men have only a single X chromosome, so they have only one copy of any gene on the X chromosome. Thus, a gene error on X definitely caused disease in men (who are XY), but women are XX, and have two copies of the gene. X-linked recessive disorders are more likely to occur than autosomal recessive disorders, because men have only one X chromosome, whereas all people have 2 copies of each autosome.

Recessive diseases often occur in genes that produce an enzyme. In a carrier, who has only one bad copy, there is often no disease, because the second gene can pull up the slack, and maintain health. In some recessive diseases, a carrier gets a mild form of the disease. For example, in X-linked recessive hemophilia, a female carrier has one bad gene on chromosome X, but the good gene on the other X chromosome produces enough of the good clotting enzyme to maintain health. The recessive disease only arises when the male has no good gene on the other chromosome (because they get a Y instead of a second good X).

X-linked recessive inheritance: These diseases arise from an error on the X chromosome, which causes disease only when there is no corresponding paired X chromosome with a good gene. However, since men are XY a man with the bad gene on the X chromosome must get the disease, because there is no second X chromosome. Since women are XX, they usually have a second good X chromosome which suppresses the bad X gene, leaving them

disease-free, but as carriers. The following patterns of inheritance are typical:

Gender bias: Typically, males are the ones who get the disease, whereas females are carriers. Men cannot be carriers because they cannot have a bad X chromosome gene without getting the disease. Women cannot get the disease, because they typically have a second good X chromosome. However, in the rare case of a daughter of an affected father and a carrier mother, then the daughter might have two bad X genes and get the disease itself (like an autosomal recessive disease).

Father to son transmission: 0% chance of disease and 100% chance of disease-free unless the mother is also a carrier (males always get their single X from the mother not father and cannot get a bad gene from the father), 0% chance of carrier (males cannot be carriers).

Father to daughter transmission: 0% chance of disease (females can only be carriers), 100% chance the female child is a carrier (because the father gives a bad X gene as there is only the bad one to give). If the mother is also a carrier, the female can be fully afflicted with the rare double-recessive female version of the disease.

Mother (carrier) to son transmission: 50% chance of disease, 50% chance disease-free, 0% chance of carrier (males cannot be carriers).

Mother to daughter: 0% chance of disease (females can only be carriers), 50% chance of female carrier, 50% chance neither affected nor carrier.

Other children: If a couple has one (male) child with the disease, what are the odds for another child having it. Usually this means the mother is a carrier, because the father cannot transmit the disease to a child (and the father would probably have noticable disease). So the risk for a second child of the same couple is probably the mother-to-son transmission risk, 50% chance of disease, and a female child cannot have the disease but has a 50% chance of being a carrier.

Mild disease in female carriers: Female carriers can have a mild form of disease, because they have a bad gene on one of their two X chromosomes, and a good gene on the other. If the disease is not totally recessive, a partial disease can result even though the woman has one good gene. In other words, if the second gene copy is not a good enough "backup", a partial level of mild disease can still result in carriers. However, most X-linked recessive diseases have symptom-free female carriers.

Y-linked Genetic Diseases

The Y chromosome is a sex-linked chromosome. Men are XY and women are XX, so only men have a Y chromosome. The Y chromosome is very small and contains few genes.

Y-linked transmission: The Y chromosome has a trivially simple inheritance pattern because women are XX and men are XY. Only men have a Y chromosome and so the Y is only passed from father to son. The Y chromosome is small and does not contain many genes. There are few genetic diseases related to genes on Y.

Male sex determination: The main Y gene is called the SRY gene, which is the master gene that specifies maleness and male features. It is the single gene that sets off the initial cascade of hormone changes that make a person male. It is not the entire Y chromosome, but just this gene that is necessary for maleness. There is evidence of this in rare diseases where the SRY gene is missing. People who are genetically male with XY chromosomes, but with a mutation or deletion of this SRY gene on the Y chromosome, will be female despite having most of the Y chromosome. And people who are genetically female with XX but also have a tiny piece of the Y chromosome with this gene, will become male despite their female-like XX chromosomes.

Introduction to Chromosome Diseases

Chromosome diseases are genetic diseases where a large part of the genetic code has been disrupted. Chromosomes are long sequences of DNA that contain hundreds or thousands of genes. Every person has 2 copies of each of the 23 chromosomes, called chromosomes 1..22 and the 23rd is the sex chromosome, which is either X and Y. Men are XY and women are XX in the 23rd chromosome pair.

Causes of chromosome diseases: Chromosomal diseases arise from huge errors in the DNA that result from having extra chromosomes, large missing sequences, or other major errors. These are usually caused by a random physical error during reproduction and are not inherited diseases (i.e. both parents are usually free of the condition).

Spontaneous chromosome errors: Most chromosomal diseases arise spontaneously from parents where neither has the disease. A large genetic mistake typically occurs in the woman's egg, which may partially explain why older women are more likely to have babies with Down syndrome.

Many chromosome errors cause the fetus to be aborted before birth, but some syndromes can be born and survive, though all typically suffer severe mental and physical defects. Down syndrome is the most common and well-known chromosome defect, but there are many.

Types of chromosome diseases: There are several common types of chromosome errors that cause disease. The effects of errors in the sex chromosomes (X and Y) differ greatly from errors in the autosomes (chromosomes 1..22). The following major classes of chromosome diseases can occur:

Trisomy conditions: Most people have 2 copies of each chromosome, but some people are born with 3 copies, which is called trisomy. Trisomy can occur in chromosomes 1..22 (autosomal trisomy) and also in the sex chromosome (see below). Down syndrome is a trisomy affecting the autosome chromosome 21.

Monosomy conditions: When a person has only one of a given chromosome, rather than a pair, this is called monosomy. These conditions are very rare for autosomes (chromosomes 1..22) because body cells without pairs do not seem to survive, but can occur in the sex chromosome (monosomy X is Turner syndrome).

Sex chromosome conditions: Typically men are XY and women are XX in the pair for the 23rd chromosome. However, sometimes people are born with only one sex chromosome (monosomy of the sex chromosome), or with three sex chromosomes (trisomy of the sex chromosome).

Translocation disorders: Partial errors in chromosomes can occur, where a person still only has a pair, but accidentally has entire sequences misplaced. These can lead to diseases similar to trisomy. For example, Translocation Down Syndrome is a subtype of Down Syndrome caused by translocation of a large sequence of a chromosome.

Subtraction disorders: The process of translocation can also cause large sequences of DNA to be lost from chromosomes. This creates diseases similar to monosomy conditions.

Mosaicisim: This refers to the bizarre situation where people have two types of cells in the body. Some cells have normal chromosomes, and some cells have a disorder such as a trisomy. One-sided chromosome disorders: For these unusual diseases it matters whether the chromosomes were inherited from the father or mother.

Non-contagiousness of chromosome diseases: All types of genetic diseases occur at birth

including chromosome diseases. You cannot catch the disease from someone else who has the disease. You are either born with the error in your chromosomes or not Genetic tests can determine whether or not a person has a chromosome disease, even as early as in the fetus by antenatal testing for genetic diseases.

Sex Chromosome Conditions

Sex chromosome defects: There are various defects of the sex chromosomes. Normally a man has XY and a woman XX. But the wrong combinations can arise with extra sex chromosomes or missing ones:

Turner syndrome (XO syndrome, monosomy X, missing Y): This should just be called the "X syndrome" because the person has an X, but no second sex chromosome. Such people are female, as there is no male Y chromosome. It is a 1-in-5000 syndrome, involving some relatively minor conditions, but usually sterility.

Klinefelter syndrome (XXY syndrome, also rarely XXXY): a 1-in-1000 disorder where the person is usually male (because of the Y chromosome), but has lower levels of testosterone and may have some female-like features (because there are two X chromosomes), and is usually sterile. The rarer XXXY syndrome may lead to retardation.

Jacobs syndrome (XYY syndrome): The person has an extra Y male chromosome. He will be male and may be largely normal, or may suffer from minor features such as excess acne and may be very tall, and in some cases behavioral complaints such as aggression. Frequency around 1-in-2000.

Triple-X (XXX, also XXXX or XXXXX): These people are females with an additional X chromosome. In rarer cases, there can even be 4 or 5 X chromosomes. They can be largely normal, or may suffer from problems such as infertility (some but not all), and reduced mental acuity. Occurs with a frequency around 1-in-700.

So there are viable combinations: XX (male), XY (female), XXY (Klinefelter), XXX, XYY, and XO (Turner). They all contain the X chromosome. Interestingly, there has been no combinations found that contain only Y: YO (Y, missing X), YY, or YYY syndromes. Not even aborted fetal embryo cells with this combination have been found. It has been suggested that there is something fundamental on the X chromosome that is needed for life.

Autosomal Trisomy Chromosome Diseases

The 22 non-sex autosome chromosomes (autosomes) can also exhibit disorders, of which the most common is trisomy (having 3 copies rather than a pair). Because these are disorders of the autosomes and not the sex chromosomes, these disorders can occur with males or females. These chromosome diseases arise rather surprisingly from an extra copy of the DNA, which makes you wonder why having 3 copies of the code bad even when the DNA code on the extra chromosome is actually correct. The condition of having 3 chromosomes is called trisomy and the most common example for autosomes is Down syndrome.

Here some details about particular autosome disorders:

Down syndrome (trisomy 21): an extra autosome creating a triplet at chromsome 21. These people are usually mentally retarded, and have physical characteristics such as an enlarged tongue and rounded flattened facial features. Frequency is around 1-in-800 but risk increases with the age of the mother to around 1-in-25 for a 45-year-old mother. The extra chromosome occurs because the mother's egg (or less commonly father's sperm) has wrongly kept both of its autosome 21 pair.

Edwards syndrome (trisomy 18): an extra autosome at chromosome 18. Most fetuses are aborted before term, but a live birth with this condition occurs with a frequency around 1-in-3000. Edwards syndrome is more severe than Down's syndrome, and includes mental retardation and numerous physical defects that often cause an early death.

Patau syndrome (trisomy 13): a very severe disorder leading to mental retardation and physical defects, occurring with a frequency around 1-in-5000. It is so severe that many babies die soon after birth.

Miscarriages caused by trisomy: So we have seen trisomies at autosomes 13, 15, 18, and 21. Trisomy at the other autosomes seems to be fatal in embryos leading to spontaneous miscarriage. The high frequency of natural miscarriages, around 1-in-5, occurs to a large extent because of chromosome errors.

Causes of trisomy: Since Down syndrome occurs more frequently in older women, one might theorize of the reason why. The most likely idea is that the problem is not during the pregnancy, but at the start, with more eggs created with poorly separated chromosomes in older women (about 1-in-5 for young women, compared to 3-in-4 for 40-year-old women).

However, another possibility is that the female body gradually loses its ability to recognize wrong cells in a fetus. But it is not an immune issue because the uterus is an immune-privileged site during pregnancy.

Partial trisomy: Down syndrome can be caused not only by a full trisomy, but also by a partial trisomy at autosome 21. Due to errors in a process called "translocation", a part of a chromosome can be wrongly attached to another pair. This creates a partial trisomy.

Another possible variant of Down's syndrome is a translocation between two pairs of chromosomes, usually part of 21 gets add to the 14th. This also causes a variant known as Translocation Down syndrome.

Mosaicism: Yet another chromosome oddity is mosaicism, where a person has different sets of chromosomes in different cells. If some cells are normal and others have trisomy 21, then Down syndrome results. Mosaicism can result from two paths. In the first method, the fetus started with trisomy 21, and then one line of cells lost the trisomy. In the second method, the fetus started normal, but somehow a cell line gained trisomy 21.

So why chromosome 21? It is one of the smaller chromosomes, and has relatively few genes (maybe 200-250). Research continues into determining why having too many of these genes, and consequent gene overexpression, leads to Down syndrome's characteristic mental and physical features.

Monosomy and Autosome Subtraction Disorders

Monosomy occurs when there is only one of a pair of chromosomes and is usually non-viable.

For example, the opposite of Down syndrome is monosomy-21, which is fatal. More common are "subtraction disorders" which occur due to missing genetic material within chromosomes, typically when a sequence of a chromosome is missing. The creation of reproductive sperm and egg cells involves a complex process that can sometimes misplace parts of a chromosome, such that one cell has an extra sequence (perhaps leading to one of the trisomy disorders if this cell becomes a child), but if a child is generated from the other cell, it may get a subtraction disorder.

Cri-du-chat syndrome (cat's cry): a subtraction disorder at autosome 5, with a missing short arm of chromosome 5, but not an entirely missing chromosome. Extremely rare at 1-in-50,000 and exhibiting severe mental retardation and physical defects including larynx problems giving the

characteristic cat-like child's cry.Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS): These are two separate genetic chromosome subtraction disorders that arise from the deletion of the same sequence on one copy of chromosome 15, specifically the sequence are "15q11-13". Prader-Willi Syndrome arises when it occurs on the father-inherited copy of chromosome 15, and Angelman Syndrome occurs if from the mother-inherited copy. This one-sided distinction between the father and mother's chromosomes is unusual, and quite important, and is discussed in detail later.

One-sided genetic disorders: Prader-Willi Syndrome and Angelman Syndrome

There are several disorders that have an odd characteristic in that it actually matters which of a pair of chromosomes is affected. Different effects arise for the chromosomes from the mother and from the father. This was a totally unexpected discovery since traditional genetic theory, particularly the "law of equivalent crosses", indicated that it did not matter which chromosome a gene was present on. However, it seems that the body does distinguish between the chromosomes that come from the father and the mother within each pair. Some genes are only activated on the chromosome that came from the mother's or father's side. It is like having male and female genes with slightly different effects. They are "imprinted" with some extra information, although exactly how this occurs is as yet unclear.

The best known examples are Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS), which both arise from the same sequence on chromosome 15. They are both rare, arising around 1-in-10,000 to 1-in-15,000 but the two diseases are related in a surprising way. For some reason, the sequence 15q11-13 is likely to be misplaced during reproductive cell creation.

Rather than causing a single disease, this error can cause two diseases. If it is deleted from the mother's egg, the child will get Angelman Syndrome; if the deletion occurs in a father's sperm, the child gets Prader-Willi Syndrome.

It is also important to note that both Prader-Willi and Angelman are actually gene disorders, not really chromosome disorders. Although the most common cause is from chromosome deletion (a non-inherited random physical occurrence), both of these diseases can arise rarely from a non-chromosome genetic inheritance. The real cause of the disease is the missing genes, rather than the chromosome-level changes. The gene for Angelman has been identified as the gene that creates the E6AP ubiquitin protein ligase 3A (UBE3A) protein, which is involved in the ubiquitination pathway (whatever that is). Inherited mutations of

the UBE3A gene do cause Angelman without any major chromosome error. The exact gene for PWS is not known since there are several genes on the 15q11-13 sequence deleted in PWS and AS. The most likely PWS gene seems to be SNRPN (small nuclear ribonucleoprotein N) gene but it is not yet certain.

PWS and AS are very distinct diseases. They are not two variants of the same disease and have significantly different mental and physical features. This makes sense since they are caused by failures of different genes. The AS gene is mother-sided and the gene(s) causing PWS are father-sided.

PWS has several features, the most notable of which is a total lack of appetite suppression leading sufferers to continual hunger and over-eating. If left uncontrolled, they will literally eat themselves to death via extreme obesity and the consequent heart or organ damage. PWS suffers may have a slightly reduced mental capacity, but are not usually significantly retarded. Other physical features include some facial features, hypogonadism (testes or ovaries), and short stature.

AS is a more several mental disorder causing retardation or at least developmental difficulty. There are usually speech problems and an inappropriately happy smiling child.

AS is caused by one gene only, despite losing several genes in chromosome deletion. Presumably, the other missing genes are compensated for by the genes on the other chromosome in the pair, but for some reason the AS gene is one-sided and cannot be activated on the father's chromosome. The UBE3A gene is only one-sided within brain cells, which explains why AS is a mental disease without physical defects. The UBE3A gene is expressed from both chromosomes in other tissues.

Because AS is a single gene disease, there are in fact several ways to get it:

Chromosome deletion (most common): loss of the 15q11-13 sequence on the mother's copy of chromosome 15, which deletes the UBE3A gene (and many others as well).

Uniparental disomy: getting both copies of chromosome 15 from the father (i.e. the pair), so there is no mother's copy of chromosome 15 at all.

Gene mutations: ordinary localize genetic mutations within the UBE3A gene, causing AS in the same way as other genetic non-chromosome diseases. However, the mutation only causes the disease if on the father's chromosome copy. A mutation in UBE3A on the mother's side does not cause AS, nor does it cause PWS, since PWS and AS involve different genes.

Imprint disorders: mutations in the genetic code that surrounds the gene, inhibiting the activation of the UBE3A gene.

Another point to note is that both male and female children equally get AS and PWS. The one-sided gender-imprinting of the gene is not affected by the gender of the child with the disease.

Does one-sidedness go back to the gender of grandparents? Must it comes from the mother's mother or father's father, or can it cross gender in the previous generation?

Some traits inherited from only one side? The one-sidedness of these diseases also raises the question of what other traits are inherited from only one parent.

Uniparental disomy: Another strange way to get both PWS or AS is called uniparental disomy. This means getting from one parent (uniparental) both pairs of chromosomes (disomy). In a pair, you get two chromosomes from one parent, none from the other. Although the majority of PWS and AS are caused by simple deletions within a chromosome, some cases arise because both copies of chromosome 15 come from the same parent. Somewhere along the path, the egg or sperm kept both of its pair of chromosome 15, and the other parent's copy was discarded. People with two mother-inherited copies of chromosome 15 get PWS and two father-inherited copies cause AS.

Uniparental disomy is interesting because the person theoretically has two good copies of chromosome 15 with no genes missing. However, it doesn't work that way. For full health, you need copies from both parents.

Also interesting is that having the entire chromosome 15 from the mother's side seems to only cause PWS, despite there being hundreds or thousands of genes on the entirety of chromosome 15. Hence, it would seem that very few genes are one- sided. If lots of genes are one-sided, then numerous diseases would arise from uniparental disomy of chromosome 15 rather than just one.

Note that uniparental disomy has been seen on several chromosomes: 4, 6, 7, 11, 14, 15, 16, and 21. Like trisomy, it also occurs more often for an older mother.

One-sided disease genes compared to X-linked recessive carriers: But how is this different from X-linked recessive disorders? Isn't inheriting Prader-Willi as an error from the mother's side the same as inheriting a recessive hemophilia gene from a mother carrier? The answer is no, not really, there are several differences. Firstly, the gender differences in

hemophilia arise because it involves a gene on the X sex chromosome, whereas one-sided genes occur on autosomes. Secondly, although X-linked recessive disorders are similar to a maternal one-sided disorder, there is no analogous X-linked or autosomal recessive inheritance pattern that matches paternal one-sided disorders.

Polygenic Diseases

Polygenic means "multiple genes" and polygenic diseases are affected by many genes. In a sense, saying that a disease is polygenic almost means "not genetic". Polygenic diseases usually have a small genetic basis, often less than 10% chance of inheritance from parents. This is often described by saying that a child inherited a "genetic predisposition" to getting a disease, but will not always get the disease (unless some other trigger occurs).

Examples of polygenetic diseases: Many of the big-name diseases are in this class of diseases including cancers, heart disease, autoimmune diseases, and many others. With most of these conditions, they are not regarded as being caused by genetics, nor are they directly inherited from parents. However, a family history of disease is a risk factor for the disease, indicating that there is some inherited risk in the genes. The genetics of this type of disease is an area of current research for all of the major diseases.

Inborn Errors of Metabolism

Inborn errors of metabolism are inherited disorders in which the body cannot metabolize the components of food (carbohydrates , proteins , and lipids

Metabolism is the biochemical process that changes food components into energy and other required molecules . These disorders may be caused by the altered activity of essential enzymes , deficiencies of the substances that activate the enzymes, or faulty transport compounds. Metabolic disorders can be devastating if appropriate treatment is not initiated promptly and monitored frequently

Inborn errors of metabolism often require diet changes, with the type and extent of the changes dependant on the specific metabolic disorder. The particular enzyme absence or inactivity for each inborn error of metabolism dictates which components are restricted and which are supplemented. Registered dietitians and physicians can help an individual assess the diet changes needed for each disease. The goals of nutrition therapy are to correct the metabolic imbalance and promote growth and development by providing adequate nutrition,

while also restricting (or supplementing) one or more nutrients or dietary components. Additional goals in some disorders include reducing the risk of brain damage, other organ damage, episodes of metabolic crisis and coma, and even death. These restrictions and supplementations are specific for each disorder, and they may include the restriction of total fats, simple sugars, or total carbohydrates.

Listed below are several of the metabolic disorders that respond to nutrition therapy. The appropriate dietary restrictions and modifications that are necessary for treatment are also listed.

Disorders of Amino Acid Metabolism

Phenylketonuria (PKU) is the most common disorder of amino acid metabolism. In this disorder the body cannot use the amino acid phenylalanine normally, and excess amounts build up in the blood. If untreated, PKU can cause mental retardation, seizures, behavior problems, and eczema . With treatment, persons with PKU have normal development and intelligence. The treatment for PKU consists of a special phenylalanine-restricted diet designed to maintain blood phenylalanine levels within an acceptable range. Medical formulas and foods, which do not contain phenylalanine, are used to provide the necessary intake of protein and other nutrients. Foods containing natural protein are prescribed in limited amounts to meet the body's requirement for phenylalanine, without providing too much.

Maple syrup urine disease (MSUD*)* is a disorder in which the body is unable to use the amino acids isoleucine, leucine, and valine in a normal way. Excessive amounts of these amino acids and their metabolites will build up in the blood and spill into the urine and perspiration, giving them the odor of maple syrup (which is how this disorder got its name). An untreated infant with MSUD may have some or all of the following symptoms: difficulty breathing, sleepiness, vomiting, irregular muscle movement, seizures, or coma, and the disease can cause death. Basic treatment involves restricting foods and infant formula that contain leucine, isoleucine, and valine. Medical formulas and foods, which contain very small amounts of leucine, isoleucine, and valine, are used to provide the necessary intake of protein and other nutrients.

Disorders of Carbohydrate Metabolism

Galactosemia is a disorder in which the body cannot break down the sugar called galactose. Galactose can be found in food, and the body can break down lactose (milk sugar) to galactose and glucose . The body uses glucose for energy. People with galactosemia lack the enzyme to break down galactose, so it builds up and becomes toxic. In reaction to this buildup of galactose the body makes some abnormal chemicals. The buildup of galactose and these chemicals can cause liver damage, kidney failure, stunted growth, mental retardation, and cataracts in the eyes.

If not treated, galactosemia can cause death. Over time, children and young adults with galactosemia can have problems with speech, language, hearing, stunted growth, and certain learning disabilities. Children who do not follow a strict diet have an increased risk of having one or more of the problems listed above. Even when a strict diet is followed, some children do not do as well as others. Most girls with galactosemia have ovarian failure. The treatment for galactosemia is to restrict galactose and lactose from the diet for life. Since galactose is a part of lactose, all milk and all foods that contain milk must be eliminated

from the diet, including foods that contain small amounts of milk products such as whey and casein. In addition, organ meats should not be eaten because they contain stored galactose.

Glycogen storage diseases require different treatments depending on the specific enzyme alteration. The most common type of glycogen storage disease is classified as type 1A. In this disorder the body is missing the enzyme that coverts the storage form of sugar (glycogen) into energy (glucose). If food is not eaten for two to four hours, blood glucose levels drop to a low level, leading to serious health problems such as seizures, poor growth, enlarged liver, high levels of some fats circulating in the blood, and high levels of uric and lactic acids in the blood. Dietary management of GSD-1A eliminates table sugar (sucrose) and fruit sugar (fructose) and limits milk sugar (lactose), as the body cannot use some sugars in these foods.

Frequent meals and snacks that are high in complex carbohydrates are recommended. In addition, often supplements of uncooked cornstarch are often eaten between meals to keep blood sugar levels stable. Eating a diet that prevents low blood sugar will promote normal growth, decrease liver enlargement, and the high blood levels of uric and lactic acids.

Disorders of fatty acid metabolism occur when the body is not able to break down fat to use as energy. The body's main source of energy is glucose, but when the body runs out of glucose, fats are used for energy. If untreated, these disorders can lead to serious complications affecting the liver, heart, eyes, and muscles. Treatment includes altering the kind and the amount of fat in the diet and frequent feedings of carbohydrate-containing foods.

Urea cycle disorders are inherited disorders of nitrogen metabolism. When protein is digested it breaks down into amino acids, and nitrogen is found in all the amino acids. Those who have these disorders cannot use nitrogen in a normal way. Dietary treatment for these disorders is to provide only the amount of protein that the body can safely use. The diet consists mostly of fruits, grains, and vegetables that contain low amounts of protein and, therefore, low amounts of nitrogen.

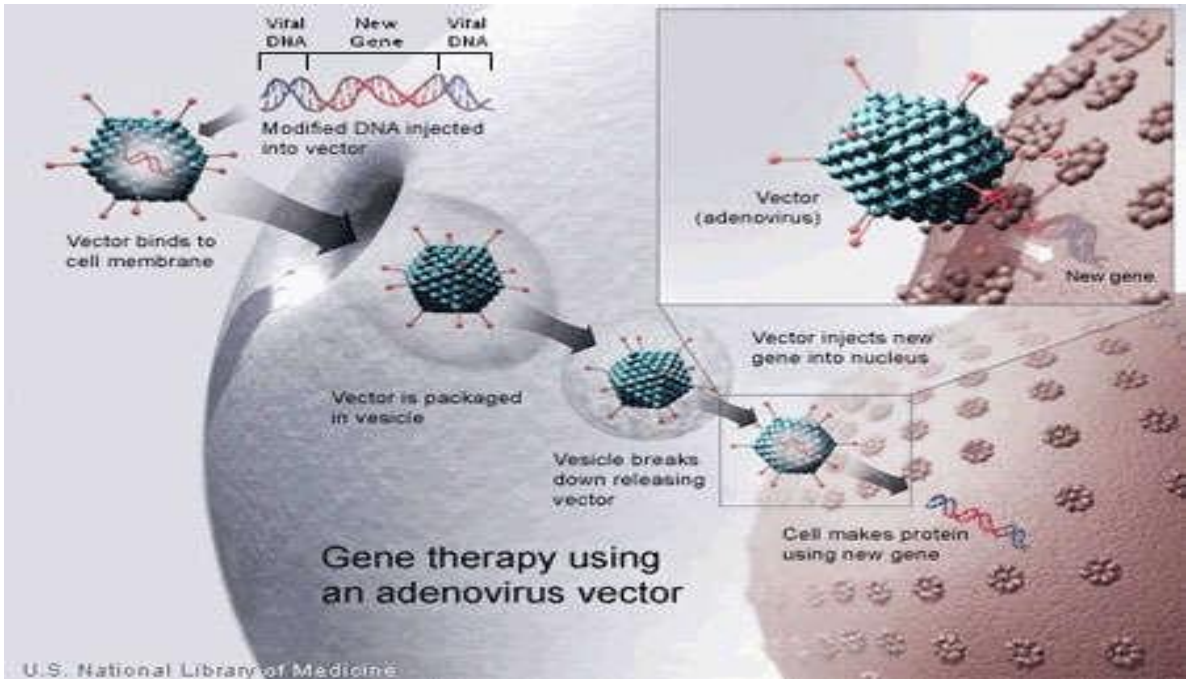
There are more than nineteen metabolic disorders that respond to nutrition therapy. The role of proper nutrition in the treatment of these disorders is crucial. Because these disorders are rare and require careful monitoring, affected individuals are best served by clinics specializing in metabolic disorders.

GENE THERAPY: INTRODUCTION AND METHODS

Gene therapy is the insertion of genes into an individual's cells and tissues to treat a disease, such as a hereditary disease in which a deleterious mutant allele is replaced with a functional one. Although the technology is still in its infancy, it has been used with some success. Antisense therapy is not strictly a form of gene therapy, but is a genetically-mediated therapy and is often considered together with other methods.

Gene therapy is a novel treatment method which utilizes genes or short oligonucleotide sequences as therapeutic molecules, instead of conventional drug compounds. This technique is widely used to treat those defective genes which contribute to disease development. Gene therapy involves the introduction of one or more foreign genes into an organism to treat hereditary or acquired genetic defects. In gene therapy, DNA encoding a therapeutic protein is packaged within a "vector", which transports the DNA inside cells within the body. The disease is treated with minimal toxicity, by the expression of the inserted DNA by the cell machinery. In 1990 FDA for the first time approved a gene therapy experiment on ADA-SCID in the United States after the treatment of Ashanti DeSilva. After that, approximately 1700 clinical

trials on patients have been performed with various techniques and genes for numerous diseases. Many diseases such as ADA-SCID, X-linked SCID, Leber's congenital amaurosis (a retinal disease), Parkinson's disease, multiple myeloma, chronic and acute lymphocytic leukemia, adrenoleukodystrophy have reported of successful clinical trials. But these are still not approved by FDA. Some other diseases on which gene therapy-based research is going on are Haemophilia, Tyrosinemia, Hyperbilirubinemia (Criglar-Nijjar Syndrom), Cystic Fibrosis and many other cancers. After 30 years of research and clinical trials, only one product called Glybera got approval in November 2012 which may be available in market in late 2013. It has the ability to cure lipoprotein lipase deficiency (LPLD) a very rare disease.



Types of gene therapy

There are several approaches for correcting faulty genes; the most common being the insertion of a normal gene into a specific location within the genome to replace a non functional gene. Gene therapy is classified into the following two types: 1. Somatic gene therapy 2. Germ line gene therapy 8-1.2 .1 Somatic Gene Therapy In somatic gene therapy, the somatic cells of a patient are targeted for foreign gene transfer. In this case the effects caused by the foreign gene is restricted to the individual patient only, and not inherited by the patient's offspring or later generations. 8-1.2 .1 Germ Line Gene Therapy Here, the functional genes, which are to be integrated into the genomes, are inserted in the germ cells, i.e., sperm or eggs. Targeting of germ cells makes the therapy heritable. 8-1.3 Gene Therapy Strategies 8-1.3.1 Gene Augmentation Therapy (GAT) In GAT, simple addition of functional alleles is used to treat inherited disorders caused by genetic deficiency of a gene product, e.g. GAT has been applied to autosomal recessive disorders. Dominantly inherited disorders are much less amenable to GAT.

Methods of gene therapy

There are mainly two approaches for the transfer of genes in gene therapy:

1. Transfer of genes into patient cells outside the body (ex vivo gene therapy)

2. Transfer of genes directly to cells inside the body (in vivo).

Gene Therapy using autologous cells: Cells are used, i.e. cells are removed from the patient, cultured in vitro, before being returned to the patient's body. In this figure in vivo and ex vivo gene therapy is diagrammatically explained.

Ex vivo gene therapy

In this mode of gene therapy genes are transferred to the cells grown in culture, transformed cells are selected, multiplied and then introduced into the patient. The use of autologous cells avoids immune system rejection of the introduced cells. The cells are sourced initially from the patient to be treated and grown in culture before being reintroduced into the same individual. This approach can be applied to the tissues like hematopoietic cells and skin cells which can be removed from the body, genetically corrected outside the body and reintroduced into the patient body where they become engrafted and survive for a long period of time.

In Vivo Gene Therapy

In vivo method of gene transfer involves the transfer of cloned genes directly into the tissues of the patient. This is done in case of tissues whose individual cells cannot be cultured in vitro in sufficient numbers (like brain cells) and/or where re-implantation of the cultured cells in the patient is not efficient. Liposomes and certain viral vectors are employed for this purpose because of lack of any other mode of selection. In case of viral vectors such type of cultured cells was often used which have been infected with the recombinant retrovirus in vitro to produce modified viral vectors regularly. These cultured cells will be called as vector-producing cells (VPCs)). The VPCs transfer the gene to surrounding disease cells. The efficiency of gene transfer and expression determines the success of this approach, because of the lack of any way for selection and amplification of cells which take up and express the foreign gene.

Vectors for gene therapy Vectors for gene therapy can be classified into two types:

1. Viral vectors

2. Non-viral

Viral vectors

Retroviruses, adenoviruses and adeno-associated viruses (AAV) some commonly used viral vectors whereas some less commonly used viral vectors are derived from the Herpes simplex virus (HSV-1), the baculovirus etc.

Non- viral vectors

It involves chemical and physical methods such as direct injection of naked plasmid DNA (particle bombardment), receptor-mediated endocytosis and gene transfer through liposomes, polymers, nano particles etc

Microinjection

Microinjection involves the delivery of foreign DNA, by the help of glass micropipette into a living cell. The cell is held against a solid support or holding pipette and micro neeedle containing the desired DNA is inserted into the cell. The tip of the pipette used is about 0.5 to 5 micro meter diameter which resembles an injection needle. For this, glass micropipette is heated until the glass becomes somewhat liquefied and is quickly stretched to ressemble a injection needle. The delivery of foreign DNA is done under a powerful microscope (micromanupulator).

Liposomes Mediated

Liposomes are spherical vesicles which are made up of synthetic lipid bilayers which imitate the structure of biological membranes. DNA to be transferred is packaged into the liposome in vitro and transferred to to the targeted tissue. The lipid coating helps the DNA to survives in vivo and enters into the cell by endocytosis. Cationic liposomes, where the positive charge on liposomes is stabilized by binding of negatively charged DNA, are popular vehicles for gene transfer in vivo.

Advantage: •

The liposomes with the foreign DNA are easy to prepare. • There is no restriction in the size of DNA that is to be transferred.

Disadvantage: •

Efficiency of gene transfer is low and transient expression of the foreign gene is obtained as they are not designed to integrate into the chromosomal DNA.

Electroporation:

In electroporation, the external electric field is applied to the protoplast, which changes the electrical conductivity and the permeability of cell membrane; and thus the exogenous molecules found in the medium are taken up to either the cytoplasm (transient transfection) or into the nucleus (stable transfection).The efficiency of electroporation can be increased by giving the cell a heat shock, prior to the application of electric field or by using small quantity of PEG while doing electroporation. See lecture 3 of module 5 for detailed explanation.

Advantage: •

By electroporation large numbers of cells can be processed at once, and thus the amount of time spent processing cells can be cut down.

Disadvantages: •

If the voltage applied is not calculated properly, the cells may damage. • If electroporation does not occur in controlled environment, the potentially harmful substances can enter the cell or the impurities from solution may enter. This is because there is no way to control what enters the cell membrane.

Advantages of Gene Therapy •

Gene therapy can cure genetic diseases by addition of gene or by removal of gene or by replacing a mutated gene with corrected gene.

• Gene therapy can be used for cancer treatment to kill the cancerous cells.

• Gene expression can be controlled.

• Therapeutic protein is continuously produced inside the body which also reduces the cost of treatment in long term.

GENETIC ENGINEERING

General outline of genetic engineering

A. DNA cleavage
B. Production of recombinant DNA
C. Cloning of the recombinant DNA
D. Screening clones

Manipulating DNA: DNA cleavage and Production of Recombinant DNA

E. restriction enzymes: molecular scissors with a twist
    1. **restriction enzymes**, also called restriction endonucleases, are enzymes that cut DNA molecules in specific places
    2. restriction enzymes vary considerably hundreds of different kinds of restriction enzymes are known (recognizing different DNA sequences) recognized sequence length varies (most common are "4-base cutters" and "6-base cutters") placement of cut varies; some leave "**sticky ends**", others "**blunt ends**" most recognized sequences are **palindromic** the sequence on one strand matches that of the complementary strand read in the opposite direction thus 5'-AGCGCT-3' would have a complementary strand 3'-TCGCGA-5' or reading from 5' to 3', 5'-AGCGCT-3'
    3. restriction enzymes are mostly from bacteria, and their natural role is to destroy DNA from invading viruses
F. making recombinant DNA
    1. restriction enzymes are used to cut up DNA of interest and a "vector" into which you want to place the DNA, making **restriction fragments**
    2. particularly when sticky ends are involved, the target DNA restriction fragment can form basepairs with the vector
    3. **DNA ligase** is then used to join the DNA strand backbones

Cloning of recombinant DNA: using vectors

G. **cloning** is the process of making many genetically identical cells from cell containing recombinant DNA
    1. the gene piece introduced in the recombinant DNA is said to be the DNA that is cloned
    2. recombinant DNA is introduced to cells by a vector; the vector is usually maintained in the altered cell line
H. a **vector** is a means of delivering recombinant DNA to an organism
    1. vectors must have a way of getting into the host organism (**transformation**)
    2. vectors must have some way of being propagated
        - some types of vectors remain free but are copied and distributed in cell division

- some type of vectors have the inserted DNA integrate all or in part with the host DNA

3. vector DNA sequence must be known enough so that restriction sites can be accurately predicted and used

I. most commonly, vectors are either **plasmids**, viruses, or **yeast artificial chromosomes** (**YACs**)
   1. **plasmids** as vectors
      - the most commonly used vectors today are plasmids
      - **plasmids** are small, circular DNA molecules with at least one replication origin
        - most bacterial cells contain several plasmids
        - some eukaryotic cells commonly have plasmids (such as the yeast *Saccharomyces cerevisiae*)
      - plasmids vary in what organisms can maintain them (largely based on the type of replication origin they carry)
      - most plasmids carry genes that are expressed, again with variations depending on the host cell
   2. viruses as vectors
      - viruses infect cells with their DNA; recombinant DNA in a virus can thus be transferred into cells
      - some of this "transduction" occurs naturally, but genetic engineers control and exploit the process
   3. **yeast artificial chromosomes** (**YACs**) as vectors
      - eukaryotes can support and maintain larger pieces of DNA as chromosomes
      - YACs have the required elements of chromosomes (centromere, telomeres) and can be used as vectors for large segments of recombinant DNA in some eukaryotes

J. vectors typically include a selectable marker and a cloning site
   1. **selectable markers** usually are a gene for a product that the host cell cannot make, such as an antibiotic resistance factor
   2. the **cloning site** on a vector is engineered with many possible sites for restriction enzyme cutting, where foreign DNA can be inserted

K. the piece of foreign DNA inserted at a cloning site is said to be **cloned**, and the combined foreign DNA + vector is called **recombinant DNA**

Screening

L. Often many clones are made with various DNA pieces inserted

M. Screening is used to find the DNA of interest; typically:
   1. a selectable marker is used to ensure that the vector is present
   2. a second type of selectable marker is tested to ensure that the vector contains inserted DNA (that is, make sure it is recombinant DNA)
   3. cells from cell colonies that pass the screens to this point are used as sources for making large numbers of cells; DNA from these cells is then subjected to other treatments to help identify cell lines containing the DNA of interest.

II. DNA libraries

A. the first step in working with the DNA of a species is to break the whole genome into manageable bits for study; this is done by creating DNA libraries

B. vectors serve as the "books" in a DNA library – each "book" has a different piece of inserted DNA

C. two main types of libraries are **genomic libraries** and **cDNA libraries**

D. genomic libraries
   1. raw genomic DNA is broken into fragments

- sometimes the breaking is done mechanically
- sometimes the breaking is done with restriction enzymes
- often a combination is used

2. the broken DNA pieces are put into vectors and then the vectors into host cells
3. cells lines are maintained for each library piece (often, the whole genome is represented multiple times in the library for completeness)
4. the cell lines are given unique identifiers, and DNA probing techniques (described later) can be used to determine what lines carry particular cloned DNA sequences

E. cDNA (complementary DNA) libraries

1. a more refined approach than genomic libraries, this type of library is based mainly on the coding regions of DNA
2. mRNAs are isolated from a cell and converted into complementary DNA using the enzyme **reverse transcriptase**
3. the cDNA is then inserted into vectors and the library is made and maintained just like a genomic library
4. different types of cDNA libraries can be made, reflecting the conditions under which cells made the original mRNAs
5. again, DNA probing techniques are used to find which lines have a cDNA of interest

III. Techniques used to manipulate and study DNA before and after cloning include: PCR, DNA gel electrophoresis, probing, DNA sequencing, and RFLPs

A. DNA sequence amplification: **PCR** (**polymerase chain reaction**) is used to get enough DNA to work with

1. DNA polymerase can build a DNA strand provided there is a template strand, a primer, and dNTPs (deoxyribonucleotides of each type: dATP, dCTP, dGTP, and dTTP)
   - **denaturation**: heating a DNA molecule will eventually **denature** ("melt") the double strands into separate single strands, breaking the hydrogen bonds between A-T and C-G basepairs; this can provide potential template strands
   - **annealing of primers:** when the DNA cools, basepairs will reform; if small, specific DNA primers are added in excess compared to the amount of target (template) DNA molecules, the DNA primers will tend to bind to the target DNA strands and keep the original double helices from reforming
   - **primer extension:** then, DNA polymerases can add dNTPs to make a complementary DNA strands, starting at the 3' ends of the primers
   - if you repeat this through a series of cycles, you will exponentially make new DNA strands that cover a specific region of DNA, defined by the specific primers used – a polymerase chain reaction, where each cycle essentially doubles the amount of your target DNA fragment
2. PCR works well only when the DNA polymerase used can withstand temperatures that melt DNA strands
   - such enzymes are found in organisms that grow under very hot conditions (such as thermophilic bacteria from hot springs)
   - these are called heat-stable DNA polymerases (the best known one is *Taq* polymerase)
3. typically, the PCR works like this:
   - DNA melting is done 94°C (just a bit under the boiling temperature of water) for a minute or less
   - DNA annealing is done at a temperature around 50°C for a minute or less, but this varies depending on the DNA primers used and their optimal annealing

temperature – you want to avoid getting too cool, where some nonspecific annealing can occur
    - DNA synthesis (primer extension) is performed at the optimal temperature for the heat-stable DNA polymerase, usually 72°C; the time given to extension is roughly 1 min per kilobase of DNA in the final target size
    - the process then moves to back to DNA melting, and the "cycle" is repeated for up to ~35 cycles
    - often, an extra melting period is put before any cycles (since larger DNA strands are harder to melt), and an extra extension period is put after all cycles end (to finish up as many strands as possible)
4. PCR greatly amplifies the target DNA sequence
    - starting with a single double-stranded DNA molecule (the minimal extreme):
        - after 4 cycles one has $2^4 = 16$ DNA molecules
        - after 20 cycles one has $2^{20} = 1{,}048{,}576$ DNA molecules
    - with wise primer choices, a segment of DNA can be amplified to make enough molecules for useful study
    - thus, given enough sequence knowledge and a small DNA sample, any piece of DNA from any individual can usually be amplified through PCR to useful quantities
    - PCR products can be used for such things as the raw material for cloning, for probing DNA libraries, and for DNA sequencing

B. DNA gel electrophoresis

1. the overall DNA molecule is negatively charged, and will migrate though a viscous material such as a gel if a voltage difference is supplied (moving toward the positive pole)
2. the speed of migration through a gel will be determined in part by the size of the DNA molecule; the longer the molecule, the slower it moves
3. thus, relative migration rate through a gel can be used to determine the approximate size of a DNA fragment
4. this mostly holds true for RNA as well, but different conditions must be used to prevent degradation of RNA (which is much more common, and much more of a problem, than DNA degradation)

C. probing

1. DNA and RNA fragments can be transferred to a filter, denatured, and incubated with probe molecules that will **hybridize** (bond by forming correct basepairs) with specific sequences
2. the probe molecules (usually DNA fragments) can be made with some nucleotides that are either radioactive or fluorescent, thus "labeling" the probe – and, when the probe is used, labeling the sites on the filter where the probe is able to hybridize
3. if DNA is on the filter and being probed, this is called a **Southern blot** or **DNA gel blot** (after the inventor)
4. if RNA is being probed, this is called a **Northern blot** or **RNA gel blot**
5. an analogous process with proteins is called a **Western blot** (there antibodies are used as probes)

D. DNA sequencing

1. a DNA sequence can be determined using special nucleotides and migration differences of DNA strands through a gel based on size
2. special ddNTPs **(dideoxyribonucleotide triphosphates**) are used for sequencing

3. when a ddNTP is incorporated into a growing DNA strand, it prevents further elongation of the DNA strand
    - there is no 3'-OH on which to add the next nucleotide, so the strand stops
    - strand length is thus set by where the stop occurs
4. sequencing typically involves 4 polymerization mixtures
    - usually, each mixture has labeled primers that allow DNA visualization
    - each mixture also has a DNA polymerase and multiple copies of single-stranded template DNA
    - each mixture also has all 4 normal dNTPs
    - the mixtures differ in the ddNTP included (one will be ddATP, one ddCTP, one ddGTP, one ddTTP); the ddNTP is included in a small amount relative to the dNTPs
    - at the end, each mixture will have a number of newly-synthesized DNA strands with a variety of sizes, but within a given mixture you will know what ddNTP is at the 3' end of each of the stands
5. the labeled mixes are run on a gel and separated by size; the gel is then read from shortest (thus fastest, and closest to the bottom) up to the longest (thus slowest, and closest to the top) fragment – a letter is assigned based on the ddNTP that was used for the mixture that was run in that gel lane

E. restriction fragment length polymorphisms (RFLPs)

1. DNA cut by restriction enzymes and run on a gel can produce distinguishable DNA bands
2. sequence differences between organisms (between species, or even within the same species) can result in different bands
3. thus, given the right restriction enzymes or set of enzymes, RFLPs can serve as a "DNA fingerprint" for an individual
4. DNA sequencing is the most reliable means of identification, and as it becomes cheaper and more available it is replacing some uses of RFLP analysis; however, RFLP analysis likely will always be quicker and cheaper than sequencing, and is still used heavily (RFLP analysis is much more reliable than older techniques like actual fingerprint matching)

IV. Applications of genetic engineering

A. **transgenic organism** - any organism with a foreign gene(s) incorporated in it uses of transgenic organisms

1. as drug makers
    - many gene products have potential medical uses; a useful protein or enzyme can often be made in bulk via genetic engineering
    - a case in point: the human insulin gene has been made in *E. coli* for over two decades (much safer than insulin from other animals, which is not identical to human insulin)
    - another example: human growth hormone – much safer than the old process of purifying from cadavers
2. as medical models
    - transgenic mice for modeling disease
    - transgenic organisms for basic research on disease-related topics such as cell division
3. as gene therapy tools – viruses to deliver DNA to human cells
4. tools used to make better tools
    - cloned restriction enzymes, *Taq* polymerase, etc.

- sometimes cloned enzymes are modified to improve them for specific uses

5. improved food sources – examples:
   - golden rice, engineered to make enough beta carotene to reduce vitamin A deficiencies in many societies
   - insect-resistant cotton, corn, etc. – express a bacterial gene that codes for a toxin that kills insects that try to feed on the plant
   - herbicide resistance

safety guidelines

6. potential misuses (both intentional and accidental) are a concern
7. stringent guidelines are in place to prevent such things as producing and releasing a "super virus," "super bacteria," or "super weed" that would become a serious medical and/or ecological disaster
8. genetically engineered organisms that are released into the environment in some way are closely monitored on a case-by-case basis – example of Bt corn
9. much concern over genetic engineering exists in the general public (especially in Europe), so things such as labeling of genetically modified foods is a controversial issue.

GENETIC COUNSELING

As members of a healthcare team, genetic counselors provide information and support to families affected by or at risk for a genetic disorder. They serve as a central resource of information about genetic disorders for other healthcare professionals, patients, and the general public. This chapter provides an overview of the role of genetic counselors and their approach to educating patients and identifying individuals/families at risk of a genetic disorder. Patient resources are also provided.

Role of Genetic Counselling

Genetic counselors help identify families at possible risk of a genetic condition by gathering and analyzing family history and inheritance patterns and calculating chances of recurrence. They provide information about genetic testing and related procedures. They are trained to present complex and difficult-to-comprehend information about genetic risks, testing, and diagnosis to families and patients. Genetic counselors can help families understand the significance of genetic conditions in relation to cultural, personal, and familial contexts. They also discuss available options and can provide referrals to educational services, advocacy and support groups, other health professionals, and community or state services. Genetic counselors can serve as a central resource of information about genetic conditions for other healthcare professionals, patients, and the general public.

Process of Genetic counselling

In general, a genetic counseling session aims to:

- Increase the family's understanding of a genetic condition • Discuss options regarding disease management and the risks and benefits of further testing and other options
- Help the individual and family identify the psychosocial tools required to cope with potential outcomes
- Reduce the family's anxiety

It is not unusual for multiple genetic counseling sessions to occur and, at a minimum, to include a pre-testing and post-testing session. During the initial genetic counseling visit, the genetic counselor will determine why the patient/family is seeking genetic counseling, identify what information they wish to obtain from the session, collect and record a family medical history, and assess and record the medical and psychosocial history of the patient.

Among the topics that may be discussed during a pre-testing session are the clinical presentation of the condition(s) the patient may be at risk for, pattern of genetic inheritance of the condition, chance of recurrence, available testing procedures and test limitations, reproductive options, and follow-up procedures, if needed. General questions relating to suggested treatment or therapy are also addressed. Referrals may be made to specialists regarding specific issues that fall outside the scope of genetic counseling practice.

If the patient decides to have genetic testing performed, the genetic counselor often acts as the point person to communicate the results. However, the post-test session involves more than the provision of medical information and often focuses on helping families cope with the emotional, psychological, medical, social, and economic consequences of the test results. In particular, psychological issues such as denial, anxiety, anger, grief, guilt, or blame are addressed, and, when necessary, referrals for in-depth psychosocial counseling are offered. Information about community resources and support groups can be provided to the patient/family.

If the genetic test is positive, testing may be considered for additional relatives of the individual. Genetic counseling referrals for other family members for risk assessment may be discussed. It may be necessary to refer relatives to other genetic counselors due to geographical and other constraints.

At the conclusion of the final genetic counseling session, the patient may receive a written summary of the major topics discussed. The summary is often provided in the form of a letter, which serves as a permanent record of the information discussed and can include additional information that became available after the final counseling session. The patient may choose to share the letter with other family members or healthcare providers.

Patient Education

Many patients rely heavily on their primary healthcare providers for information related to their condition. In general, though, patients will require information providers may not have. Before providing patients with any educational materials, providers should be sure to check that the information is current and produced by a credible source.

Books and pamphlets are appreciated by patients, even those who are web-savvy. Patient advocacy groups generally provide the best and most up-to-date information. The organizations listed on the following page are excellent sources of information about genetic diseases that can be helpful to patients.