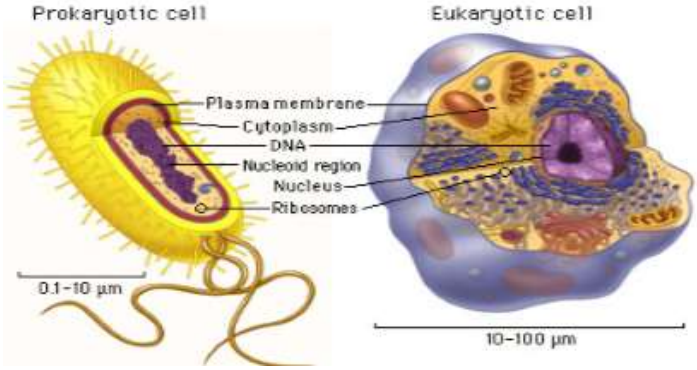
CELL AND MOLECULAR BIOLOGY

Cells: Prokaryote vs Eukaryote



Cells have evolved two different architectures:

- Prokaryote "style"
- Eukaryote "style"

Prokaryotic cells were here first and for billions of years were the only form of life on Earth. All prokaryotic organisms are unicellular

Eukaryotic cells appeared on earth long after prokaryotic cells but they are much more advanced. Eukaryotic organisms unlike prokaryotic can be unicellular or multicellular.

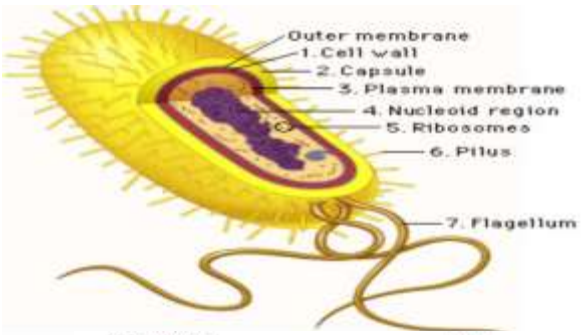
Characteristics of Prokaryotes

- Prokaryotes are the simplest type of cell.
- Oldest type of cell appeared about four billion years ago.
- Prokaryotes are the largest group of organisms
- Prokaryotes unicellular organisms that are found in all environments.
- Prokaryotes do not have a nuclear membrane. Their circular shaped genetic material dispersed throughout cytoplasm.
- Prokaryotes do not have membrane-bound organelles.
- Prokaryotes have a simple internal structure.
- Prokaryotes are smaller in size when compared to Eukaryotes.
- Shapes of Prokaryotes

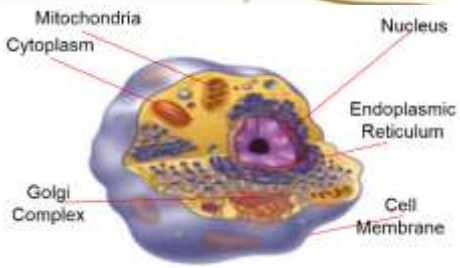
  Cocci = spherical (round)

  Bacillus = (rod shaped)

  Spirilla = helical (spiral)

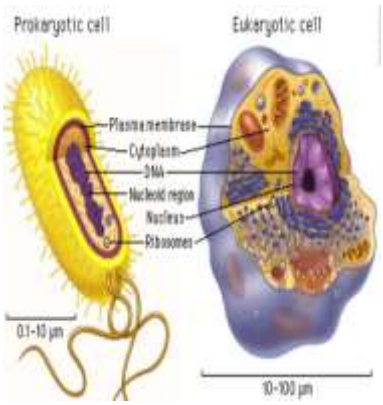


Characteristics of eukaryotes



- Eukaryotic cells appeared approximately one billion years ago
- Eukaryotes are generally more advanced than prokaryotes
- Nuclear membrane surrounds linear genetic material (DNA)
- Unlike prokaryotes, eukaryotes have several different parts.
- Prokaryote's organelles have coverings known as membranes.
- Eukaryotes have a complex internal structure.
- Eukaryotes are larger than prokaryotes in size .

Similarities between prokaryote and eukaryote cells



- Both types of cells have cell membranes (outer covering of the cell)
- Both types of cells have ribosomes
- Both types of cells have DNA
- Both types of cells have a liquid environment known as the cytoplasm

Differences between prokaryote and eukaryote cells

Prokaryotes	***Eukaryotes***
Organelles lack a membrane	Organelles covered by a membrane
Ribosomes are the only organelles	Multiple organelles including ribosomes
Genetic material floats in the cytoplasm (DNA and RNA)	Membrane covered Genetic material
Circular DNA	Linear DNA
Unicellular	May be multicellular or unicellular
Cells are smaller in size	Cells are larger in size
Has larger number of organisms	Has smaller number of organisms
Appeared 4 billion years ago	Appeared 1 billion years ago

Prokaryote cells are smaller and simpler

- Commonly known as bacteria
- 10-100 microns in size
- Single-celled (unicellular) or
- Filamentous (strings of single cells)

Prokaryote cells are simply built (example: E. coli)

- capsule: slimy outer coating
- cell wall: tougher middle layer
- cell membrane: delicate inner skin
- cytoplasm: inner liquid filling
- DNA in one big loop
- pilli: for sticking to things
- flagella: for swimming
- ribosomes: for building proteins

Prokaryote lifestyle

- unicellular: all alone
- colony: forms a film
- filamentous: forms a chain of cells

Prokaryote Feeding

- Photosynthetic: energy from sunlight
- Disease-causing: feed on living things
- Decomposers: feed on dead things

Eukaryotes are bigger and more complicated

- Have organelles
- Have chromosomes
- can be multi-cellular
- include animal and plant cells

Organelles are membrane-bound cell parts

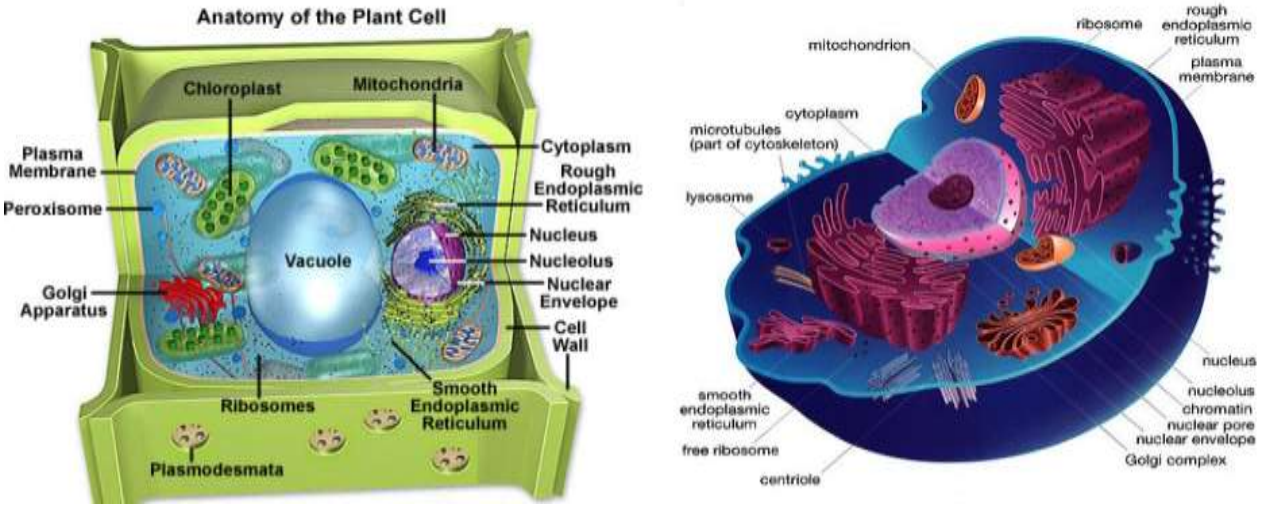
Mini "organs" that have unique structures and functions located in cytoplasm

Cell structure

- Cell membrane >> delicate lipid and protein skin around cytoplasm found in all cells
- Nucleus >> membrane-bound sac evolved to store the cell's chromosomes(DNA) & has pores: holes
- Nucleolus >> inside nucleus , location of ribosome factory , made of RNA
- Mitochondrion >> makes the cell's energy , the more energy the cell needs, the more mitochondria it has

- Ribosomes >> build proteins from amino acids in cytoplasm, may be free-floating or may be attached to ER, made of RNA
- Endoplasmic reticulum >> may be smooth: builds lipids and carbohydrates or may be rough: stores proteins made by attached ribosomes
- Golgi Complex >> takes in sacs of raw material from ER, sends out sacs containing finished cell products
- Lysosomes >> sacs filled with digestive enzymes, digest worn out cell parts, digest food absorbed by cell
- Centrioles >> pair of bundled tubes, organize cell division
- Cytoskeleton >> made of microtubules, found throughout cytoplasm, gives shape to cell & moves, Structures found in plant cells
- Cell wall >> very strong, made of cellulose, protects cell from rupturing, glued to other cells next door
- Vacuole >> huge water-filled sac, keeps cell pressurized, stores starch
- Chloroplasts >> filled with chlorophyll,
- turn solar energy into food energy

Difference between Animal & Plant Cell



Structure	Animal cells	Plant cells
cell membrane	Yes	yes
nucleus	Yes	yes
nucleolus	yes	yes
ribosomes	yes	yes
ER	yes	yes
Golgi	yes	yes
centrioles	yes	no
cell wall	no	yes
mitochondria	yes	yes
cholorplasts	no	yes
One big vacuole	no	yes
cytoskeleton	yes	Yes

Eukaryote cells can be multicellular

- The whole cell can be specialized for one job
- cells can work together as tissues
- Tissues can work together as organs

Advantages of each kind of cell architecture

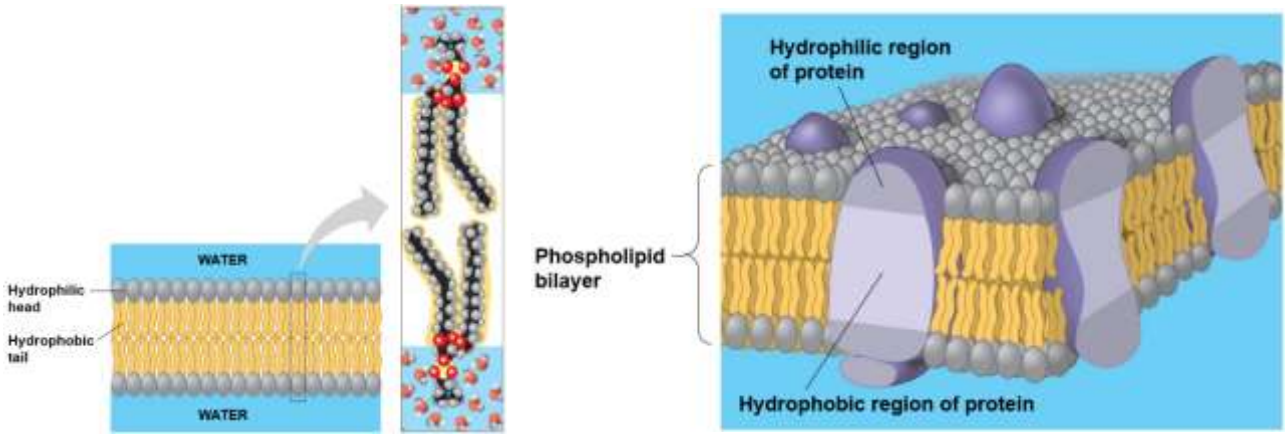
Prokaryotes	Eukaryotes
simple and easy to grow	can specialize
fast reproduction	Multi-cellularity
all the same	can build large bodies

PLASMA MEMBRANE

- The plasma membrane is the boundary that separates the living cell from its nonliving surroundings
- The plasma membrane exhibits selective permeability, allowing some substances to cross it more easily than others

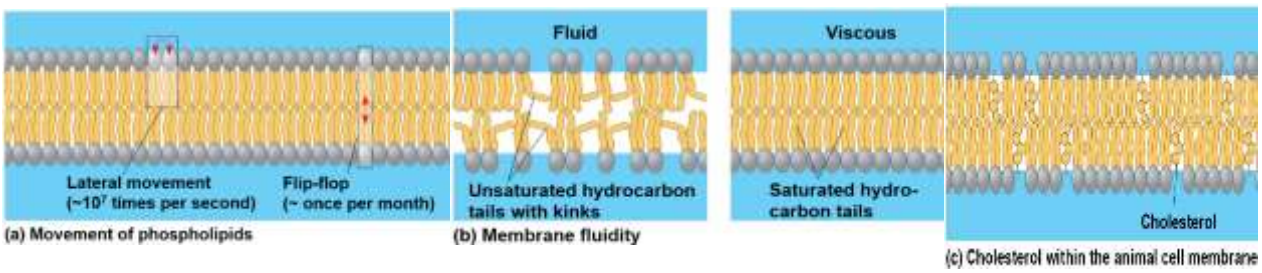
Cellular membranes are fluid mosaics of lipids and proteins

- Phospholipids are the most abundant lipid in the plasma membrane
- Phospholipids are amphipathic molecules, containing hydrophobic and hydrophilic regions
- The fluid mosaic model states that a membrane is a fluid structure with a "mosaic" of various proteins embedded in it

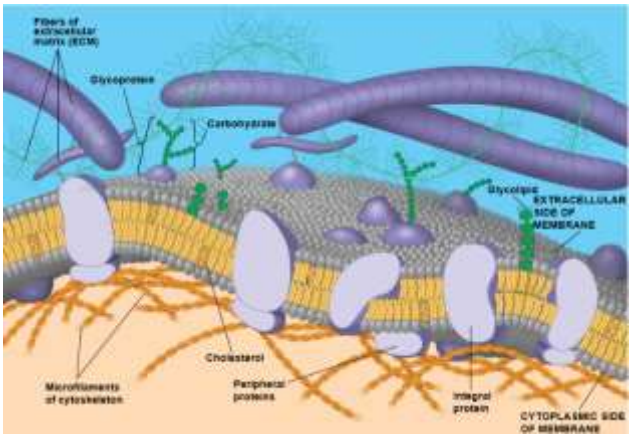


The Fluidity of Membranes

- Phospholipids in the plasma membrane can move within the bilayer
- Most of the lipids, and some proteins, drift laterally
- Rarely does a molecule flip-flop transversely across the membrane
- As temperatures cool, membranes switch from a fluid state to a solid state
- The temperature at which a membrane solidifies depends on the types of lipids
- Membranes rich in unsaturated fatty acids are more fluid than those rich in saturated fatty acids
- Membranes must be fluid to work properly; they are usually about as fluid as salad oil
- The steroid cholesterol has different effects on membrane fluidity at different temperatures
- At warm temperatures (such as 37°C), cholesterol restrains movement of phospholipids
- At cool temperatures, it maintains fluidity by preventing tight packing



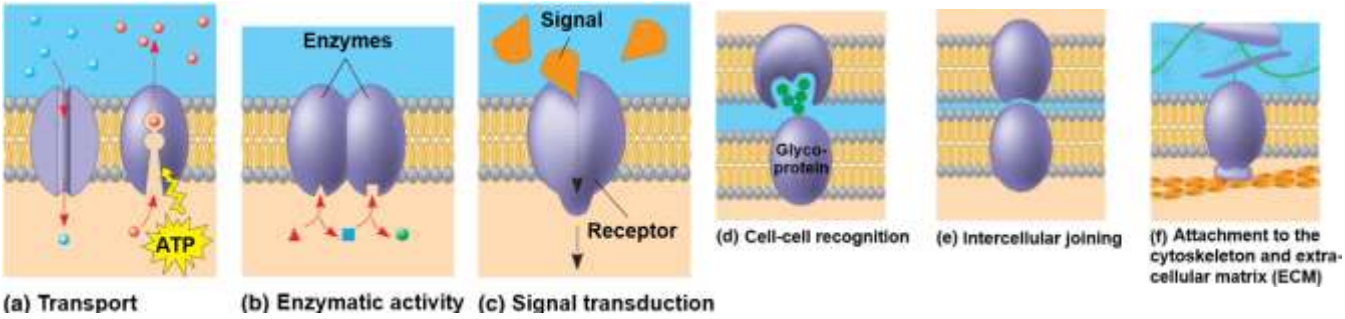
Memebrane proteins and their Functions



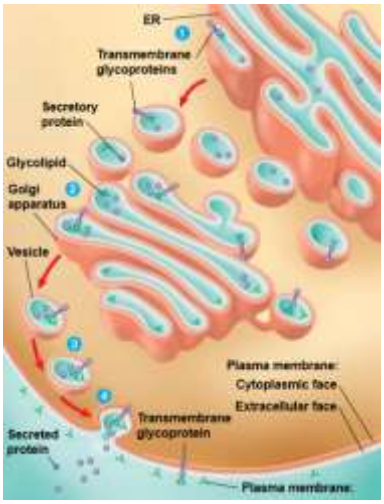
- A membrane is a collage of different proteins embedded in the fluid matrix of the lipid bilayer
- Proteins determine most of the membrane's specific functions
- Peripheral proteins are not embedded
- Integral proteins penetrate the hydrophobic core and often span the membrane
- Integral proteins that span the membrane are called transmembrane proteins
- The hydrophobic regions of an integral protein consist of one or more stretches of nonpolar amino acids, often coiled into alpha helices

Six major functions of membrane proteins:

- Transport
- Enzymatic activity
- Signal transduction
- Cell-cell recognition
- Intercellular joining
- Attachment to the cytoskeleton and extracellular matrix (ECM)



The Role of Membrane Carbohydrates in Cell-Cell Recognition



- Cells recognize each other by binding to surface molecules, often carbohydrates, on the plasma membrane
- Membrane carbohydrates may be covalently bonded to lipids (forming glycolipids) or more commonly to proteins (forming glycoproteins)
- Carbohydrates on the external side of the plasma membrane vary among species, individuals, and even cell types in an individual

Synthesis and Sidedness of Membranes

- Membranes have distinct inside and outside faces
- The asymmetrical distribution of proteins, lipids and associated carbohydrates in the plasma membrane is determined when the membrane is built by the ER and Golgi apparatus

Membrane structure results in selective permeability

- A cell must exchange materials with its surroundings, a process controlled by the plasma membrane
- Plasma membranes are selectively permeable, regulating the cell's molecular traffic

The Permeability of the Lipid Bilayer

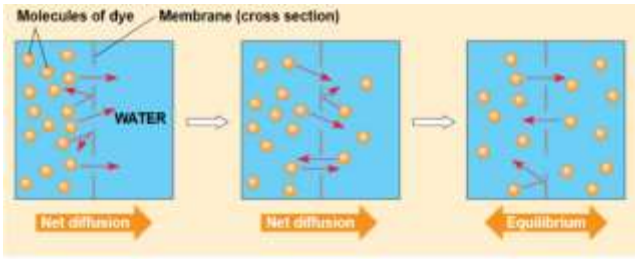
- Hydrophobic (nonpolar) molecules, such as hydrocarbons, can dissolve in the lipid bilayer and pass through the membrane rapidly
- Polar molecules, such as sugars, do not cross the membrane easily

Transport Proteins

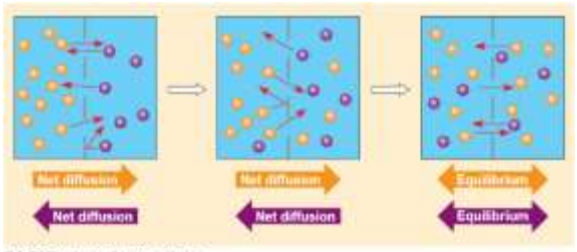
- Transport proteins allow passage of hydrophilic substances across the membrane
- Some transport proteins, called channel proteins, have a hydrophilic channel that certain molecules or ions can use as a tunnel
- Channel proteins called aquaporins facilitate the passage of water
- Other transport proteins, called carrier proteins, bind to molecules and change shape to shuttle them across the membrane
- A transport protein is specific for the substance it moves

Passive transport is diffusion of a substance across a membrane with no energy investment

- Diffusion is the tendency for molecules to spread out evenly into the available space
- Although each molecule moves randomly, diffusion of a *population* of molecules may exhibit a *net* movement in one direction
- At dynamic equilibrium, as many molecules cross one way as cross in the other direction
- Substances diffuse down their concentration gradient, the difference in concentration of a substance from one area to another
- No work must be done to move substances down the concentration gradient
- The diffusion of a substance across a biological membrane is passive transport because it requires no energy from the cell to make it happen



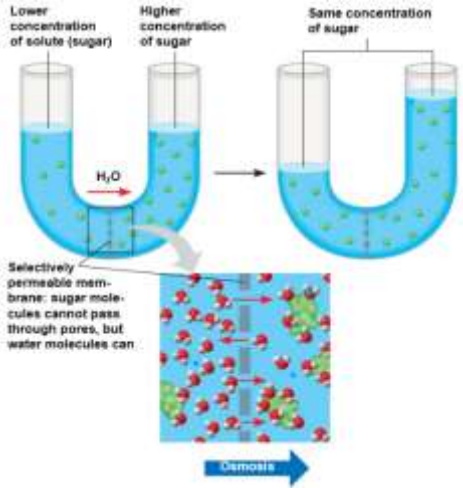
(a) Diffusion of one solute



(b) Diffusion of two solutes

Effects of Osmosis on Water Balance

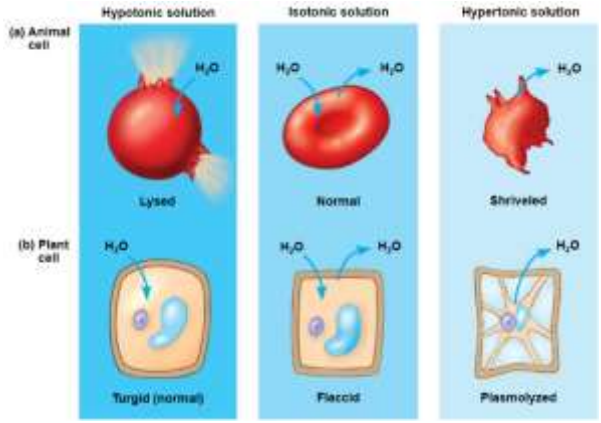
- Osmosis is the diffusion of water across a selectively permeable membrane
- The direction of osmosis is determined only by a difference in *total* solute concentration
- Water diffuses across a membrane from the region of lower solute concentration to the region of higher solute concentration



Water Balance of Cells Without Walls

- Tonicity is the ability of a solution to cause a cell to gain or lose water
  - Isotonic solution: solute concentration is the same as that inside the cell; no net water movement across the plasma membrane
  - Hypertonic solution: solute concentration is greater than that inside the cell; cell loses water
  - Hypotonic solution: solute concentration is less than that inside the cell; cell gains water
- Animals and other organisms without rigid cell walls have osmotic problems in either a hypertonic or hypotonic environment
- To maintain their internal environment, such organisms must have adaptations for osmoregulation, the control of water balance

- The protist *Paramecium*, which is hypertonic to its pond water environment, has a contractile vacuole that acts as a pump

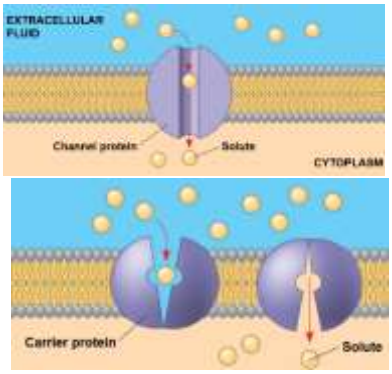


Water Balance of Cells with Walls

- Cell walls help maintain water balance
- A plant cell in a hypotonic solution swells until the wall opposes uptake; the cell is now turgid (firm)
- If a plant cell and its surroundings are isotonic, there is no net movement of water into the cell; the cell becomes flaccid (limp), and the plant may wilt
- In a hypertonic environment, plant cells lose water; eventually, the membrane pulls away from the wall, a usually lethal effect called plasmolysis

Facilitated Diffusion: Passive Transport Aided by Proteins

- In facilitated diffusion, transport proteins speed movement of molecules across the plasma membrane
- *Channel proteins* provide corridors that allow a specific molecule or ion to cross the membrane
- *Carrier proteins* undergo a slight change in shape that translocates the solute-binding site across the membrane

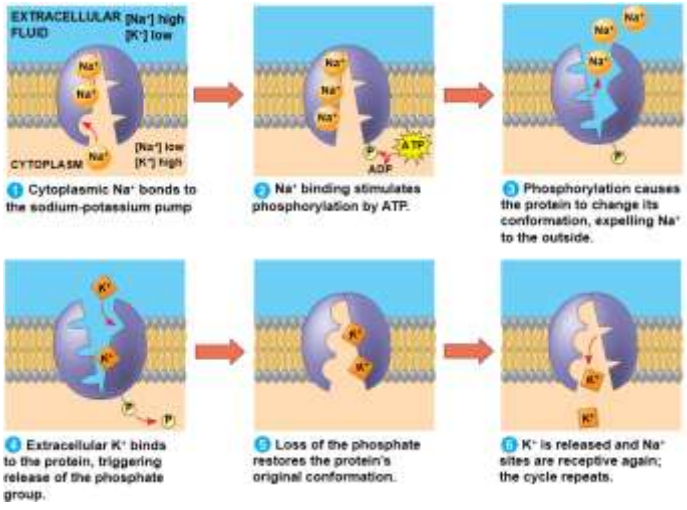


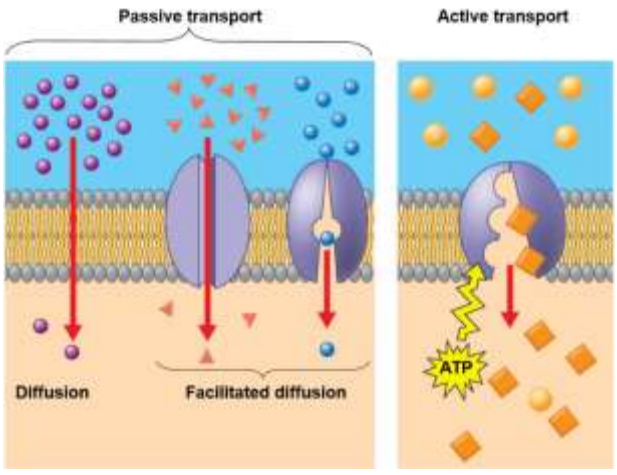
Factors Affecting Diffusion Rate

- Steepness of concentration gradient >> Steeper gradient, faster diffusion
- Molecular size >> Smaller molecules, faster diffusion
- Temperature >> Higher temperature, faster diffusion

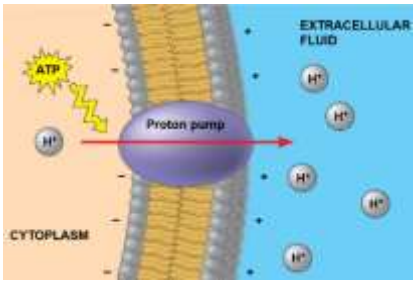
Active Transport

- Active transport moves substances against their concentration gradient
- Active transport requires energy, usually in the form of ATP
- Active transport is performed by specific proteins embedded in the membranes
- The sodium-potassium pump is one type of active transport system

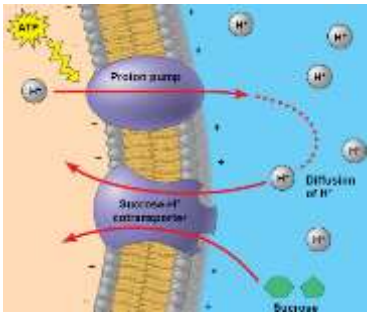




Maintenance of Membrane Potential by Ion Pumps



- Membrane potential is the voltage difference across a membrane
- Two combined forces, collectively called the electrochemical gradient, drive the diffusion of ions across a membrane:
- A chemical force (the ion's concentration gradient)
- An electrical force (the effect of the membrane potential on the ion's movement)
- An electrogenic pump is a transport protein that generates the voltage across a membrane
- The main electrogenic pump of plants, fungi, and bacteria is a proton pump

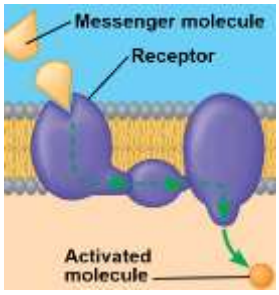


Cotransport: Coupled Transport by a Membrane Protein

- Cotransport occurs when active transport of a solute indirectly drives transport of another solute
- Plants commonly use the gradient of hydrogen ions generated by proton pumps to drive active transport of nutrients into the cell

Many membrane proteins are enzymes

- This is especially important on the membranes of organelles.



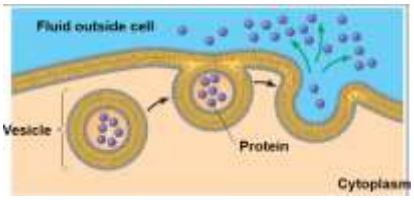
Signal transduction (receptor) proteins

- bind hormones and other substances on the outside of the cell.
- Binding triggers a change inside the cell.
- *Called signal transduction*
- Example: The binding of insulin to insulin receptors causes the cell to put glucose transport proteins into the membrane.

Bulk Flow

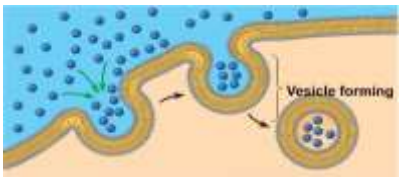
- Vesicles are used to transport large particles across the PM.
- Requires energy
- Types: Exocytosis, Endocytosis (Phagocytosis, pinocytosis, receptor-mediated)

Exocytosis



- Cytoplasmic vesicle merges with the PM and releases its contents
- Example:
  - Golgi body vesicles merge with the PM an release their contents
  - How nerve cells release neurotransmittors

Endocytosis



- PM sinks inward, pinches off and forms a vesicle
- Vesicle often merges with Golgi for processing and sorting of its contents

Endocytosis - terms

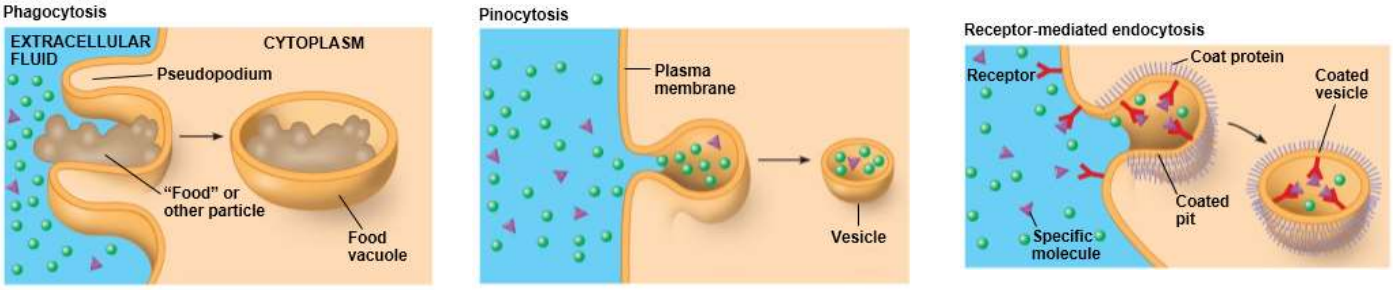
- Phagocytosis – cell eating
  - Membrane sinks in and captures solid particles for transport into the cell
  - Examples: Solid particles often include: bacteria, cell debris, or food
- Pinocytosis – cell drinking
  - Cell brings in a liquid

Endocytosis - *comments*

- Phagocytosis and pinocytosis are not selective
  - Membrane sinks inward and captures whatever particles/fluid present.
  - Vesicle forms and merges with the Golgi body…

Receptor Mediated Endocytosis

- Receptor Mediated Endocytosis is a highly specific form of endocytosis.
- Receptor proteins on the outside of the cell bind specific substances and bring them into the cell by endocytosis
- Receptor proteins on PM bind specific substances *(vitamins, hormones..)*
- Membrane sinks in and forms a pit called a coated pit
- Pit pinches closed to form a vesicle around bound substances
- Cytoskeleton aids in pulling in the membrane and vesicle formation

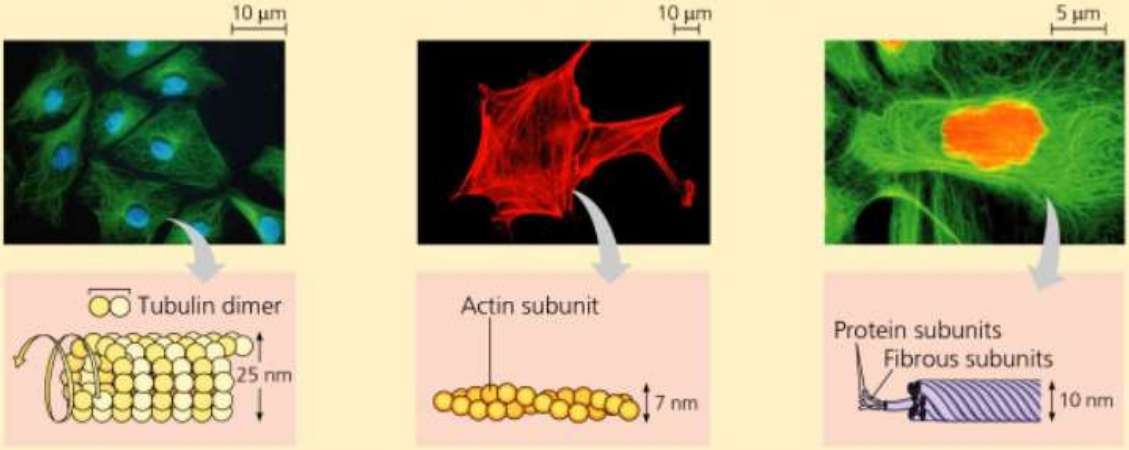


Cytoskeleton

- The cytoskeleton is the structure consisting of fibrous proteins that occur in the cytoplasm and maintain the shape of the cell.
- Microtubules – function in cell division and serve as a "temporary support" for other organelles.
- Actin microfilaments are thin threads that function in cell division and cell motility.
- Intermediate filaments are between the size of the microtubules and the actin filaments.
- gives the cell shape,
- anchors some organelles and directs the movement of others,
- may enable the entire cell to change shape or move.
- may play a regulatory role, by mechanically transmitting signals from the cell's surface to its interior.

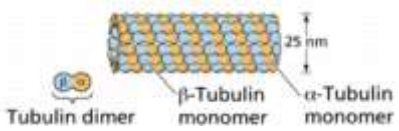
Table 7.2 The Structure and Function of the Cytoskeleton

Property	Microtubules	Microfilaments (Actin Filaments)	Intermediate Filaments
Structure	Hollow tubes; wall consists of 13 columns of tubulin molecules	Two intertwined strands of actin	Fibrous proteins supercoiled into thicker cables
Diameter	25 nm with 15-nm lumen	7 nm	8–12 nm
Protein subunits	Tubulin, consisting of α-tubulin and β-tubulin	Actin	One of several different proteins of the keratin family, depending on cell type
Main functions	Maintenance of cell shape (compression-resisting "girders") Cell motility (as in cilia or flagella) Chromosome movements in cell division Organelle movements	Maintenance of cell shape (tension-bearing elements) Changes in cell shape Muscle contraction Cytoplasmic streaming Cell motility (as in pseudopodia) Cell division (cleavage furrow formation)	Maintenance of cell shape (tension-bearing elements) Anchorage of nucleus and certain other organelles Formation of nuclear lamina



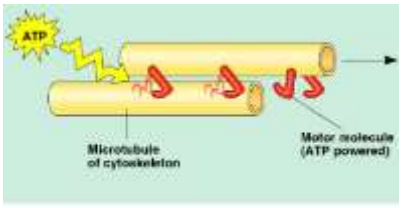
Role of microtubules

- Hollow tubes with wall that consists of 13 columns of tubulin molecules (25 nm in diameter)
- Involved in:
  - cell shape maintenance (compression resistance)
  - cell motility (as in cilia or flagella)
  - chromosome movement in cell division
  - Organelle movements

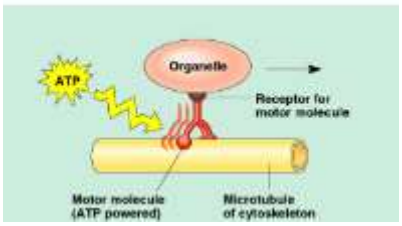


Motor molecules and the cytoskeleton

- The microtubules and microfilaments interact with proteins called motor molecules.
- Motor molecules change their shapes, moving back and forth something like microscopic legs.
- ATP powers these conformational changes.



- The motor molecule releases at its free end and then grips at a site further along a microtubule or microfilament. For example, a sliding of neighboring microtubules moves cilia and flagella. In muscle cell contraction, motor molecules slide microfilaments rather than microtubules.



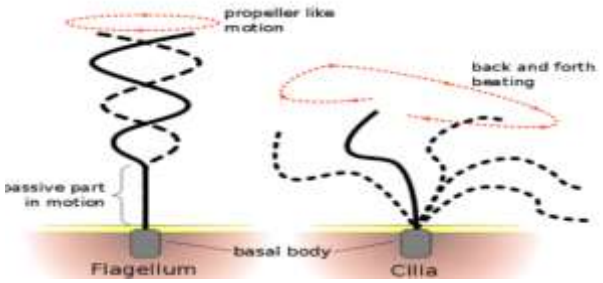
- Motor molecules can also attach to receptors on organelles such as vesicles and enable the organelles to "walk" along microtubules of the cytoskeleton. For example, vesicles containing neurotransmitters migrate to the tips of axons, the long extensions of nerve cells that release transmitter molecules as chemical signals to adjacent nerve cells.
- Kinesin moves organelles towards periphery (+), and Dinein towards the nucleus (-).

Centrosome containing a pair of centrioles

- An animal cell has a pair of centrioles within its centrosome,
- the region near the nucleus where the cell's microtubules are initiated.
- The centrioles, each about 250 nm (0.25 μm) in diameter, are arranged at right angles to each other, and each is made up of nine sets of three microtubules (TEM).

Flagella and Cilia

- Locomotive appendages that protrude from some cells.
- A specialized arrangement of microtubules responsible for their beating

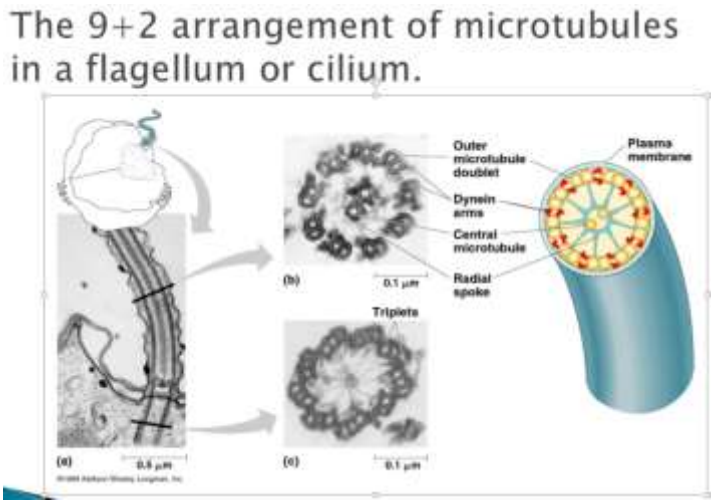


A comparison of the beating of flagella and cilia

- flagellum has a snakelike motion driving a cell in the same direction as the axis of the flagellum.
- Propulsion of a sperm cell is an example of flagellate locomotion (SEM).
- The cilia of Paramecium beat at a rate of about 40 to 60 strokes per second.
- Cilia have a back-and-forth motion, alternating active strokes with recovery strokes.
- This moves the cell, or moves a fluid over the surface of a stationary cell.

Ultrastructure

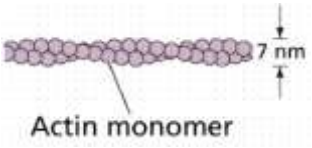
- The basal body anchoring the cilium or flagellum to the cell has a ring of nine microtubule triplets.
- The nine doublets of the cilium extend into the basal body, where each doublet joins another microtubule to form the ring of nine triplets.
- The two central microtubules of the cilium terminate above the basal body (TEM).



Dynein – motor protein

- Responsible for the bending movements of cilia and flagella
- The dynein arms of one microtubule doublet grip the adjacent doublet, pull, release, and then grip again.
- The action of the dynein arms causes the doublets to bend.

Actin components of the cytoskeleton.



- Microfilaments – actin filaments.
- They are built from molecules of a globular protein – actin.
- A microfilament is a twisted double chain of actin subunits (7 nm in diameter)

Role of microfilaments

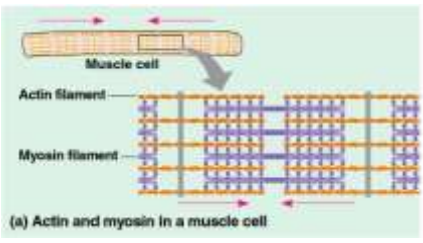
- Maintenance of cell shape (as a tension-bearing elements)
- Changes in cell shape
- Muscle contraction
- Cytoplasmic streaming
- Cell motility
- Cell division – cleavage furrow formation

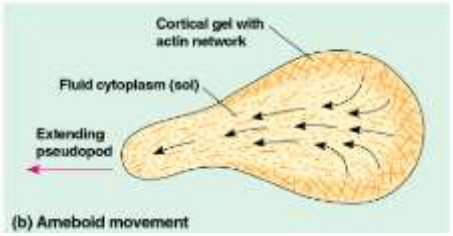
A structural role of microfilaments

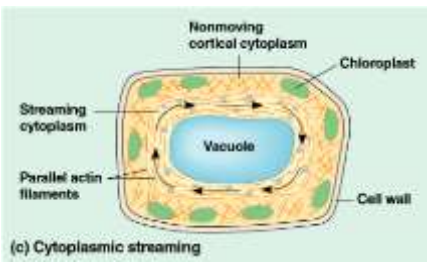
- The surface area intestinal cell is increased by its many microvilli,
- cellular extensions reinforced by bundles of microfilaments.
- These actin filaments are anchored to a network of intermediate filaments

Microfilaments and motility

- In muscle cells, actin filaments (orange) lie parallel to thick myosin filaments (purple).Myosin acts as a motor molecule. The teamwork of many such sliding filaments enables the entire muscle cell to shorten.
- In a crawling cell (ameboid movement), actin is organized into a network in the gel-like cortex (outer layer).This contraction forces the interior fluid into the pseudopod, where the actin network has been weakened.The pseudopod extends until the actin reassembles into a network.
- In cytoplasmic streaming, a layer of cytoplasm cycles around the cell, moving over a carpet of parallel actin filaments.Myosin motors attached to organelles in the fluid cytosol may drive the streaming by interacting with the actin.

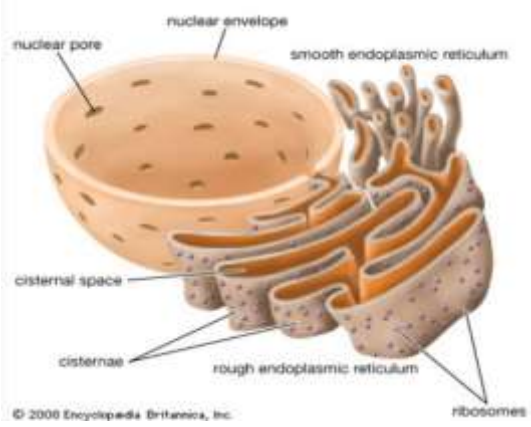






Role of intermediate filaments

- Fibrous proteins supercoiled into thicker cables (8-12 nm)
- Depending on the cell type, it is presented by one of the several different proteins of the keratin family
- Responsible for:
  - maintenance of cell shape (tension-bearing elements)
  - anchorage of nucleus and certain other organelles
  - formation of nuclear lamina



Endoplasmic Reticulum

- Cytoplasm of eukaryotic cells contain a network of interconnecting membranes. This extensive structure is called endoplasmic reticulum.
- It consists of membranes with smooth appearance in some areas and rough appearance in some areas- Smooth endoplasmic reticulum and rough endoplasmic reticulum.

Biomedical importance

Rough Endoplasmic Reticulum

- These membranes enclose a lumen.
- In this lumen newly synthesized proteins are modified.
- Rough appearance is due to the presence of ribosomes attached on its cytosolic side(outer side).
- These ribosomes are involved in the biosynthesis of proteins.
- The synthesizedproteins are either incorporated into the membranes or into the organelles.
- Special proteins are present that are called **CHAPERONES**. Theses proteins play a role in proper folding of newly synthsied proteins.
- Protein glycosylation also occurs in ER i.e. the carbohydrates are attached to the newly synthesized proteins.

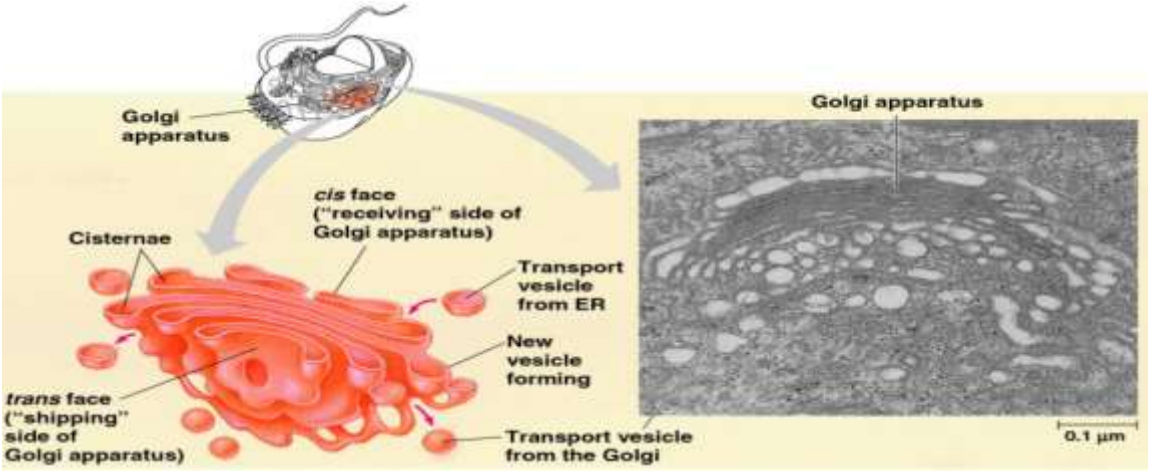
Smooth Endoplasmic Reticulum

- Smooth endoplasmic reticulum is involved in lipid synthesis.
- Cholesterol synthesis
- Steroid hormones synthesis.
- Detoxification of endogenous and exogenous substances.
- The enzyme system involved in detoxification is called Microsomal Cytochrome P450 monooxygenase system(xenobiotic metabolism).
- ER along with Golgi apparatus is involved in the synthesis of other organelles –lysosomes & Peroxisomes.
- Elongation of fatty acids e.g. Palmitic acid 16 C- Stearic acid 18 C.
- Desaturation of fatty acids.
- Omega oxidation of fatty acids.

Golgi Apparatus

- Golgi complex is a network of flattened smooth membranous sacs- cisternae and vesicles.
- The membrane of each cisterna separates its internal space from the cytosol
- One side of the Golgi, the *cis* side, receives material by fusing with vesicles, while the other side, the *trans* side, buds off vesicles that travel to other sites.
- These are responsible for the secretion of proteins from the cells(hormones, plasma proteins, and digestive enzymes).
- It works in combination with ER.
- Enzymes in golgi complex transfer carbohydrate units to proteins to form of glycoporoteins, this determines the ultimate destination of proteins.
- Golgi is the major site for the synthesis of new membrane, lysosomes and peroxisomes.
- It plays two major roles in the membrane synthesis:
  - processing of oligosaccharide chains of the membranes (all parts of the GA participates).
  - sorting of various proteins prior to their delivery(Trans Golgi network).
- During their transit from the cis to trans pole, products from the ER are modified to reach their final state.
- This includes modifications of the oligosaccharide portion of glycoproteins.

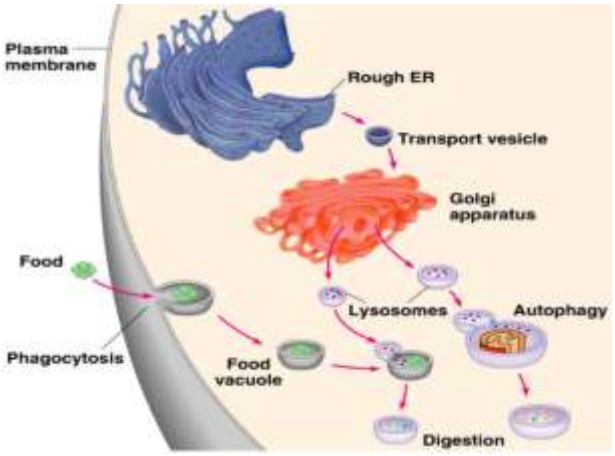
- The Golgi can also manufacture its own macromolecules, including pectin and other noncellulose polysaccharides.
- During processing material is moved from cisterna to cisterna, each with its own set of enzymes.
- Finally, the Golgi tags, sorts, and packages materials into transport vesicles.



Lysosomes

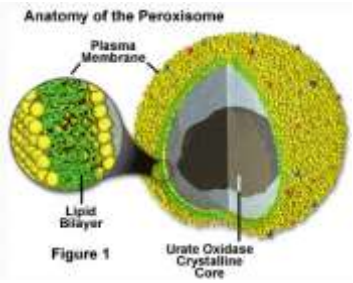
- These are responsible for the intracellular digestion of both intra and extracellular substances.
- They have a single limiting membrane.
- They have an acidic pH- 5
- They have a group of enzymes called Hydrolases. Lysosomal enzymes can hydrolyze proteins, fats, polysaccharides, and nucleic acids.

Biomedical importance



- The enzyme content varies in different tissues according to the requirement of tissues or the metabolic activity of the tissue.
- Lysosomal membrane is impermeable and specific translocations are required.
- Vesicles containing external material fuses with lysosomes, form primary vesicles and then secondary vesicles or digestive vacuoles.
- Lysosomes are also involved in autophagy.
- Products of lysosomal digestion are released and reutilised.
- Indigestible material accumulates in the vesicles called residual bodies and their material is removed by exocytosis.
- Some residual bodies in non dividing cells contain a high amount of a pigmented substance called Lipofuscin.
- Also called age pigment or wear–tear pigment.
- In some genetic disease individual lysosomal enzymes are missing and this lead to the accumulation of that particular substance.
- Such lysosomes gets enlarged and they interfere the normal function of the cell.
- Such diseases are called lysosomal storage diseases
- Most importantt is I-cell disease (inclusion-cell disease).

Peroxisomes



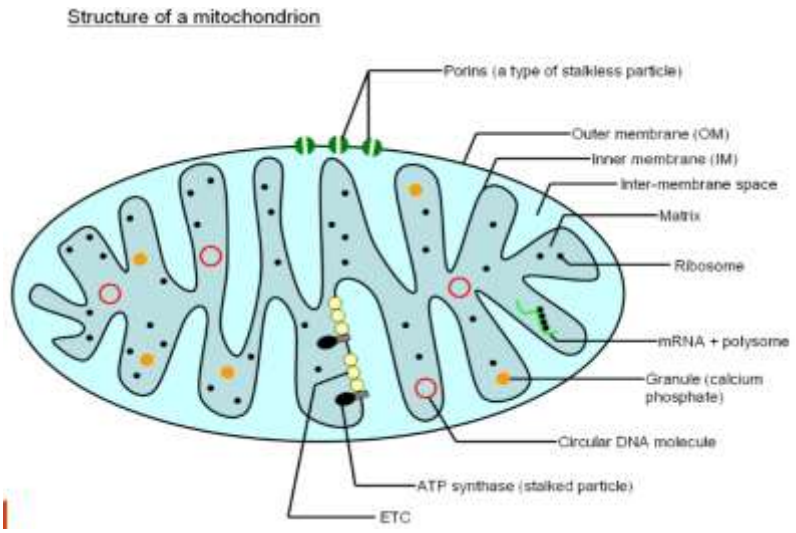
- Called Peroxisomes because of their ability to produce or utilize H_2O_2. Peroxisomes contain enzymes that can transform hydrogen into oxygen, creating hydrogen peroxide as a waste product. This oxygen can be used to brake down macromolecules into smaller molecules that can be used for cellular respiration.
- They are small, oval or spherical in shape.
- They have a fine network of tubules in their matrix.
- About 50 enzymes have been identified.The number of enzymes fluctuates according to the function of the cells.

Biomedical importance

- Xenobiotics leads to the proliferation of Peroxisomes in the liver.
- Have an important role in the breakdown of lipids, particularly long chain fatty acids.
- Synthesis of glycerolipids.
- Synthesis of glycerol ether lipids.
- Synthesis of isoprenoids.
- Synthesis of bile.
- Oxidation of D- amino acids.
- Oxidation of Uric acid to allantoin (animals)
- Oxidation of Hydroxy acids which leads to the formation of H_2O_2.
- Contain catalase enzyme, which causes the breakdown of H_2O_2 .
- Diseases associated: Most important disease is Zellweger Syndrome. There is absence of functional peroxisomes. This leads to the accumulation of long chain fatty acids in the brain, decreased formation of plasmalogens (type of phospholipid), and defects of bile acid formation.

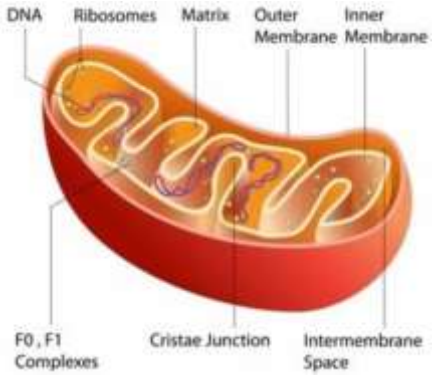
Mitochondrias

- Mitochondria The organelle that releases energy in the cell. (The powerhouse of the cell)
- Mitochondria produce ATP using energy stored in food molecules.

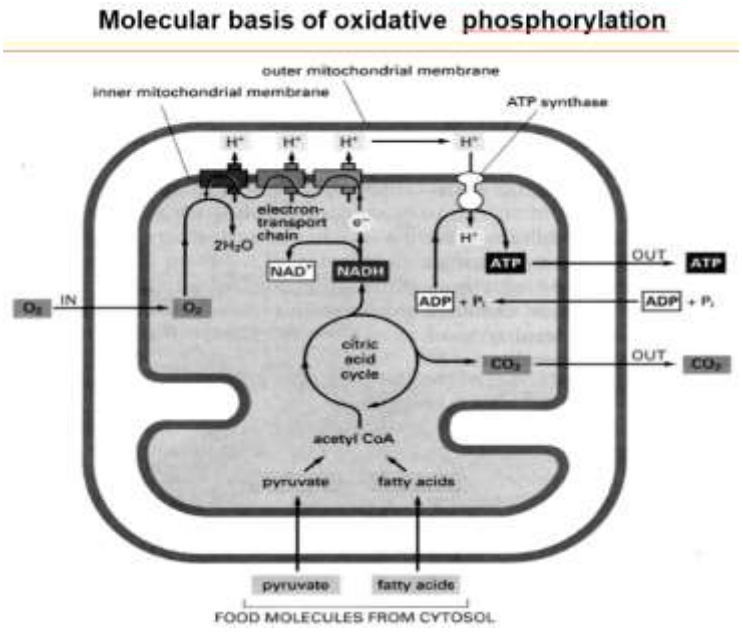


Structure

- Mitochondria have a double membrane structure
- There is a single outer membrane and a folded inner membrane
- Sac with two inner compartments which are separated by the inner membrane.
- The first compartment is between the outer and inner membranes.
- The outer compartment is inside the inner membrane.
- The outer mitochondrial membrane is composed of about 50% phospholipids by weight and contains a variety of enzymes involved in such diverse activities as the elongation of fatty acids, oxidation of epinephrine (adrenaline), and the degradation of tryptophan.
- The inner membrane contains proteins with three types of functions:
- those that carry out the oxidation reactions of the respiratory chain
- ATP synthase, which makes ATP in the matrix
- specific transport proteins that regulate the passage of metabolites into and out of the matrix.
- Intermembrane space: Contains several enzymes use ATP to phosphorylate other nucleotides.



- Matrix: Enzymes; Mit DNA, Ribosomes, etc.

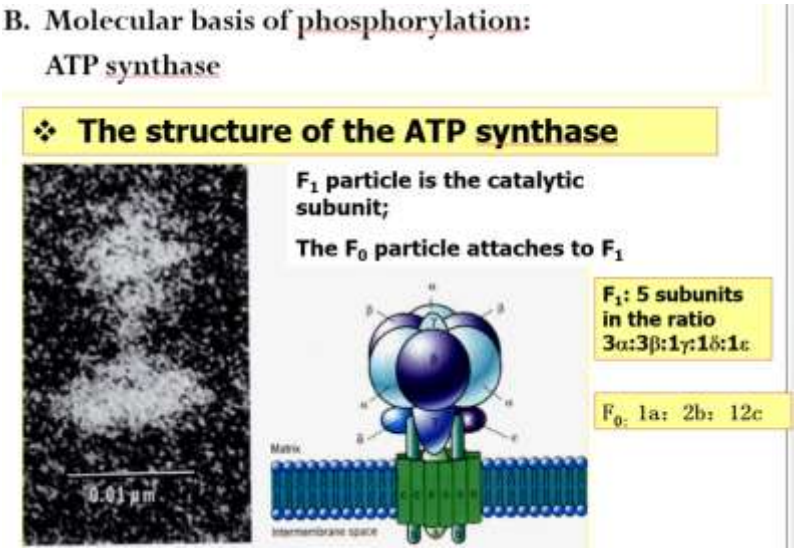


Function

- Mitochondria are the site of most of the energy production in eukaryotic cells.
- They use complex molecules and oxygen to produce a high energy molecule know as ATP (Adenosine Triphosphate)
- process called **aerobic respiration**
- Energy production the mitochondria has been called the "powerhouse of the cell".
- Mitochondria are very abundant in cells that require lots of energy.
- E.g. Muscle

Uniqueness Of Mitochondrion

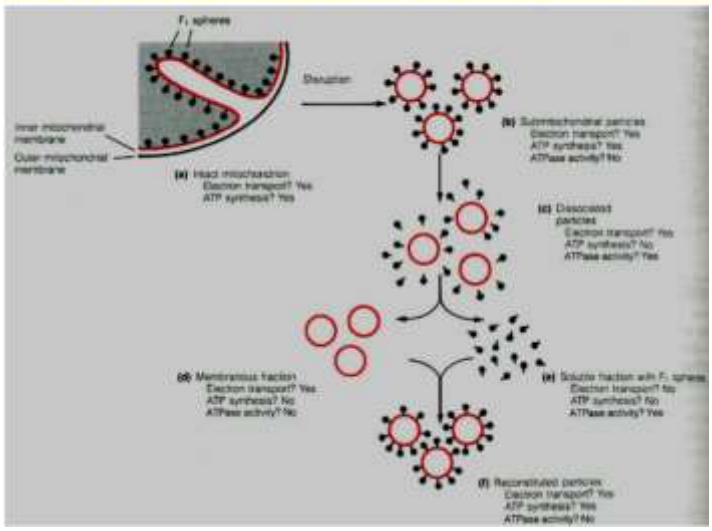
- Mitochondria are very unique in several regards
- have their own circular DNA
- Have their own Ribosomes.
- (The DNA in the cell nucleus does not code for the construction of mitochondria).
- All the mitochondria in your body came from your mother.
- Mitochondria are not part of the genetic code in the nucleus of your cells.
- Fathers only give genes to their children.
- Mothers give genes and cytoplasm to their children in their egg cells.
- Since mitochondria are in the cytoplasm and reproduce themselves they only are inherited from mothers
- Geneticists have used this curious feature of mitochondria to study maternal family lines and rates of evolution



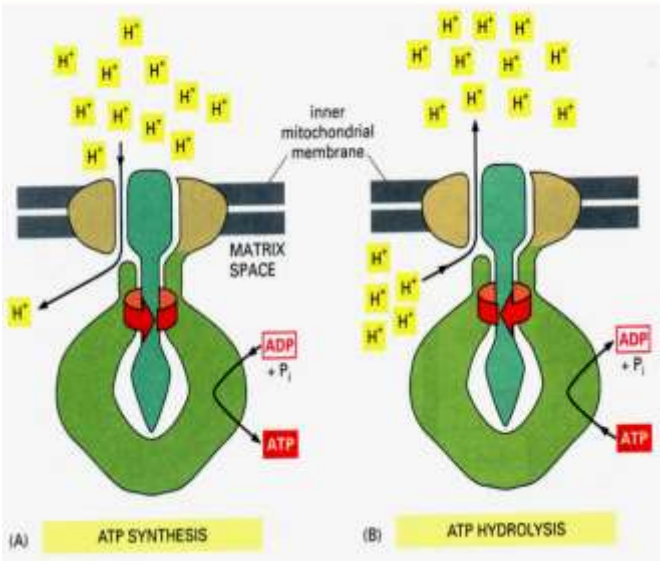
- Although the primary function of mitochondria is to convert organic materials into cellular energy in the form of ATP, mitochondria play an important role in many metabolic tasks, such as:
  - Apoptosis-cell death
  - Cellular proliferation
  - Regulation of the cellular redox state
  - Heme synthesis
  - Steroid synthesis
  - Heat production (enabling the organism to stay warm).

- 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 a variety of mitochondrial diseases.

❖F1 particles have ATP synthase activity



❖ The ATP synthase is a reversible coupling device



Glyoxysomes

- A specialized type of peroxisome found only in plants
- Contain some of same enzymes (catalase, fatty acid oxidase), but others as well
- Plant seedlings rely on stored fatty acids to provide energy & material to form new plant
- Glyoxylate cycle , is a cycle occurring in the glyoxysomes
- A primary metabolic activity in these germinating seedlings is the conversion of stored fatty acids to carbohydrate
- Stored fatty acid disassembly produces acetyl CoA & it condenses with oxaloacetate to form citrate

- Citrate is then converted to glucose by a series of glyoxylate cycle enzymes found in glyoxysomes

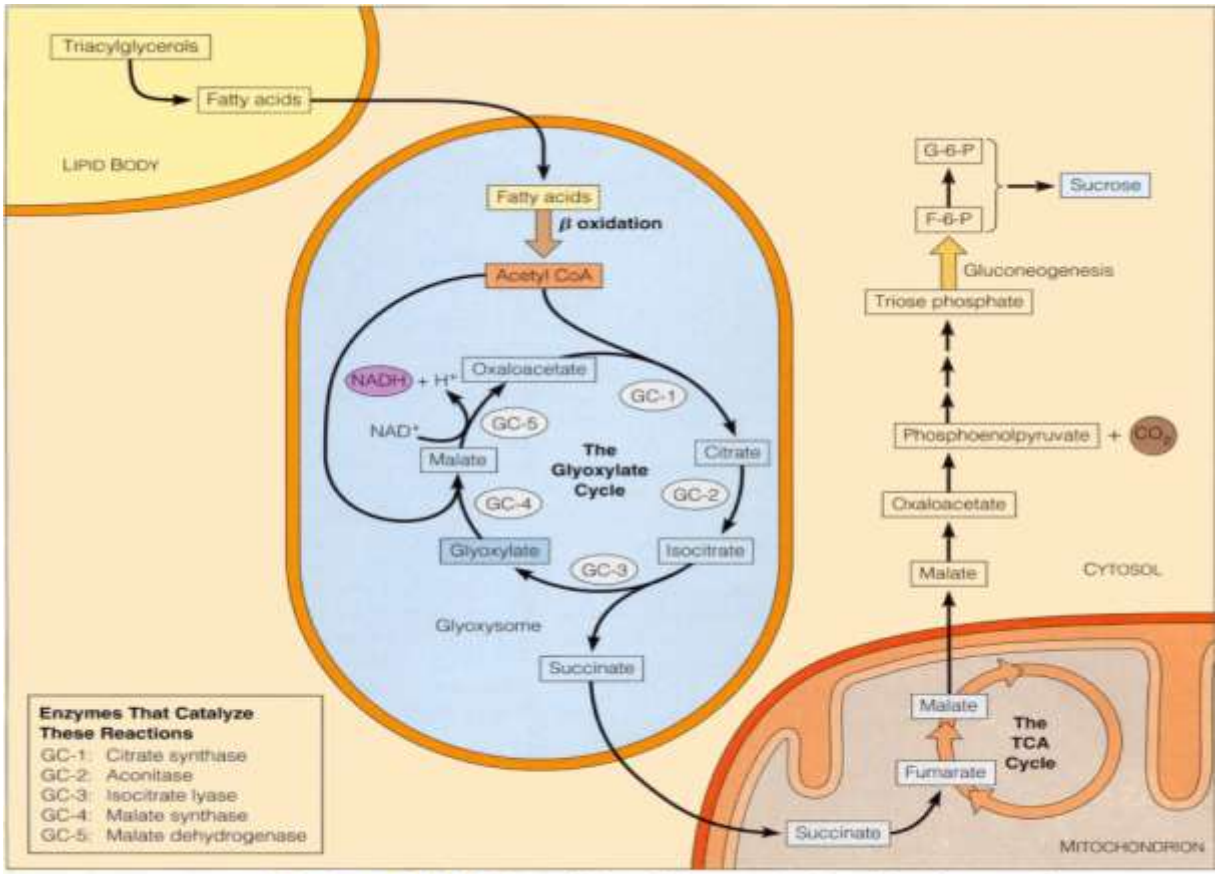
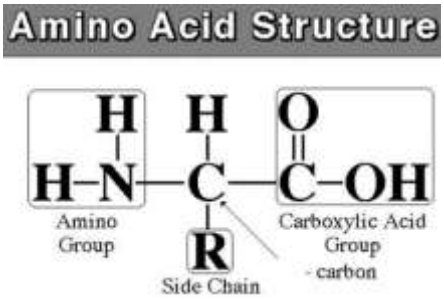


Figure 14A-2 The Glyoxylate Cycle and Gluconeogenesis in Fat-Storing Seedlings.

PROTEIN SYNTHESIS



DNA

- DNA contains genes, sequences of nucleotide bases
- These Genes code for polypeptides (proteins)
- Proteins are used to build cells and do much of the work inside cells

Genes & Proteins

- Proteins are made of amino acids linked together by peptide bonds
- 20 different amino acids exist

Polypeptides

Amino acid chains are called polypeptides

DNA Begins the Process

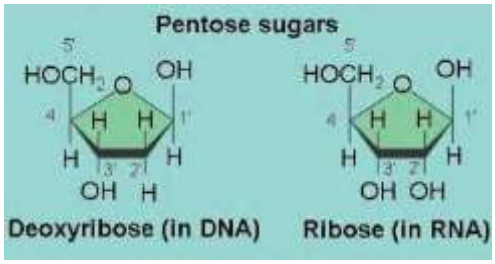
- DNA is found inside the nucleus
- Proteins, however, are made in the cytoplasm of cells by organelles called ribosomes
- Ribosomes may be free in the cytosol or attached to the surface of rough ER

Starting with DNA

- DNA 's code must be copied and taken to the cytosol
- In the cytoplasm, this code must be read so amino acids can be assembled to make polypeptides (proteins)
- This process is called PROTEIN SYNTHESIS

Roles of RNA and DNA

- DNA is the MASTER PLAN
- RNA is the BLUEPRINT of the Master Plan

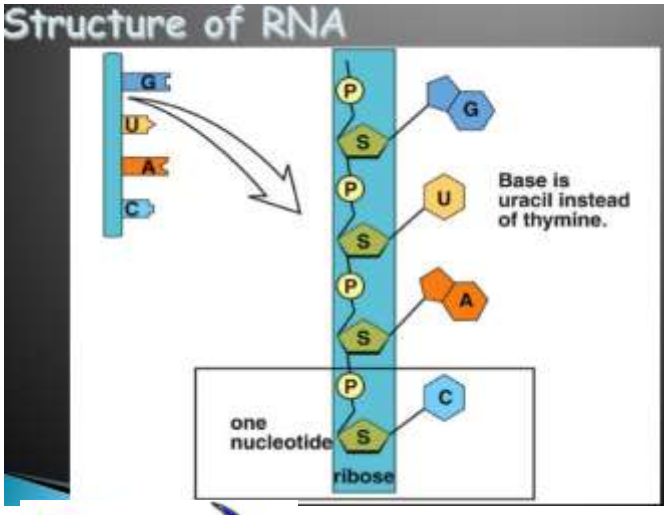


RNA Differs from DNA

- RNA has a sugar ribose
- DNA has a sugar deoxyribose

Other Differences

- RNA contains the base uracil (U) while DNA has thymine (T)
- RNA molecule is single-stranded while DNA is double-stranded

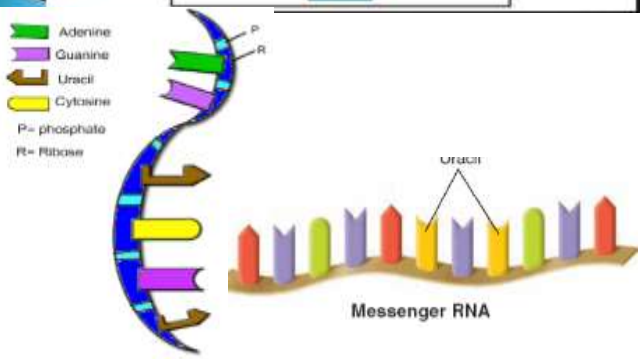


Three Types of RNA

- Messenger RNA (mRNA) copies DNA's code & carries the genetic information to the ribosomes
- Ribosomal RNA (rRNA), along with protein, makes up the ribosomes
- Transfer RNA (tRNA) transfers amino acids to the ribosomes where proteins are synthesized

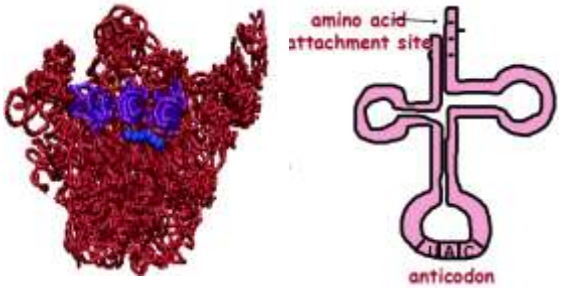
Messenger RNA

- Long Straight chain of Nucleotides
- Made in the Nucleus
- Copies DNA & leaves through nuclear pores
- Contains the Nitrogen Bases A, G, C, U (no T)
- Carries the information for a specific protein
- Made up of 500 to 1000 nucleotides long
- Sequence of 3 bases called codon
- AUG – methionine or start codon
- UAA, UAG, or UGA – stop codons



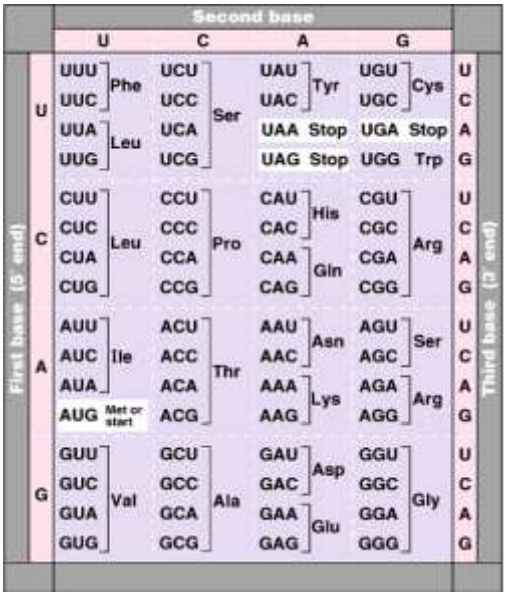
Ribosomal RNA (rRNA)

- rRNA is a single strand 100 to 3000 nucleotides long
- Globular in shape
- Made inside the nucleus of a cell
- Associates with proteins to form ribosomes
- Site of protein Synthesis



The Genetic Code

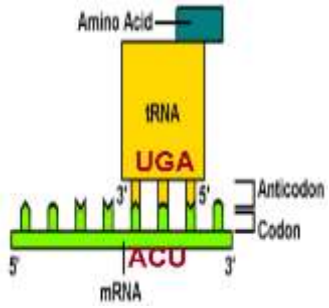
- A codon designates an amino acid
- An amino acid may have more than one codon
- There are 20 amino acids, but 64 possible codons
- Some codons tell the ribosome to *stop* translating



Transfer RNA (tRNA)

- Clover-leaf shape
- Single stranded molecule with attachment site at one end for an amino acid
- Opposite end has three nucleotide bases called the anticodon

Codons and Anticodons



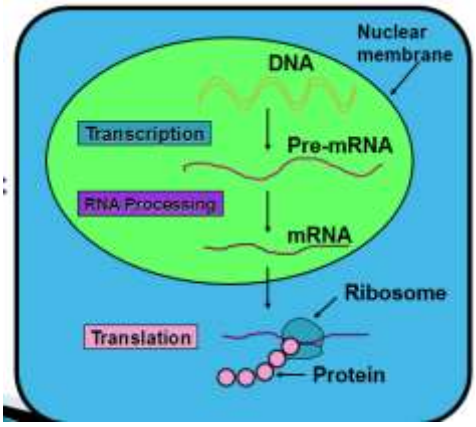
The 3 bases of an anticodon are complementary to the 3 bases of a codon

Example: Codon ACU

Anticodon UGA

Pathway to Making a Protein

DNA → mRNA → tRNA (ribosomes) → Protein



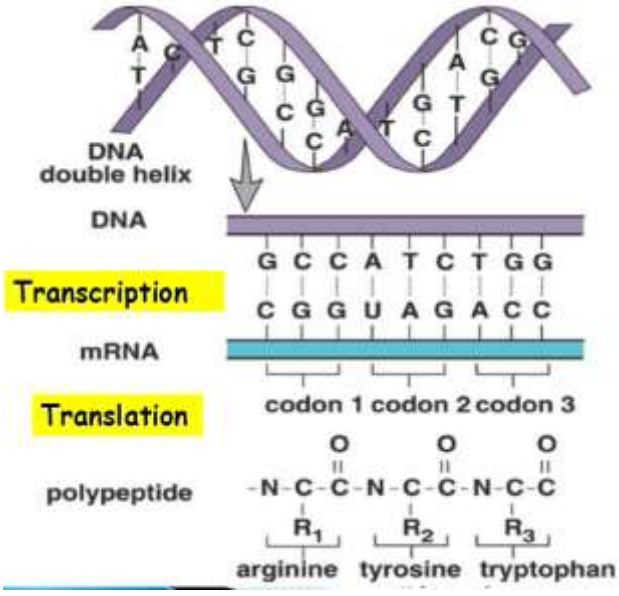
Protein Synthesis

- The production or synthesis of polypeptide chains (proteins)
- Two phases:
  Transcription & Translation
- mRNA must be processed before it leaves the nucleus of eukaryotic cells

Translation

Translation is the process of decoding the mRNA into a polypeptide chain

Ribosomes read mRNA three bases or 1 codon at a time and construct the proteins



Ribosomes

Made of a large and small subunit

Composed of rRNA (40%) and proteins (60%)

Have two sites for tRNA attachment --- P and A

Step 1- Initiation

mRNA transcript start codon AUG attaches to the small ribosomal subunit

Small subunit attaches to large ribosomal subunit

Step 2 - Elongation

As ribosome moves, two tRNA with their amino acids move into site A and P of the ribosome

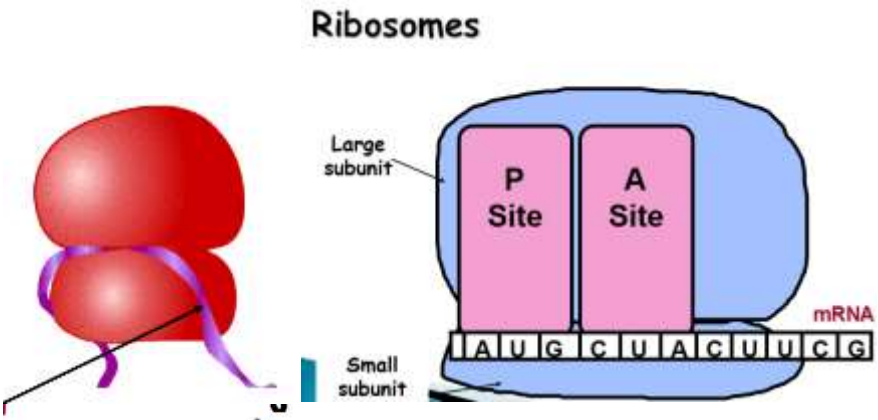
Peptide bonds join the amino acids

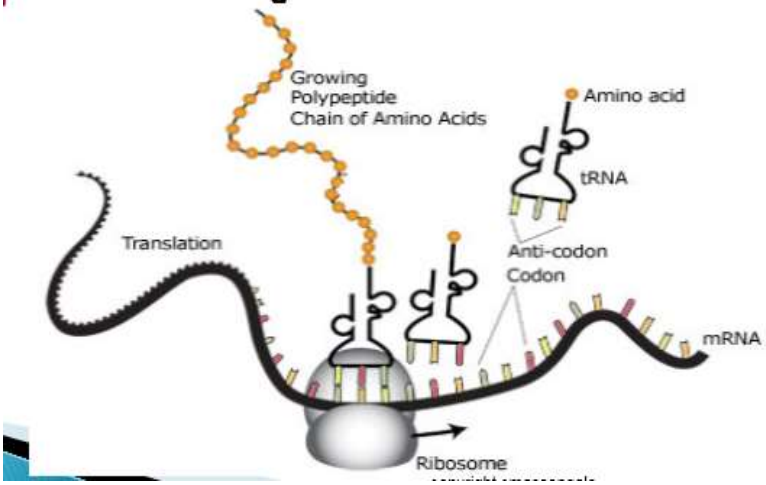
End Product –The Protein!

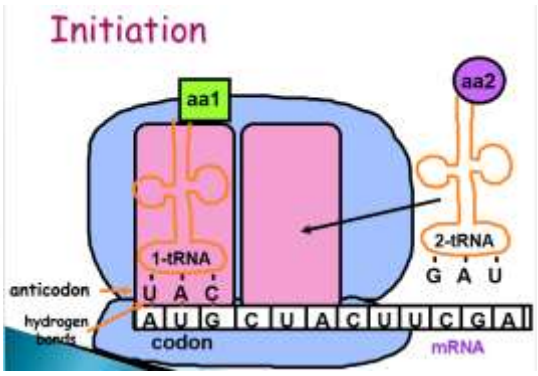
The end products of protein synthesis is a primary structure of a protein

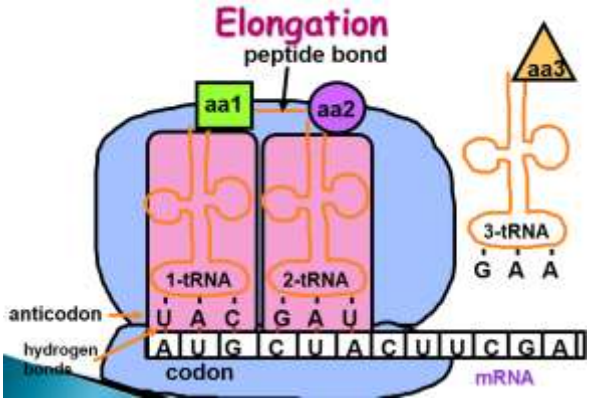
A sequence of amino acid bonded together by peptide bonds

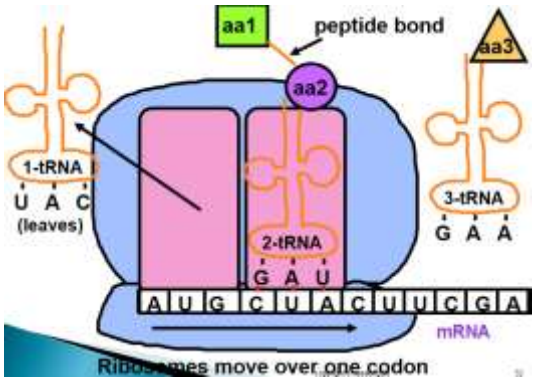
Messenger RNA (mRNA)

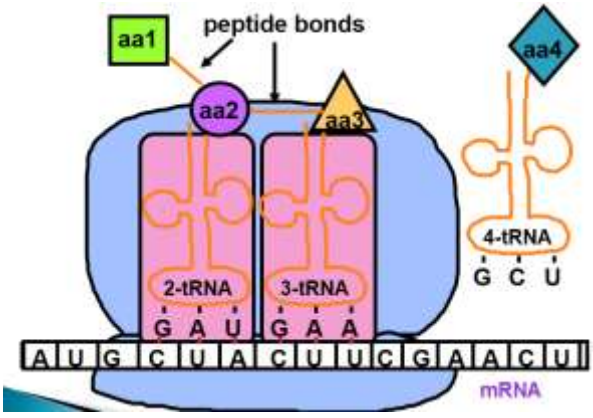


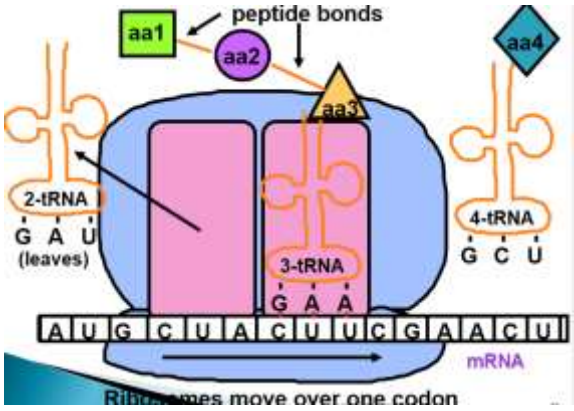


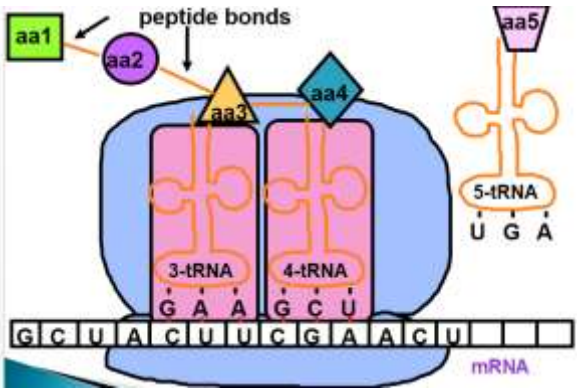


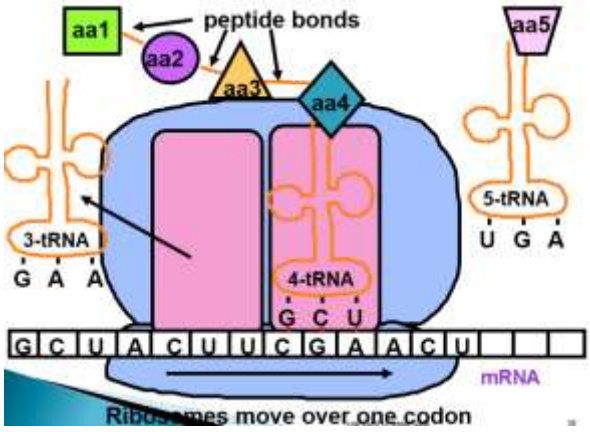


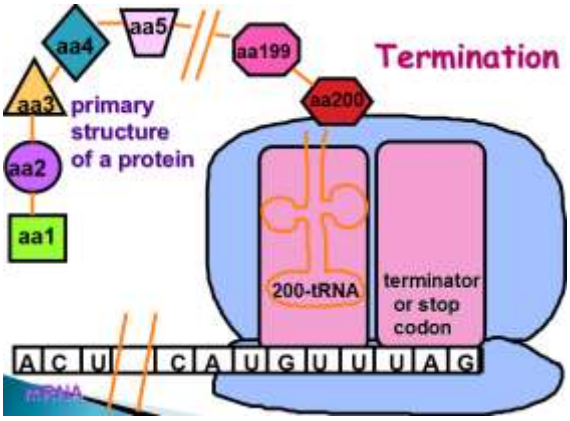


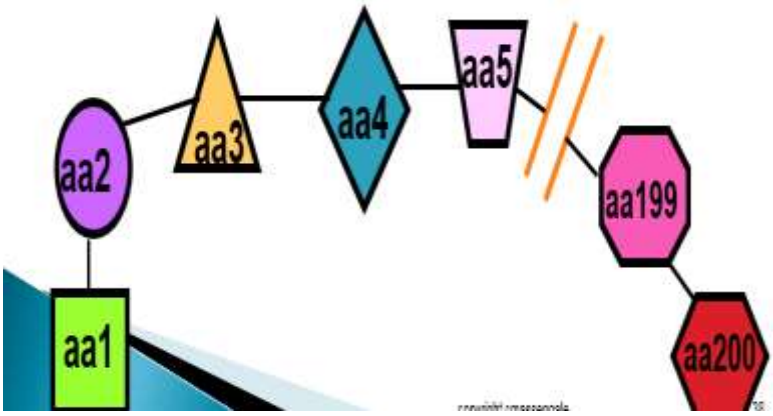


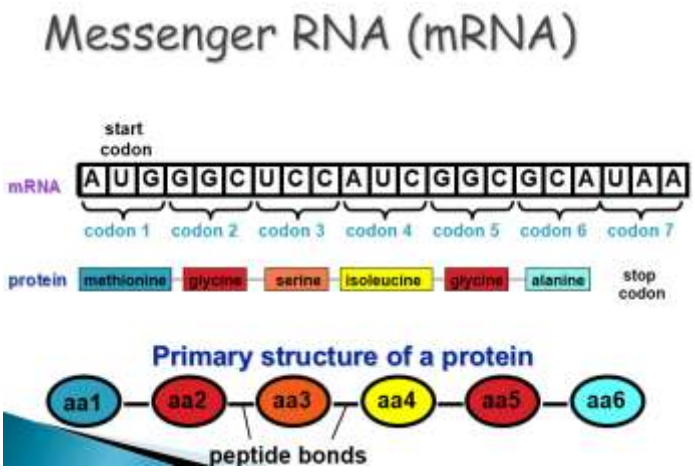








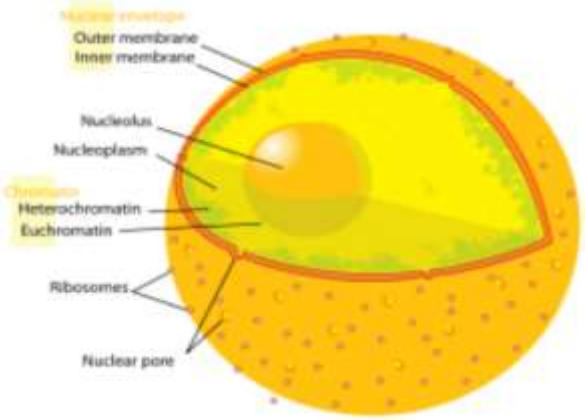




NUCLEUS

The nucleus is the largest cellular organelle in animals. In mammalian cells, the average diameter of the nucleus is approximately 6 micrometers (μm), which occupies about 10% of the total cell volume. The viscous liquid within it is called nucleoplasm, and is similar in composition to the cytosol found outside the nucleus. It appears as a dense, roughly spherical organelle.

Eukaryotic cells contain a nucleus.



It has got two membranes- nuclear envelope.

- ✖ Outer membrane is continuous with the membrane of endoplasmic reticulum.
- ✖ Nuclear envelope has numerous pores. That permit controlled movement of particles and molecules between the nuclear matrix and cytoplasm.

Most proteins, ribosomal subunits, and some RNAs are transported through the pore complexes in a process mediated by transport factors known as karyopherins. Those karyopherins that mediate movement into the nucleus are also called importins, while those that mediate movement out of the nucleus are called exportins.

The space between the membranes is called the Perinuclear space and is continuous with the RER lumen.

the nuclear lamina, a meshwork within the nucleus that adds mechanical support, much like the cytoskeleton supports the cell as a whole.

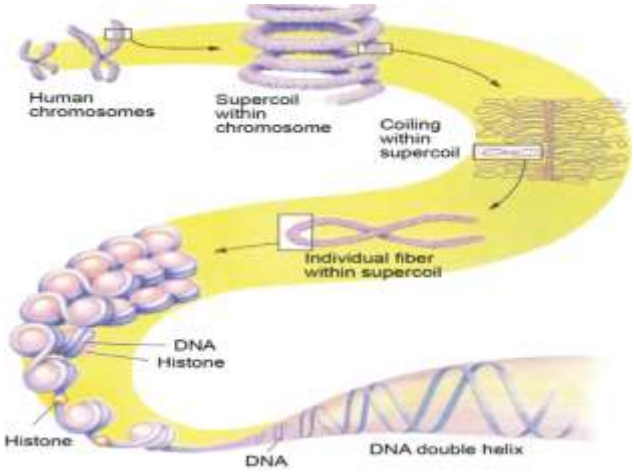
Nucleus has got a major sub compartment- nucleolus.

Deoxyribonucleic acid (DNA) is located in the nucleus. It is the storehouse of genetic information.

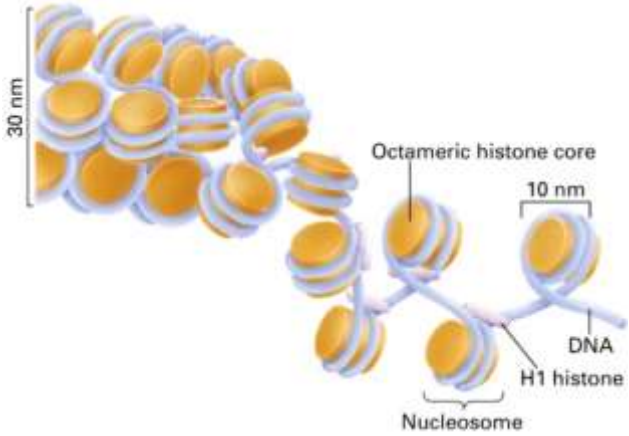
Present as DNA- protein complex –Chromatin, which is organized into chromosomes.

A typical human cell contains 46 chromosomes.

To pack it effectively it requires interaction with a large number of proteins. These are called histones.



They order the DNA into basic structural unit called Nucleosomes. Nucleosomes are further arranged into more complex structures called chromosomes



CHROMATIN:

It is the substance of chromosomes and each chromosome represents the DNA in a condensed form. It is the combination of DNA and proteins. These proteins are called histones.

- There are five classes of histones- H1,H2A, H2B, H3, H4.These proteins are positively charged and they interact with negatively charged DNA.
- Two molecules each of H2A, H2B, H3 and H4 form the structural core of the nucleosome. Around this core the segment of DNA is Wound nearly twice. Neighboring nucleosomes are joined by linker DNA. H1 is associated with linker DNA.

Biomedical importance

Nucleus contains the biochemical processes involved in the Replication of DNA before mitosis.

Involved in the DNA repair.

Transcription of DNA – RNA synthesis.

Translation of DNA- Protein synthesis.

NUCLEOLUS- involved in the processing of rRNA and ribosomal units

After being produced in the nucleolus, ribosomes are exported to the cytoplasm where they tran slate mRNA.

Antibodies to certain types of chromatin organization, particularly nucleosomes, have been associated with a number of autoimmune diseases, such as systemic lupus erythematosus, multiple sclerosis These are known as anti-nuclear antibodies (ANA).

Gene expression

Gene expression first involves transcription, in which DNA is used as a template to produce RNA. In the case of genes encoding proteins, that RNA produced from this process is messenger RNA (mRNA), which then needs to be translated by ribosomes to form a protein. As ribosomes are located outside the nucleus, mRNA produced needs to be exported.

Polynucleated cells contain multiple nuclei.

In humans, skeletal muscle cells, called myocytes, become polynucleated during development; the resulting arrangement of nuclei near the periphery of the cells allows maximal intracellular space for myofibrils.

Multinucleated cells can also be abnormal in humans; for example, cells arising from the fusion of monocytes and macrophages, known as giant multinucleated cells, sometimes accompany inflammation and are also implicated in tumor formation.

Since the nucleus is the site of transcription, it also contains a variety of proteins which either directly mediate transcription or are involved in regulating the process. These proteins include helicases that unwind the double-stranded DNA molecule to facilitate access to it.

RNA polymerases that synthesize the growing RNA molecule, topoisomerases that change the amount of supercoiling in DNA, helping it wind and unwind, as well as a large variety of transcription factors that regulate expression.

Processing of pre-mRNA

Newly synthesized mRNA molecules are known as primary transcripts or pre-mRNA. They must undergo post-transcriptional modification in the nucleus before being exported to the cytoplasm.

mRNA that appears in the nucleus without these modifications is degraded rather than used for protein translation.

The three main modifications are 5' Capping, 3' Polyadenylation, and RNA splicing.

Nuclear transport

Macromolecules, such as RNA and proteins, are actively transported across the nuclear membrane in a process called the Ran-GTP nuclear transport cycle.

The entry and exit of large molecules from the nucleus is tightly controlled by the nuclear pore complexes. Although small molecules can enter the nucleus without regulation, macromolecules such as RNA and proteins require association karyopherins called importins to enter the nucleus and exportins to exit.

Cargo proteins that must be translocated from the cytoplasm to the nucleus contain short amino acid sequences known as nuclear localization signals which are bound by importins, while those transported from the nucleus to the cytoplasm carry nuclear export signals bound by exportins.

Assembly and disassembly

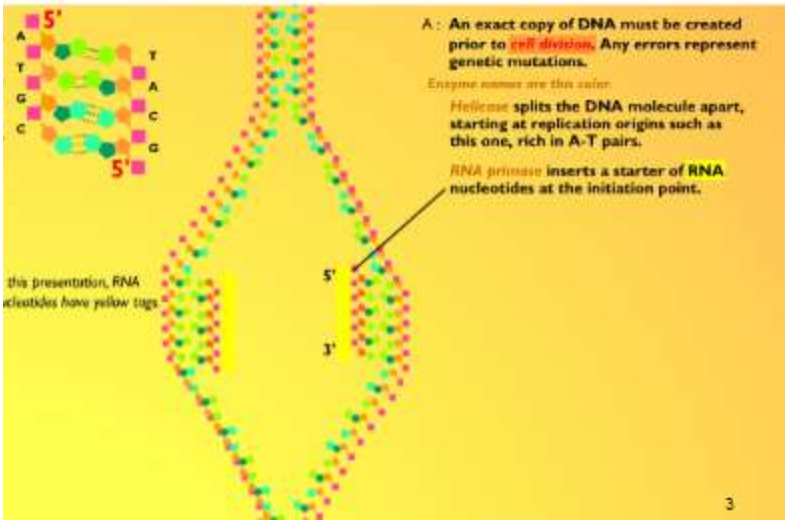
During its lifetime a nucleus may be broken down, either in the process of cell division or as a consequence of apoptosis, a regulated form of cell death. During these events, the structural components of the nucleus —the envelope and lamina— are systematically degraded.

Anucleated and polynucleated cells

Although most cells have a single nucleus, some eukaryotic cell types have no nucleus, and others have many nuclei. This can be a normal process, as in the maturation of mammalian red blood cells, or a result of faulty cell division.

Anucleated cells contain no nucleus and are therefore incapable of dividing to produce daughter cells. The best-known anucleated cell is the mammalian red blood cell, or erythrocyte, which also lacks other organelles such as mitochondria and serves primarily as a transport vessel to ferry oxygen from the lungs to the body's tissues.

There are two types of chromatin –Euchromatin and Heterochromatin. Euchromatin is the less compact DNA form, and contains genes that are frequently expressed by the cell. The other type, heterochromatin, is the more compact form, and contains DNA that are infrequently transcribed.



DNA Replication

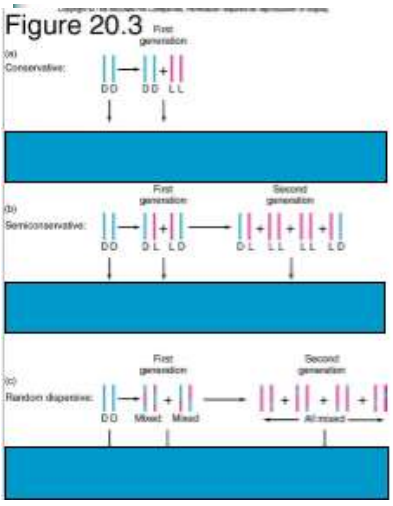
In DNA replication, the two strands of a helix separate and serve as templates for the synthesis of new strands (nascent strands), so that one helix gives rise to two identical "daughter" helices. Hypothetically, there could be three possible ways that DNA replication occur:

- Conservative replication: One daughter helix gets both of the old (template) strands, and the other daughter helix gets both of the new (nascent) strands
- Semiconservative: Each daughter helix gets one old strand and one new strand
- Dispersive: The daughter helices are mixes of old and new

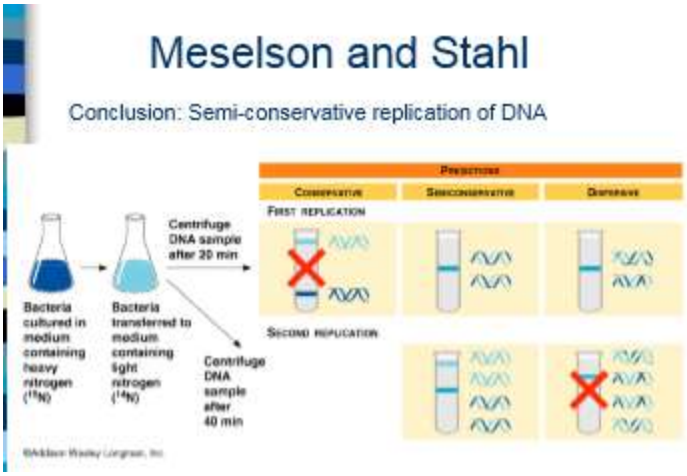
Two major lines of experiment in the mid 1950s – early 1960s demonstrated that DNA replication is semiconservative, both in prokaryotes and eukaryotes:

- Meselson and Stahl demonstrated semiconservative replication in *Escherichia coli* in 1958
- Taylor, Woods, and Hughes demonstrated semiconservative replication in *Vicia faba* (broad bean) in 1957
- Experiments with other organisms support semiconservative replication as the universal mode for DNA replication

Models of Replication



PREDICTED DENSITIES OF NEWLY REPLICATED DNA MOLECULES ACCORDING TO THE THREE HYPOTHESES ABOUT DNA REPLICATION



Replication in *E. coli*

DNA replication is semiconservative and requires a template

Deoxynucleoside triphosphates (dNTPs) (dATP, dTTP, dGTP, dCTP) are the "raw materials" for the addition of nucleotides to the nascent strand

Nucleotides are added only to the 3′ end of a growing nascent chain; therefore, the nascent chain grows only from the 5′ ® 3′ direction

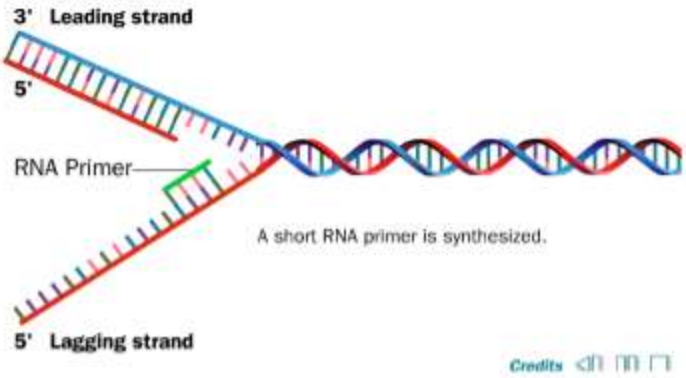
The addition of nucleotides to a growing chain is called chain elongation

Addition of nucleotides to a nascent chain is catalyzed by a class of enzymes called DNA-directed DNA polymerases (or DNA polymerases, for short)

***E. coli* has three DNA polymerases (I, II, and III)**

DNA polymerase I was discovered in the mid 1950s by Arthur Kornberg (it was originally simply called "DNA polymerase"DNA polymerase I has three different enzymatic activities:

- 5′ ® 3′ polymerase activity (elongation)
- 3′ exonuclease activity (proofreading function)
- 5′ exonuclease activity (primer excision)



The 3′ exonuclease activity of DNA polymerase I performs a "proofreading" function: it excises mismatched bases at the 3′ end, reducing the frequency of errors (mutations)

The 5′ exonuclease activity is responsible for RNA primer excision

By the late 1960s, biologists suspected that there must be additional DNA polymerases in *E. coli* (to account for the rate of replication observed in experiments)

In the early 1970s, DNA polymerases II and III were discovered

DNA polymerases II and III each have two enzymatic activities:

- 5′ ® 3′ polymerase activity (elongation)
- 3′ exonuclease activity (proofreading)

Neither has the 5′ exonuclease activity

DNA polymerase III is the enzyme responsible for most of the nascent strand elongation in *E. coli*

DNA polymerase can only elongate existing chains; it cannot initiate chain synthesis

- Nascent strand initiation requires the formation of a short RNA primer molecule
- The RNA primers are synthesized by RNA primase (a type of 5′ ® 3′ RNA polymerase, capable of initiating nascent chain synthesis from a DNA template; uses ribose NTPs as nucleotide source)
- The primers are eventually excised by the 5′ exonuclease activity of DNA polymerase I

Replication begins at a location on the chromosome called the origin of replication (ori), and proceeds bidirectional.

As the DNA helix unwinds from the origin, the two old strands become two distinctive templates:

- the 3′ ® 5′ template,
- and the 5′ ® 3′ template
- Replication on the 3′ ® 5′ template is continuous (leading strand synthesis), proceeding into the replication fork
- Replication on the 5′ ® 3′ template is discontinuous, resulting in the synthesis of short nascent segments (lagging strand or Okazaki fragments), each with its own primer
- After primer excision is complete, nascent segments are "sealed" (the final phosphodiester bond is formed) by DNA ligase

- DNA polymerase III may be able to synthesize both the leading and lagging strands simultaneously by having the 5´ ® 3´ template to fold back.

Several proteins are required to unwind the helix

- Helicases

dnaA protein recognizes the origin , binds, and begins the separation of the helix

dnaB dissociates from dnaC; the dnaB is responsible for moving along the helix at the replication fork, "unzipping" the helix

- DNA gyrase

Makes temporary single-stranded "nicks" (single PDE bond breaks) in one of the two template strands to relieve the torsional stress and supercoiling caused by the unwinding of the helix

- Single-stranded binding proteins (SSBPs)

Bind to the unwound strands of the template, stabilizing the single-stranded state long enough for

Transcription

Transcription is the DNA-directed synthesis of RNA

RNA synthesis

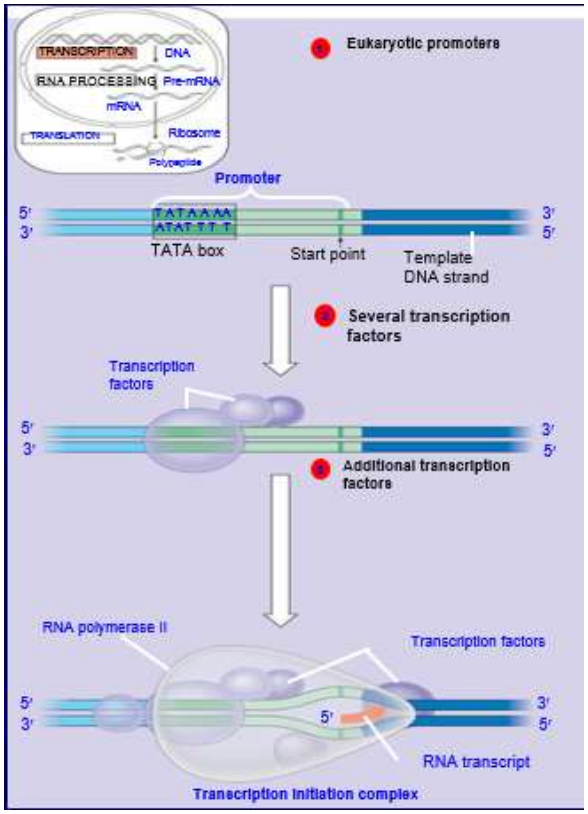
- Is catalyzed by RNA polymerase, which pries the DNA strands apart and hooks together the RNA nucleotides
- Follows the same base-pairing rules as DNA, except that in RNA, uracil substitutes for thymine

RNA

- RNA is single stranded, not double stranded like DNA
- RNA is short, only 1 gene long, where DNA is very long and contains many genes
- RNA uses the sugar ribose instead of deoxyribose in DNA
- RNA uses the base uracil (U) instead of thymine (T) in DNA.

Types of RNA and their functions

1. Messenger RNA (mRNA) carries information specifying amino acid sequences of proteins from DNA to ribosomes
2. Transfer RNA (tRNA) serves as adaptor molecule in protein synthesis translates mRNA codons into amino acids
3. Ribosomal RNA (rRNA) plays catalytic (ribozyme) roles and structural roles in ribosomes
4. Primary transcript serves as a precursor to mRNA, rRNA or tRNA before being processed by splicing or cleavage. Some intron RNA acts as a ribozyme catalyzing its own splicing
5. Small nuclear RNA (snRNA) plays structural and catalytic roles in spliceosomes. The complexes of protein and RNA that splice pre-mRNA



Synthesis of an RNA Transcript

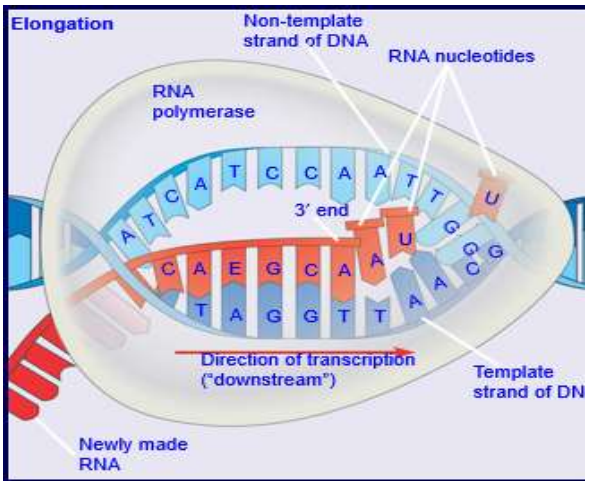
The stages of transcription are

Initiation Elongation Termination

Initiation

- Promoters signal the initiation of RNA synthesis
- Transcription factors help eukaryotic RNA polymerase recognize promoter sequences

Elongation



- RNA polymerase synthesizes a single strand of RNA against the DNA template strand (anti-sense strand), adding nucleotides to the 3' end of the RNA chain
- As RNA polymerase moves along the DNA it continues to untwist the double helix, exposing about 10 to 20 DNA bases at a time for pairing with RNA nucleotides

Termination

- Specific sequences in the DNA signal termination of transcription
- When one of these is encountered by the polymerase, the RNA transcript is released from the DNA and the double helix can zip up again.

Post termination RNA processing

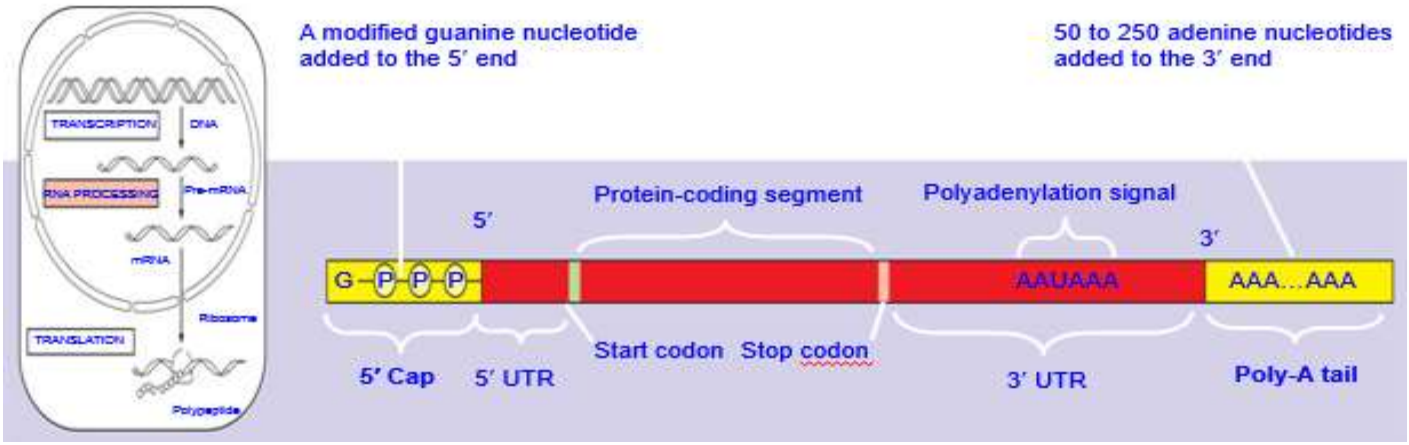
- Most eukaryotic mRNAs aren't ready to be translated into protein directly after being transcribed from DNA, mRNA requires processing.
- Transcription of RNA processing occur in the nucleus. After this, the messenger RNA moves to the cytoplasm for translation.
- The cell adds a protective cap to one end, and a tail of A's to the other end. These both function to protect the RNA from enzymes that would degrade
- Most of the genome consists of non-coding regions called introns
- Non-coding regions may have specific chromosomal functions or have regulatory purposes
- Introns also allow for alternative RNA splicing
- Thus, an RNA copy of a gene is converted into messenger RNA by doing 2 things:
    - Add protective bases to the ends

- Cut out the introns

Alteration of mRNA Ends

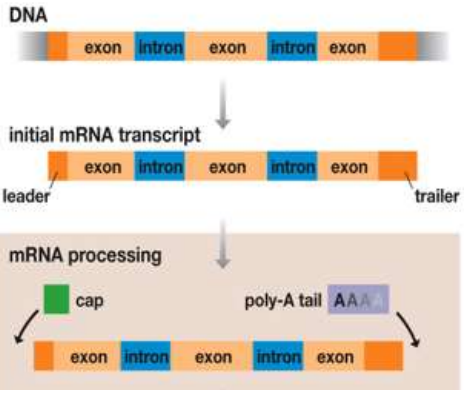
Each end of a pre-mRNA molecule is modified in a particular way

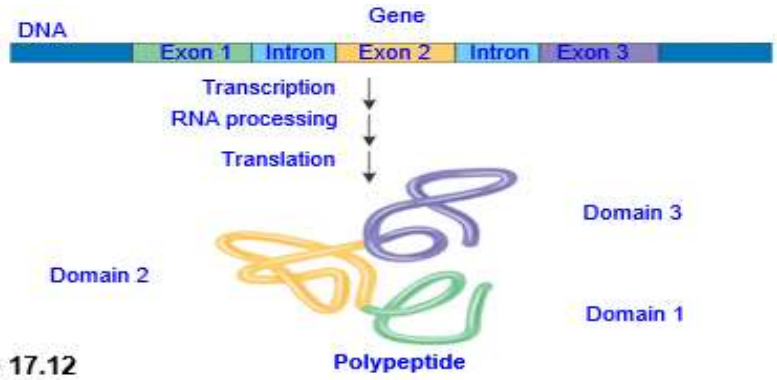
- The 5′ end receives a modified nucleotide cap
- The 3′ end gets a



RNA Processing- Splicing

- The original transcript from the DNA is called pre-mRNA.
- It contains transcripts of both introns and exons.
- The introns are removed by a process called splicing to produce messenger RNA (mRNA)
- Proteins often have a modular architecture consisting of discrete structural and functional regions called domains
- In many cases different exons code for the different domains in a protein





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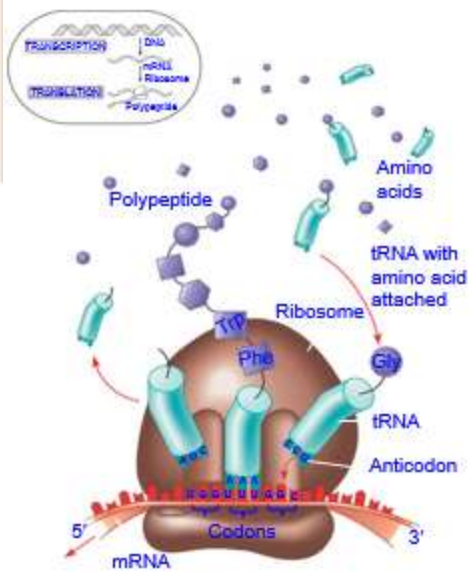
Translation

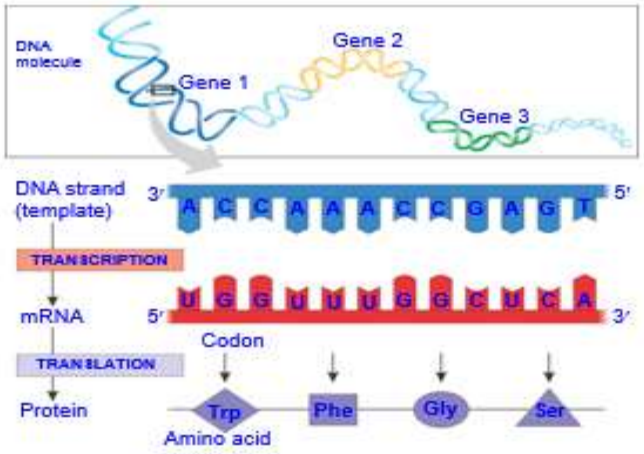
- Translation is the RNA-directed synthesis of a polypeptide
- Translation involves
  - mRNA
  - Ribosomes - Ribosomal RNA
  - Transfer RNA
  - Genetic coding - codons

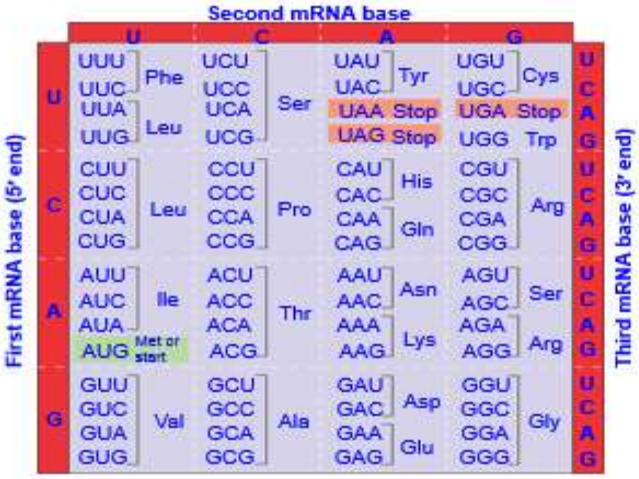


The Genetic Code

- Genetic information is encoded as a sequence of nonoverlapping base triplets, or codons
- Codons: 3 base code for the production of a specific amino acid, sequence of three of the four different nucleotides
- Since there are 4 bases and 3 positions in each codon, there are 4 x 4 x 4 = 64 possible codons

- 64 codons but only 20 amino acids, therefore most have more than 1 codon
- 3 of the 64 codons are used as STOP signals; they are found at the end of every gene and mark the end of the protein
- One codon is used as a START signal: it is at the start of every protein
- Universal: in all living organisms
- A codon in messenger RNA is either translated into an amino acid or serves as a translational start/stop signal

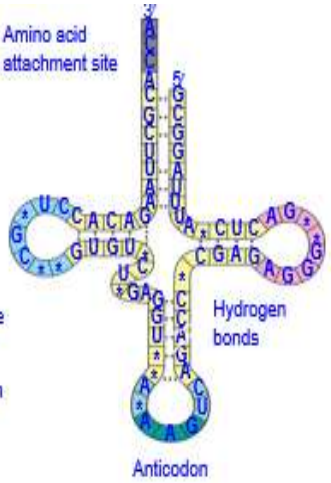




Transfer RNA

- Consists of a single RNA strand that is only about 80 nucleotides long
- Each carries a specific amino acid on one end and has an anticodon on the other end
- A special group of enzymes pairs up the proper tRNA molecules with their corresponding amino acids.
- tRNA brings the amino acids to the ribosomes,
- 3 dimensional tRNA molecule is roughly "L" shaped

The "anticodon" is the 3 RNA bases that matches the 3 bases of the codon on the mRNA molecule



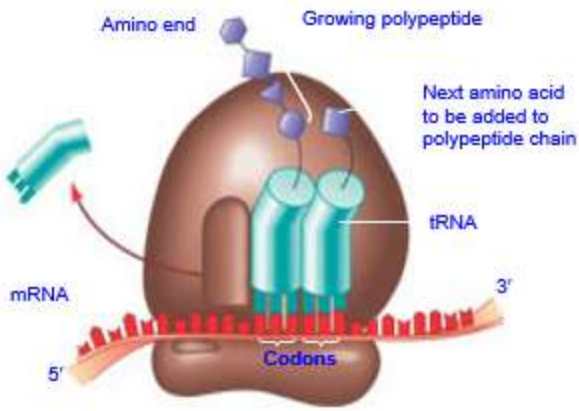
(a) **Two-dimensional structure.** The four base-paired regions and three loops are characteristic of all tRNAs, as is the base sequence of the amino acid attachment site at the 3′ end. The anticodon triplet is unique to each tRNA type. (The asterisks mark bases that have been chemically modified, a characteristic of tRNA.)

Ribosomes

- Ribosomes facilitate the specific coupling of tRNA anticodons with mRNA codons during protein synthesis
- The 2 ribosomal subunits are constructed of proteins and RNA molecules named ribosomal RNA or rRNA

Building a Polypeptide

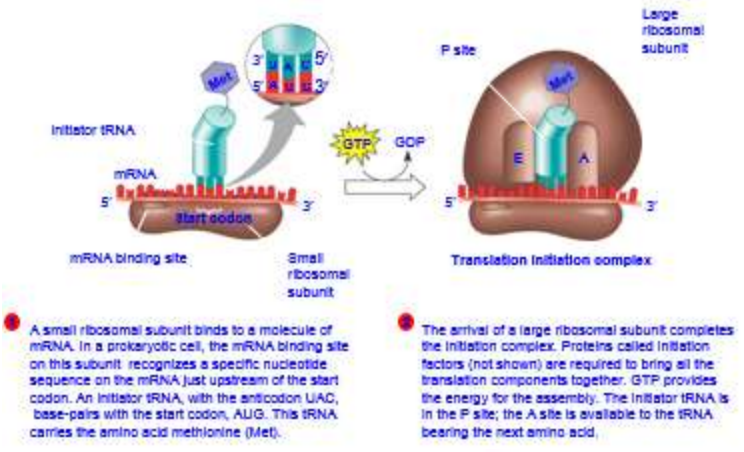
- We can divide translation into three stages
  Initiation, Elongation, Termination
- The AUG start codon is recognized by methionyl-tRNA or Met
- Once the start codon has been identified, the ribosome incorporates amino acids into a polypeptide chain
- RNA is decoded by tRNA (transfer RNA) molecules, which each transport specific amino acids to the growing chain



- Translation ends when a stop codon (UAA, UAG, UGA) is reached

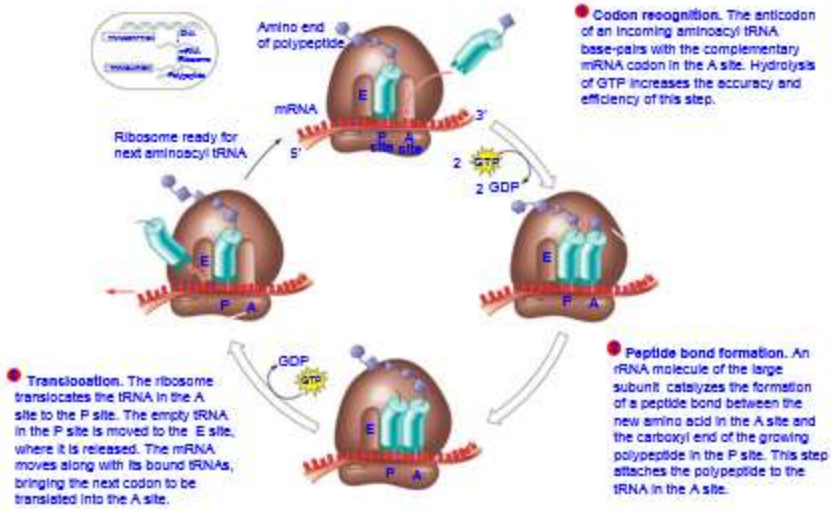
Initiation of Translation

The initiation stage of translation brings together mRNA, tRNA bearing the first amino acid of the polypeptide, and two subunits of a ribosome



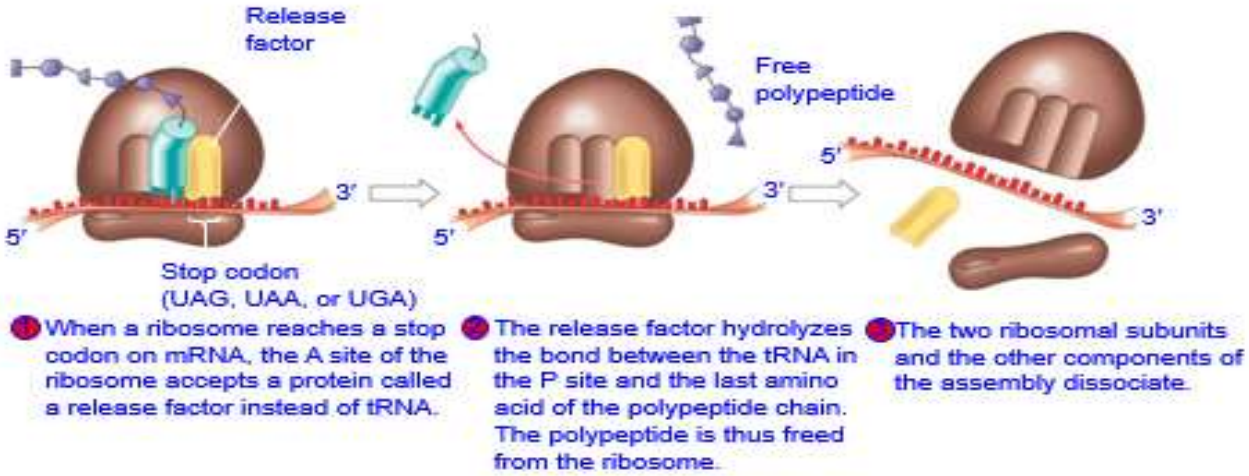
Elongation of the Polypeptide Chain

In the elongation stage, amino acids are added one by one to the preceding amino acid



Termination of Translation

- The final step in translation is termination. When the ribosome reaches a STOP codon, there is no corresponding transfer RNA.
- Instead, a small protein called a "release factor" attaches to the stop codon.
- The release factor causes the whole complex to fall apart: messenger RNA, the two ribosome subunits, the new polypeptide.
- The messenger RNA can be translated many times, to produce many protein copies.



Post-translation

- The new polypeptide is now floating loose in the cytoplasm if translated by a free ribosome.
- It might also be inserted into a membrane, if translated by a ribosome bound to the endoplasmic reticulum.
- Polypeptides fold spontaneously into their active configuration, and they spontaneously join with other polypeptides to form the final proteins.
- Sometimes other molecules are also attached to the polypeptides: sugars, lipids, phosphates, etc. All of these have special purposes for protein function.

Mutation Causes and Rate

- The natural replication of DNA produces occasional errors. DNA polymerase has an editing mechanism that decreases the rate, but it still exists.
- Typically genes incur base substitutions about once in every 10,000 to 1,000,000 cells.
- Since we have about 6 billion bases of DNA in each cell, virtually every cell in your body contains several mutations.
- However, most mutations are neutral: have no effect.
- Only mutations in cells that become sperm or eggs—are passed on to future generations.
- Mutations in other body cells only cause trouble when they cause cancer or related diseases.

Point mutations

- Point mutations involve alterations in the structure or location of a single gene. Generally, only one or a few base pairs are involved.
- Point mutations can significantly affect protein structure and function
- Point mutations may be caused by physical damage to the DNA from radiation or chemicals, or may occur spontaneously
- Point mutations are often caused by mutagens

Mutagens

- **Mutagens** are chemical or physical agents that interact with DNA to cause mutations.
- Physical agents include high-energy radiation like X-rays and ultraviolet light
- Chemical mutagens fall into several categories.
  - Chemicals that are base analogues that may be substituted into DNA, but they pair incorrectly during DNA replication.
  - Interference with DNA replication by inserting into DNA and distorting the double helix.
  - Chemical changes in bases that change their pairing properties.
  - Most carcinogens are mutagenic and most mutagens are carcinogenic.

Viral Mutagens

- Scientists have recognized a number of *tumor viruses* that cause cancer in various animals, including humans
- About 15% of human cancers are caused by viral infections that disrupt normal control of cell division
- All tumor viruses transform cells into cancer cells through the integration of viral nucleic acid into host cell DNA.

Point Mutation

The change of a single nucleotide in the DNA's template strand leads to the production of an abnormal protein

Types of Point Mutations

Point mutations within a gene can be divided into two general categories

1. Base-pair substitutions 2. Base-pair insertions or deletions

Substitutions

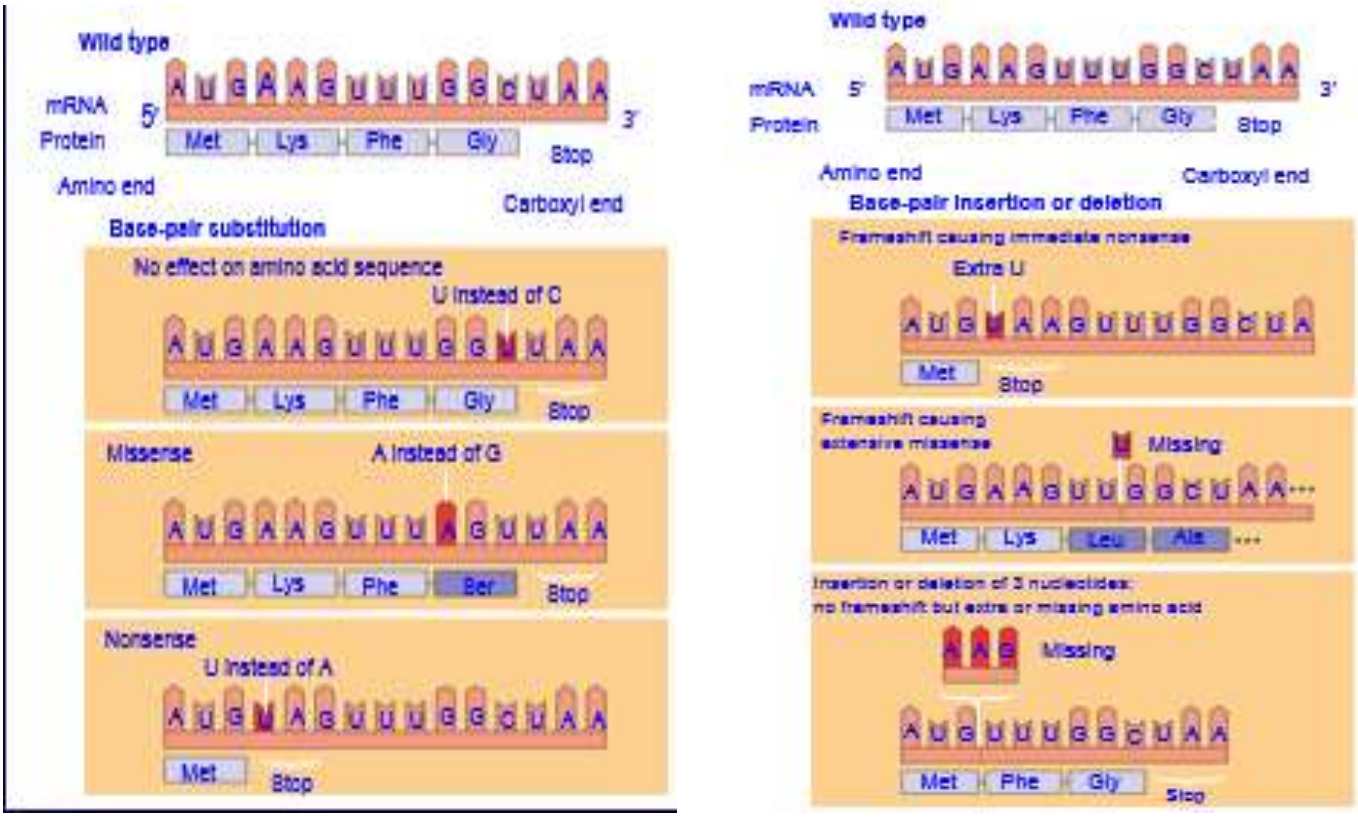
A base-pair substitution is the replacement of one nucleotide and its partner with another pair of nucleotides

- Silent - changes a codon but codes for the same amino acid
- Missense - substitutions that change a codon for one amino acid into a codon for a different amino acid
- Nonsense -substitutions that change a codon for one amino acid into a stop codon

Insertions and deletions

Are additions or losses of nucleotide pairs in a gene

May produce frame shift mutations that will change reading frame of the gene,and alter all codons downstream from the mutation.



Gene Expression

It is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in non-protein coding genes such as rRNA genes or tRNA genes, the product is a functional RNA.

Classification of gene with respect to their Expression:

On the basis of expression gene can be classified to two kinds as below:-

1. **Constitutive (house-keeping) genes:**

They are expressed at a fixed rate, irrespective to the cell condition. Their structure is simpler

2. **Controllable genes**:

They are expressed only when needed. Their amount may increase or decrease with respect to their basal level in different condition. Their structure is relatively complicated with some response elements

Several steps in the gene expression process may be modulated,

Expression of genes can be modulated at different steps including the

1. Transcription,
2. RNA splicing
3. Translation,
4. Post-translational modification of a protein.

Types of regulation of gene expression

Regulation of gene expression is of two types

1. **Positive regulation or induction :**

When the expression of genetic is quantitatively increased by the presence of specific regulatory element is known as positive regulation. Element modulating positive regulation is known as inducer, activator or positive regulator.

2. **Negative regulation or repression.**

When the expression of genetic information diminished by the presence of specific regulatory element. The element or molecule mediating the negative regulation is said to be repressor.

Key feature of both regulation types

One key feature of both systems is that a single mRNA is transcribed with multiple translation stop codons. The proteins that can be translated from the mRNA are the enzymes required for a specific pathway. This type of mRNA is called a polycistronic mRNA and is totally unique to prokaryotes.

Gene expression in prokaryotes

Prokaryotes only transcribe genes that their end-proteins are needed at the time. They do this in order to save up energy and increase efficiency. The regulation of gene expression is depended mainly on their immediate environment, for example on the presence and absence of nutrients. In prokaryotes such as Escherichia coli (E. coli), regulation of gene expression occurs primarily at the level of transcription and, in general, is mediated by the binding of trans-acting proteins to cis-acting regulatory elements on their single DNA molecule (chromosome). Regulating the first step in the expression of a gene is an efficient approach, insofar as energy is not wasted making unneeded gene products.

Operons, the principle of gene regulation

An operon is a functioning unit of genomic DNA containing a cluster of genes under the control of a single promoter.

Operon is a genetic regulatory system found in bacteria and their viruses in which genes coding for functionally related proteins are clustered along the DNA. This feature allows protein synthesis to be controlled coordinately in response to the needs of the cell. By providing the means to produce proteins only when and where they are required, the operon allows the cell to conserve energy which is an important part of an organism's life strategy.

In bacteria, genes that encode for proteins with closely related functions are found grouped along with cis-acting regulatory elements that determine the transcription of these genes, thus these genes are regulated in a coordinated way. These clusters of genes are called operons, and their transcription product is a single polycistronic mRNA. Organization of genes in operons contributes to the regulation of gene expression.

Types of operon

On the basis of activity of operon, they are classified into two classes:-

Inducible operons:

They include genes that encode for enzymes that take part in metabolic pathways and the expression of the gene is controlled by the substrate. Example is the "Lac Operon".

Repressible operons:

They include genes that encode for enzymes involved in biosynthetic pathways, and the expression of the gene is controlled by the end-product of the pathway. Example is the "Trp Operon".

Generalized structure of operon

Generally an operon consists of promoter, operator, structural gene and regulator.

Promoter

A promoter is a nucleotide sequence in the DNA that initiates transcription of a particular gene. Promoters are located near the transcription start sites of genes, on the same strand of the DNA towards the 5' region of the sense strand. Promoters can be about 100–1000 base pairs long.

Operators

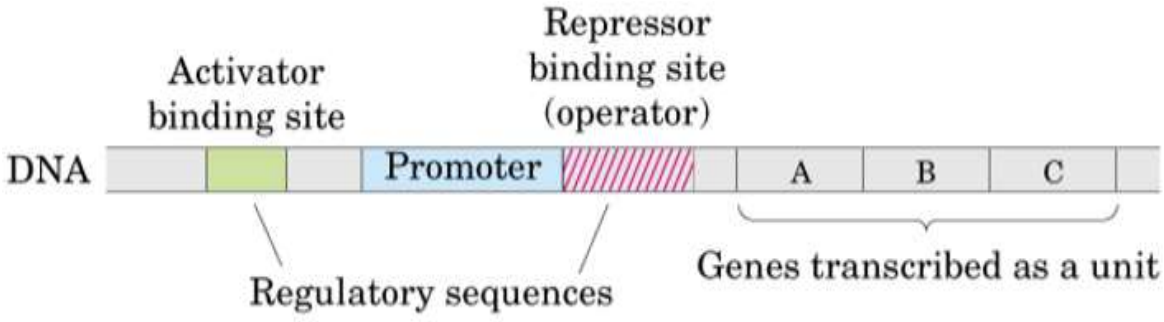
These are segments of DNA that regulate the activity of the structural genes of the operon. It is a nucleotide sequence located in between the promoter and the genes.

Structural Genes

A structural gene is a gene that codes for any RNA or protein product other than a regulatory factor (i.e. regulatory protein). It may code for a structural protein, an enzyme, or an RNA molecule not involved in regulation. These genes are needed for the morphological or functional traits of the cell. The structural genes are mainly concerned with the synthesis of a polypeptide chain.

Regulator

These nucleotide sequences control the operator gene in cooperation with certain compounds called inducers and co-repressors present in the cytoplasm. A regulator gene is not necessarily adjacent to its controlling operator gene. The regulator gene codes for and produces a protein substance called repressor. The repressor substance combines with the operator gene to repress its action. A regulator gene controls an operon, but is not the actual part of the operon.



The lac Operon - an inducible system

The first control system for enzyme production worked out at the molecular level described the control of enzymes that are produced in response to the presence of the sugar lactose in E. coli cell. The work was performed by Jacob and Monod for which they were awarded the Nobel Prize. The following is the pathway that leads to the production of glucose and galactose.

Lactose ——β- galactosidase——→ glucose + galactose

The lactose (lac) operon contains the genes that code for three proteins involved in the catabolism of the disaccharide lactose:

- The lac Z gene codes for β-galactosidase, which hydrolyzes lactose to galactose and glucose;
- the lac Y gene codes for a permease, which facilitates the movement of lactose into the cell;
- the lac A gene codes for thiogalactoside transacetylase, which acetylates lactose.

The most direct way to control the expression of a gene is to regulate its rate of transcription. Gene transcription begins at a particular nucleotide. RNA polymerase actually binds to that particular site called the promoter. In bacteria, the promoter sequence is TATAAT (TATA box).

Lac operon , summarized

Each of the three enzymes synthesized in response to lactose is encoded by a separate gene. The three genes are arranged in cycle on the bacterial chromosome. In the absence of lactose, the repressor protein encoded by the "I gene "binds to the lac operator and prevents transcription. Binding of allolactose to the repressor causes it to leave the operator. This enables RNA polymerase to transcribe the three genes of the operon. The single mRNA molecule that results is then translated into the three proteins. The lac repressor binds to operator. Most of the operator is downstream of the promoter. When the repressor is bound to the operator, RNA polymerase is unable to proceed downstream with its task of gene transcription.

The gene encoding the lac repressor is called the lac I gene. It happens to be located just upstream of the lac promoter. However, its precise location is probably not important because it achieves its effect by means of its protein product, which is free to diffuse throughout the cell. And, in fact, the genes for some repressors are not located close to the operators they control.

Across the DNA, the repressor protein can move along it until it meets the operator sequence. Now an allosteric change in the tertiary structure of the protein allows the same amino acids to establish bonds mostly hydrogen bonds with particular bases in the operator sequence.

The lac repressor is made up of four identical polypeptides and bears a site that enable it to recognize and bind to the lac operator. Another part of the repressor contains sites that bind to allolactose. When allolactose unites with the repressor, it causes a change in the shape of the molecule, so that it can no longer remain attached to the DNA sequence of the operator. Thus, when lactose is added to the culture medium,

- it causes the repressor to be released from the operator
- RNA polymerase can now begin transcribing the 3 genes of the operon into a single molecule of messenger RNA.

Hardly does transcription begin, before ribosomes attach to the growing mRNA molecule and move down it to translate the message into the three proteins. The punctuation codons — UAA, UAG, or UGA — are needed to terminate translation between the portions of the mRNA coding for each of the three enzymes.

Catabolite repression of the lac operon

Absence of the lac repressor is essential but not sufficient for effective transcription of the lac operon. The activity of RNA polymerase also depends on the presence of another DNA-binding protein called catabolite activator protein or CAP. However, CAP can bind to DNA only when cAMP is bound to CAP. So when cAMP levels in the cell are low, CAP fails to bind DNA and thus RNA polymerase cannot begin its work, even in the absence of the repressor. So the lac operon is under both negative repression and positive CAP control (induction).

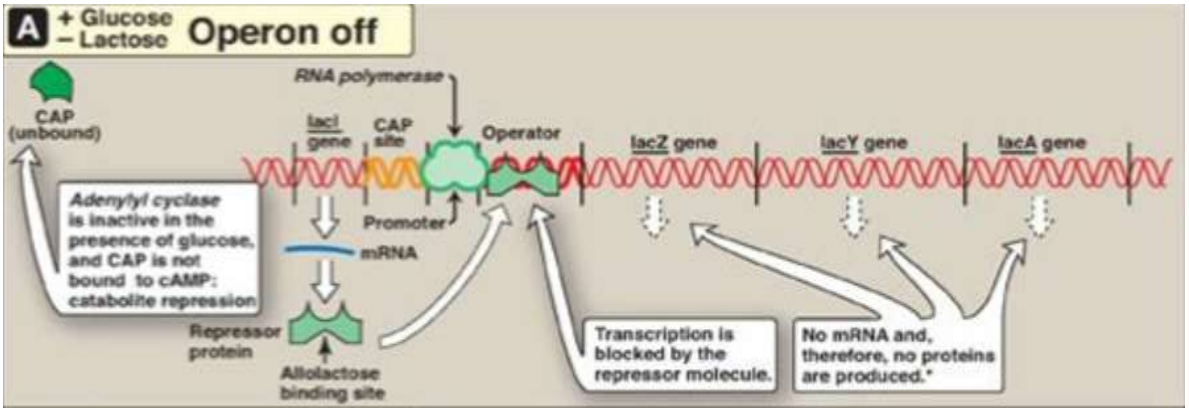
Although the presence of lactose removes the repressor but the presence of glucose lowers the level of cAMP in the cell and thus removes CAP. Without CAP, binding of RNA polymerase is inhibited even though there is no repressor to interfere.The binding of the CAP-cAMP complex to the promoter site is required for transcription of the lac operon.

The presence of this complex is closely associated with the presence of glucose in the cell. As the concentration of glucose increases the amount of cAMP decreases. As the cAMP decreases, the amount of complex decreases. This decrease in the complex inactivates the promoter, and the lac operon is turned off. Because the CAP-cAMP complex is needed for transcription, the complex exerts a positive induction control over the expression of the lac operon

Different conditions of lac operon

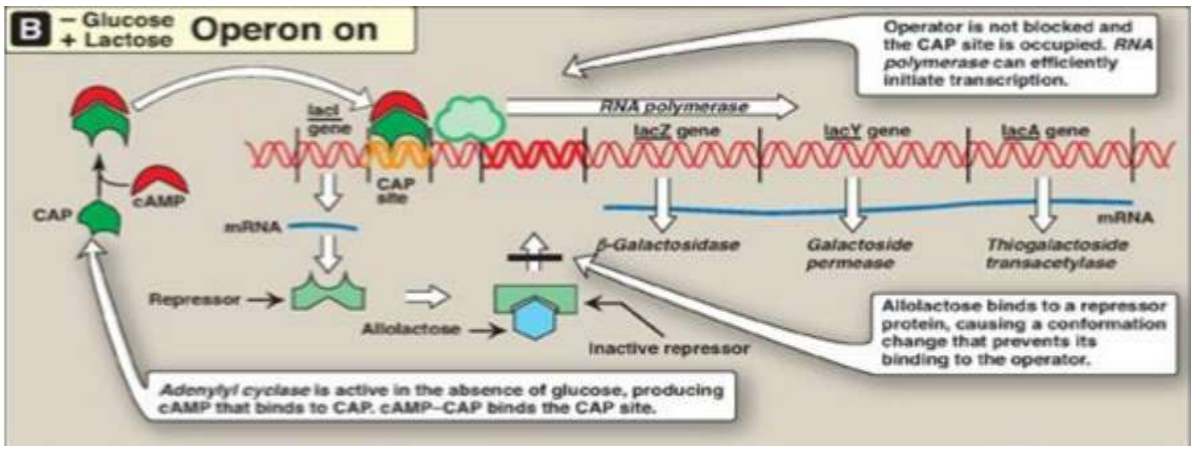
1) When only glucose is available:

In this case, the lac operon is repressed (turned off). Repression is mediated by the repressor protein binding to the operator site. Binding of the repressor interferes with the progress of RNA polymerase and blocks transcription of the structural genes. This is an example of negative regulation.



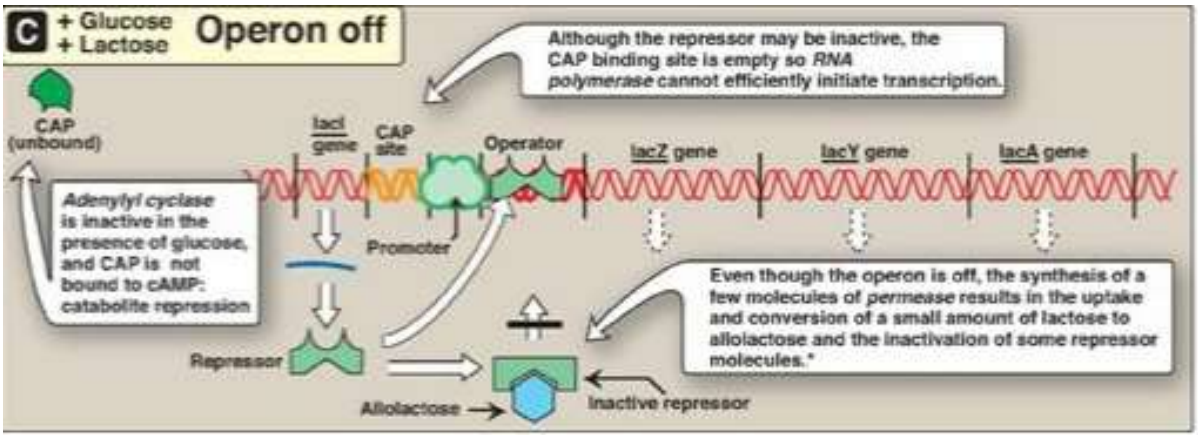
2) When only lactose is available:

In this case, the lac operon is induced (maximally expressed, or turned on). A small amount of lactose is converted to an isomer, allolactose. This compound is an inducer that binds to the repressor protein, changes its conformation so that it can no longer bind to the operator. In the absence of glucose, adenylyl cyclase is active, and sufficient quantities of cAMP are made and bind to the CAP protein. The cAMP–CAP complex binds to the CAP site, causing RNA polymerase to more efficiently initiate transcription at the promoter site. This is an example of positive regulation.



3) When both glucose and lactose are available:

In this case, transcription of the lac operon is negligible, even if lactose is present at a high concentration. Adenylyl cyclase is inhibited in the presence of glucose (a process known as catabolite repression) so no cAMP–CAP complex forms, and the CAP site remains empty. RNA polymerase is, therefore, unable to effectively initiate transcription, even though the repressor may not be bound to the operator region. Consequently, the three structural genes of the operon are not expressed.



The trp Operon - a repressible system

The trp operon in E. coli was the first repressible operon discovered in 1953 by Jacques Monod and colleagues. Tryptophan (Trp) operon is inhibited by a chemical (tryptophan). The operon is regulated so that when tryptophan is present in the environment, the genes for tryptophan synthesis are not expressed.

Components of trp operon

The trp operon consists of the following

- 5 structural genes

These genes (trp A, trp B, trp C, trp D, trp E) code for the enzyme tryptophan synthesis pathway.

- A promoter

The DNA segment where RNA polymerase binds and start transcription.

- Operator:

DNA segment found between the promoter and structural genes. It determines if transcription will take place. If the operator is turned "on", transcription will occur.

The trp operon is an example of repressible negative regulation of gene expression. Within the operon's regulatory sequence, the operator is blocked by the repressor protein in the presence of tryptophan thereby preventing transcription and is liberated in tryptophan's absence thereby allowing transcription.

The trp operon of E. coli controls the biosynthesis of tryptophan in the cell from the initial precursor chorismic acid. This operon contains genes for the production of five proteins which are used to produce three enzymes.

1. Enzyme *anthranilate synthetase*.

The product of trp E and trp D, which catalyzes first two reactions in trptophan pathway

2. Enzyme *indole glycerolphosphate synthetase*.

The product of trp C, which catalyzes the next two steps in tryptophan pathway

3. Enzyme *tryptophan synthetase*.

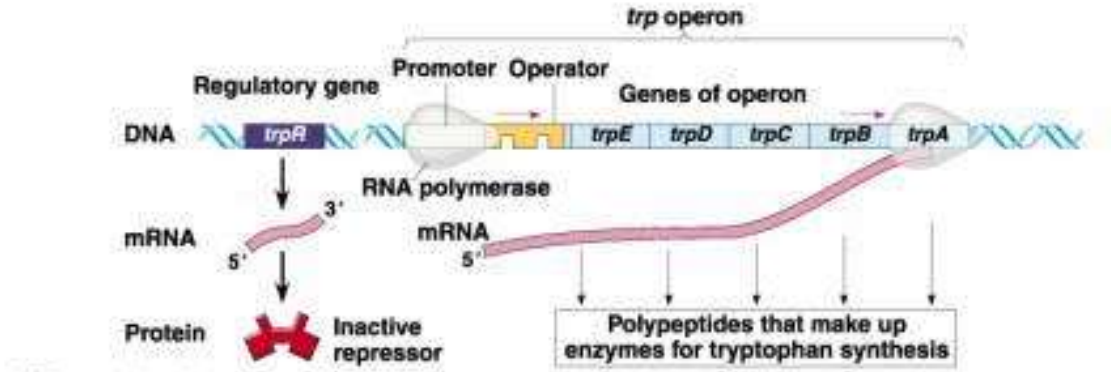
The product of trp B and trp A, which produces tryptophan from indole-glycerol phosphate and serine.

Repression

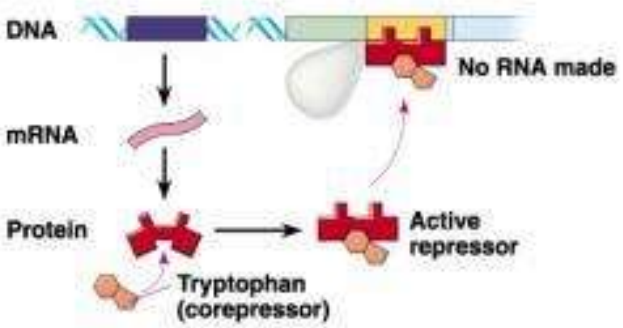
The operon operates by a negative repressible feedback mechanism. The repressor for the trp operon is produced upstream by the trp R gene, which is constitutively expressed at a low level.

When tryptophan is present, these tryptophan repressor bind to tryptophan, causing a change in the repressor conformation, allowing the repressor to bind to the operator. This prevents RNA polymerase from binding to and transcribing the operon, so tryptophan is not produced from its precursor. Here tryptophan acts as a co-represser. This is called as positive repression.

When tryptophan is absent, the repressor is in its inactive conformation and cannot bind the operator region, so transcription is permitted by the repressor. This is called as negative induction.



(a) Tryptophan absent, repressor inactive, operon on



(b) Tryptophan present, repressor active, operon off