Lecture 1

Introduction to the Study

of Cell Biology

The discovery of cells

•Because of their small size, cells can only be observed with the aid of a microscope.

•Spectacles were first made in Europe in the thirteenth century.

•The first compound (double-lens) light microscopes were constructed by the end of the sixteenth century.

•By the mid-1600s, a handful of pioneering scientists had used their handmade microscopes to uncover a world that would never have been revealed to the naked eye.

•Robert Hooke, an English microscopist discovered the cell at age 27, and he was awarded the position of curator of the Royal Society of London.

•He took a dead piece of cork and observed empty compartments in it under the microscope, and named them cell.

•Anton van Leeuwenhoek examined a drop of pond water under the microscope and he observed the crawling microscopic entities which he named as “animalcules”.

•He was also the first to describe various forms of bacteria, which he obtained from water in which pepper had been soaked and from scrapings of his teeth.

•In 1838, Matthias Schleiden, a German botanist, concluded that, plants were made of cells and that the plant embryo arose from a single cell.

•In 1839, Theodor Schwann, a German zoologist and colleague of Schleiden's, published a comprehensive report on the cellular basis of animal life.

•Schwann concluded that the cells of plants and animals are similar structures and proposed these two views of the cell theory:

1.All organisms are composed of one or more cells.

2.The cell is the structural unit of life.

(a)

(b) (a)One of Robert Hooke's more ornate compound (double-lens) microscopes.(Inset) Hooke's drawing of a thin slice of cork, showing the honeycomb-like network of "cells."

(b) Single-lens microscope used by Anton van Leeuwenhoek to observe bacteria and other microorganisms. The biconvex lens, which was capable of magnifying an object approximately 270 times and providing a resolution of approximately 1.35 m, was held between two metal plates.

Basic properties of cells

- Cells Are Highly Complex and Organized
- Cells Possess a Genetic Program and the Means to Use It
- Cells Are Capable of Producing More of Themselves

- Cells Acquire and Utilize Energy
- Cells Carry Out a Variety of Chemical Reactions
- Cells Engage in Mechanical Activities
- Cells Are Able to Respond to Stimuli
- Cells Are Capable of Self-Regulation

Lecture 2

Introduction to Molecular Biology

Molecular Biology

(BIO-302)

•In the broader terms, definition of molecular biology includes all aspects of the study of life from a molecular perspective.

•More precisely, the term “Molecular Biology” refers to the biology of the molecules related to genes, gene products and heredity.

•In other words, the term molecular biology is often substituted for a more appropriate term, Molecular Genetics.

•So molecular biology grew out of the disciplines of genetics and biochemistry.

•In the present age, world is in the midst of two scientific revolutions. One is information technology and the other is Molecular Biology.

•Both deal with the handling of large amounts of information.

•Molecular Biology has revolutionized the biological sciences as well especially in the fields of Health Sciences and Agricultural Sciences.

Lecture 3

Introduction to Prokaryotic and Eukaryotic Cells

Two fundamentally different classes of cells

On the base of their size and the types of internal structures, or organelles, cells are divided into two classes.

The structurally simpler, prokaryotic cells include bacteria

The structurally more complex eukaryotic cells include protists, fungi, plants, and animals.

A Comparison of Prokaryotic and Eukaryotic Cells

Features held in common by the two types of cells:

1. Plasma membrane of similar construction

2. Genetic information encoded in DNA using identical genetic code

3. Similar mechanisms for transcription and translation of genetic information, including similar ribosomes

4. Shared metabolic pathways (e.g., glycolysis and TCA cycle)

5. Similar apparatus for conservation of chemical energy as ATP (located in the plasma membrane of prokaryotes and the mitochondrial membrane of eukaryotes)

6. Similar mechanism of photosynthesis (between cyanobacteria and green plants)

7. Similar mechanism for synthesizing and inserting membrane Proteins

8. Proteasomes (protein digesting structures) of similar construction (between archaebacteria and eukaryotes)

A Comparison of Prokaryotic and Eukaryotic Cells

Features of eukaryotic cells not found in prokaryotes:

1.Division of cells into nucleus and cytoplasm, separated by a nuclear envelope containing complex pore structures

2. Complex chromosomes composed of DNA and associated proteins that are capable of compacting into mitotic structures

3. Complex membranous cytoplasmic organelles (includes endoplasmic reticulum, Golgi complex, lysosomes, endosomes, peroxisomes, and glyoxisomes)

4. Specialized cytoplasmic organelles for aerobic respiration (mitochondria) and photosynthesis (chloroplasts)

5. Complex cytoskeletal system (including microfilaments, intermediate filaments, and microtubules) and associated motor proteins

6. Complex flagella and cilia

7. Ability to ingest fluid and particulate material by enclosure within plasma

membrane vesicles (endocytosis and phagocytosis)

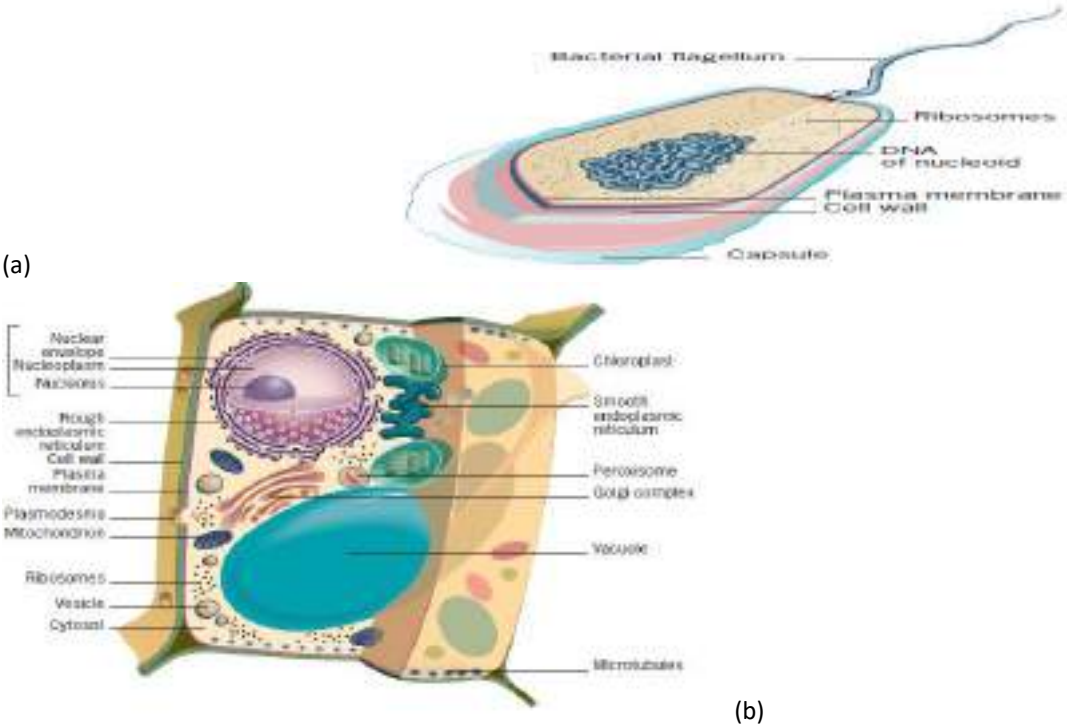
8. Cellulose-containing cell walls (in plants)

9. Cell division using a microtubule-containing mitotic spindle that separates chromosomes

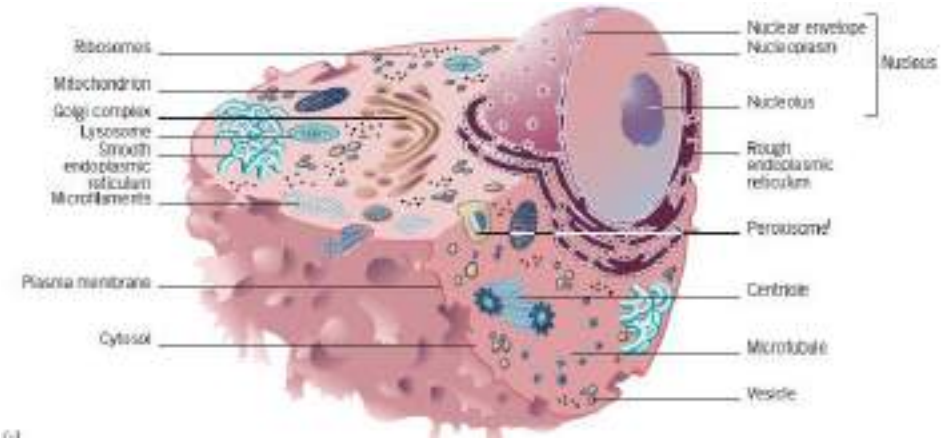
10. Presence of two copies of genes per cell (diploidy), one from each parent

11. Presence of three different RNA synthesizing enzymes (RNA polymerases)

12. Sexual reproduction requiring meiosis and fertilization



Schematic diagrams of a "generalized" bacterial (a), plant (b) cells

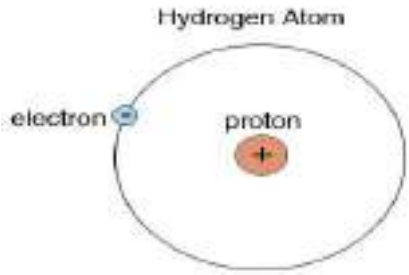


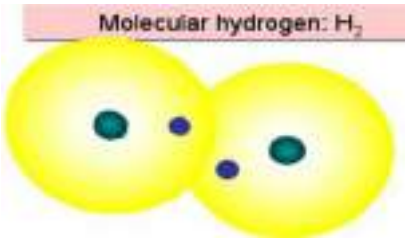
Schematic diagrams of a "generalized" animal cell

Lecture 4

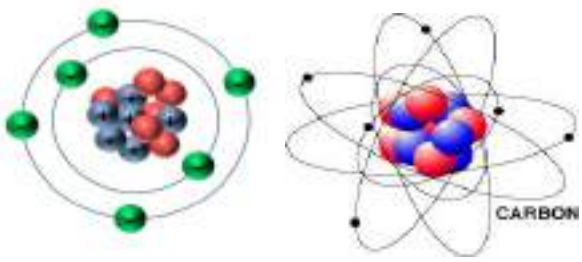
Composition of Matter

Atomic Structure



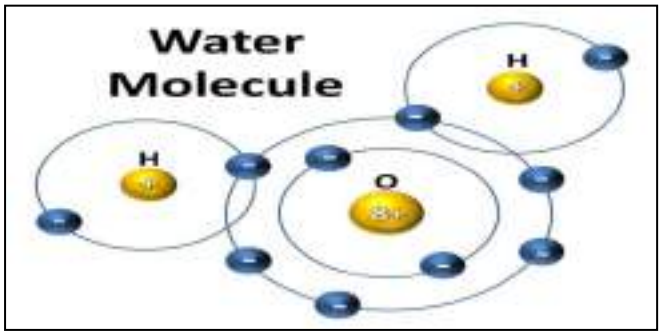


Carbon Atom



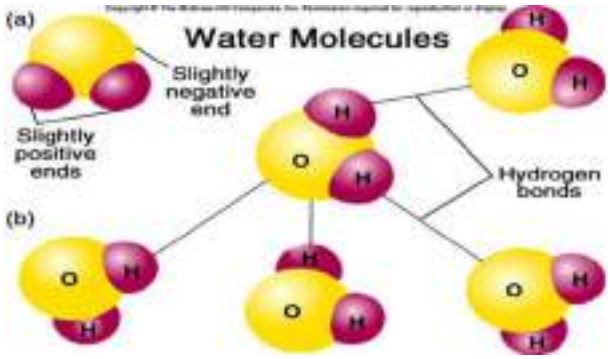
Electronegativity

The tendency of an atom to attract electrons towards itself.

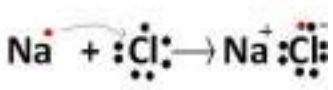


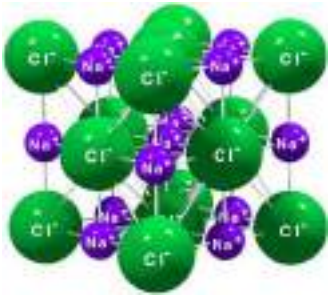
Polarity of H2O

Results in Hydrogen Bonding



Ionic Bond

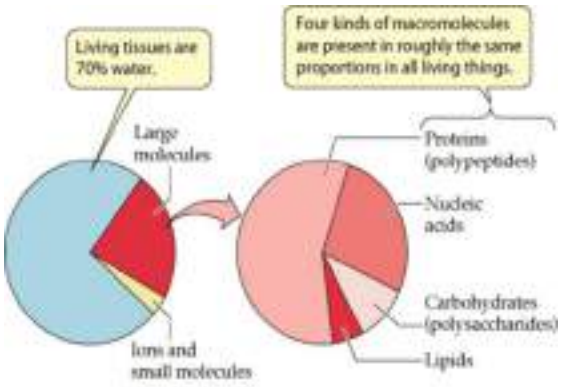




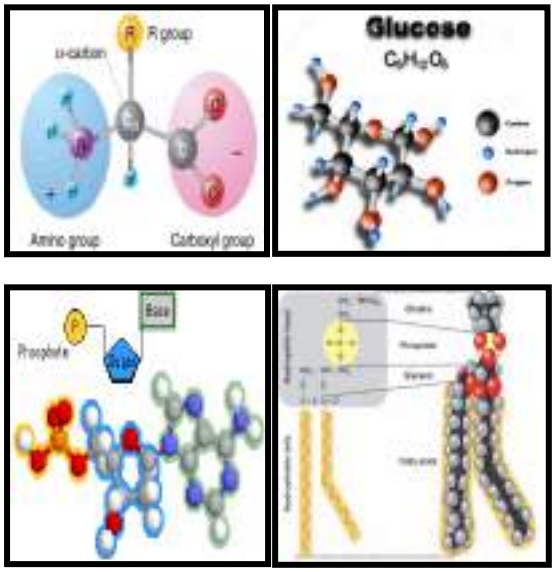
Lecture 5

Molecules of life

Composition of Life



Building Blocks of Life



Polymers &Monomers

Macromolecules are polymers of smaller monomers molecules

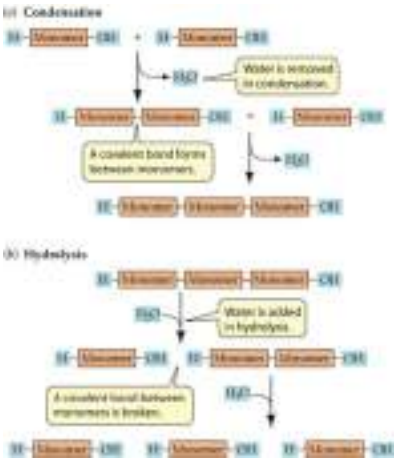
Condensation reactions:

Monomers join H2O Removal

Hydrolysis reactions:

Use water to break polymers into monomers.

Condensation & Hydrolysis



Lecture 6

Unit of Life

Cell Structure

Cellular compartments

Organelles

Structure & Function

All organisms are composed of cells the basic unit of life and all cells come from preexisting cells.

Not a random collection of molecules

Life

Collection of macromolecules that can perform unique functions because the are enclosed in structural compartment that provides consistency (homeostasis).

Lecture 7

Journey into the Cell

Unit of Life

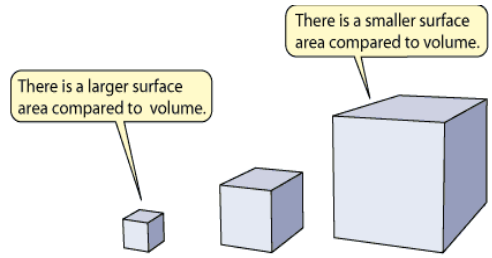
View animation

Lecture 8

Size Matters

Cell Structure

Cells are small to maintain large surface area to volume ratio



	1-mm cube	2-mm cube	4-mm cube
Surface area	6 sides × 1^2 = 6 mm^2	6 sides × 2^2 = 24 mm^2	6 sides × 4^2 = 96 mm^2
Volume	1^3 = 1 mm^3	2^3 = 8 mm^3	4^3 = 64 mm^3
Surface area-to-volume ratio	6/1	3/1	1.5/1

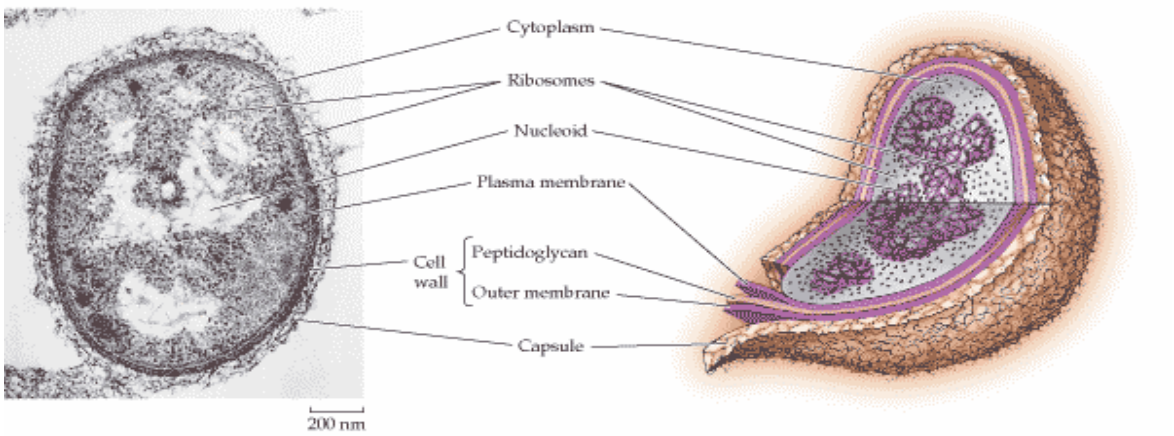
Prokaryotic Cells

No membrane enclosed internalcompartments.

Plasma membrane regulates traffic (barrier).

Nucleoid region contains DNA.

Most have cell wall.



Prokaryotic Cells

Special Features

Cyanobacteria Chlorophyll containing have folds of plasma membrane, other have mesosomes (energy).

Some have actin like filaments and other have Flagella made-up of Flagellin.

Lecture 9

Plasma Membrane

Introduction

The cells are separated from the external world by a thin, fragile structure called plasma membrane.

It consists of a lipid bilayer with embedded proteins.

It is only 5 to 10 nm wide.

The basic function of the cell membrane is to protect the cell from its surroundings.

The cell membrane controls the movement of substances in and out of cells and organelles. In this way, it is selectively permeable to ions and organic molecules

The cell membrane controls the movement of substances in and out of cells and organelles. In this way, it is selectively permeable to ions and organic molecules.

In addition, cell membranes are involved in a variety of cellular processes

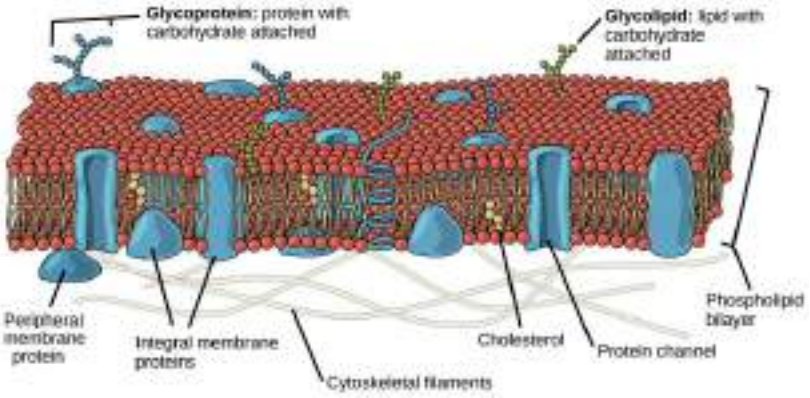
such as;

1.Cell adhesion

2.Ion conductivity

3.Cell signalling

4.Serve as the attachment surface for several structures, including the cell wall, glycocalyx, and cytoskeleton.



Lecture 10

An overview of membrane functions

1. Compartmentalization

It allows specialized activities to proceed without external interference and also enables cellular activities to be regulated independently of one another.

2. Scaffold for biochemical activities

Membranes provide the cell with an extensive framework or scaffolding for effective interactions between reactants of a reaction**.**

3. Providing a selectively permeable barrier

Membranes prevent the unrestricted exchange of molecules from one side to the other.

4. Transporting solutes

The membrane's transport machinery allows the transport of specific ions, thereby establishing ionic gradients across itself. This capability is especially critical for nerve and muscle cells.

5. Responding to external signals

The plasma membrane plays a critical role in the response of a cell to external stimuli, a process known as signal transduction.

6. Intercellular interaction

The plasma membrane allows cells to recognize and signal one another, to adhere when appropriate, and to exchange materials and information.

7. Energy transduction

Membranes are intimately involved in the processes by which one type ofenergy is converted to another type through chloroplasts and mitochondria.

Lecture 11

A brief history of studies on plasma membrane structure

The first insights into the chemical nature of the outer boundary layer of a cell were obtained by Ernst Overton of the University of Zürich during the 1890s.

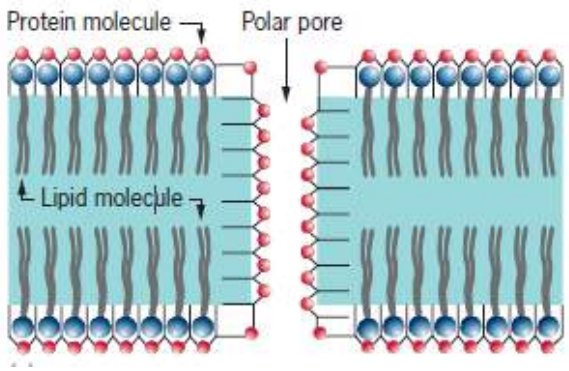
The first proposal that cellular membranes might contain a lipid bilayer was made in 1925 by two Dutch scientists, E. Gorter and F. Grendel.

In the 1920s and 1930s, cell physiologists obtained evidence that there must be more to the structure of membranes than simply a lipid bilayer.

In 1935, Hugh Davson and James Danielli proposed that the plasma membrane was composed of a lipid bilayer that was lined on both its inner and outer surface by a layer of globular proteins.

Davson and Danielli revised their model in the early 1950s to account for the selective permeability of the membranes they had studied In the revised version.

They suggested that, in addition to the outer and inner protein layers, the lipid bilayer was also penetrated by protein-lined pores, which could provide conduits for polar solutes and ions to enter and exit the cell.



A revised 1954 version of the Davson-Danielli model showing the lipid bilayer.

Fluidmosaic model proposed in 1972 by S. Jonathan Singer and Garth Nicolson of the University of California, San Diego.

In the fluid-mosaic model, which has served as the “central dogma” of membrane biology for more than three decades, the lipid bilayer remains the core of the membrane, but attention is focused on the physical state of the lipid.

Lecture 12

Chemical composition of membranes

Membranes are lipid–protein assemblies in which the components are held together in a thin sheet by non-covalent bonds.

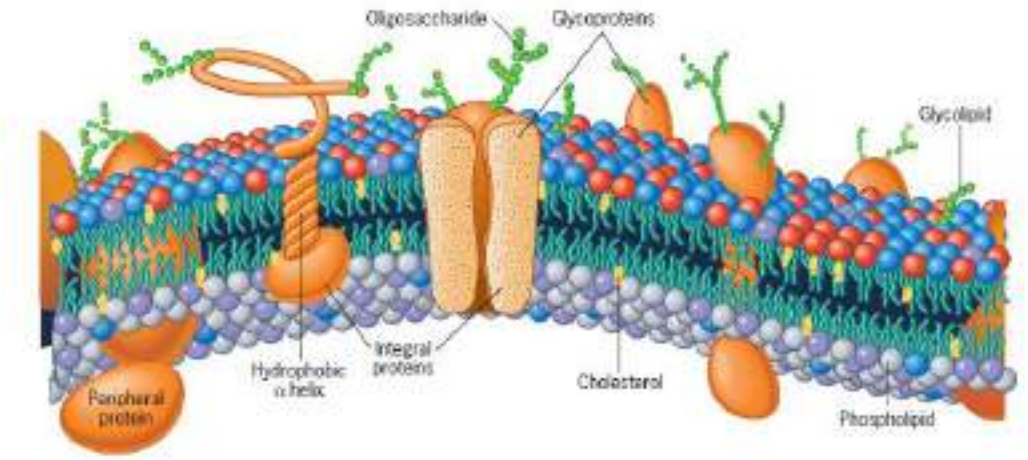
The core of the membrane consists of a sheet of lipids arranged in a bimolecular layer.

The lipid bilayer serves primarily as a structural backbone of the membrane and provides the barrier that prevents random movements of water-soluble materials into and out of the cell.

Each type of differentiated cell contains a unique complement of membrane proteins, which contributes to the specialized activities of that cell type.

The ratio of lipid to protein in a membrane varies, depending on;

1. The type of cellular membrane (plasma vs. endoplasmic reticulum vs. Golgi).
2. The type of organism (bacterium vs. plant vs. animal).
3. The type of cell (cartilage vs. muscle vs. liver).



A current representation of the plasma membrane showing the same basic organization as that proposed by Singer and Nicolson. The external surface of most membrane proteins, as well as a small percentage of the phospholipids, contain short chains of sugars, making them glycoproteins and glycolipids. Those portions of the polypeptide chains that extend through the lipid bilayer typically occur as helices composed of hydrophobic amino acids. The two leaflets of the bilayer contain different types of lipids as indicated by the differently colored head groups.

Lecture 13

Membrane Lipids

Membranes contain a wide diversity of lipids, all of which are amphipathic; that is, they contain both hydrophilic and hydrophobic regions.

There are three main types of membrane lipids:

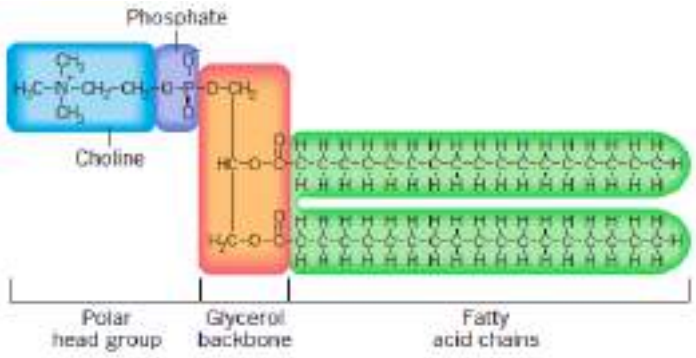
1. Phosphoglycerides
2. Sphingolipids
3. Cholesterol.

Phosphoglycerides

Most membrane lipids contain a phosphate group, which makes them phospholipids.

And most membrane phospholipids are built on a glycerol backbone, they are calledphosphoglycerides.

Membrane glycerides are diglycerides—only two of the hydroxyl groups of the glycerol are esterified to fatty acids; the third is esterified to a hydrophilic phosphate group.



Phospholipid phosphatidylcholine

The molecule consists of a glycerol backbone whose hydroxyl groups are covalently bonded to two fatty acids and a phosphate group. The negatively charged phosphate is also bonded to a small, positively charged choline group. The end of the molecule that contains the phosphorylcholine is hydrophilic, whereas the opposite end, consisting of the fatty acid tail, is hydrophobic.

Lecture 14

Sphingolipids

A less abundant class of membrane lipids, called sphingolipids.

These are derivatives of sphingosine, an amino alcohol that contains a long hydrocarbon chain.

Sphingolipids consist of sphingosine linked to a fatty acid by its amino group. This molecule is a ceramide.

Various sphingosine-based lipids have additional groups esterified to the terminal alcohol of the sphingosine moiety.

If the substitution is phosphorylcholine, the molecule is sphingomyelin, which is the only phospholipid of the membrane that is not built with a glycerol backbone.

If the substitution is a carbohydrate, the molecule is a glycolipid.

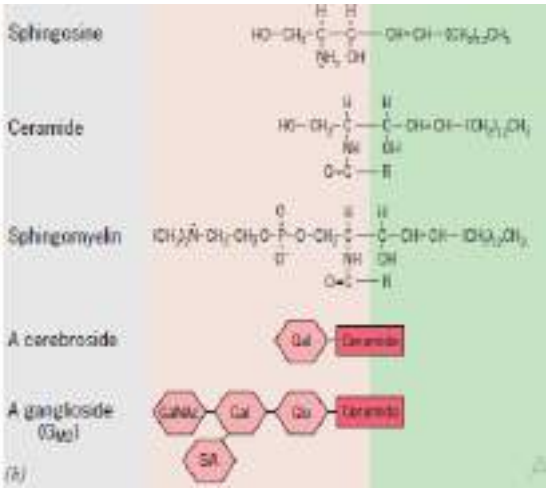
If the carbohydrate is a simple sugar, the glycolipid is called a cerebroside;

If it is a small cluster of sugars, the glycolipid is called a ganglioside.

Since all sphingolipids have two long, hydrophobic hydrocarbon chains at one end and a hydrophilic region at the other, they are also amphipathic

These are basically similar in overall structure to the phosphoglycerides.

The structures of sphingolipids



Sphingomyelin is a phospholipid; cerebrosides and gangliosides are glycolipids. The green portion of each lipid, which represents the hydrophobic tail(s) of the molecule, is actually much longer than the hydrophilic head group

Lecture 15

Membrane Lipids (Cholesterol)

Cholesterol

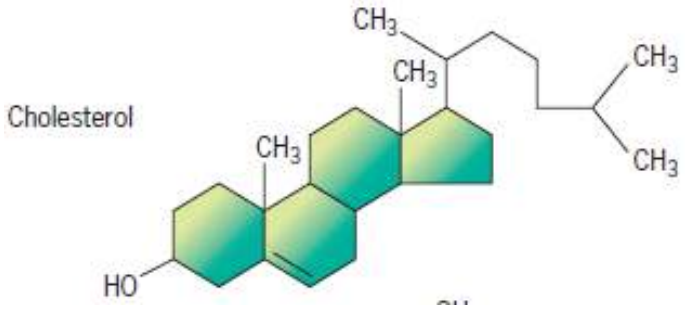
Lipid component of certain membranes is the sterol cholesterol.

In certain animal cells cholesterol may constitute up to 50 percent of the lipid molecules in the plasma membrane.

Cholesterol is absent from the plasma membranes of most plant and all bacterial cells.

Cholesterol molecules are oriented with their small hydrophilic hydroxyl group toward the membrane surface and the remainder of the molecule embedded in the lipid bilayer.

The hydrophobic rings of a cholesterol molecule are flat and rigid, and they interfere with the movements of the fatty acid tails of the phospholipids



Cholestrol molecule

Lecture 16

Membrane Carbohydrates

Depending on the species and cell type, the carbohydrate content of the plasma membrane ranges between 2 and 10 percent by weight.

More than 90 percent of the membrane's carbohydrate is covalently linked to proteins to form glycoproteins

Remaining 10 percent carbohydrate is covalently linked to lipids to form glycolipids.

All of the carbohydrate of the plasma membrane faces outward into the extracellular space.

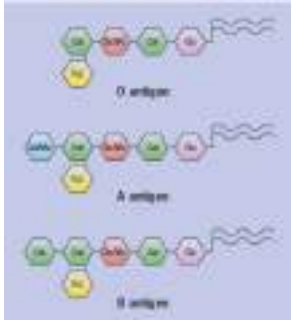
The addition of carbohydrate, or glycosylation, is the most complex of all types of protein modifications.

The carbohydrate of glycoproteins is present as short, branched hydrophilic oligosaccharides, typically having fewer than about 15 sugars per chain.

In contrast to most high-molecular-weight carbohydrates (such as glycogen, starch,or cellulose), the oligosaccharides attached to membrane proteins and lipids can display considerable variability in composition and structure.

The carbohydrates of the glycolipids of the red blood cell plasma membrane determine whether a person's blood type is A, B, AB, or O.

A person having blood type A has an enzyme that adds an N-acetylgalactosamine to the end of the chain, whereas a person with type B blood has an enzyme that adds galactose to the chain terminus. People with AB blood type possess both enzymes, whereas people with O blood type lack enzymes capable of attaching either terminal sugar.



Blood-group antigens. Whether a person has type A, B, AB, or O blood is determined by a short chain of sugars covalently attached to membrane lipids and proteins of the red blood cell membrane. The

oligosaccharides attached to membrane lipids (forming a ganglioside) that produce the A, B, and O blood types are shown here. A person with type AB blood has gangliosides with both the A and B structure.

(Gal, galactose; GlcNAc, N-acetylglucosamine; Glu,

glucose; Fuc, fucose; GalNAc,

O-acetylgalactosamine.)

Lecture 17

Membrane proteins

The structure and functions of membrane proteins

Depending on the cell type and the particular organelle within that cell, a membrane may contain hundreds of different proteins.

Each membrane protein has a defined orientation relative to the cytoplasm, so that the properties of one surface of a membrane are very different from those of the other surface.

The asymmetry of membrane proteins is referred to as membrane "sidedness."

In the plasma membrane, for example, those parts of membrane proteins that interact with other cells or with extracellular substances project outward into the extracellular space, whereas those parts of membrane proteins that interact with cytoplasmic molecules project into the cytosol.

Membrane proteins can be grouped into three distinct classes distinguished by the intimacy of their relationship to the lipid bilayer; These are

1.Integral Protein

2.Peripheral protein

3.Lipid-Anchored Protein

Integral proteins

Integral proteins are transmembrane proteins; that is, they pass entirely through the lipid bilayer and thus have domains that protrude from both the extracellular and cytoplasmic sides of the membrane.

Some integral proteins have only one membrane-spanning segment, whereas others are multispanning.

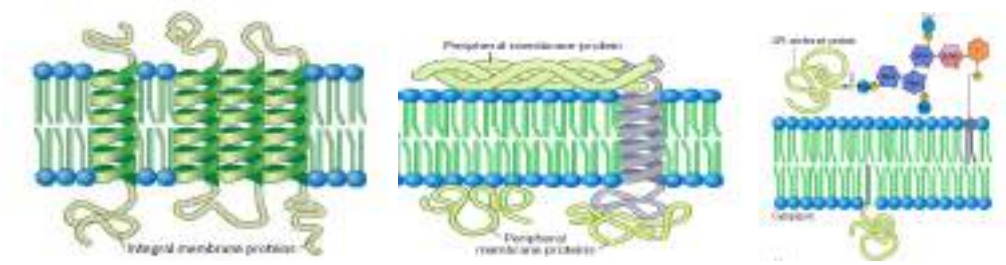
Genome-sequencing studies suggest that integral proteins constitute 20–30 percent of all encoded proteins.

Peripheral proteins

Peripheral proteins that are located entirely outside of the lipid bilayer, on either the cytoplasmic or extracellular side, yet are associated with the surface of the membrane by noncovalent bonds.

Lipid-anchored proteins

Lipid-anchored proteins that are located outside the lipid bilayer, on either by the extracellular or cytoplasmic surface, but are covalently linked to a lipid molecule that is situated within the bilayer.



(a) (b)

(a) Integral proteins typically contain one or more transmembrane helices.

(b) Peripheral proteins are noncovalently bonded to the polar head groups of the lipid bilayer and/or to an integral membrane protein.

(c) Lipid-anchored proteins are covalently bonded to a lipid group that resides within the membrane. The lipid can be phosphatidylinositol, a fatty acid, or a prenyl group (a long-chain hydrocarbon built from five carbon isoprenoid units).

I, inositol; GlcNAc, N-acetylglucosamine; Man, mannose; Etn, ethanolamine; GPI, glycosylphosphatidylinositol.

Lecture 18

Membrane lipids and membrane fluidity

The physical state of the lipid of a membrane is described by its fluidity (or viscosity).

If the temperature of the bilayer is kept relatively warm (e.g., 37C), the lipid exists in a relatively fluid state.

If the temperature is slowly lowered, a point is reached where the bilayer distinctly changes .

The lipid is converted from a liquid crystalline phase to a frozen crystalline gel in which the movement of the phospholipid fatty acid chains is greatly restricted. The temperature at which this change occurs is called the transition temperature.

(a) (b)

The structure of the lipid bilayer depends on the temperature.

(a)Above the transition temperature, the lipid molecules and their hydrophobic tails are free to move in certain directions, even though they retain a considerable degree (a) (b) of order.

(b)Below the transition temperature, the movement of the molecules is greatly restricted, and the entire bilayer can be described as a crystalline gel.

Importance of Membrane Fluidity

1.Membrane fluidity provides a perfect compromise between a rigid, ordered structure in which mobility would be absent and a completely fluid, nonviscous liquid in which the components of the membrane could not be oriented and structural organization and mechanical support would be lacking.

2.Fluidity allows for interactions to take place within the membrane.

3.Fluidity also plays a key role in membrane assembly.

Following cellular processes depends upon movement of membrane components;

1. Cell movement
2. Cell growth

3. Cell division
4. Formation of intercellular junctions
5. Secretion
6. Endocytosis

Lecture 19

Diffusion

The movement of substances across cell membranes

In a sense, the plasma membrane has a dual function.

1. On one hand, it must retain the dissolved materials of the cell so that they do not simply leak out into the environment,

2. while on the other hand, it must allow the necessary exchange of materials into and out of the cell.

The lipid bilayer of the membrane is ideally suited to prevent the loss of charged and polar solutes from a cell.

There are basically two means for the movement of substances through a membrane:

1.Passively by diffusion

2.Actively by an energy-coupled transport process.

Both types of movements lead to the net flux of a particular ion or compound.

The term net flux indicates that the movement of the substance into the cell (influx) and out of the cell (efflux) is not balanced, but that one exceeds the other.

Diffusion

Diffusion is a spontaneous process in which a substance moves from a region of high concentration to a region of low concentration, eventually eliminating the concentration difference between the two regions.

A membrane may be permeable to a given solute either

(1) because that solute can pass directly through the lipid bilayer

(2) because that solute can traverse an aqueous pore that spans the membrane.

It is evident that the greater the lipid solubility, the faster the penetration.

Measure of the polarity (or nonpolarity) of a substance is its partition coefficient, which is the ratio of its solubility in a nonpolar solvent.

Another factor determining the rate of penetration of a compound through a membrane is its size. If two molecules have approximately equivalent partition coefficients, the smaller molecule tends to penetrate the lipid bilayer of a membrane more rapidly than the larger one.

Lecture 20

Diffusion of Water through Membranes

Water molecules move much more rapidly through a cell membrane than do dissolved ions or small polar organic solutes, which are essentially non penetrating.

Because of this difference in the penetrability of water versus solutes, membranes are said to be semipermeable.

Water moves readily through a semipermeable membrane from a region of lower solute concentrate on to a region of higher solute concentration. This process is called osmosis.

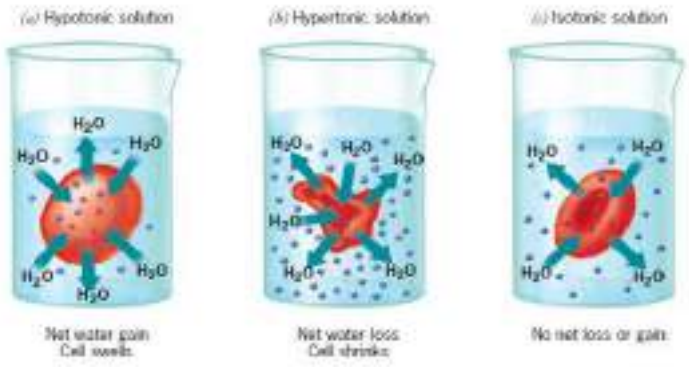
The compartment of higher solute concentration is said to be hypertonic (or hyperosmotic) relative to the compartment of lower solute concentration, which is described as being hypotonic (or hypoosmotic).

When a cell is placed into a hypotonic solution, the cell rapidly gains water by osmosis and swells.

Conversely, a cell placed into a hypertonic solution rapidly loses water by osmosis and shrinks.

Once the internal solute concentration (which includes a high concentration of dissolved proteins) equals the external solute concentration, the internal and external fluids are isotonic (or isosmotic).

The effects of differences in the concentration of solutes on opposite sides of the plasma membrane.



(a)A cell placed in a hypotonic solution (one having a lower solute concentration than the cell) swells because of a net gain of water by osmosis.

(b)A cell in a hypertonic solution shrinks because of a net loss of water by osmosis.

(c)A cell placed in an isotonic solution maintains a constant volume because the inward flux of water is equal to the outward flux.

Lecture 21

The Diffusion of Ions through Membranes

The lipid bilayer of biological membranes is highly impermeable to charged

substances, including small ions such as Na, K, Ca2, and Cl.

In 1955, Alan Hodgkin and Richard Keynes of Cambridge University first

proposed that cell membranes contain ion channels.

Ion channels are openings in the membrane that are permeable to specific ions.

Three major categories of gated channels are distinguished:

1. Voltage-gated channels

2. Ligand-gated channels

3. Mechano-gated channels

1.Voltage-gated channels whose conformational state depends on the difference in ionic charge on the two sides of the membrane.

2. Ligand-gated channels whose conformational state depends on the binding of a specific molecule (the ligand), which is usually not the solute that passes through the channel.

For example, neurotransmitters, such as acetylcholine, act on the outer surface of certain cation channels, while cyclic nucleotides, such as cAMP, act on the inner surface of certain calcium ion channels.

3. Mechano-gated channels whose conformational state depends on mechanical forces (e.g., stretch tension) that are applied to the membrane. Members of one family of cation channels, for example, are opened by the movements of stereocilia on the hair cells of the inner ear in response to sound or motions of the head.

Lecture 22

Facilitated Diffusion

Substances always diffuse across a membrane from a region of higher concentration on one side to a region of lower concentration, but they do not always diffuse through the lipid bilayer or through a channel.

In many cases, the diffusing substance first binds selectively to a membrane-spanning protein, called a facilitative transporter, that facilitates the

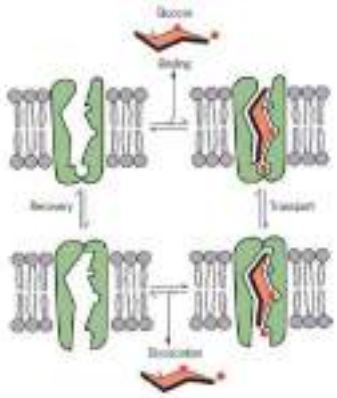
diffusion process.

Because they operate passively, that is, without being coupled to an energy-releasing system, facilitated transporters can mediate the movement of solutes equally well in both directions.

The direction of net flux depends on the relative concentration of the substance on the two sides of the membrane.

Facilitated diffusion

A schematic model for the facilitated diffusion of glucose depicts the alternating conformation of a carrier that exposes the glucose binding site to either the inside or outside of the membrane.



Lecture 23

Active Transport

The endergonic movement of ions or other solutes across the membrane against a concentration gradient is coupled to an exergonic process, such as the hydrolysis of ATP, the absorbance of light, the transport of electrons, or the flow of other substances down their gradients.

Like facilitated diffusion, active transport depends on integral membrane proteins.

Proteins that carry out active transport are often referred to as “pumps.”

Coupling Active Transport to ATP Hydrolysis

In 1957, Jens Skou, a Danish physiologist, discovered an ATP-hydrolyzing enzyme in the nerve cells of a crab that was only active in the presence of both Na and K ions.

Skou proposed, that this enzyme, which was responsible for ATP hydrolysis, was the same protein that was active in transporting the two ions; the enzyme was called the Na/K-ATPase, or the sodium–potassium pump.

The ratio of NaK pumped by the Na/K-ATPase is not 1:1.

For each ATP hydrolyzed, three sodium ions are pumped out as two potassium ions are

The Na/K-ATPase.

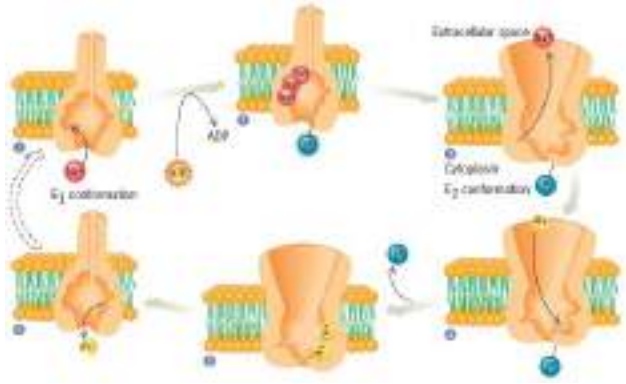
Simplified schematic model of the transport cycle. Sodium ions

(1) bind to the protein on the inside of the membrane. ATP is hydrolyzed, and the phosphate is transferred to the protein (2)changing its conformation (3) and allowing

sodium ions to be expelled to the external space. Potassium ions then bind to the protein (4), and the phosphate group is subsequentl lost (5), which causes the protein to snap back to its original conformation, allowing the potassium ions to diffuse into the cell (6). The cation binding sites are located deep within the

transmembrane domain, which consists of 10 membrane-spanning helices.

pumped in.



Lecture 24

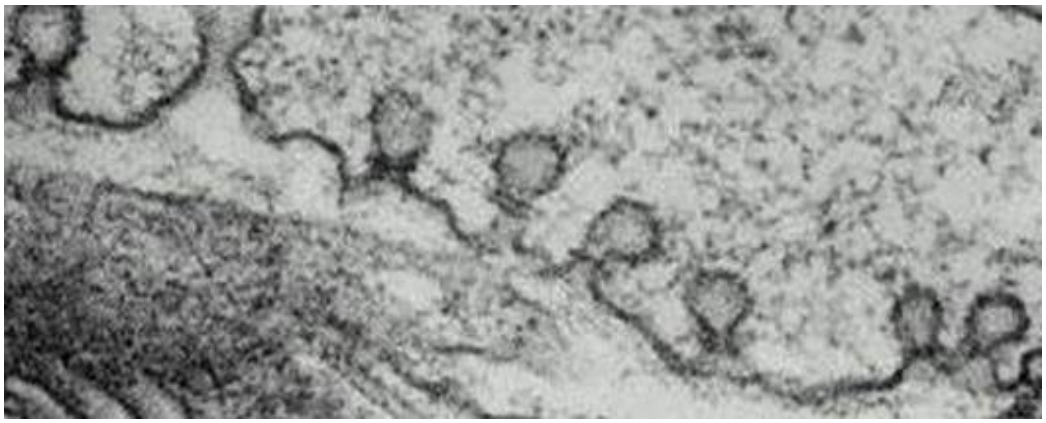
Endocytosis

The material to be ingested is progressively enclosed by a small portion of the plasma membrane, which first invaginates and then pinches off to form an endocytic vesicle containing the ingested substance or particle

Phagocytosis involves the ingestion of large particles microorganisms or dead cells via large vesicles called phagosomes (>250 nm in diameter)

Pinocytosis involves ingestion of fluid & solutes via small pinocytic vesicles (about 100 nm in diameter)

Most eucaryotic cells are continually ingesting fluid and solutes by pinocytosis; large particles are most efficiently ingested by specialized phagocytic cells



In mammals, three classes of white blood cells act as professional phagocytes macrophages, neutrophils, and dendritic cells

These cells develop from hemopoietic stem cells and they defend us against infection by ingesting invading microorganisms.

Macrophages also scavenge senescent cells and cells that have died by apoptosis.

In quantitative terms, the latter function is by far the most important: our macrophages phagocytose more than 1011 senescent red blood cells every day.

Particles first bind to the surface of the phagocyte.

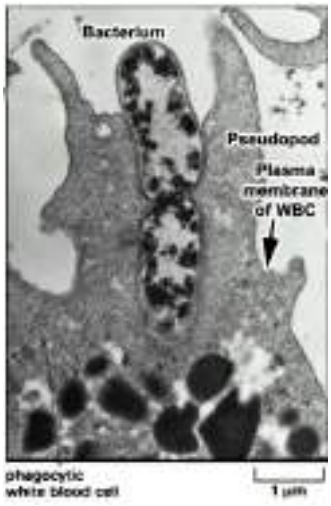
Phagocytes have a variety of specialized surface receptors that are functionally linked to the phagocytic machinery of the cell.

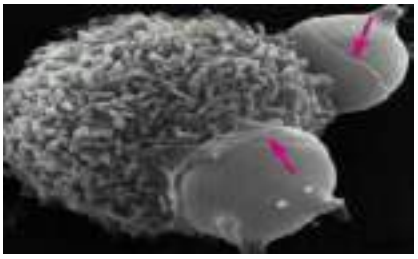
Phagocytosis is a triggered process, requiring that receptors be activated that signal the cell interior and initiate the response

Best-characterized triggers are antibodies, which protect us by binding to the surface of infectious microorganisms to form a coat in which the tail region of each antibody molecule, called the Fc region, is exposed on the exterior

This antibody coat is recognized by specific Fc receptors on the surface of macrophages and neutrophils, whose binding induces the phagocytic cell to extend pseudopods that engulf the particle and fuse at their tips to form a phagosome.

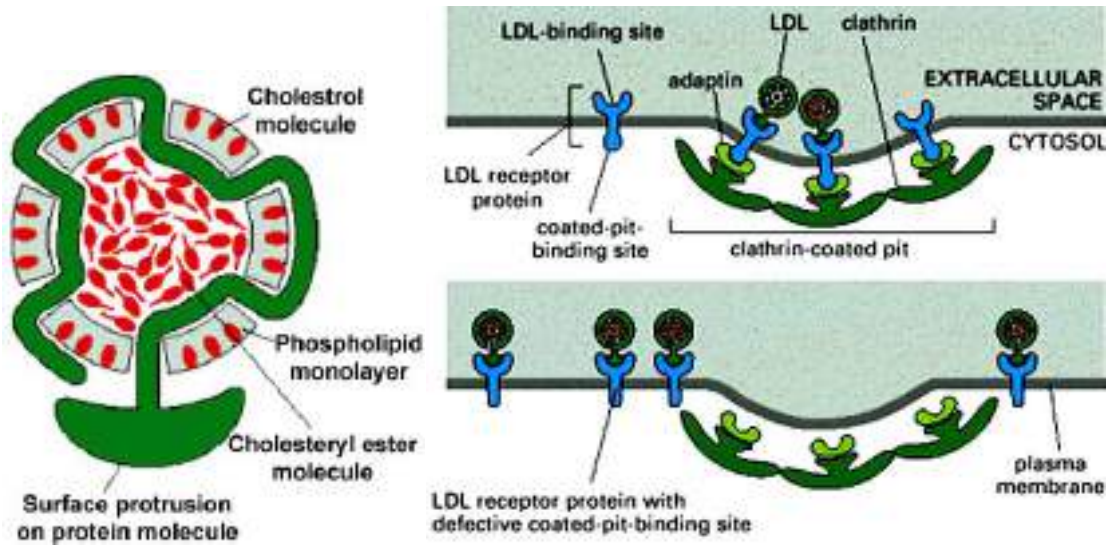
Apoptotic cells lose the asymmetric distribution of phospholipids in their plasma membrane as a consequence, negatively charged phosphatidylserine, which is normally confined to the cytosolic leaflet of the lipid bilayer, is now exposed on the outside of the cell, where it triggers the phagocytosis of the dead cell

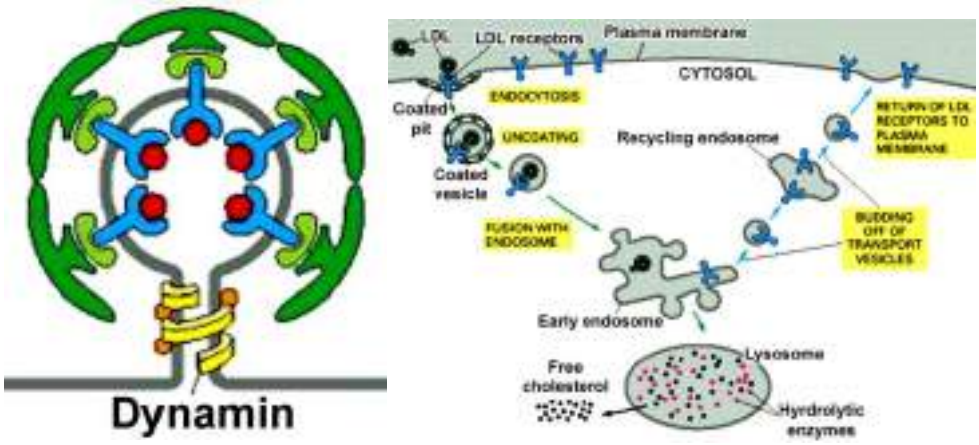




Low-density lipoprotein (LDL) particle

Each particle contains a core of about 1500 cholesterol molecules esterified to long-chain fatty acids





Lecture 25

Phagocytosis

Phagocytosis (“cell eating”) is carried out extensively by a few types of cells.

Uptake of relatively large particles (0.5 m diameter) from the environment.

Many single-celled protists, such as amoebas and ciliates, trap food particles and smaller organisms and enclosing them within folds of the plasma membrane.

The folds fuse to produce a vacuole (or phagosome) that pinches off inwardly from the plasma membrane.

The phagosome fuses with a lysosome, and the material is digested within the resulting phagolysosome.

In most animals, phagocytosis is a protective mechanism rather than a mode of feeding.

Mammals possess a variety of “professional” phagocytes, including macrophages and neutrophils.

Once inside the phagocyte, microorganisms may be killed by lysosomal enzymes or by oxygen free radicals.

The engulfment of particulate material is driven by contractile activities of the actin-containing microfilaments

Some bacterial species hijack the phagocytic machinery to promote their own survival in the body.

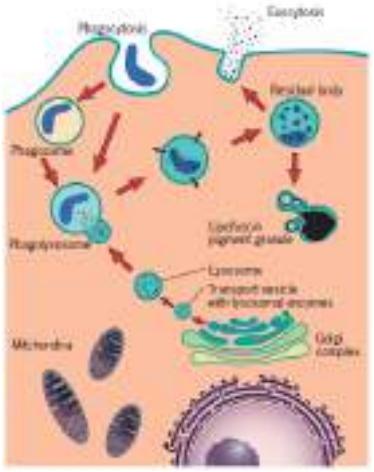
If the phagosome does become highly acidic, the bacterium is able to maintain its own physiological pH despite the lowered pH of its surrounding medium.

Examples

The bacterium responsible for Q fever, Coxiella burnetii, becomes enclosed in a phagosome that does fuse with a lysosome, but neither the acidic environment nor the lysosomal enzymes can destroy the pathogen.

3. Listeria monocytogenes, a bacterium that causes meningitis, produces proteins that destroy the integrity of the lysosomal membrane, allowing the bacterium to escape into the cell's cytosol.

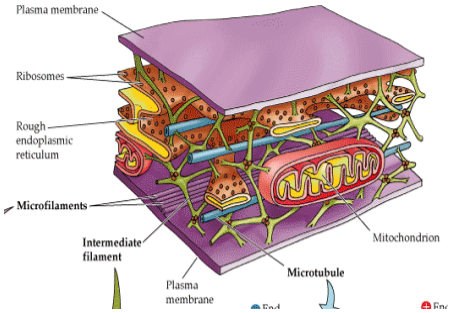
A summary of the phagocytic pathway.

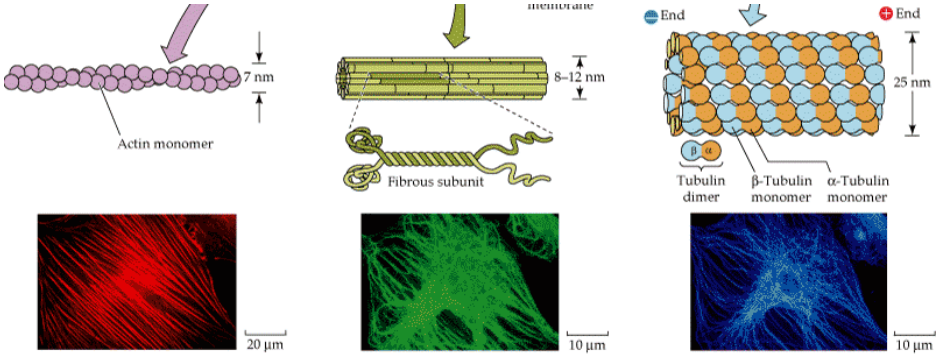


Lecture 26

Cytoskeleton

Shape & Function





Microfilaments are made up of strands of the protein actin and often interact with strands of other proteins. They change cell shape and drive cellular motion, including contraction, cytoplasmic streaming, and the "pinched" shape changes that occur during cell division. Microfilaments and myosin strands together drive muscle action.

Intermediate filaments are made up of fibrous proteins organized into tough, ropelike assemblages that stabilize a cell's structure and help maintain its shape. Some intermediate filaments help to hold neighboring cells together. Others make up the nuclear lamina.

Microtubules are long, hollow cylinders made up of many molecules of the protein tubulin. Tubulin consists of two subunits, α-tubulin and β-tubulin. Microtubules lengthen or shorten by adding or subtracting tubulin dimers. Microtubule shortening moves chromosomes. Interactions between microtubules drive the movement of cells. Microtubules serve as "tracks" for the movement of vesicles.

Actin

Actin (cortical)

Stabilizes cell shape.

Generates movement

local.

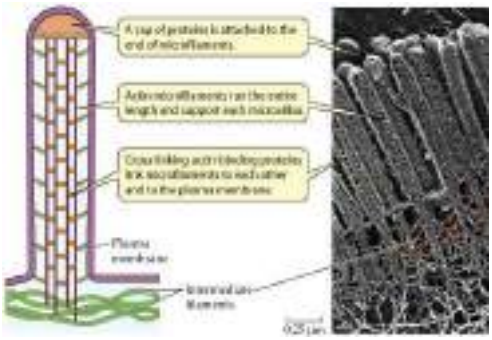
Muscle contraction.

Constriction ring in

cell divission (Cytokinesis).

Cytoplasmic strreaming.

Actin for Support



Lecture 27

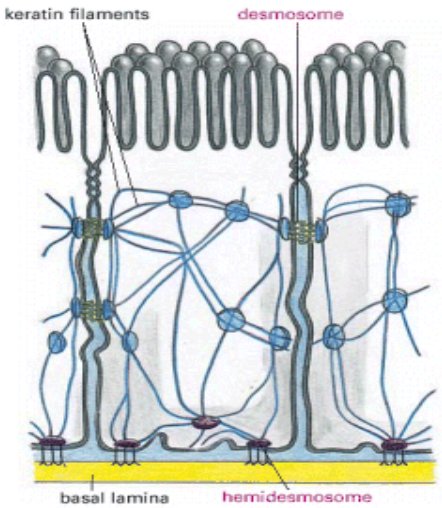
Cytoskeleton 2

IF stabilize cell structure (hold organelles).

Stabilize and maintain tissue Rigidity (desmosomes).

Lamins in nucleus. Resist tention.

IF



Microtubules

Rigid internal skeleton resist compression.

Tracks along for motor proteins.

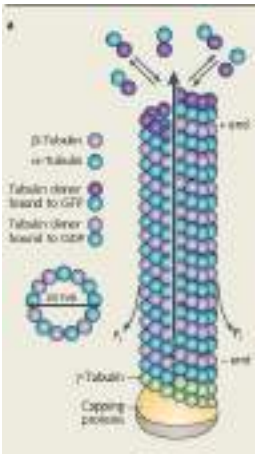
Tubulin is a dimer of

α-tubulin and

β-tubulin.

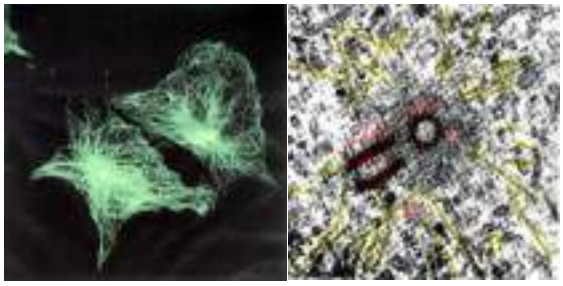
The two ends different plus (+) end & minus (–) end (cat Tail & dynamic).

Microtubules 13



Centrosome

Microtubule organizing center



Lecture 28

Types of cytoskeleton

The cytoskeleton is composed of three well-defined filamentous structures

1.Microtubules are long, hollow, unbranched tubes composed of subunits of

the protein tubulin.

2.Microfilaments are solid, thinner structures, often organized into a branching network and composed of the protein actin.

3.Intermediate filaments are tough, ropelike fibers composed of a variety of related proteins.

Each of the three types of cytoskeletal filaments is a polymer of protein subunits held together by weak, noncovalent bonds.

Properties of Microtubules, Intermediate Filaments, and Actin Filaments

	Microtubules	Intermediate filaments	Actin filaments
Subunits incorporated into polymer	GTP-αβ-tubulin heterodimer	~70 different proteins	ATP-actin monomers
Preferential site of incorporation	+ End (β-tubulin)	Internal	+ End (barbed)
Polarity	Yes	No	Yes
Enzymatic activity	GTPase	None	ATPase
Motor proteins	Kinesins, dynein	None	Myosins
Major group of associated proteins	MAPs	Plakins	Actin-binding proteins
Structure	Stiff, hollow, inextensible tube	Tough, flexible, extensible filament	Flexible, inextensible helical filament
Dimensions	25 nm outer diam.	10–12 nm diam.	8 nm diam.
Distribution	All eukaryotes	Animals	All eukaryotes
Primary functions	Support, intracellular transport, cell organization	Structural support	Motility, contractility
Subcellular distribution	Cytoplasm	Cytoplasm + nucleus	Cytoplasm

Overview of the major functions of the cytoskeleton

1.It provides structural support that can determine the shape of the cell and resist forces that tend to deform.

2.It is responsible for positioning the various organelles within the interior of the cell.

3.It direct the movement of materials and organelles within cells.

4.It helps in movement of cells from one place to another. Single-celled organisms move with the aid of specialized, microtubule-containing locomotor organelles (cilia and flagella).

5.It serves as an essential component of the cell's division machinery.

Lecture 29

MICROTUBULES

Structure and Composition

Microtubules are hollow, relatively rigid, tubular structures.

They occur in nearly every eukaryotic cell.

They have an outer diameter of 25 nm and a wall thickness of approximately 4 nm.

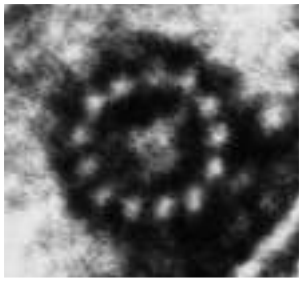
The wall of a microtubule is composed of globular proteins arranged in longitudinal rows, termed protofilaments.

Microtubules are consist of 13 protofilaments.

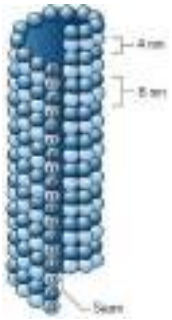
Each protofilament is assembled from dimeric building blocks consisting of one α-tubulin and one β-tubulin subunit.

The two types of globular tubulin subunits have a similar three-dimensional structure and fit tightly together.

All of the protofilaments of a microtubule have the same polarity. Consequently, the entire polymer has pollarity.



Electron micrograph of a cross section of a microtubule of a juniperus root tip cell revealing the 13 subunits arranged within the wall of the tubule.



Longitudinal section of microtubule

Functions

In animal cells, microtubules extend in a radial array outward around the nucleus, giving these cells their round, flattened shape.

Microtubules serves as skeletal elements which is obvious from its role in cilia and flagella and the axons of nerve cells.

In plant cells, microtubules play role in maintaining cell shape.

They help in maintaining the internal organization of cells.

Lecture 30

Intermediate Filaments

Introduction

The term "intermediate filaments" refers only to the diameter of the fibers, and bears no relationship to position or function.

Intermediate filaments are about 11 nm in diameter.

Intermediate filaments are strong, flexible ropelike fibers that provide mechanical strength to cells.

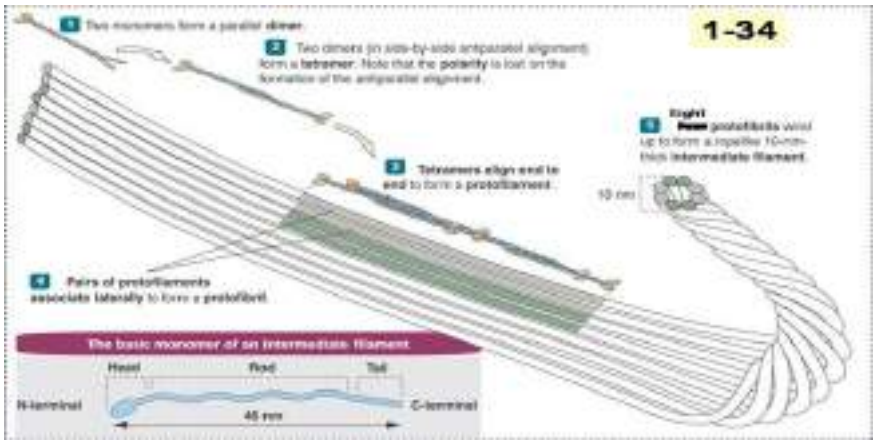
IFs are a chemically heterogeneous group of structures that, in humans, are encoded by approximately 70 different genes.

Structure

The structural subunit of an intermediate filament is a dimeric fibrous protein which makes it fairly rigid.

These are non-polarized, in contrast to the polarized nature of microfilaments and microtubules. Thus intermediate filaments do not possess (+) or (-) ends, but are symmetrical.

To make an intermediate filament, these tetramers lengthen, and eight join laterally to form the 11 nm intermediate filament. The length may be very great.



Functions

The tight association between protofilaments provide intermediate filaments with a high tensile strength, therefore found in particularly durable structures such as hair, scales and fingernails.

Intermediate filaments is to create cell cohesion and prevent the acute fracture of epithelial cell sheets under tension.

Lecture 31

Microfilaments

Introduction

Microfilaments are approximately 8 nm in diameter and composed of globular subunits of the protein actin.

In the presence of ATP, actin monomers polymerize to form a flexible, helical filament.

An actin filament is essentially a two-stranded structure with two helical grooves running along its length.

The terms actin filament, F-actin, and microfilament are basically synonyms for this type of filament.

Functions

Microfilament functions include;

1.Cytokinesis

2.Amoeboid movement

3.Cell motility in general

4.Changes in cell shape

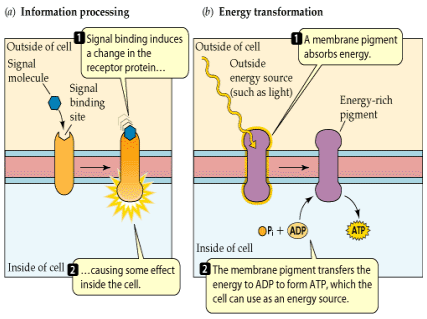
5.Endocytosis and exocytosis

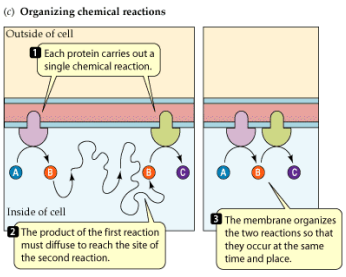
7. Cell contractility and mechanical stability.

Lecture 32

Membranes

More Than Barriers

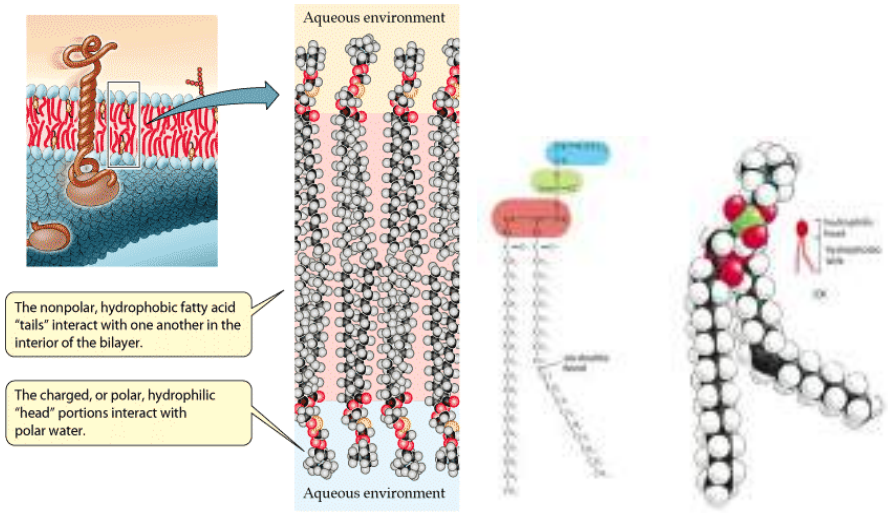




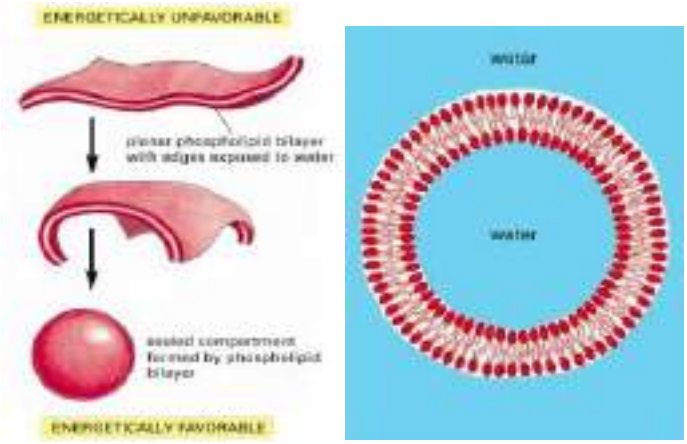
Fatty acid legth:14-24Cs

One tail Unsaturated Cis

Spontaneous Assembly



Free Energy Dictates



Features

Phospholipids coexist with water form a bilayer

Dynamic structures:

parts move & change.

Vital physiological roles:

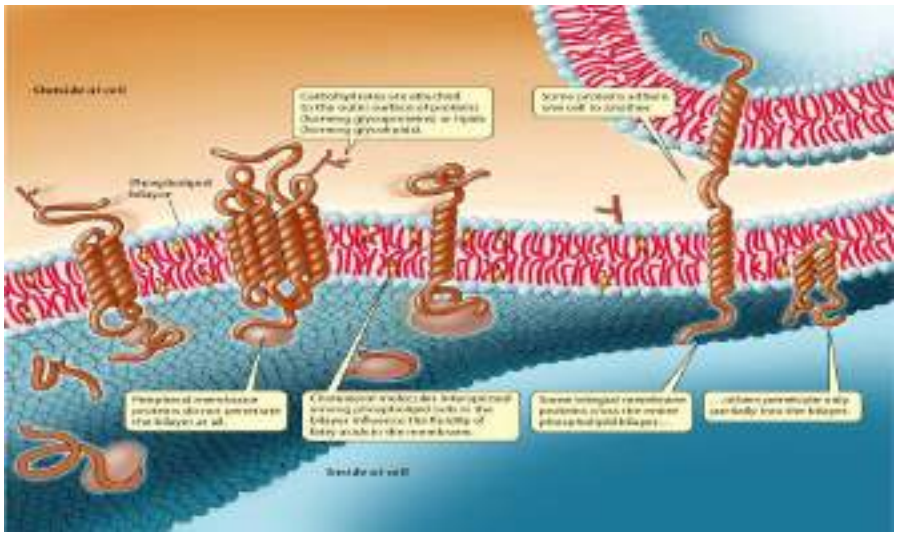
cell cell & cell interaction with environmental

Physical integrity: lipids,

create a barrier to hydrophilic materials such as water and ions

Lecture 33

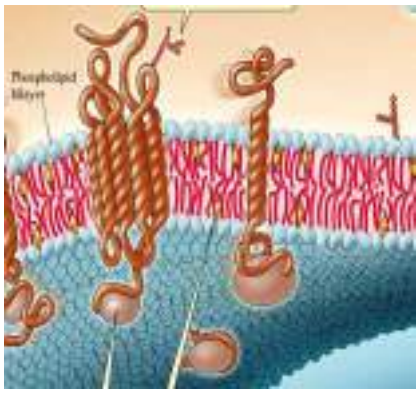
Membranes Component



Lipid “lake” in which a variety of proteins “float”

Each membran has a set of proteins suitable to the specialized function of the cell or organelle it surrounds.

Carbohydrates attached either to lipids/protein molecules



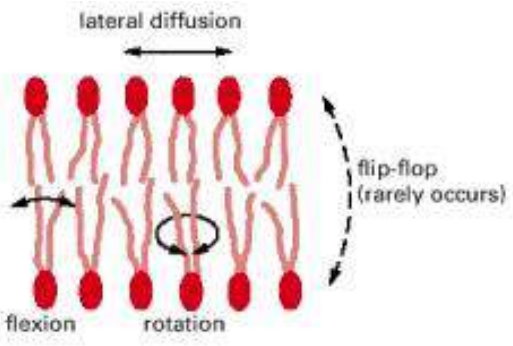
Fatty acids interact with each other & the polar regions face aqueous environment outside

Movement

A phospholipid molecule travels from one end the other in about 1 second

Phospholipid molecule flip overs seldom (polar part of molecule hardly ever moves through the hydrophobic interior

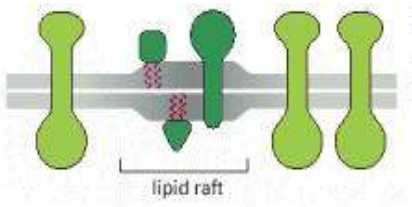
Inner and outer halves of the bilayer have different phospholipids



Lipid Rafts

Some proteins migrate freely others “anchored” to specific semisolid region different lipid composition (very long fatty acid chains)

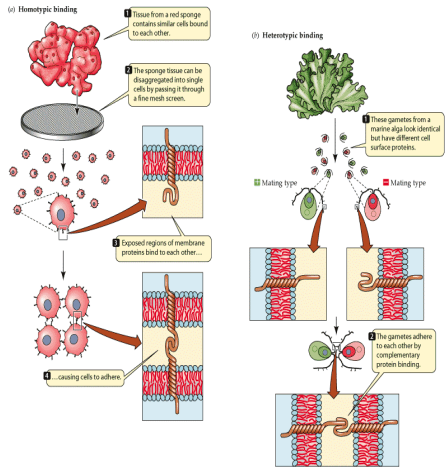
Longer Trans-membrane domain



Lecture 34

Recognition & Adhesion

Tissue Formation

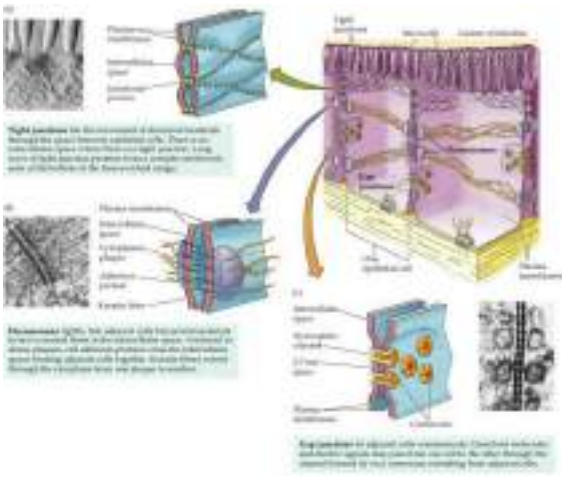


Cell recognition:

One cell specifically binds to another cell

Cell adhesion:

Relationship between the two cells is “cemented”



Tight Junctions

(a)Prevent the passage of molecules through

the spaces between cells

(b)Restricting the migration of lipid molecules

& membrane proteins

Passive Transport

Does not require any input of energy to drive the process

Active Transport

Requires input of energy to drive the process

Lecture 35

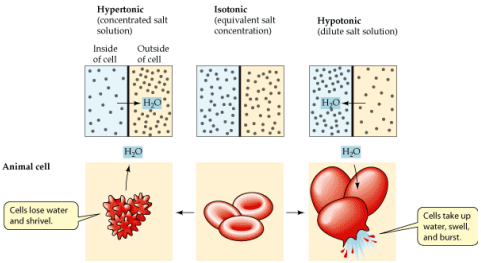
Transport Across

Membrane

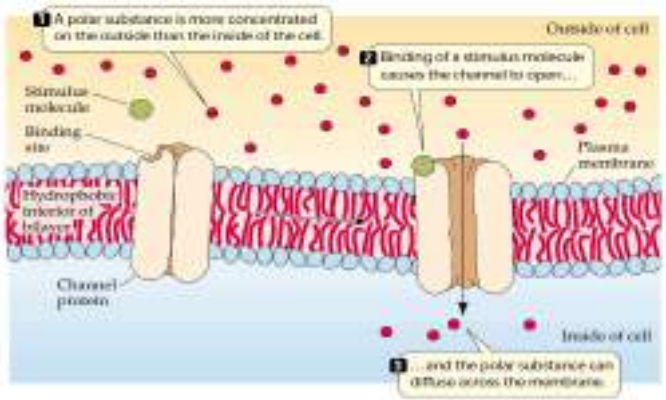
Aqua-porins

Water molecules move through membranes By Osmosis (diffusion).

Number of solute particles not on the kinds of particles.



The Two Domains

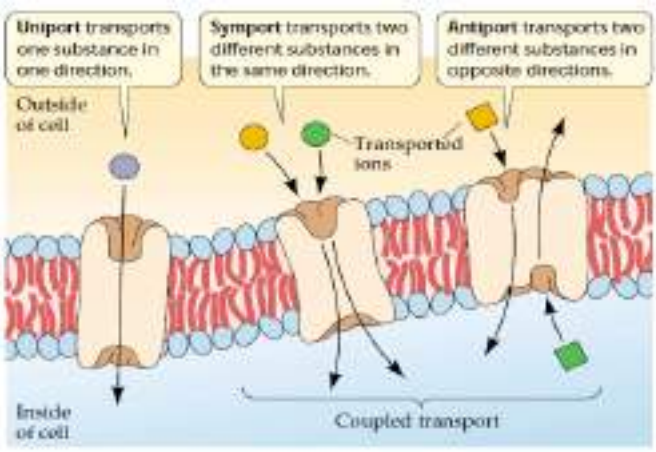


Polar amino acids and water on the inside of the channel pore (to bind to the polar or charged substance and allow it to pass through)

Nonpolar amino acids on the outside of the macromolecule (to allow the channel protein to insert itself into the lipid bilayer)

Primary active transport: ATP required directly.

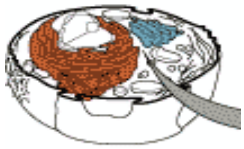
Secondary active transport: ATP not used directly; ion con

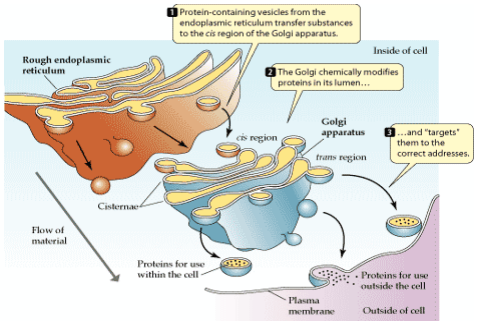


Lecture 36

The Endoplasmic Reticulum

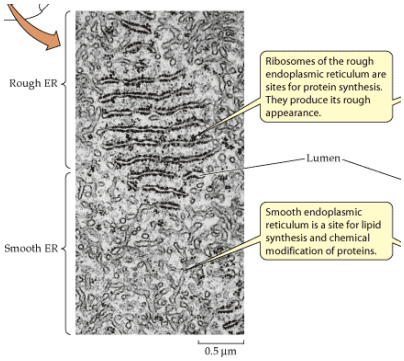
RER + SER





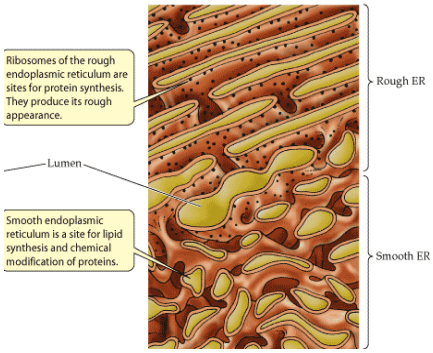
RER

Segregates newlysynthesized Proteins



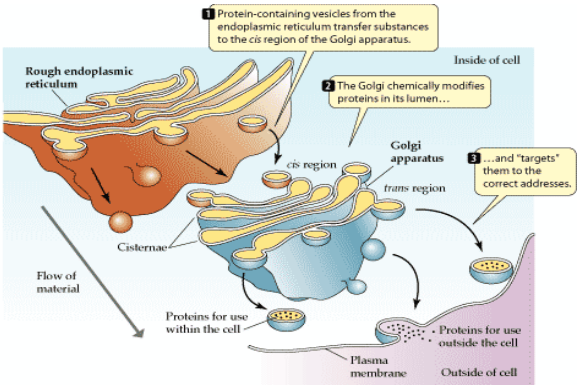
RER

Chemically modifies Proteins (Glycosylation address)



SER

Glycogen hydrolysis secreting cells have abundant SER



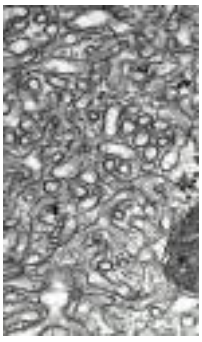
Lecture 37

ER Transport

Introduction

The Endoplasmic Reticulum

Smooth ER



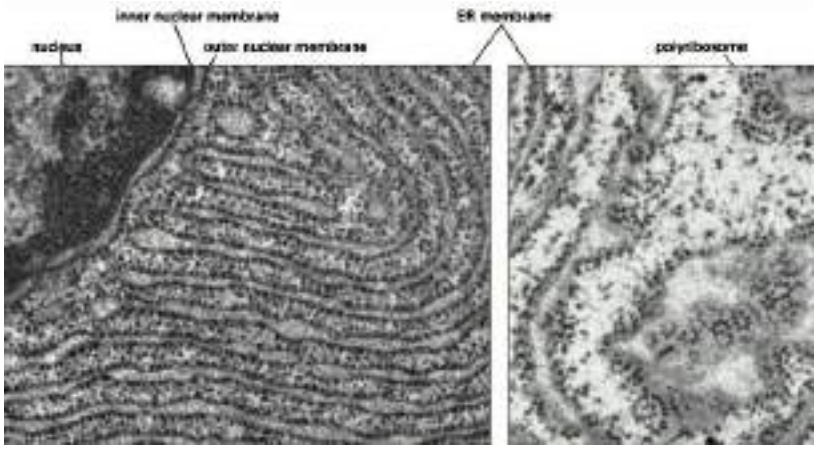
Regions of ER that lack bound ribosomes are called smooth endoplasmic reticulum, or smooth ER in the great majority of cells, such regions are scanty and are often partly smooth and partly rough

Smooth ER contains ER exit sites from which transport vesicles carrying newly synthesized proteins and lipids bud off for transport to the Golgi apparatus

Smooth ER

In hepatocytes smooth ER doubles in surface area during detoxification of both lipid-soluble drugs and various harmful compounds produced by metabolism e.g.phenobarbital

ER in most eucaryotic cells stores Ca2+ e.g. Muscle cells, have an abundant specialized smooth ER, “sarcoplasmic reticulum”, which sequesters Ca2+ from the cytosol



The ribosome that is synthesizing the protein is directly attached to the ER

membrane creating regions termed rough endoplasmic reticulum

All eucaryotic cells have an endoplasmic reticulum (ER) which is organized into a netlike branching tubules and flattened sacs throughout the cytosol

The tubules and sacs are interconnected the ER membrane encloses a single internal space

This highly convoluted space is called the ER lumen or the ER cisternal space.

The ER membrane separates the ER lumen from the cytosol, and it mediates the selective transfer of molecules between these two compartments

The ER captures selected proteins from the cytosol as they are being synthesized

Two types:

1)transmembrane proteins, partly translocated across the ER membrane and become embedded in it,

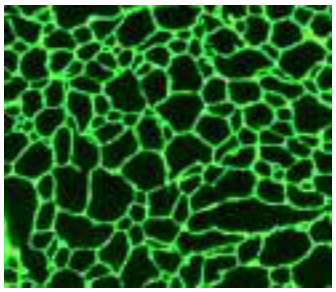
2)water-soluble proteins, which are fully translocated across the ER membrane and are released into the ER lumen

Some of the transmembrane proteins function in the ER, but many are destined to reside in the plasma membrane or the membrane of another organelle, water-soluble proteins are destined either for the lumen of an organelle or for secretion.

All of these proteins, have same kind of signal sequence & are translocated by similar mechanisms

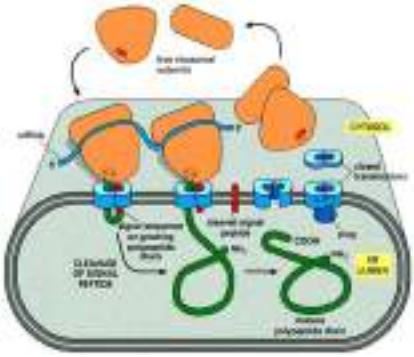
ER protein import is a co-translational process the import of proteins into mitochondria, chloroplasts, nuclei, and peroxisomes, is posttranslational processes

One end of the protein is usually translocated into the ER as the rest of the polypeptide chain is being made, the protein is never released into the cytosol and therefore is never in danger of folding up before reaching the translocator in the ER membrane



Lecture 38

ER Transport



Signal Sequences Were First Discovered in Proteins Imported into the Rough ER

Signal-Recognition Particle (SRP)

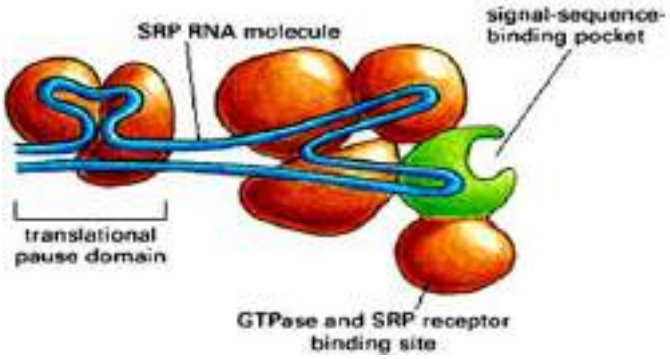
The ER signal sequence is guided to the ER by 2components:

1) SRP which cycles between the ER membrane and the cytosol & binds to the signal sequence,

2) SRP receptor in the ER membrane

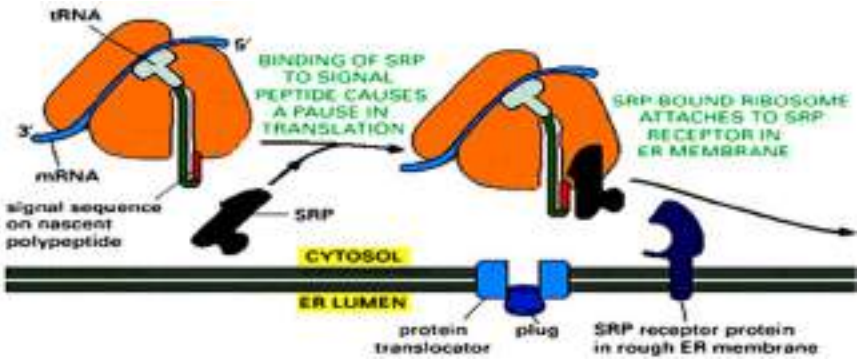
The SRP consisting of six different polypeptide chains

bound to a single small RNA molecule



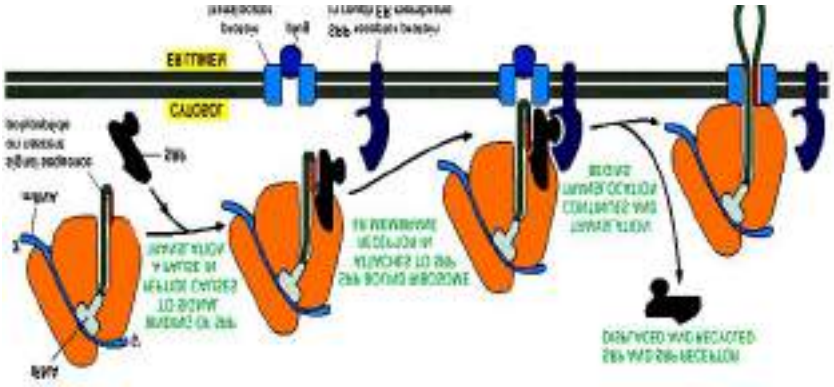
The SRP binds to the ER signal sequence as soon as the peptide has emerged from the ribosome causes a pause in protein synthesis, ensuring that the protein is not released into the cytosol

This is safety mechanisim especially for lysosomal hydrolases that could wreak havoc in the cytosol



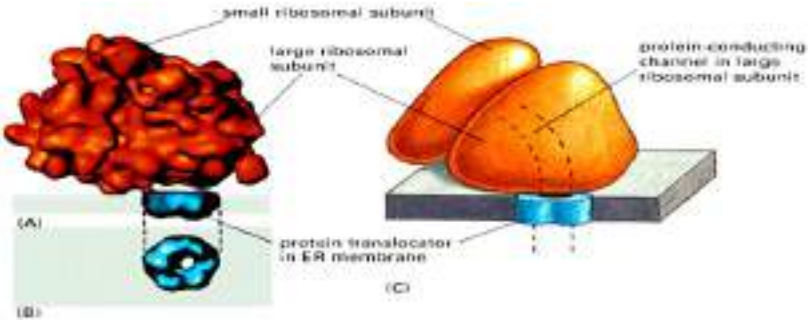
the pause presumably gives the ribosome enough time to bind to the ER

SRP-ribosome complex binds to the SRP receptor, this interaction brings the SRP-ribosome complex to a protein translocator SRP & SRP receptor are then released, and the growing polypeptide chain is transferred across the membrane



The Translocator

The translocator Sec61 complex forms a water-filled pore in the membrane through which the polypeptide chain traverses the membrane, it consists of 4 protein which assemble into a donut like structure



When a ribosome binds, the central hole in the translocator lines up with a tunnel in the large ribosomal subunit through which the growing polypeptide chain exits from the ribosome

The bound ribosome forms a tight seal with the translocator, such that the space inside the ribosome is continuous with the lumen of the ER and no molecules can escape from the ER

The pore in the translocator cannot be open permanently, however; if it were, Ca2+ would leak out of the ER when the ribosome detaches

A lumenal ER protein serves as a plug or that the translocator itself can rearrange to close the pore when no ribosome is bound

Thus, the pore is a dynamic structure that opens only transiently when a ribosome with a growing polypeptide chain attaches to the ER membrane

The signal sequence in the growing polypeptide chain triggers the opening of the pore: after the signal sequence is released from the SRP and the growing chain has reached a sufficient length, the signal sequence binds to a specific site inside the pore itself, thereby opening the pore

An ER signal sequence is therefore recognized twice: first, by an SRP in the cytosol, and then by a binding site in the ER protein translocator

This may help to ensure that only appropriate proteins enter the lumen of the ER

Some proteins, however, are imported into the ER after their synthesis has been completed, demonstrating that translocation does not always require ongoing translation

Lecture 39

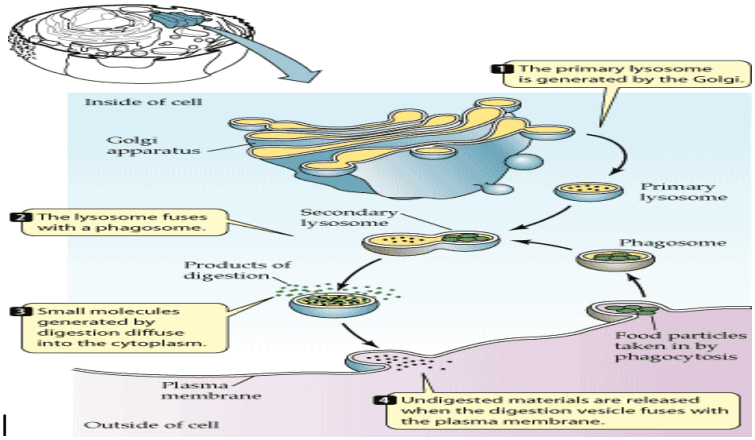
Golgi & Lysosomes

Golgi Apparatus

Receives materials from the rough ER and modifies them.

Concentrates sorts and packages proteins and sends them to other destinations.

Manufactures poly-saccharides for the plant wall

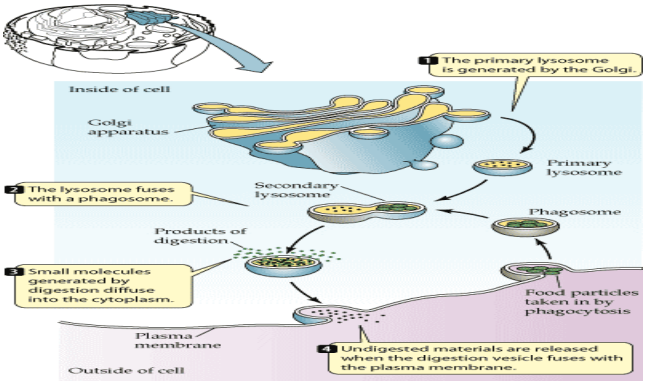


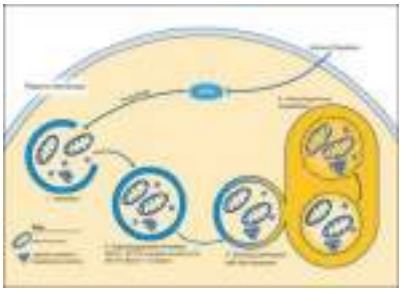
Lysosomes

Garbage Disposal Contain many digestive enzymes.

Fuse with phagosomes (autophagy).

Secondary lysosomes, where engulfed materials are digested phagocytosis.





Lecture 40

Vesicle Transport

Golgi Apparatus

The Golgi complex is a major site of carbohydrate synthesis, as well as a sorting and dispatching station for the products of the ER.

Many of the cell's polysaccharides are made in the Golgi, including the pectin and hemicellulose of the cell wall in plants and most of the glycosamino-glycans of the extracellular matrix in animals

Golgi apparatus also lies on the exit route from the ER, and a large proportion of the carbohydrates that it makes are attached as oligosaccharide side chains to the many proteins & lipids that ER sends

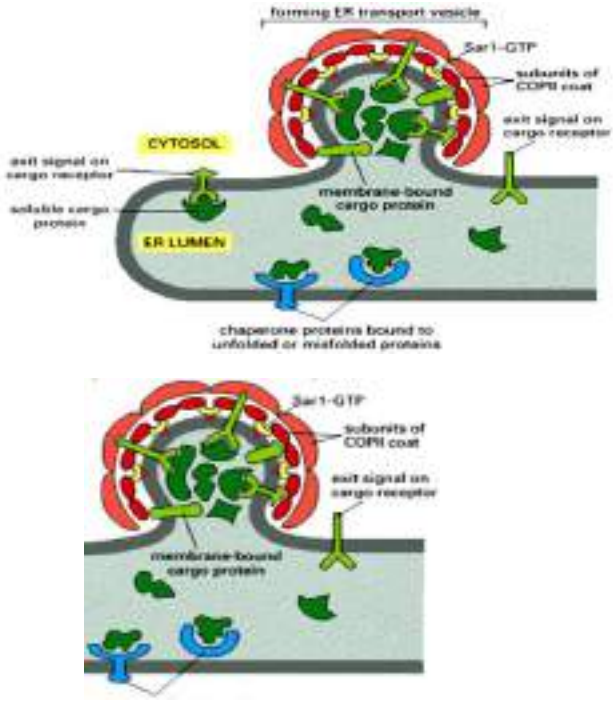
A subset of these oligosaccharide groups serve as tags to direct specific proteins into vesicles that then transport them to lysosomes

Most proteins and lipids, after acquiring their appropriate oligosaccharides in the Golgi, are recognized in other ways for targeting into the transport vesicles going to other destinations

Proteins that enter the ER and are destined for the Golgi apparatus are packaged into small COPII-coated transport vesicles which, bud from specialized regions of the ER called ER exit sites whose membrane lacks bound ribosomes

Some cargo proteins are actively recruited into such vesicles, where they become concentrated, these cargo proteins display exit (transport) signals on their surface that are recognized by complementary receptor proteins that become trapped in the budding vesicle by interacting with components of the COPII coat

Cargo proteins display exit (transport) signals on their surface that are recognized by complementary receptor proteins that become trapped in the budding vesicle by interacting with components of the COPII coat, some ER resident proteins without such exit signals can also get packaged in vesicles .



The ERGIC53 protein

To exit from the ER, proteins must be properly folded and completely assembled

Misfolded or incompletely assembled are retained in the ER, where they are bound to chaperone proteins

The ERGIC53 protein serves as a receptor for packaging some secretory proteins into COPII-coated vesicles

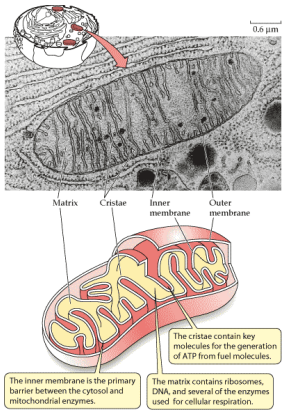
Its role in protein transport was identified because humans who lack it owing to an inherited mutation have lowered serum levels of two secreted blood-clotting factors (Factor V and Factor VIII) and therefore bleed excessively

The ERGIC53 protein is a lectin that binds mannose on Factor V and Factor VIII proteins, thereby packaging the proteins into transport vesicles in the ER

Lecture 41

Mitochondria

Make ATP Breakdown fuel



Some protists have one, liver more than a thousand and egg a few hundred thousand MT.

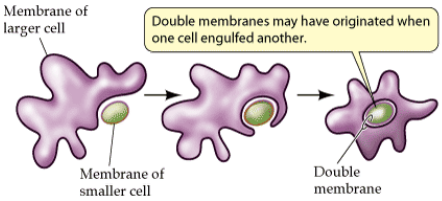
Mitochondria and chloroplasts contain their own DNA and ribosomes for making some of their own proteins

Endosymbiosis

Mutual benefits permitted this symbiotic relationship

Photosynthesis

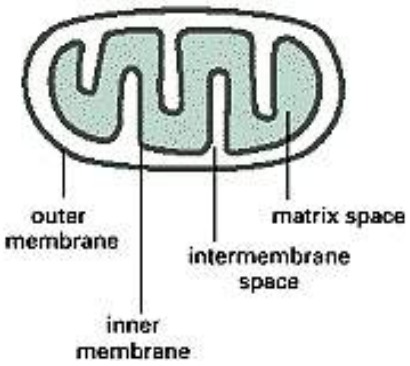
Detoxification of O2



Lecture 42

Protein Transport into Mitochondria

Mitochondria Structure



Mitochondria is double-membrane-enclosed organelle

It contains its own DNA, ribosomes, and other components required for protein synthesis, most of its proteins are encoded in the cell nucleus and imported from the cytosol

There are two subcompartments in mitochondria: the internal matrix space and the intermembrane space

Mitochondrial Import

Proteins imported into the matrix of mitochondria are usually taken up from the cytosol within seconds or minutes of their release from ribosomes.

Mitochondrial proteins are first fully synthesized as precursor proteins in the cytosol & then translocated into mitochondria by a posttranslational mechanism

Mitochondrial precursor proteins have a signal sequence at their N terminus that is rapidly removed after import by a protease (the signal peptidase) in the mitochondrial matrix

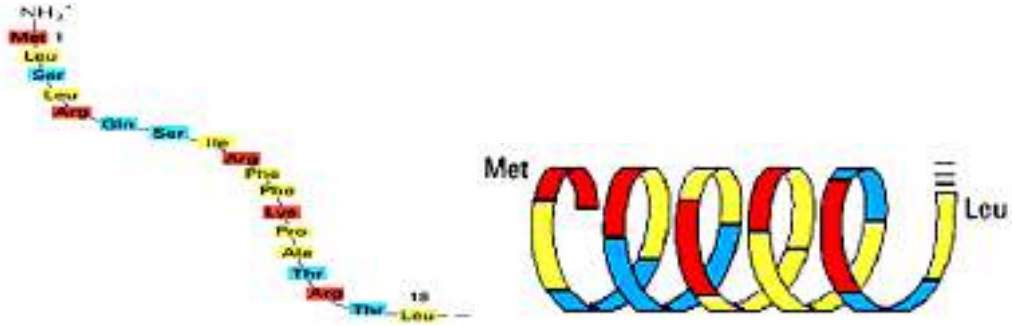
The signal sequences are both necessary and sufficient for import of the proteins that contain them genetic engineering signals linked to cytosolic protein to direct the protein into the mitochondrial matrix

Targeting Sequence

Matrix signal sequences have propensity to fold into an amphipathic α helix, in which positively charged residues are clustered on one side of the helix, while uncharged hydrophobic residues are clustered on the opposite side

This configuration—rather than a precise amino acid sequence—is recognized by specific receptor proteins that initiate protein translocation

Signal sequence



A signal sequence for mitochondrial protein import

When the signal sequence is folded as an α helix, the positively charged residues (red) are seen clustered on one face of helix, while the nonpolar residues (yellow) are clustered on opposite face amphipathic α helix, is recognized by receptor proteins on mitochondrial surface

Signal Sequence

Mitochondrial precursor proteins do not fold into their native structures after they are synthesized; instead, they remain unfolded through interactions with other proteins in the cytosol.

Lecture 43

Lysosomal Transpot

Lysosomes are membrane-enclosed compartments filled with hydrolytic enzymes that are used for the controlled intracellular digestion of macromolecules

They contain 40 types of hydrolytic enzymes, including proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases they are all are acid hydrolases

For optimal activity they require an acid environment, and the lysosome provides this by maintaining a pH of about 5.0 in its interior

The contents of the cytosol are doubly protected against attack by the cell's own digestive system

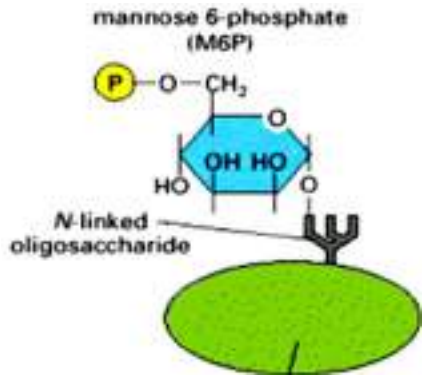
Lysosomal hydrolases and membrane proteins are synthesized in the rough ER and transported through the Golgi apparatus to the lysosome.

The transport vesicles that deliver these proteins to lysosomes bud from the trans Golgi network

These vesicles incorporate the lysosomal proteins & exclude other proteins being packaged into different transport vesicles for delivery elsewhere

Lysosomal proteins are recognized and selected in the trans Golgi network with the required accuracy

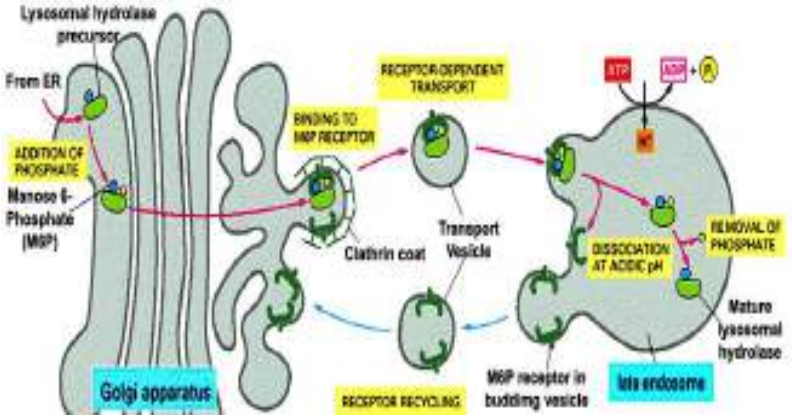
Lysosomal hydrolases carry a unique marker in the form of mannose 6-phosphate (M6P) groups,.



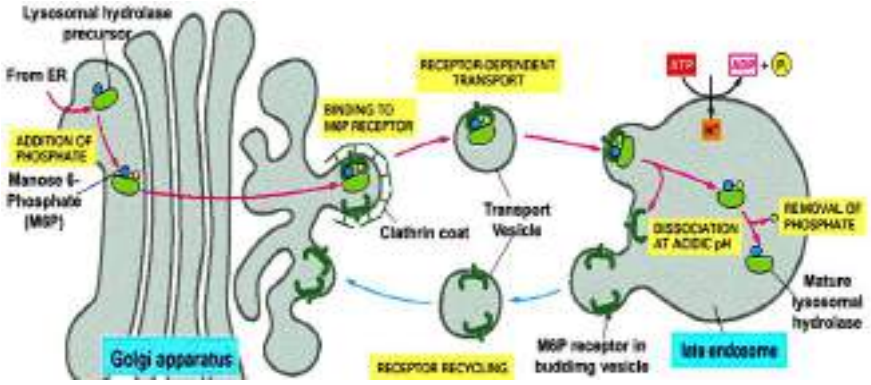
M6P is added to N-linked oligosaccharides of the soluble lysosomal enzymes in the lumen of the C-Golgi

The M6P groups are recognized by trans-membrane M6P receptor proteins, which are present in the T-Golgi

Receptors bind to lysosomal hydrolases on the lumenal side of the membrane and to adaptins to assemble clathrin coats on the cytosolic side



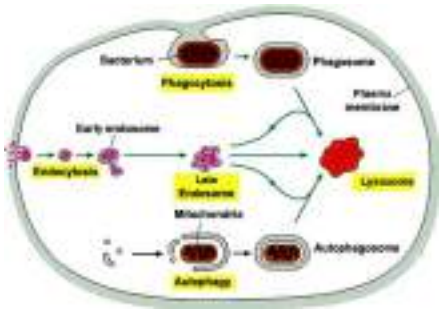
Receptors help package the hydrolases into clathrin-coated vesicles that bud from the T-Golgi network Vesicles contents are subsequently delivered to Lysosomes



M6P receptor binds its specific oligosaccharide at pH 6.5 in the T-Golgi network & releases it at pH 6, late endosomes M6P receptors are retrieved into transport

vesicles from late endosomes the receptors are returned to the T-Golgi for reuse.

Lysosome function



Inclusion-cell (I-cell) Disease

In this disease almost all of the hydrolytic enzymes are missing from the lysosomes of fibroblasts, and their undigested substrates accumulate in lysosomes, which consequently form large “inclusions” in the cells

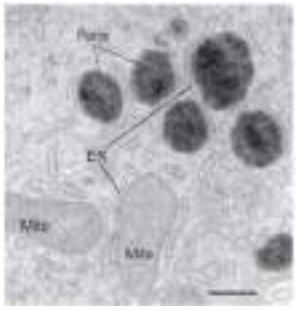
In I-cell disease patients, all the hydrolases missing from lysosomes are found in the blood

Because they fail to be sorted properly in the Golgi apparatus, the hydrolases are secreted rather than transported to lysosomes.

The Retrieval Process

Lecture 44

Peroxisomes & Glyoxysome



Introduction

Electron micrograph of a section of a rat liver cell, showing peroxisomes.

Peroxisomes also called as microbodies are simple,membrane-bound vesicles.

They have a diameter of 0.1 to 1.0 m.

It may contain a dense, crystalline core of oxidative enzymes.

Peroxisomes are multifunctional organelles containing more than 50 enzymes.

These are involved in oxidation of very-long-chain fatty acids.

These organelles were named “peroxisomes” because they are the site of synthesis and degradation of hydrogen peroxide (H_2O_2), a highly reactive and toxic oxidizing agent.

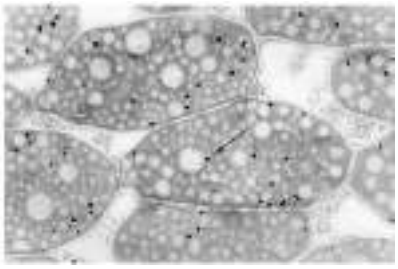
Hydrogen peroxide is produced by a number of peroxisomal enzymes, including urate oxidase, glycolate oxidase, and amino acid oxidases, that utilize molecular oxygen to oxidize their respective substrates.

The H_2O_2 generated in these reactions is rapidly broken down by the enzyme catalase, which is present in high concentrations in these organelles.

Glyoxysome

Plant seedlings contain a specialized type of peroxisome, called a glyoxysome.

Plant seedlings rely on stored fatty acids to provide the energy and material to form a new plant.



Glyoxysome localization within plant seedlings.

Functions

One of the primary metabolic activities of glyoxisomes in germinating seedlings

is the conversion of stored fatty acids to carbohydrate.

Following steps are involved in this conversion;

1.Disassembly of stored fatty acids generates acetyl CoA

2.Acetyl CoA condenses with oxaloacetate (OAA) to form citrate

3.Citrate is then converted into glucose by a series of enzymes of the glyoxylate cycle localized in the glyoxysome.

Lecture 45

Ribosome

Protein & RNA

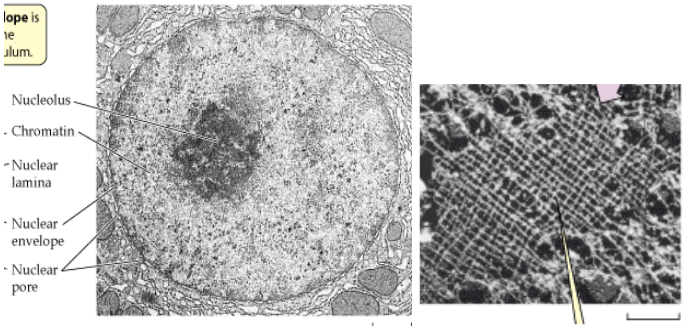
Free or attached to ER involved in protein synthesis.

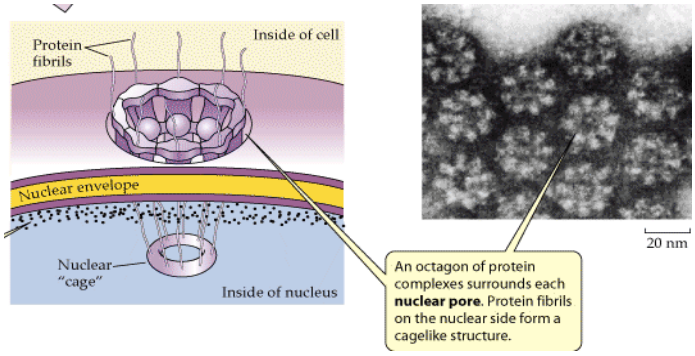
Mitochondria & Chloroplasts.

Lecture 46

The Nucleus

Nuclear EMs



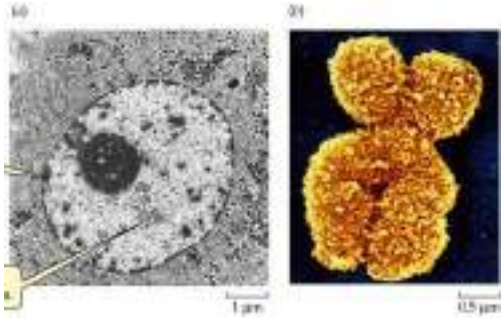


Chromatin

Euchromatin

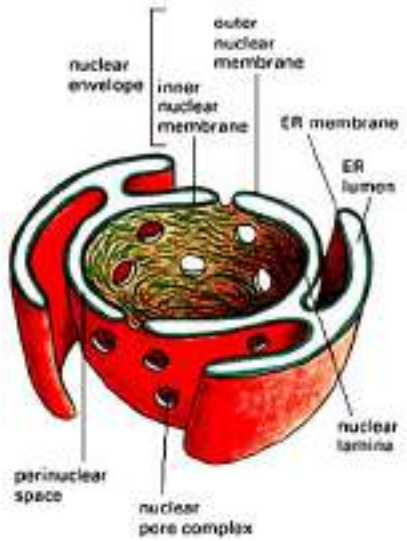
Hetrochromatin

Chromosome



Lecture 47

Nuclear Transport



Nuclear envelope encloses DNA & defines nuclear compartment

Envelope consists of 2 concentric membranes that are penetrated by

nuclear pore complexes

The inner & outer membranes are continuous & maintain distinct protein compositions.

The inner nuclear membrane contains specific proteins that act as binding sites for chromatin & protein meshwork of the nuclear lamina that provides structural support.

The inner membrane is surrounded by the outer nuclear membrane, which is continuous with the membrane of the ER

The outer nuclear membrane is studded with ribosomes engaged in protein synthesis

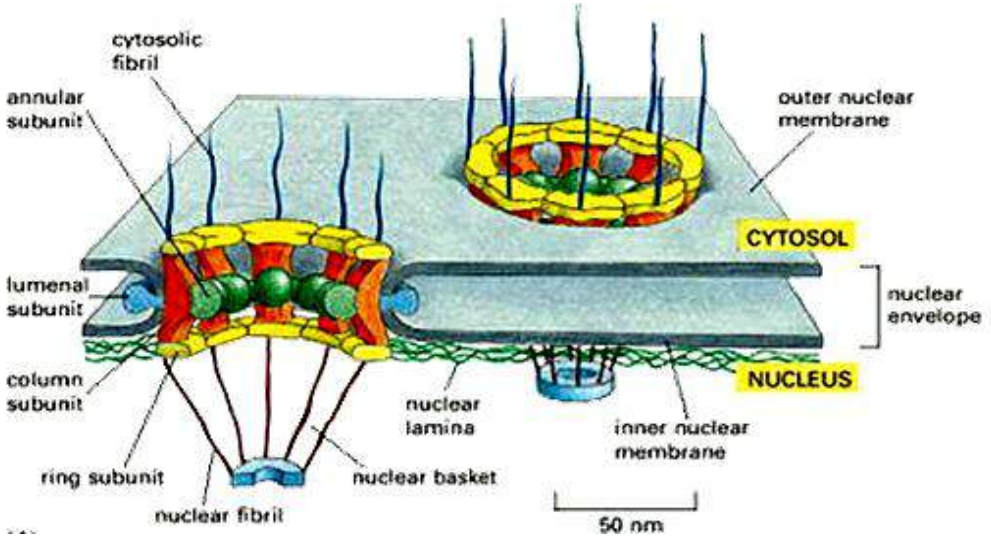
Many proteins that function in nucleus—including histones, DNA and RNA polymerases, gene regulatory proteins, & RNA-processing proteins—are selectively imported into the nuclear compartment from the cytosol, At the same time, tRNAs and mRNAs are synthesized in the nuclear compartment and then exported to cytosol

Bidirectional traffic between the cytosol and the nucleus

Like the import process, the export process is selective; mRNAs, for example, are exported only after they have been properly modified by RNA-processing reactions in the nucleus

Ribosomal proteins, are made in the cytosol, imported into the nucleus—where they assemble with newly made ribosomal RNA into particles—and are then exported again to the cytosol as part of a ribosomal subunit

Nuclear pore complexes

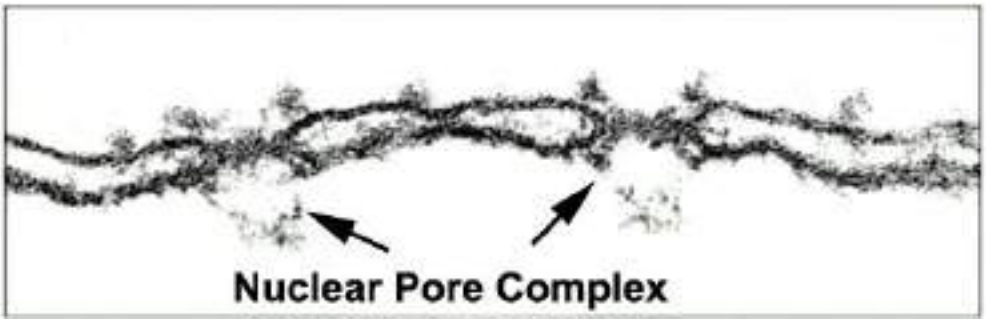


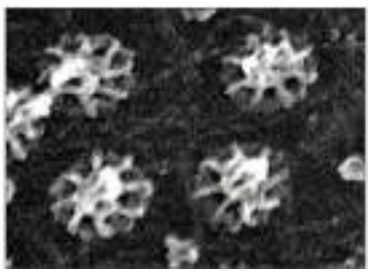
Eukaryotic nuclear envelope is perforated by large, elaborate structures called nuclear pore complexes which selectively facilitate nuclear transport

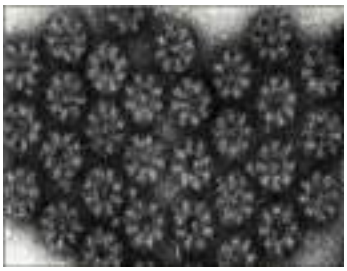
Each pore complex contains one or more open aqueous channels through which small water-soluble molecules can passively diffuse

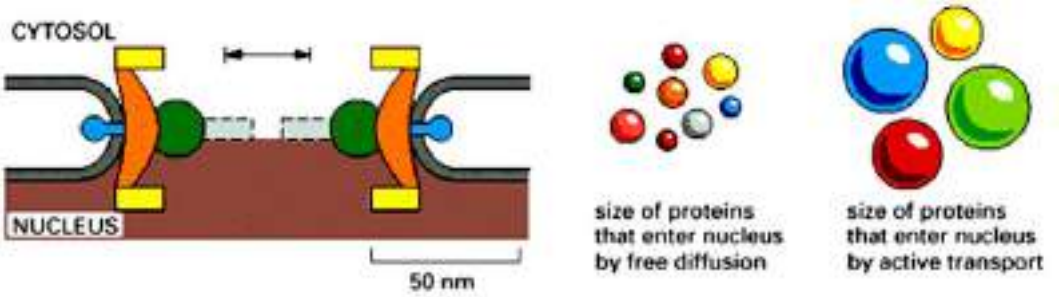
Each complex has a molecular mass of about 125 million & is composed of aprox 50 different proteins, called nucleoporins, arranged octagonal symmetry

Small molecules (5000 daltons or less) diffuse in so fast that the nuclear envelope can be considered to be freely permeable to them, a protein of 17,000 daltons takes 2 minutes to proteins larger than 60,000 daltons are unable to enter the nucleus.









Nuclear pore contains a pathway for free diffusion 9 nm in diameter & 15 nm long, this channel occupies a small fraction of total volume of the pore complex.

The more active the nucleus is in transcription, the greater the number of pore complexes

The nuclear envelope of a typical mammalian cell contains 3000–4000 pore complexes

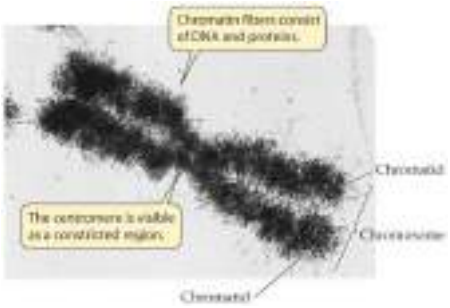
Cells synthesizing DNA, need to import about 106 histone molecules from the cytosol every 3 minutes to package DNA into chromatin, ie each pore complex needs to transport about 100 histone molecules/minute

Rapidly growing cell need to transport about 6 large & small ribosomal subunits/minute from nucleus, where they are produced, to the cytosol.

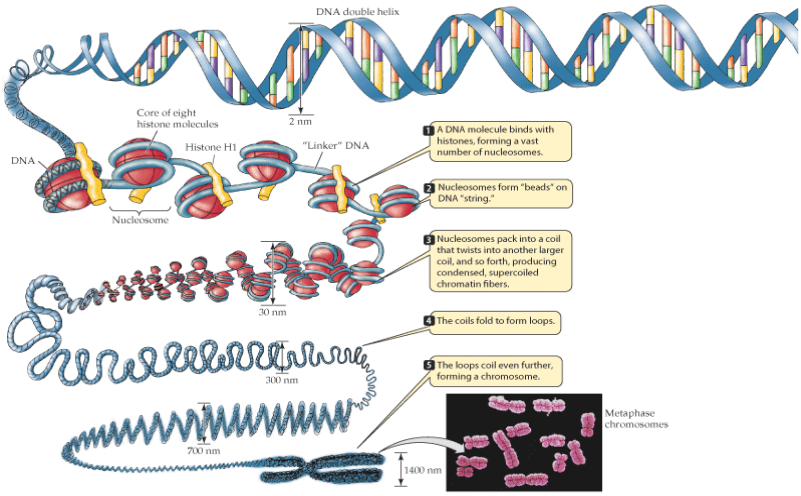
Lecture 48

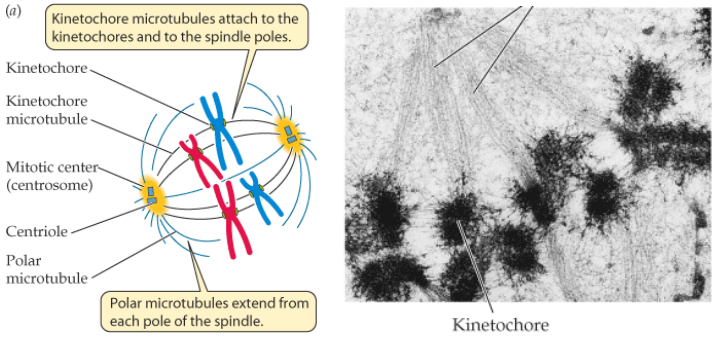
Chromosome

Anatomy



A chromosome is an organized structure of DNA and protein

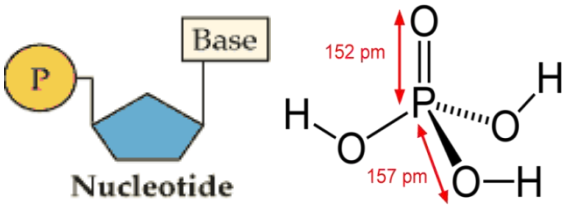


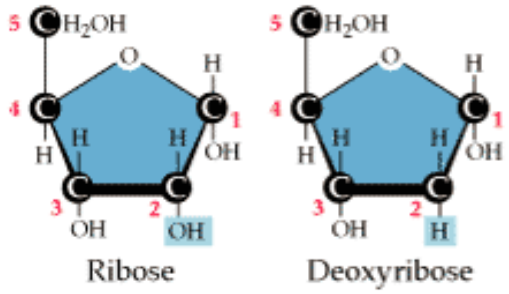


Lecture 49

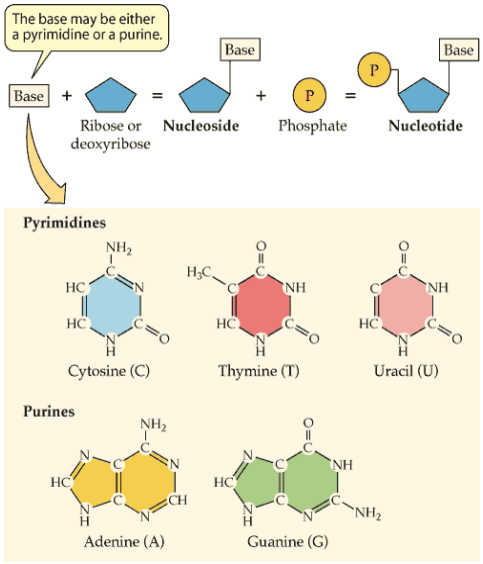
Nucleic Acids

DNA & RNA

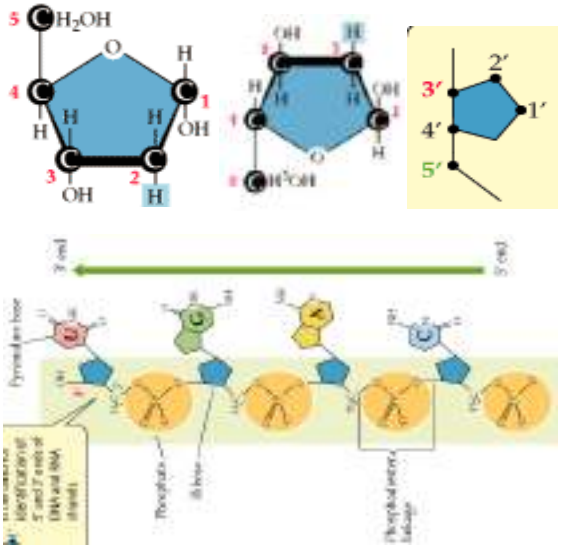




The Bases



Prime Ends (3'& 5')



Lecture 50

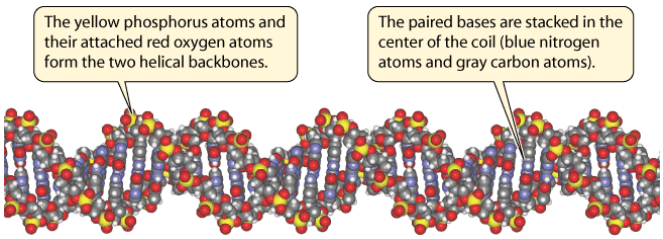
RNA & DNA

3.3 Distinguishing RNA from DNA

NUCLEIC ACID	SUGAR	BASES
RNA	Ribose	Adenine Cytosine Guanine Uracil
DNA	Deoxyribose	Adenine Cytosine Guanine Thymine

DNA Uniform Width

Information in Sequence not Shape



Chicken vs. Egg: What Came 1st

Protein or DNA

Genetic information codes for enzymes

Enzymes are required to decipher genetic information and replicate it

RNA Genetic Material & Enzyme

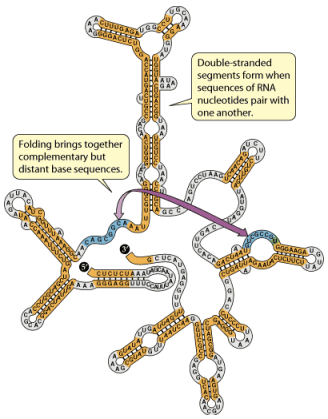
Many viruses use RNA as their hereditary material

RNAs can achieve chemical catalysis, like enzymes:

ribosome the active site is composed entirely of RNA (peptide bond formation)

RNA: Information in Sequence and Shape

Why Then DNA?



Lecture 51

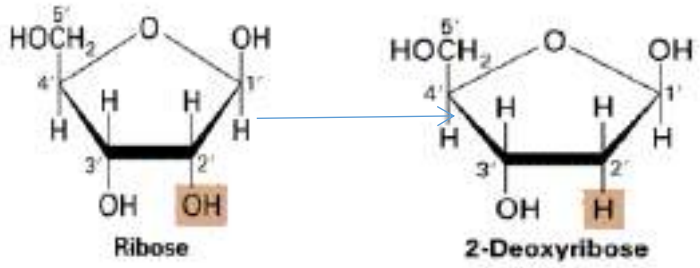
Chemical composition of DNA

DNA is a polymer of Deoxyribonucleotides.

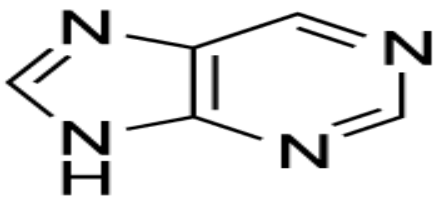
Deoxyribonucleotide is composed of three components:

- Deoxyribose
- Nitrogenous Base
- Phosphoric acid

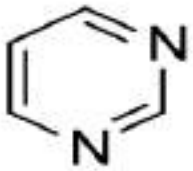
Deoxyribose (a pentose sugar derivative)



Nitrogenous bases



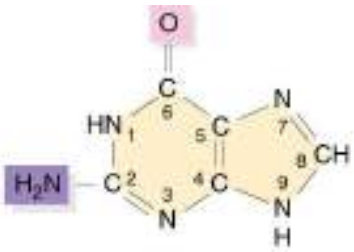
purine



pyrimidine

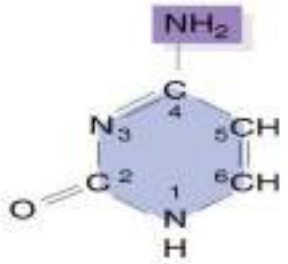
Purines

Adenine (A)

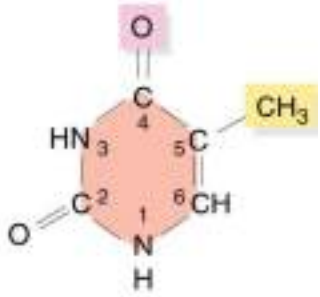


Guanine (G)

Pyrimidines

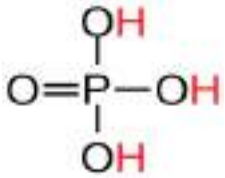


Cytosine (C)



Thymine (T)

Phosphoric acid



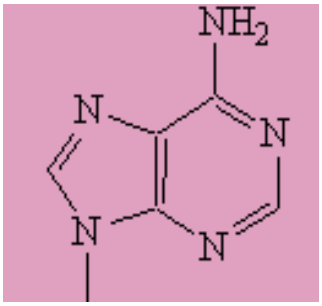
H_3PO_4

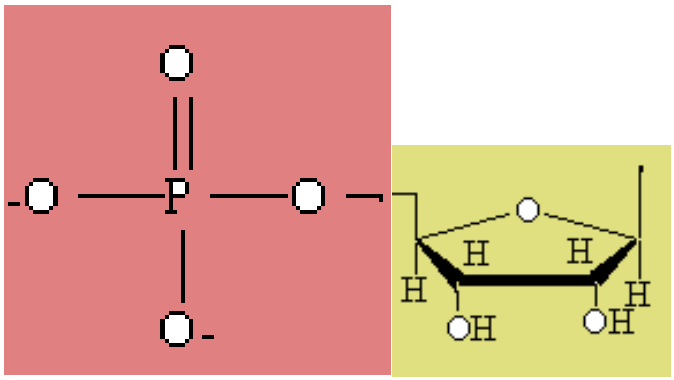
Lecture 52

Nucleoside & Nucleotide

A molecule containing all these three components is called a nucleotide.

While a molecule without the phosphate group is called a nucleoside.





Nucleotide = Nucleoside + Phophoric acid

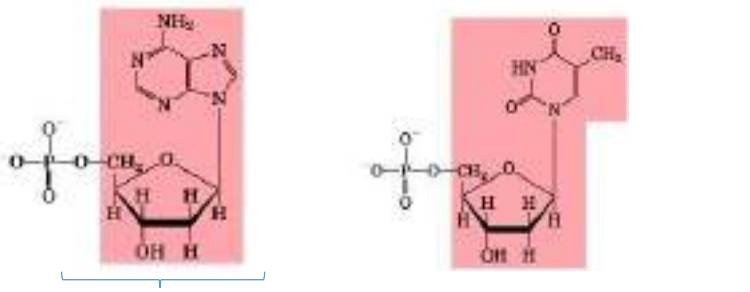
&

Nucleoside = Nucleotide – Phoshoric acid

Lecture 53

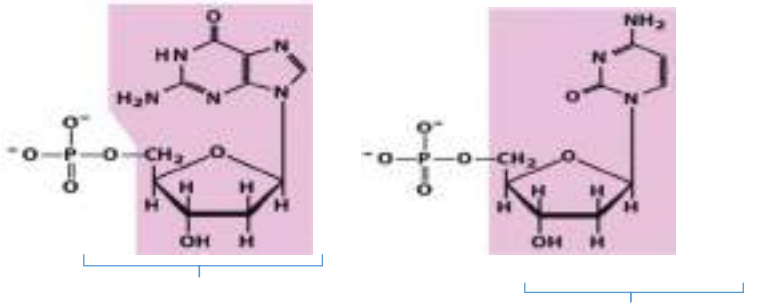
Types of Deoxyribonucleotides

There are four types of Deoxy-ribonucleotides.



Deoxyadenosine Deoxythymidine

Deoxyadenylate Deoxythymidylate



Deoxyguanosine Deoxycytidine

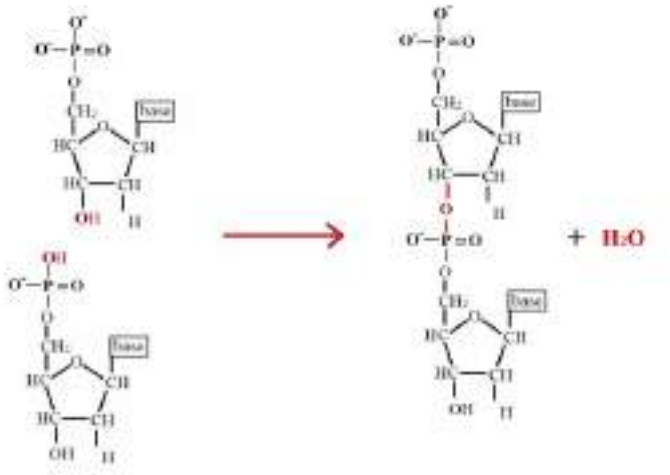
Deoxyguanylate Deoxycytidylate

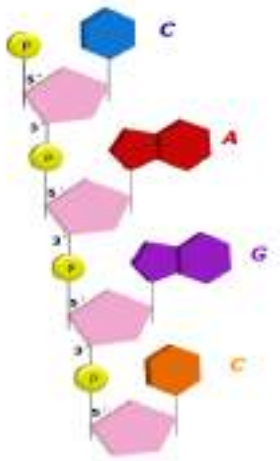
These four deoxy-ribonucleotides make the structural units of DNA

Lecture 54

How do Deoxyribonucleotides Join?

The successive nucleotides of DNA are joined together through phospho-diester linkages.





Lecture 5

Structure of DNA

Work of Chargaff

(Late 1940s)

The discovery of the structure of DNA is one of the greatest events in the history of science.

Erwin Chargaff and his colleagues provided a most important clue to the structure of DNA.

The work of Chargaff led him to following conclusions, also called “Chargaff Rules”:-

1.Base composition of DNA varies from one species to another.

2. The DNA isolated from different tissues of the same species have the same base composition

3. The base composition of DNA in a given species does not change with an organism's age, nutritional state, or changing environment

4. In DNA, the number of adenosine residues is equal to the number of thymidine (A=T) and the number of guanosine residues is equal to the number of cytidine (G=C).

It means that the sum of the purine residues equals the sum of the pyrimidine residues (AG=TC).

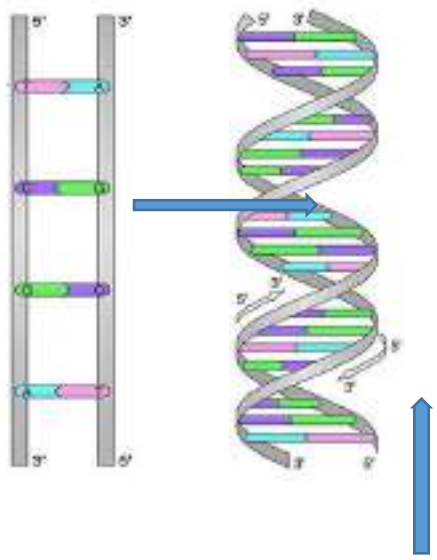
Lecture 56

Work of Watson & Crick

James Watson and Francis Crick postulated a three dimensional model of DNA structure in 1953.

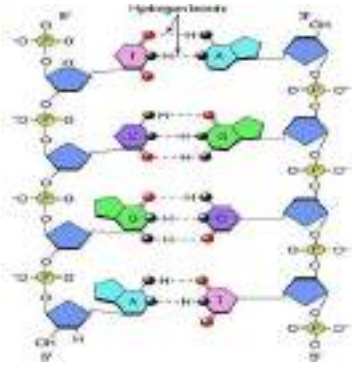
The major features of this model are as follow:-

1. DNA consists of two helical polynucleotide strands which are wound around the same axis to form a right handed double helix.

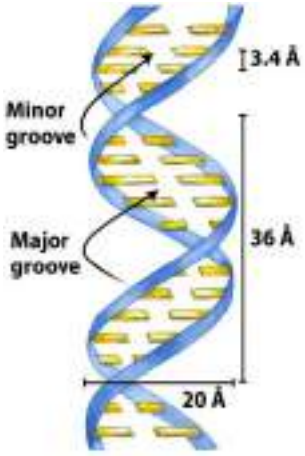


2. The hydrophilic backbones of alternating deoxyribose and phosphate groups are on the outside of the double helix, facing the surrounding water.

3. The nitrogenous bases of both strands are stacked inside the double helix lying perpendicular to the long axis of the helix.

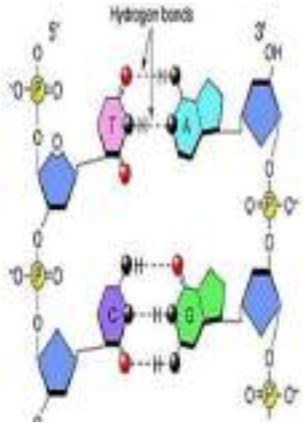


4.The pairing and coiling of the two strands create a major groove and minor groove on the surface of the helix.



4. Each nucleotide base of one strand is paired in the same plane with a base of the other strand

6. They also found that G pairs with C and A pairs with T due to the reason that they fit best within the structure.



5. They also found that G pairs with C and A pairs with T due to the reason that they fit best within the structure.

6. The two strands in DNA are present in antiparallel orientation i.e; their 5,3-phosphodiester bonds run in the opposite directions

7. The two antiparallel strands of double-helical DNA are complementary to each other.

Lecture 57

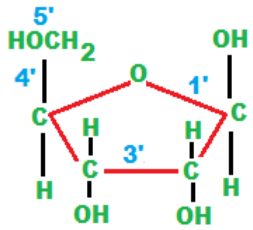
Chemical composition of RNA

RNA (Ribonucleicacid) is a polymer of ribonucleotides.

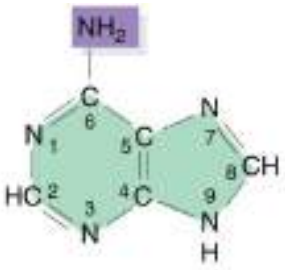
Each ribonucleotide is composed of three components;

1. A ribose sugar
2. A Nitrogenous Base
3. A Phosphoric aci

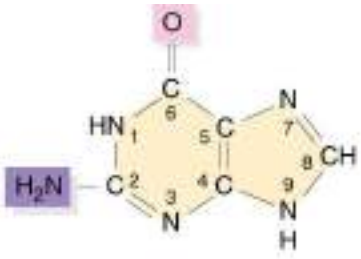
Ribose (a pentose sugar)



Nitrogenous Bases

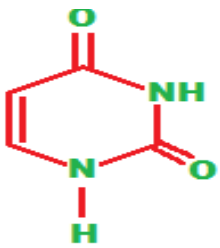


Adenine (A)



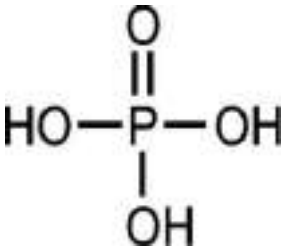
Guanine (G)

Cytosine (C)

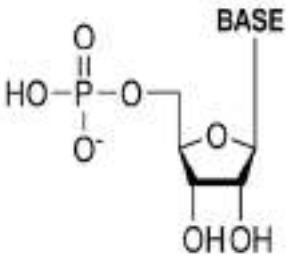


Uracil (U)

Phosphoric acid



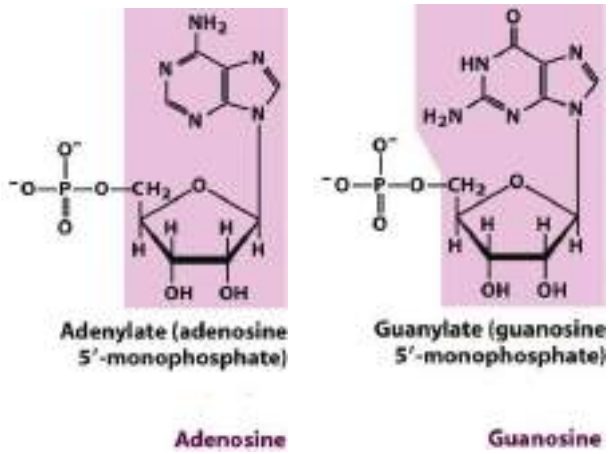
A Ribonucleotide

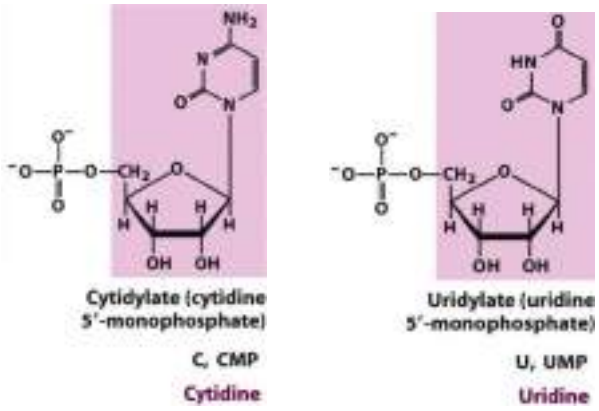


Lecture 58

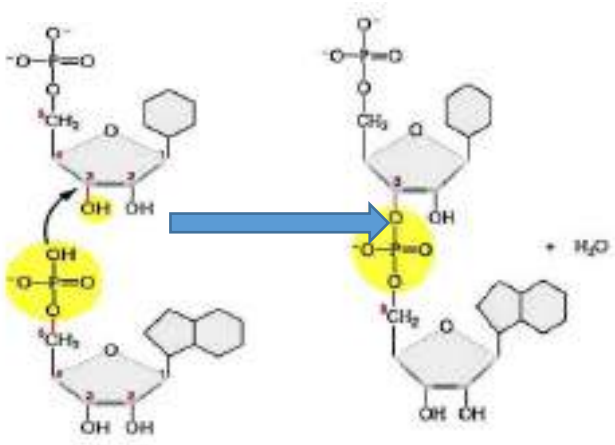
Types of Ribonucleotides

There are mainly four types of ribonucleotides depending upon the types of nitrogenous bases present in RNA.

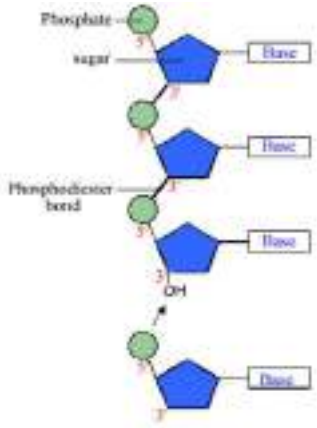




How do Ribonucleotides Join?



A Poly-Ribonucleotide



Lecture 59

Types of RNAs

There are mainly three types of Ribonucleic acids (RNAs) present in the cells of living organisms.

Messenger RNA (mRNA)

Transfer RNA (tRNA)

Ribosomal RNA (rRNA)

Messenger RNA (mRNA)

It is the type of RNA that carries genetic information from DNA to the protein biosynthetic machinery of the ribosome.

It provides the templates that specify amino acid sequences in polypeptide chains.

The process of forming mRNA on a DNA template is known as transcription.

It may be monocistronic or polycistronic.

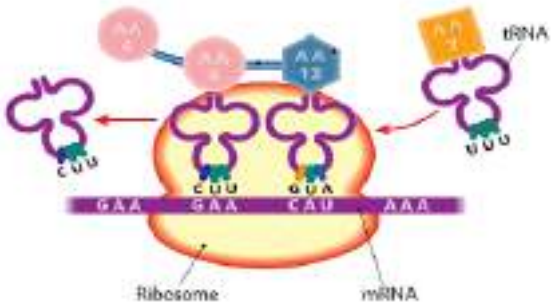
The length of mRNA molecules is variable and it depends on the length of gene.

Transfer RNA (tRNA)

Transfer RNAs serve as adapter molecules in the process of protein synthesis.

They are covalently linked to an amino acid at one end.

They pair with the mRNA in such a way that amino acids are joined to a growing polypeptide in the correct sequence.



Ribosomal RNA (rRNA)

Ribosomal RNAs are components of ribosomes.

rRNA is a predominant material in the ribosomes constituting about 60% of its weight.

It has a number of functions to perform in the ribosomes.

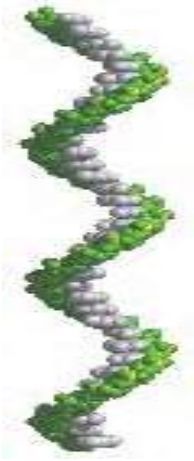
Lecture 60

Structures of RNAs

mRNA is always single stranded when it is formed from DNA.

But this single strand assumes a double helical conformation soon after its formation.

This confirmation is achieved mainly due to base stacking interactions.



Self-complementary sequences may occur in the RNA molecules which produce more complex structures.

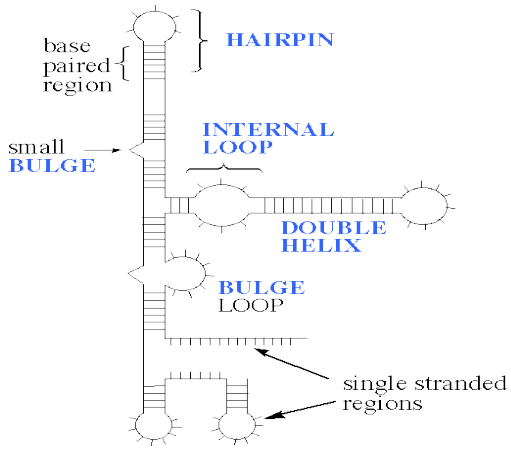
So RNA can base-pair with complementary regions of either RNA or DNA.

RNA has no any regular secondary structure that serves as a reference point.

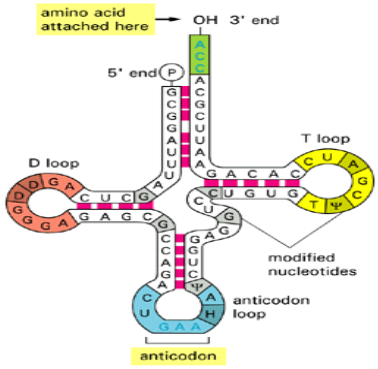
The three-dimensional structures of many RNAs are complex and unique.

Breaks in the helix caused by mismatched or unmatched bases in one or both strands are common and result in bulges or internal loops.

Hairpin loops form between nearby self-complementary sequences.



Transfer RNA (tRNA)



Lecture 61

Nature of Genetic Material

After establishment of the fact that genes are the physical units located on the chromosomes. A major problem for the biologists was to find out the molecules responsible for carrying the hereditary information.

Characteristics of Genetic Material

Genetic material must contain complex information.

Genetic material must replicate faithfully.

Genetic material must encode phenotype.

Three sets of experiments provided a pivotal evidence that DNA rather than protein, is the hereditary material.

Griffith’s Experiments

(1928)

Avery’s Experiments

(1944)

Hershey-Chase

experiments (1952)

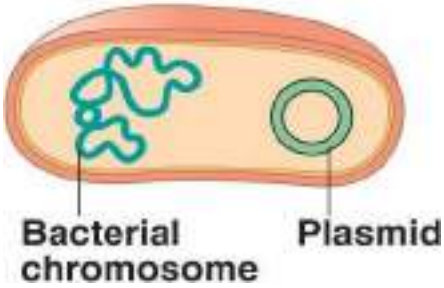
Lecture 62

Organization of Genetic Material in Bacteria

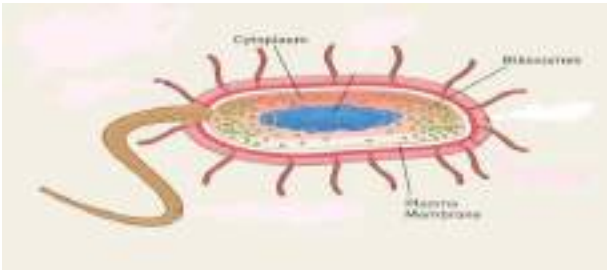
Bacteria typically have a single circular chromosome consisting of a single circular molecule of DNA with associated proteins.

The bacterial chromosome is a very long (up to 1mm).

It is looped and folded and attached at one or several points to the plasma membrane proteins.



Specific proteins interact with the bacterial DNA to form a highly condensed nucleoprotein complex called the nucleoid.

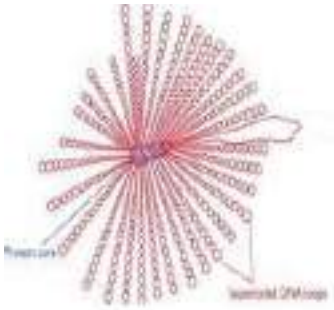


Bacterial chromatin can be released from the cell by gentle lysis of the cell.

Electron micrograph of the chromatin reveals that it consists of multiple loops which emerge from a central region of the chromatin.

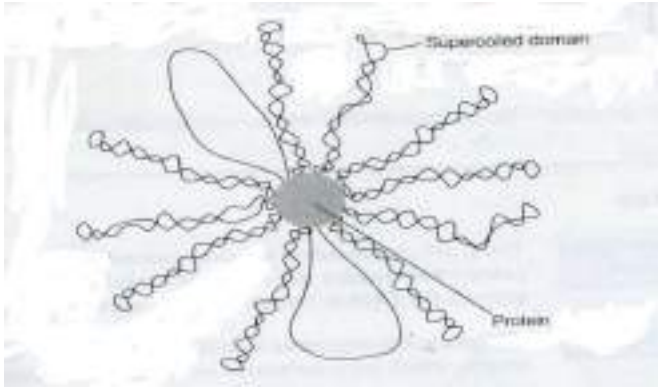
Some of the loops are super-coiled while some are relaxed.

Relaxed loops are formed as a result of a nick introduced into super-coiled loops by a cellular DNase.



If a super-coiled DNA molecule receives a nick, the strain of under-winding is immediately removed, and all the super-coiling is lost.

Studies confirm that continued nuclease treatment increases number of relaxed loops.



The bacterial DNA is arranged in super-coiled loops that are fastened to a central protein matrix, so that each loop is topologically independent from all the others.

So a nick that causes one super-coiled loop to relax would have no effect on other super-coiled loops.

The super-coiled loops are dynamic structures which change during cell growth & division.

An E. coli chromosome is estimated to have about 400 super-coiled loops.

Each loop has an average length of about 10-20 kbp.

The DNA compaction in a bacterial cell is contributed by super-coiling of loops, macromolecular crowding and DNA-binding proteins.

Lecture 63

Organization of Genetic Material in Bacteria

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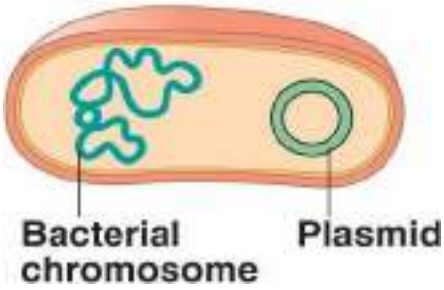

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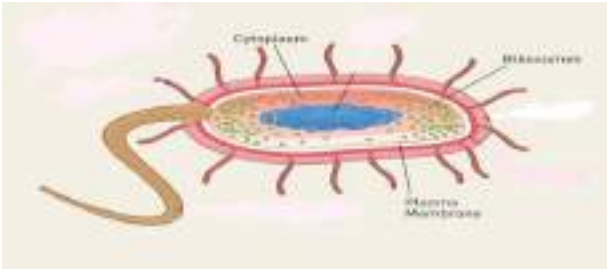

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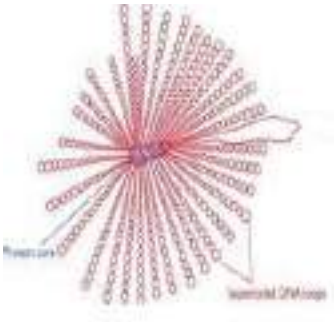

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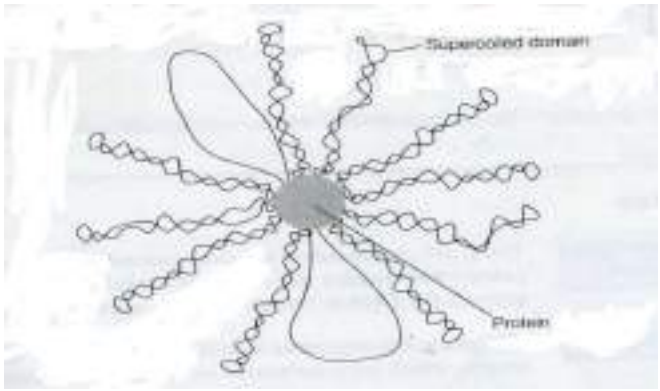

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Lecture 64

Organization of Genetic Material in Eukaryotes

The genetic material (DNA) of eukaryotic organisms is organized in the form of chromosomes.

The chromosomes of eukaryotic cells are larger and more complex than those of prokaryotes.

Each un-replicated chromosome consists of a single molecule of DNA.

If stretched out, some human chromosomes would be several centimetres long.

To package such a tremendous length of DNA into this small volume, each DNA molecule is coiled again and again and tightly packed around histone proteins.

As eukaryotic chromosomes are not circular, so instead of super-coiling, the mechanism of packaging involves winding the DNA around special proteins, the histones.

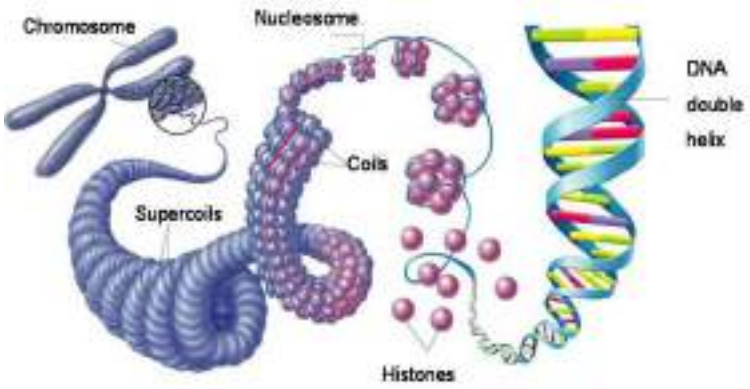
DNA with bound histones in the eukaryotes is called as chromatin.

Chromatin consists of roughly spherical subunits, the nucleosomes, each containing approx. 200 bp of DNA and nine histones.

A condensed mitotic chromosome is about 50,000 times shorter than fully extended DNA.

Highly condensed chromatin is known as heterochromatin.

The more extended form is known as euchromatin.



Lecture 65

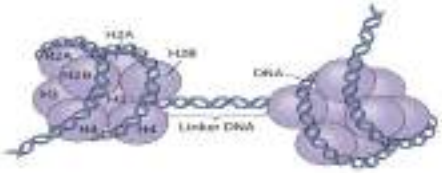
Histone Proteins

Most abundant proteins in the chromatin are histones.

There are nine types of histones including two each of H2A, H2B, H3 and H4 and one of H1.

These histones fall in five major classes i.e., H1, H2A, H2B, H3 and H4.

A typical human cell contains about 60 million copies of each kind of histone.



All histones have a high percentage of arginine and lysine but the lysine-to-arginine ratio differs in each type of histone.

The positively charged side chains of lysine and arginine enable histones to bind to the negatively charged phosphate groups of the DNA.

The electrostatic attraction is an important stabilizing force in the chromatin.

Lecture 66

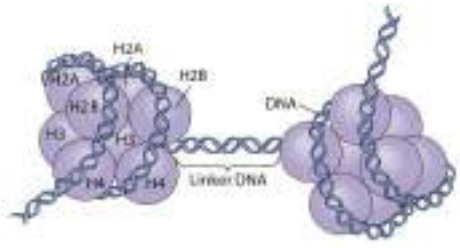
The Nucleosome

The uncondensed chromatin resembles beads on a string when viewed under the electron microscope.

Each bead is a nucleoprotein complex called nucleosome.

Each nucleosome is formed by winding DNA fibre around a protein assembly consisting of eight histone molecules.

The DNA connecting two nucleosomes is called linker DNA.



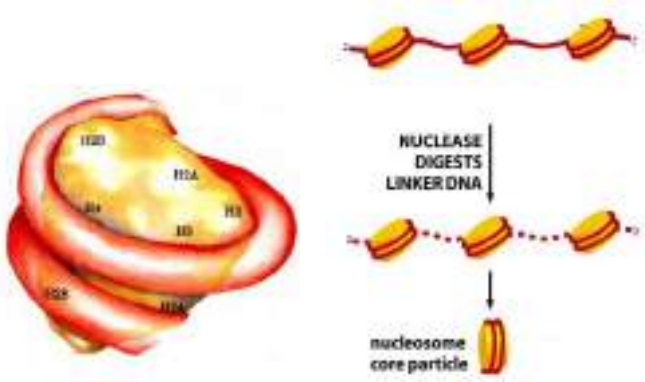
The size of the linker DNA between the nucleosomes varies among different organisms and even different organs of the same organism.

The length of DNA wrapped around nucleosomes also varies from one organism to the other ranging from about 170-240 bp.

Prolonged nuclease digestion of chromatin cleaves additional nucleotides.

The structure that remains is the nucleosome core particle.

The nucleosome core particle consists of an octameric protein complex (two copies of each H2A, H2B, H3 & H4) with a 146 bp DNA fragment wound around it.



Lecture 67

Cell Division

Life Span & Continuity

All living organisms have a limited life span.

The essence of life is to propagate.

Cells are the basic unit of life.

Cell division is the basic process that ensures the continuity of life.

The Necessity

Unicellular organisms propagate by splitting into two cells.

Multicellular organisms:replace damaged or senescent cells.

Billions of cells die each day in humans, (blood epithelia)

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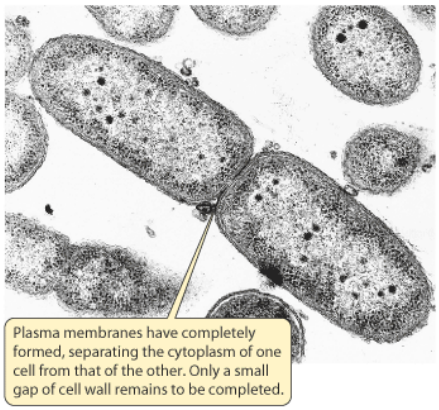
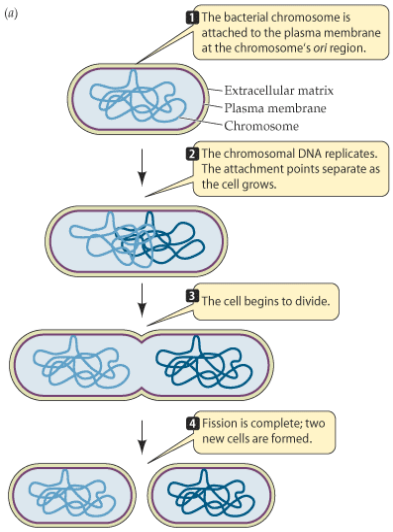
Meiosis: Organisms shuffle genes

Even the Prokaryotes Do It



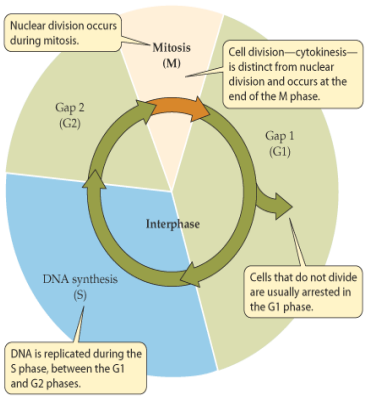
Favorable Conditions

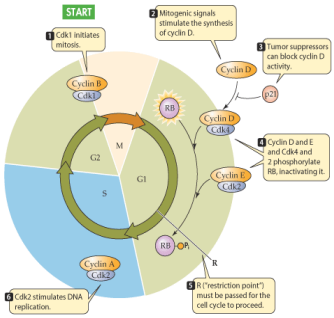
20 - 40 min



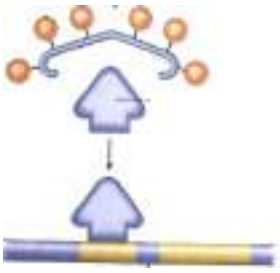
Lecture 68

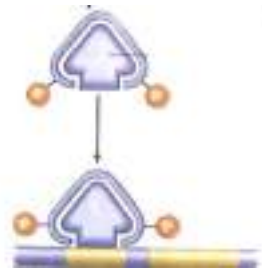
Cell Cycle





RB RB

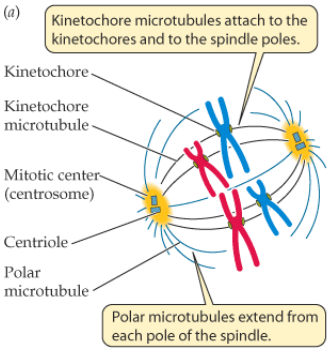


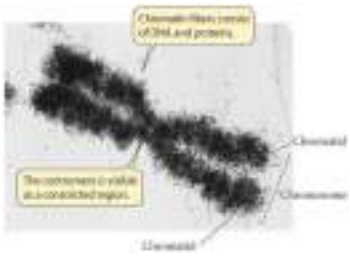


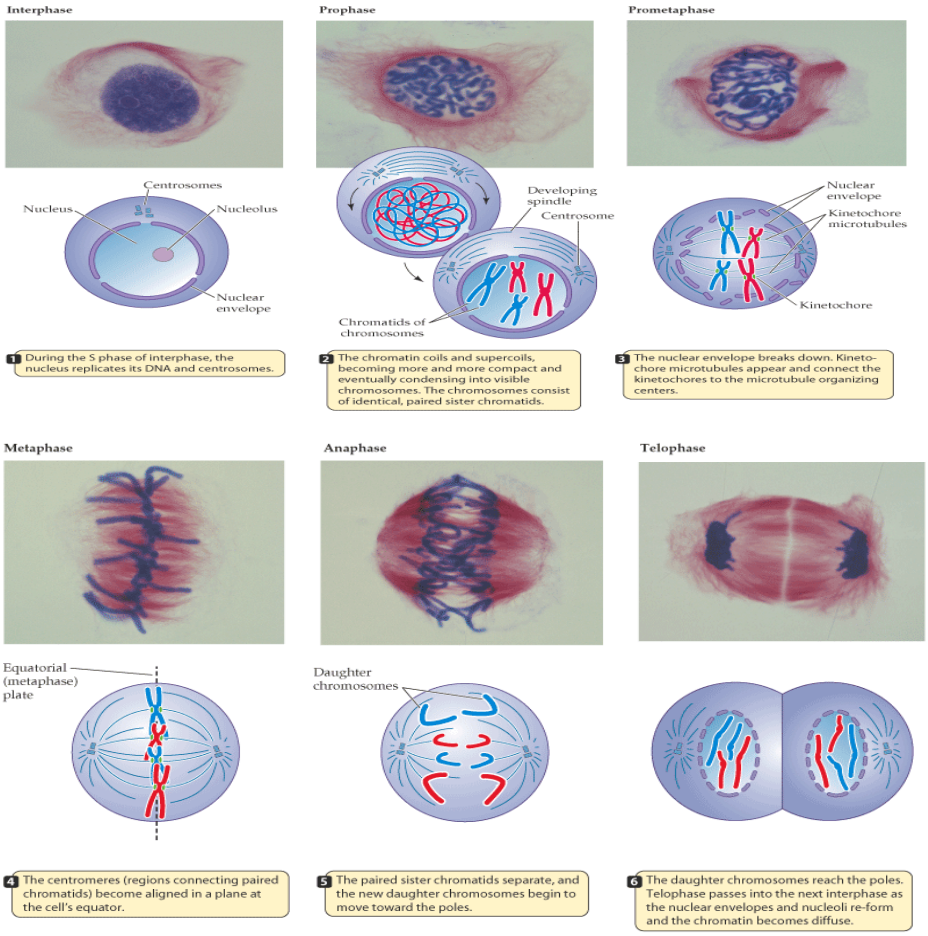
E2F E2F

Lecture 69

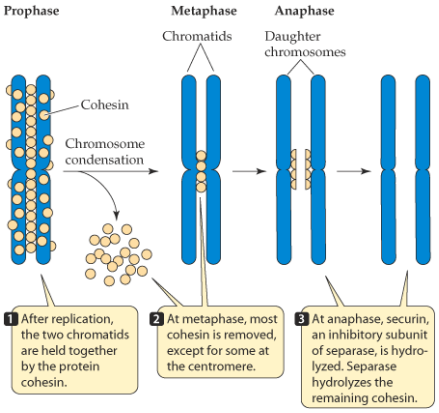
Mitosis



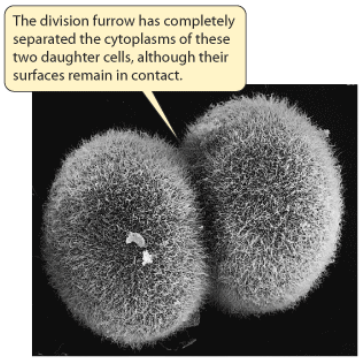




The Glue



Cytokinesis



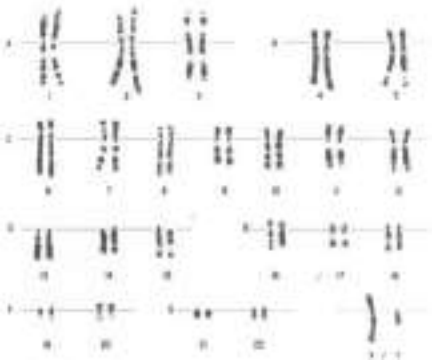
Lecture 70

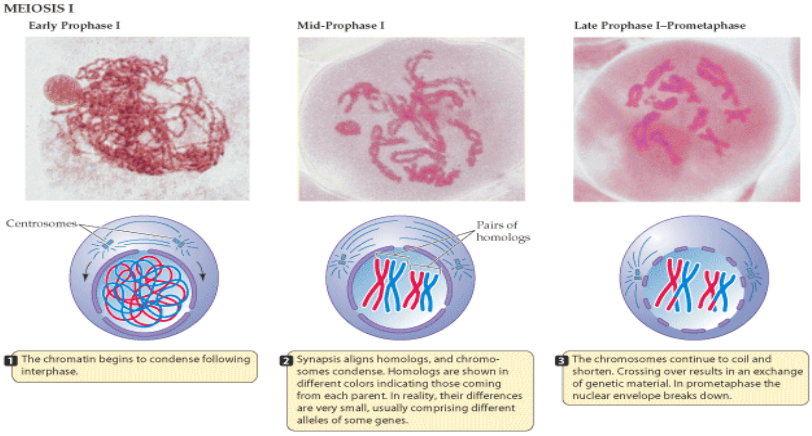
Mitosis

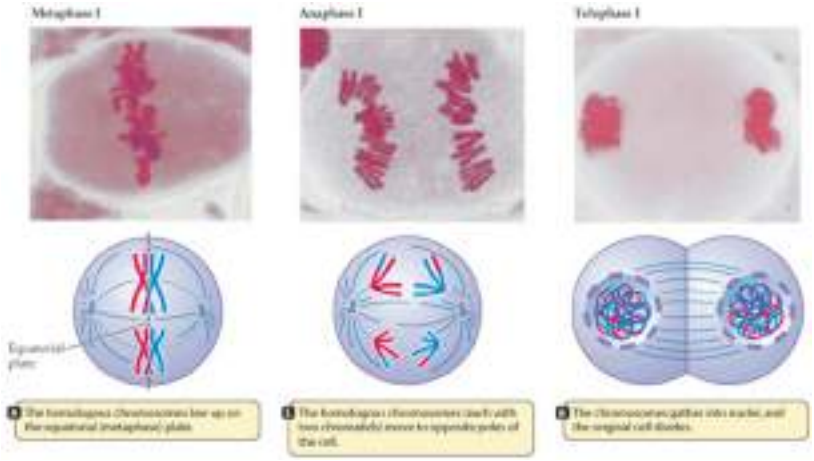
Lecture 71

Meiosis I

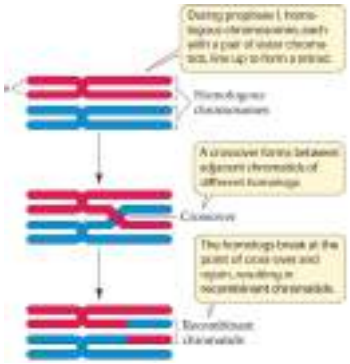
Karyotype





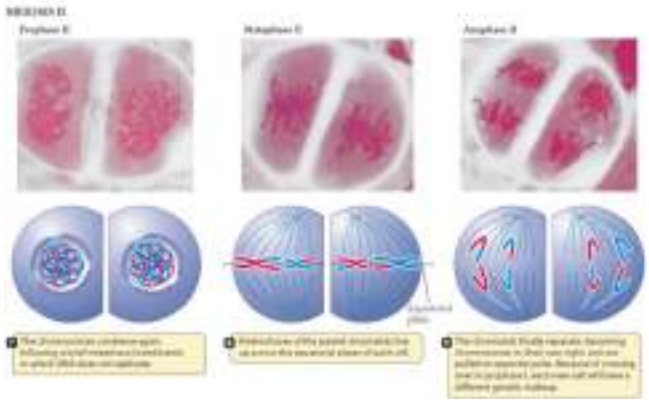


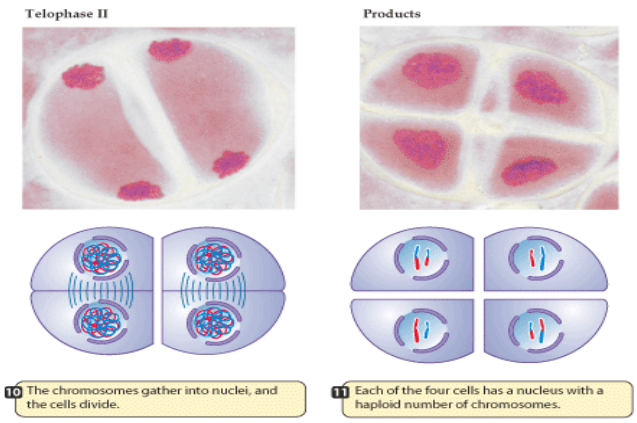
Genetically Diverse Chromosomes



Lecture 72

Meiosis II





Features

Reduces chromosome number from diploid to haploid

Ensures each of the haploid products has a complete set of chromosomes

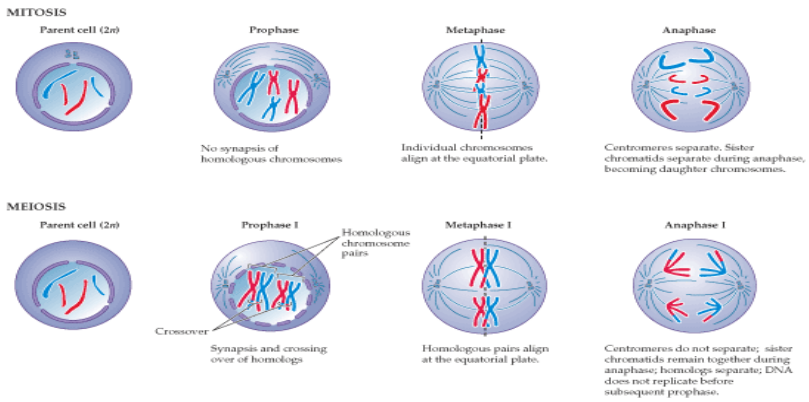
Promotes genetic diversity among the products.

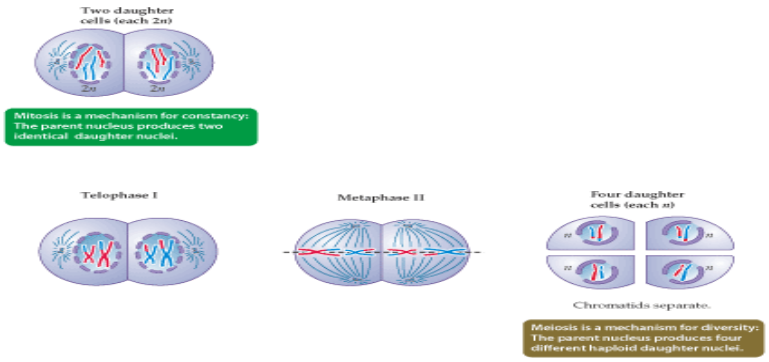
Lecture 73

Meiosis II

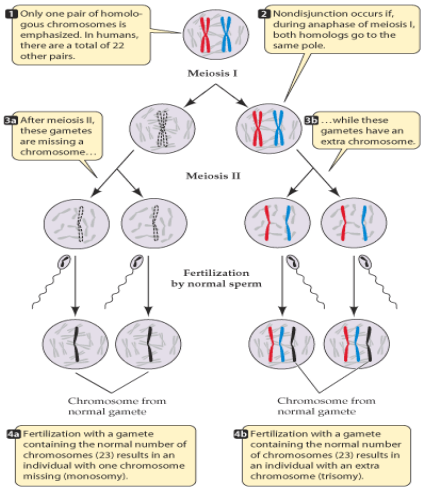
Lecture 74

The Comparison





The Common Mistake



Lecture 75

Replication of DNA

The double-helical model for DNA includes the concept that the two strands are complementary.

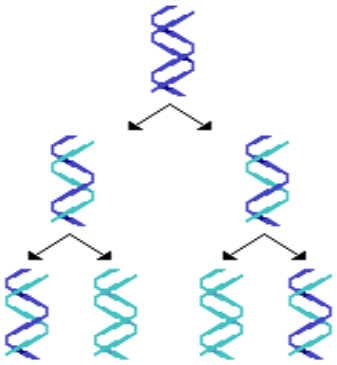
Thus, each strand can in principle serve as the template for making its own partner.

A number of models were proposed to explain the mode of replication of DNA.

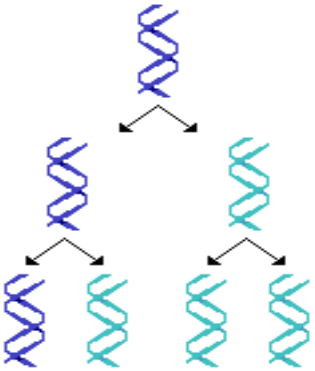
But the semiconservative model for DNA replication is the correct one.

The Watson–Crick model for DNA replication proposed that the two parental strands separate and that each then serves as a template for a new progeny strand.

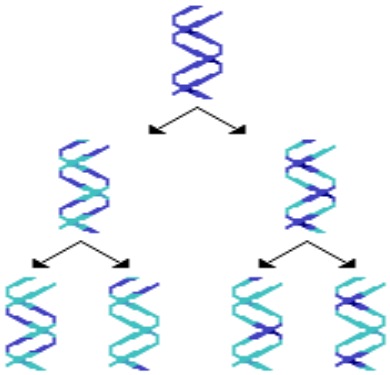
This is called semiconservative replication because each daughter duplex has one parental strand and one new strand which means that one of the parental strands is “conserved” in each daughter duplex.



Another potential mechanism is conservative replication, in which the two parental strands stay together and somehow produce another daughter helix with two completely new strands.



Yet another possibility is dispersive replication, in which the DNA becomes fragmented so that new and old DNAs coexist in the same strand after replication.



Lecture 76

Experiment of Meselson & Stahl

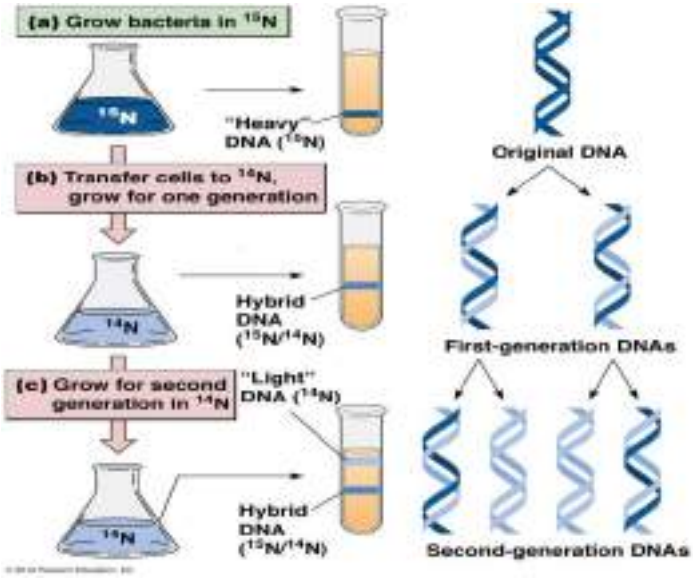
In 1958, Matthew Meselson and Franklin Stahl performed a classic experiment to distinguish among these three possibilities.

They labeled E. coli DNA with heavy nitrogen (15N) by growing cells in a medium enriched in this nitrogen isotope.

This made the DNA denser than normal.

Then they switched the cells to an ordinary medium containing primarily 14N, for various lengths of time.

Finally, they subjected the DNA to density gradient centrifugation to determine the density of the DNA.



Lecture 77

Chemistry of DNA Synthesis

Two key substrates are required for the synthesis of DNA to proceed

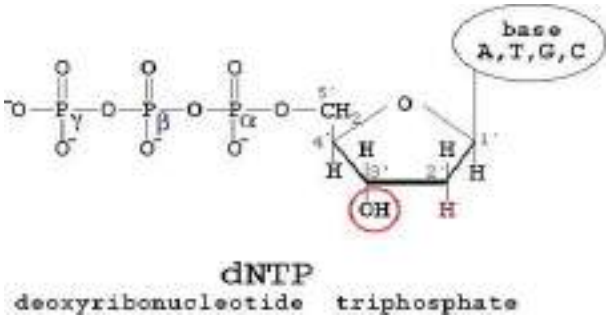
Deoxynucleoside triphosphates

Primer:template junction

Four deoxynucleoside triphospahtes namely dGTP, dCTP, dATP & dTTP are required.

Nucleoside triphosphates have three phosphoryl groups attached to the 5’ hydroxyl of deoxyribose.

The innermost phosphoryl group is called the α-phosphate whereas the middle and outermost groups are called β- and ɣ- phosphates.



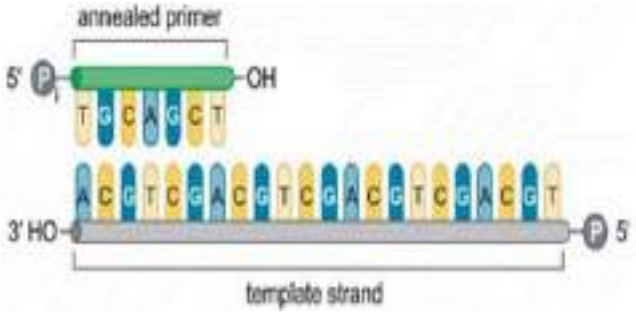
The second important substrate for DNA synthesis is a particular arrangement of single stranded DNA (ssDNA) and double stranded DNA (dsDNA).

This particular arrangement is called a primer:template junction.

It has two components:-

The Template

The Primer

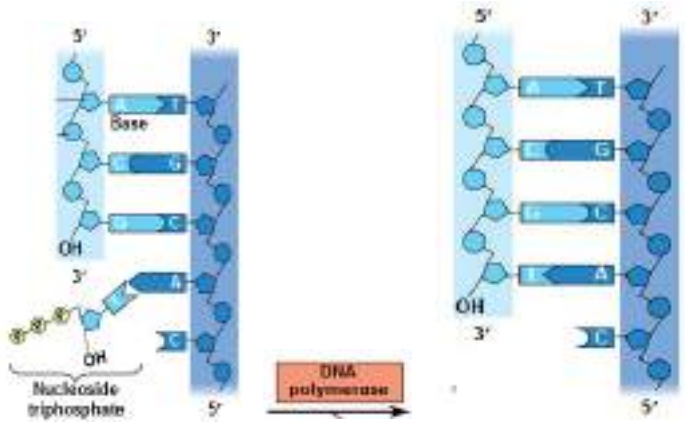


The new chain of DNA grows by extending the 3’ end of the primer.

The phosphodiester bond is formed in an SN2 reaction.

In this reaction, the hydroxyl group of the 3’ end of the primer attacks the α-phosphoryl group of the incoming nucleoside triphosphate.

The leaving group of the reaction is pyrophosphate which arises from the release of β- and ɣ- phosphates of the nucleoside.



The template strand directs which of the four nucleoside triphosphates is added.

The incoming nucleoside triphosphate base pairs with the template strand.

What is the driving force for the addition of nucleotide to a growing polynucleotide chain?

The free energy for this reaction is provided by the rapid hydrolysis of the pyrophosphate into two phosphate groups by an enzyme known as pyrophosphatase.

The net result of nucleotide addition and pyrophosphate hydrolysis is the simultaneous breaking of two high energy phosphate bonds.

Photo draw

Therefore, DNA synthesis is a coupled process.

This reaction is highly favourable with high value of Keq which means that its an irreversible reaction.

Lecture 78

Chemistry of DNA synthesis

Lecture 79

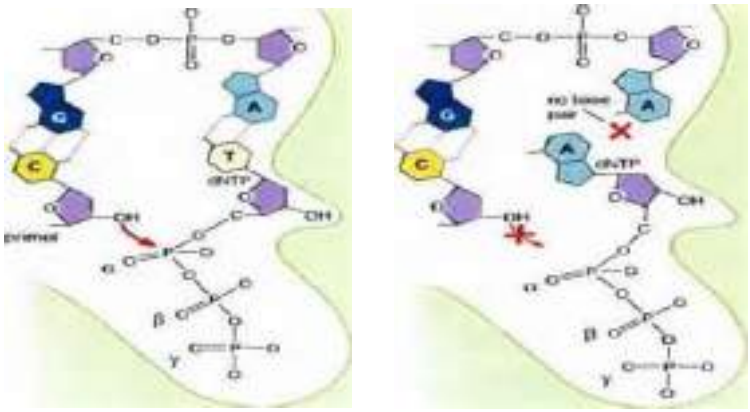
Mechanism of DNA Polymerase

The synthesis of DNA is catalyzed by an enzyme DNA polymerase.

It uses a single active site to catalyze the addition of any of four deoxynucleoside triphosphates.

DNA polymerase monitors the ability of the incoming nucleotide to form an A:T or G:C base pair, rather than detecting the exact nucleotide that enters the active site.

Only when a correct nucleotide comes, the 3'-OH of the primer and the α-phosphate of the nucleotide align in optimum position for catalysis to take place.



Incorrect base pairing leads to dramatically lower rate of nucleotide addition as a result of catalytically unfavourable alignment of these substrates.

DNA polymerase shows an impressive ability to distinguish between ribonucleoside

(rNTPs) and deoxyribonucleoside triphosphates (dNTPs).

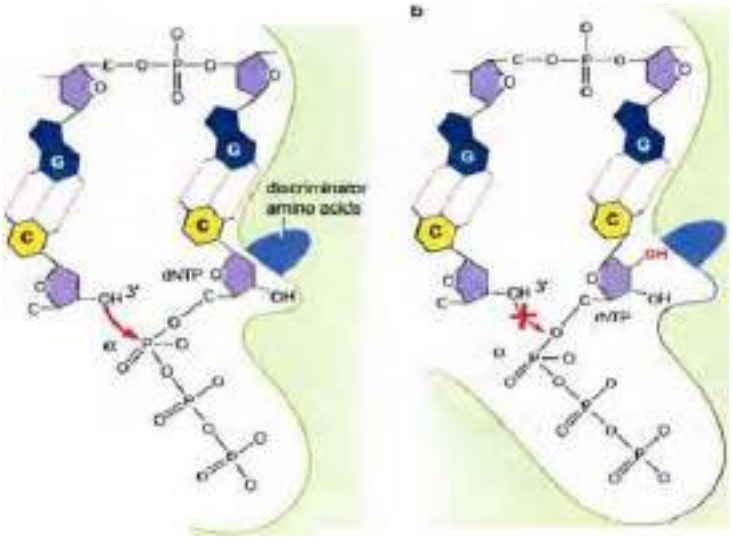
Although rNTPs are present at approx. ten-fold higher concentration in the cell, yet their incorporation rate is 1000-folds lower than dNTPs.

This discrimination is mediated by the steric exclusion of rNTPs from the active site of DNA polymerase.

In DNA polymerase, the nucleotide-binding pocket cannot accommodate a 2’-OH on the in-coming nucleotide.

This space is occupied by two amino acids that make van der Waals contacts with the deoxyribose ring.

These amino acids are called discriminator amino acids.



Lecture 80

THE REPLICATION FORK

In the cell, both strands of the DNA duplex are replicated at the same time.

So it requires separation of the two strands of the double helix to create two template DNAs.

The junction between the newly separated template strands and the unreplicated duplex DNA is known as the Replication Fork.

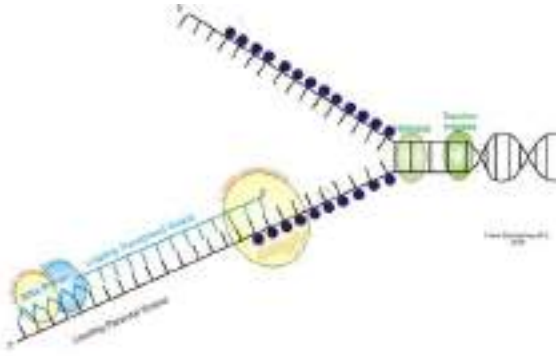
The replication fork moves continuously towards the duplex region of unreplicated DNA.

As the fork moves, it creates two ssDNA templates that each directs the synthesis of a complementary DNA strand.

The antiparallel nature of DNA creates a complication for the simultaneous replication of the two exposed templates at the replication fork.

Because DNA is synthesized only by elongating a 3’ end, only one of the two exposed templates can be replicated continuously as the replication fork moves.

The newly synthesized DNA strand directed by this template is known as the leading strand.



Synthesis of the newDNA strand directed by the other ssDNA template is more complicated.

This template directs the DNA polymerase to move in the opposite direction of the replication fork.

The new DNA strand directed by this template is known as the lagging strand.

This strand of DNA must be synthesized in a discontinuous fashion.

Synthesis of the lagging strand must wait for movement of the replication fork to expose a substantial length of template before it can be replicated.

Each time a substantial length of the template is exposed, DNA synthesis is initiated and continues until it reaches the 5’ end of the previous newly synthesized fragment of lagging strand DNA.

The resulting short fragments of new DNA formed on the lagging strand are called Okazaki fragments.

They vary in length from 1000 to 2000 nucleotides in bacteria and from 100 to 400 nucleotides in eukaryotes.

Photo lagging strand

Shortly after being synthesized, Okazaki fragments are covalently joined together to generate a continuous, intact strand of new DNA.

Okazaki fragments are therefore transient intermediates in DNA replication.

Lecture 81

THE RNA PRIMER

All DNA polymerases require a primer with a free 3’-OH.

They cannot initiate the synthesis of new DNA strand de novo.

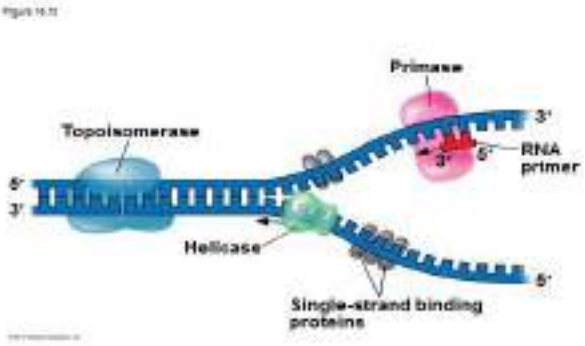
Then how?

To accomplish this, the cell takes advantage of the ability of RNA polymerases to do what DNA polymerases cannot:

start new RNA chains de novo.

Primase is a specialized RNA polymerase dedicated to making short RNA primers (5–10 nucleotides long) on a ssDNA template.

These primers are then extended by DNA polymerase.



Both the leading and lagging strands require primase to initiate DNA synthesis.

Each leading strand requires only a single RNA primer.

The discontinuous synthesis of the lagging strand means that new primers are needed for each Okazaki fragment.

Synthesis of the lagging strand can require hundreds of Okazaki fragments and their associated RNA primers.

Primase activity is dramatically increased when it associates with another protein that acts at the replication fork called DNA Helicase.

This protein unwinds the DNA at the replication fork, creating an ssDNA template that can be acted on by primase.

Lecture 82

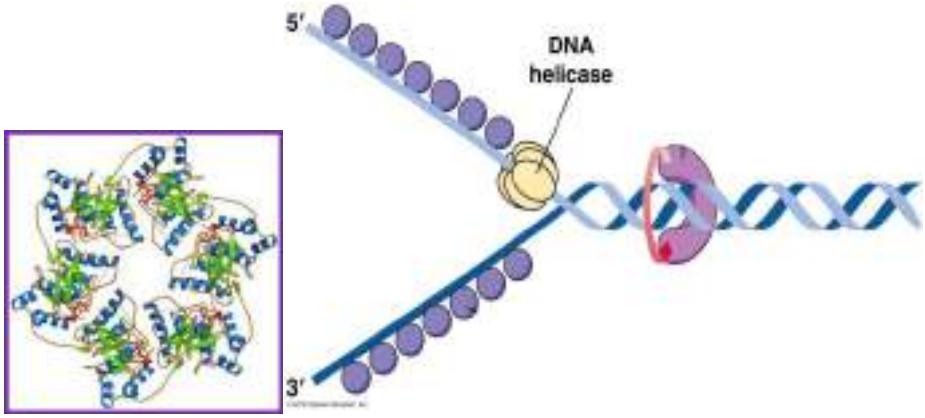
THE DNA HELICASE

DNA polymerases are unable to separate the two strands of duplex DNA.

Therefore a third class of enzymes, called DNA Helicases catalyze the separation of the two strands of duplex DNA at the replication fork.

DNA helicases are hexameric proteins that assume the shape of a ring.

This ring encircles one of the two single strands at the replication fork adjacent to the single-stranded:double-stranded junction.



DNA helicases found at replication forks exhibit high processivity because they encircle the DNA.

They associate with the DNA and unwind multiple base pairs of DNA.

Release of the helicase from the DNA therefore requires the opening of the hexameric protein ring, which is a rare event.

However, the helicase can dissociate when it reaches the end of the DNA strand.

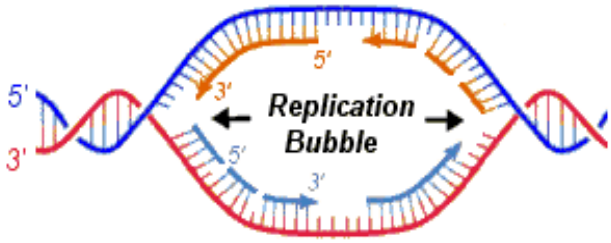
Of course, this arrangement of enzyme and DNA poses problems for the binding of the DNA helicase to the DNA strand in the first place.

Thus, there are specialized mechanisms that open the DNA helicase (hexameric) ring and place it around the DNA before re-forming the ring.

Each DNA helicase moves along ssDNA in a defined direction.

This property is referred to as the polarity of the DNA helicase.

DNA helicases can have a polarity of either 5’ 3’ or 3’ 5’. This direction is always defined according to the strand of DNA bound rather than the strand that is displaced.



Lecture 83

TOPOISOMERASES

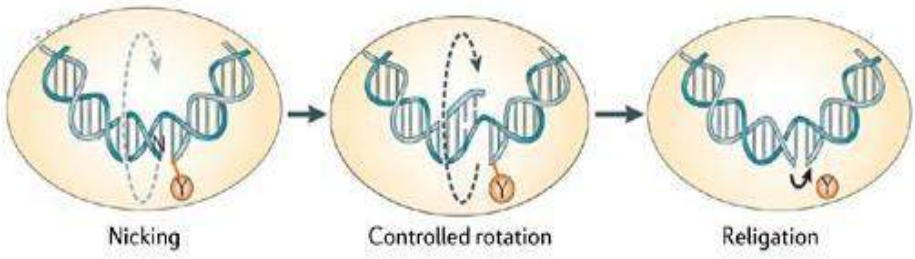
The action of DNA helicase results in the introduction of supercoils in the DNA duplex just ahead of the replication fork.

As the strands of DNA are separated at the replication fork, the dsDNA in front of the fork becomes increasingly positively supercoiled.

If there were no mechanism to relieve the accumulation of these supercoils, the replication machinery would grind to a halt due to the mounting strain placed on the DNA in front of the replication fork.

These supercoils are removed by the action of Topoisomerases that act on the unreplicated dsDNA in front of the replication fork.

These enzymes do this by breaking either one or both strands of the DNA without letting go of the DNA and passing the same number of DNA strands through the break.



This action relieves the accumulation of supercoils.

In this way, topoisomerases act as a “swivelase” that prevents the accumulation of supercoils ahead of the replication fork.

Lecture 84

INITIATION OF REPLICATION

The initial formation of a replication fork requires the separation of the two strands of the DNA duplex to provide the ssDNA.

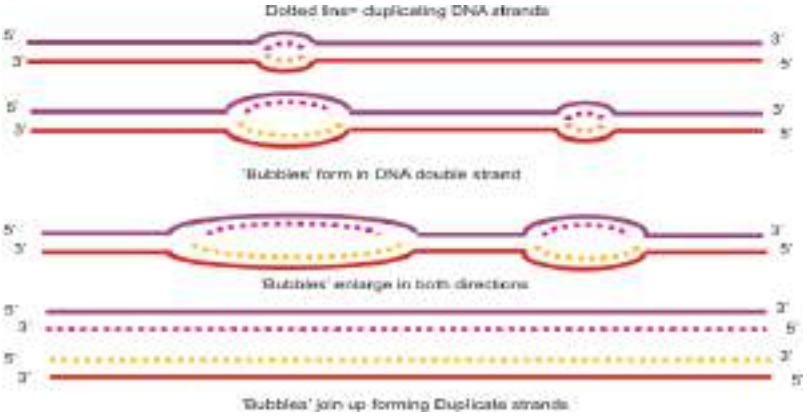
ssDNA is required for DNA helicase binding and to act as a template for thesynthesis of both the RNA primer and new DNA.

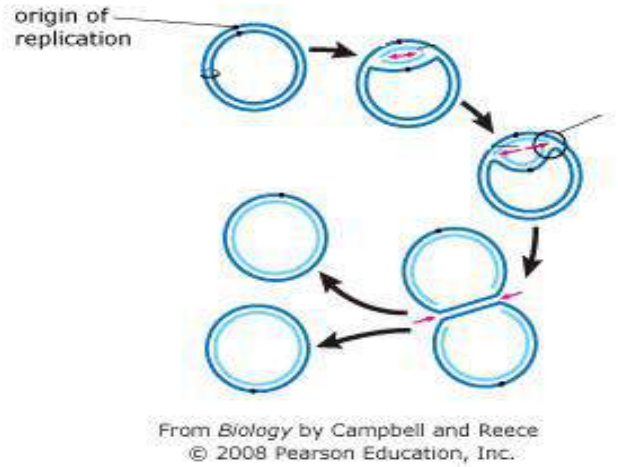
Although DNA strand separation (DNA unwinding) is most easily accomplished at chromosome ends, but DNA synthesis generally initiates at internal regions.

As the circular chromosomes lack the chromosome ends so it makes internal DNA unwinding essential for the replication initiation.

The specific sites at which DNA unwinding and initiation of replication occur are called Origins of Replication.

Depending on the organism, there may be as few as one or as many as thousands of origins per chromosome.





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Lecture 85

THE REPLICON MODEL

How the initiation of replication takes place?

It was explained by Francois Jacob, Sydney Brenner and Jacques Cuzin in 1963.

This is called as The Replicon Model of replication Initiation.

They defined all of the DNA replicated from a particular origin of replication as a replicon.

As the single chromosome found in E. coli cells has only one origin of replication, the entire chromosome is a single replicon.

In contrast, the presence of multiple origins of replication divides each eukaryotic chromosome into multiple replicons; one for each origin of replication.

The replicon model proposed two components that controlled the initiation of replication; the replicator and the initiator.

The replicator is defined as the cis-acting DNA sequences that are sufficient to direct the initiation of DNA replication.

This is in contrast to the origin of replication, which is the physical site on the DNA where the DNA is unwound and DNA synthesis initiates.

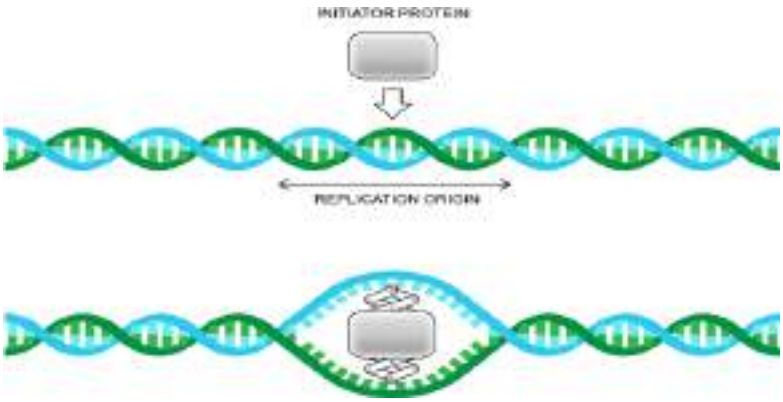
Although the origin of replication is always part of the replicator, sometimes the origin of replication is only a fraction of the DNA sequences required to direct the initiation of replication (the replicator).

The second component of the replicon model is the initiator protein.

This protein specifically recognizes a DNA element in the replicator and activates the initiation of replication.

All initiator proteins select the sites that will become origins of replication.

The initiator protein is the only sequence-specific DNA binding protein involved in the initiation of replication.



All the remaining proteins other than initiator protein, required for replication initiation do not bind to a DNA sequence specifically

Lecture 86

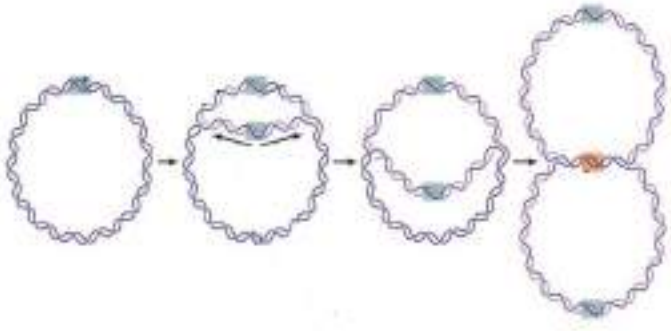
FINISHING REPLICATION

Completion of DNA replication requires a set of specific events.

These events are different for circular chromosomes and linear chromosomes.

In case of circular chromosome, the conventional replication fork machinery replicates the entire molecule, but the resulting daughter molecules

are topologically linked to each other.



While in case of linear chromosome, the replication fork machinery cannot complete replication of the very ends of linear chromosomes

Therefore, organisms containing linear chromosomes have developed novel strategies to replicate their chromosome ends.

After replication of a circular chromosome is complete, the resulting daughter DNA molecules remain linked together as catenanes.

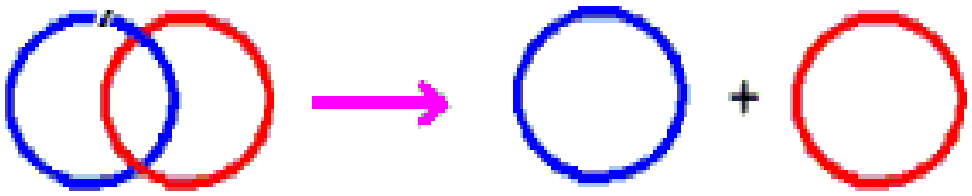
To segregate these chromosomes into separate daughter cells, the two circular DNA molecules must be disengaged from each other or “decatenated.”

This separation is accomplished by the action of Type II Topoisomerases.

Lecture 87

TYPE II TOPOISOMERASES

Type II Topoisomerases are the enzymes which have the ability to break a dsDNA molecule and pass a second dsDNA molecule through this break



So this reaction can easily decatenate the two circular daughter chromosomes and allow their segregation into separate cells.

The activity of type II topoisomerases is also critical to the segregation of large linear molecules.

Although there is no inherent topological linkage after the replication of a linear molecule, the large sized chromosomes necessitates the intricate folding of the DNA into loops which are attached to a protein scaffold.

These attachments lead to many of the same problems that circular chromosomes have after replication.

So type II topoisomerases allow these linked DNAs to be separated.

So as in the case of circular chromosomes, type II topoisomerases also allow these linked DNAs to be separated.

Lecture 88

TELOMERASE

The requirement for an RNA primer to initiate all new DNA synthesis creates a dilemma for the replication of the ends of linear chromosomes.

This is called as the end replication problem.

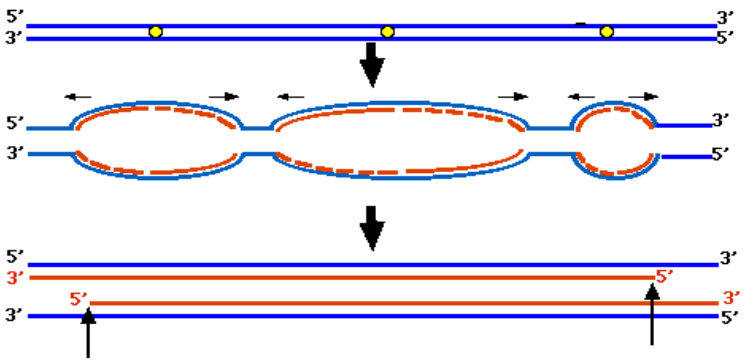
This difficulty is not observed during the duplication of the leading-strand template.

Because it requires only one RNA primer which completes the DNA synthesis up to extreme terminus of the strand.

In contrast, the requirement for multiple primers to complete lagging-strand synthesis means that a complete copy of its template cannot be made.

Even if the end of the last RNA primer for Okazaki fragment synthesis anneals to the final base of the lagging-strand template.

Once this RNA molecule is removed, there will remain a short region (the size of the RNA primer) of un-replicated ssDNA at the end of the chromosome.



Telomerase is a remarkable enzyme that includes multiple protein subunits and an RNA component.

Like all other DNA polymerases, telomerase acts to extend the 3’ end of its DNA substrate.

But unlike most DNA polymerases, telomerase does not need an exogenous DNA template to direct the addition of new dNTPs.

Instead, the RNA component of telomerase serves as the template for adding the telomeric sequence to the 3’ terminus at the end of the chromosome

Telomerase specifically elongates the 3’-OH of telomeric ssDNA sequences using its own RNA as a template.

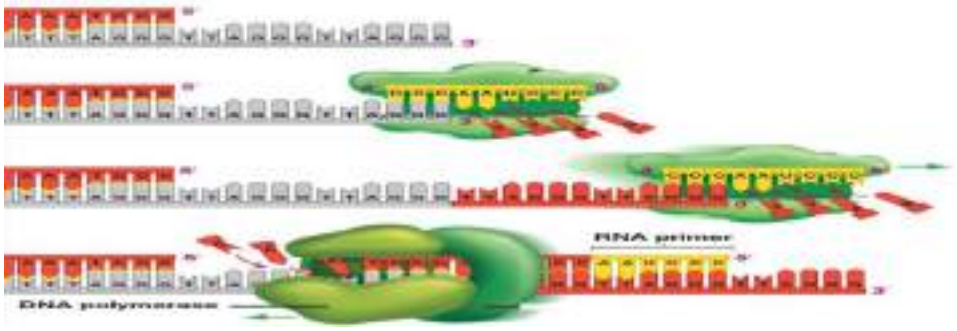
As a result, the newly synthesized DNA is single-stranded.

So when telomerase acts on the 3’ end of the telomere, it extends only one of the two strands of the chromosome.

How is then 5’ end extended?

This is accomplished by the lagging-strand DNA replication machinery.

By providing an extended 3’ end, telomerase provides additional template for the lagging-strand replication machinery.By synthesizing and extending RNA primers using the telomerase extended 3’ end as a template, the cell can effectively increase the length of the 5’ end of the chromosome as well.



Lecture 89

The Central Dogma

The central dogma outlines the flow of genetic information during growth and division of the cells.

Genetic information flows from DNA to RNA to protein during cell growth.

In addition, all living cells must replicate their DNA when they divide.

During cell division each daughter cell receives a copy of the genome of the parent cell.

Replication is the process by which two identical copies of DNA are made from an original molecule of DNA.

So Replication occurs in the cells prior to cell division.

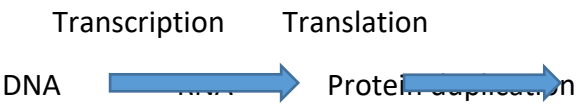
An important point is that information does not flow from protein to RNA or DNA.

However, flow of information from RNA “backwards” to DNA is possible in certain special circumstances due to the operation of reverse transcriptase.

By the end of 1953, the working hypothesis was adopted that chromosomal DNA functions as the template for the synthesis of RNA molecules.

These RNA molecules, the subsequently move to the cytoplasm, where they determine the arrangement of amino acids within proteins.

In 1956, Francis Crick referred to this pathway for the flow of genetic information as the Central Dogma.



An important point in the above equation is that the two arrows are unidirectional which means that RNA sequences are never determined by protein templates nor was DNA then imagined ever to be made on RNA templates.

The idea that proteins never serve as templates for RNA has stood the test of time.

However, RNA chains sometimes do act as templates for the synthesis of DNA chains of complementary sequence.

Such reversals of the normal flow of information are very rare events compared with the enormous number of RNA molecules made on DNA templates.

Thus, the central dogma as originally proclaimed more than 50 years ago still remains essentially valid.

Lecture 90

Synthesis of RNA upon DNA Templates

As mRNA was being discovered, the first of the enzymes that synthesize (or transcribe) RNA using DNA templates was being independently isolated in the labs of

biochemists Jerard Hurwitz and Samuel B. Weiss.

These enzymes called RNA Polymerases function only in the presence of DNA, which serves as the template upon which single-stranded RNA chains are made, and use the nucleotides ATP, GTP, CTP, and UTP as precursors.

These enzymes make RNA using appropriate segments of chromosomal DNA as their templates.

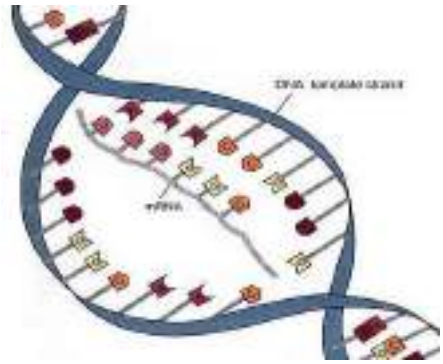
Direct evidence that DNA lines up the correct ribonucleotide precursors came from seeing how the RNA base composition varied with the addition of DNA molecules of different AT/GC ratios

In every enzymatic synthesis, the RNA AU/GC ratio was roughly similar to the DNA's AT/GC ratio.

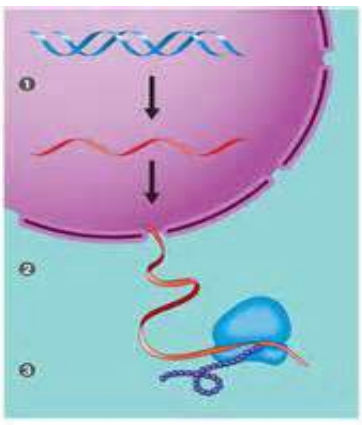
During transcription, only one of the two strands of DNA is used as a template to make RNA.

This makes sense, because the messages carried by the two DNA strands, being complementary but not identical, are expected to code for completely different polypeptides.

The synthesis of RNA always proceeds in a fixed direction, beginning at the 5' end and concluding with the 3'-end nucleotide.



By this time, there was firm evidence for the postulated movement of RNA from the DNA-containing nucleus to the ribosome-containing cytoplasm of eukaryotic cells.



By briefly exposing cells to radioactively labelled precursors, then adding a large excess of unlabeled ribonucleotides (a “pulse chase” experiment), mRNA synthesized during a short time window was labelled.

These studies showed that mRNA is synthesized in the nucleus.

Within an hour, most of this RNA had left the nucleus and was observed in the cytoplasm.

Lecture 91

TRANSCRIPTION

Up to this point, we have learnt that how the genome has been maintained i,e., how the genetic material is organized, protected, and replicated.

We now turn to the question of how that genetic material is expressed—that is, how the series of bases in the DNA directs the production of the RNAs and proteins that perform cellular functions and define cellular identity

The basic processes responsible for gene expression include transcription, RNA processing, and translation.

Transcription is chemically and enzymatically, very similar to DNA replication.

Both involve enzymes that synthesize a new strand of nucleic acid complementary to a DNA template strand.

However, the main difference is that in case of transcription, the new strand is made from ribonucleotides rather than deoxyribonucleotides.

There are some other important differences include:-

RNA Polymerase (the enzyme that catalyzes RNA synthesis) does not need a primer; rather, it can initiate transcription de novo.

The RNA product does not remain base-paired to the template DNA strand.

Transcription is less accurate than replication (one mistake/10,000 nucleotides, compared with one / 10 million). This difference reflects the lack of extensive proofreading mechanisms for transcription.

Transcription selectively copies only certain parts of the genome and makes any number of copies of that section. In contrast, replication must copy the entire genome and do so once (and only once) every cell division.

Lecture 92

RNA POLYMERASES

RNA polymerase performs essentially the same reaction in all cells, from bacteria to humans that is the synthesis of RNA.

From bacteria to mammals, the cellular RNA polymerases are made up of multiple subunits.

Bacteria have only a single RNA polymerase, whereas eukaryotic cells have three: RNA polymerases I, II, and III (RNA Pol I, II, and III).

Recently, two more DNA-dependent RNA polymerases have been identified in recent years, and have been called as Pol IV and Pol V.

These are found only in plants, where they transcribe small interfering RNAs.

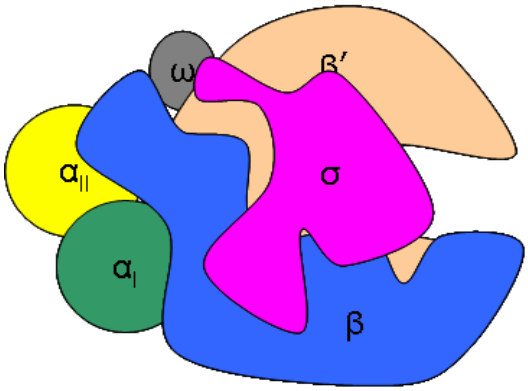
The bacterial RNA polymerase core enzyme alone is capable of synthesizing RNA and comprises two copies of the □ subunit and one each of the β, β' and □ subunits. This enzyme is closely related to the eukaryotic polymerases.

The structure of a bacterial RNA polymerase core enzyme is similar to that of the yeast Pol II enzyme.

Overall, the shape of each enzyme resembles a crab claw.

This is reminiscent of the "hand" structure of DNA polymerases.

The two pincers of the crab claw are made up predominantly of the two largest subunits of each enzyme (β and β').



The active site, which is made up of regions from both these subunits, is found at the base of the pincers within a region called the "active center cleft".

Lecture 93

Transcription Proceeds in Series of Steps

To transcribe a gene, RNA polymerase proceeds through a series of well defined steps grouped into three phases:

Initiation

Elongation

Termination

Initiation

A promoter is the DNA sequence that initially binds to the RNA polymerase (together with any initiation factors required).

Once formed, the promoter– polymerase complex undergoes structural changes required for initiation to proceed.

Then the DNA around the point where transcription will start unwinds.

The base pairs are disrupted, producing a “transcription bubble” of single-stranded DNA.

Again, like DNA replication, transcription always occurs in a 5’-to-3’ direction: the new ribonucleotide is added to the 3’ end of the growing chain.

However, unlike replication, only one of the DNA strands acts as a template on which the RNA strand is built.

The initiation can itself be broken down into a series of defined steps.

The first step is the initial binding of polymerase to a promoter to form what is called a closed complex.

In this form, the DNA remains double-stranded, and the enzyme is bound to one face of the helix

In the second step of initiation, the closed complex undergoes a transition to the open complex in which the DNA strands separate over a distance of 13 bp around the start site to form the transcription bubble.

In the next stage of initiation, polymerase enters the phase of initial transcription followed by promoter escape.

The opening up of the DNA frees the template strand.

The first two ribonucleotides are brought into the active site, aligned on the template strand, and joined together.

In the same way, subsequent ribonucleotides are incorporated into the growing RNA chain.

Incorporation of the first 10 or so ribonucleotides is a rather inefficient process, and at that stage, the enzyme often releases short transcripts (each of less than 10 or so nucleotides) and then begins synthesis again.

In this phase, the polymerase–promoter complex is called the initial transcribing complex.

Once an enzyme makes a transcript longer than 10 nucleotides, it is said to have escaped the promoter.

At this point, it has formed a stable ternary complex, containing enzyme, DNA, and RNA.

This is the transition to the elongation phase.

Lecture 94

Transcription Proceeds in Series of Steps

Elongation

Once the RNA polymerase has synthesized a short stretch of RNA (10 bases), it shifts into the elongation phase.

During elongation, the enzyme performs an impressive range of tasks in addition to the catalysis of RNA synthesis.

1) It unwinds the DNA in front and re-anneals it behind.

2) It dissociates the growing RNA chain from the template as it moves along.

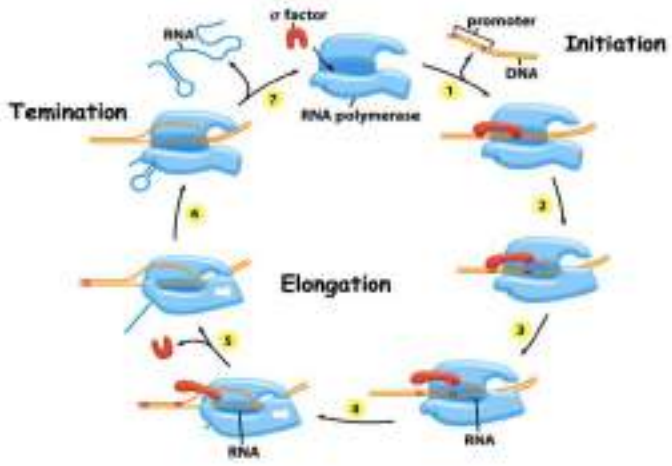
3) And it performs the proofreading functions.

Recall that during replication, in contrast, several different enzymes are required to catalyze a similar range of functions.

Termination

Once the polymerase has transcribed the length of the gene (or genes), it must stop and release the RNA product (as well as dissociating from the DNA itself). This step is called termination.

In some cells, specific, well characterized sequences trigger termination. In others, it is less clear what instructs the enzyme to cease transcribing and dissociate from the template.



Lecture 95

Transcription Cycle in Bacteria

The bacterial core RNA polymerase can, in principle, initiate transcription at any point on a DNA molecule.

It, however, initiates transcription only at promoters.

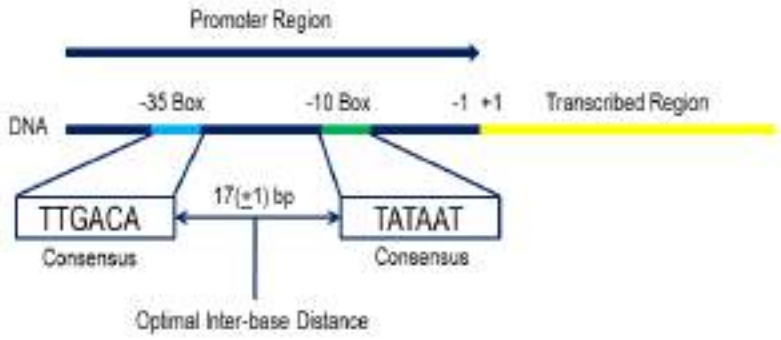
It is the addition of an initiation factor called σ that converts core enzyme (α2 ββ'□) into the form that initiates only at promoters. This form of the enzyme is called the RNA polymerase holoenzyme.

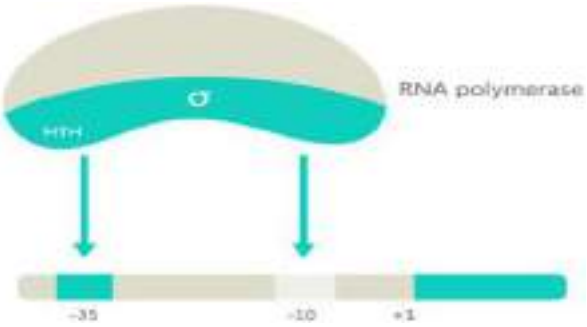
In the case of Escherichia coli, the predominant σ factor is called σ70.

Promoters recognized by polymerase have two conserved sequences each of 6 nucleotides, separated by a nonspecific stretch of 17–19 nucleotides.

The two defined sequences are centered, respectively, at ~10 bp and at ~35 bp upstream of the site where RNA synthesis starts.

The sequences are thus called the minus 35 and minus 10 regions or element.





The σ70 factor can be divided into four regions called σ region 1 through σ region 4.

The regions that recognize the –10 and –35 elements of the promoter are regions 2 and 4, respectively.

Two helices within region 4 form a common DNA-binding motif called a helix-turn-helix.

One of these helices inserts into the major groove and interacts with bases in the –35 region.

The other helix lies across the top of the groove, making contacts with the DNA backbone.

Helix-turn-helix

The –10 region has a more elaborate role in transcription initiation, because it is within that element that DNA melting is initiated in the transition from the closed to the open complex.

Thus, the region of σ that interacts with the –10 region is doing more than simply binding DNA.

The a helix involved in recognition of the –10 region contains several essential aromatic amino acids that can interact with bases on the non-template strand in a manner that stabilizes the melted DNA.

Two bases in the non-template strand are flipped out and inserted into pockets within the σ protein where they make favorable contacts that stabilize the unwound state of the promoter region.

The σ subunit is positioned within the holoenzyme structure in such a way as to make feasible the recognition of various promoter elements.

Thus, the DNA-binding regions point away from the body of the enzyme, rather than being embedded.

Lecture 96

Transition to the Open Complex

Transition to the Open Complex involves structural changes in RNA Polymerase and in the Promoter DNA.

The initial binding of RNA polymerase to the promoter DNA in the closed complex leaves the DNA in double-stranded form.

The next stage in initiation requires the enzyme to become more intimately engaged with the promoter, in the open complex.

The transition from the closed to the open complex involves structural changes in the enzyme and the opening of the DNA double helix to reveal the template and non-template strands.

This “melting” occurs between positions –11 and +2, with respect to the transcription start site.

In case of the bacterial enzyme bearing σ70, this transition is often called isomerization.

This transition does not require energy derived from ATP hydrolysis and is instead the result of a spontaneous conformational change in the DNA– enzyme complex to a more energetically favourable form.

Two bases in the non-template strand of the –10 element flip out from their base-stacking interactions and instead insert into pockets within the σ protein where they make more favourable interactions.

By stabilizing the single-stranded form of the –10 element, these interactions drive melting of the promoter region.

Isomerization is essentially irreversible and, once complete, typically guarantees that transcription will subsequently initiate.

Formation of the closed complex, in contrast, is readily reversible.

The examination of the structure of the holoenzyme in more detail reveals the active site of the enzyme, which is made up of regions from both the β and β’ subunits, is found at the base of the pincers within the active center cleft.

Lecture 97

Transition to the Open Complex

There are five channels into the enzyme, as shown in the illustration of the open complex.

The NTP-uptake channel allows ribonucleotides to enter the active center.

The RNA-exit channel allows the growing RNA chain to leave the enzyme as it is synthesized during elongation.

The remaining three channels allow DNA entry and exit from the enzyme.

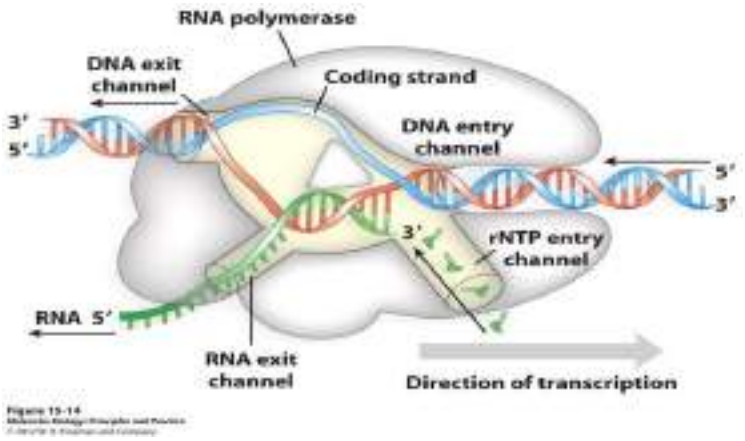
The downstream DNA (i.e., DNA ahead of the enzyme, yet to be transcribed) enters the active center cleft in double-stranded form through the downstream DNA channel (between the pincers).

Within the active center cleft, the DNA strands separate from position +3.

The non-template strand exits the active center cleft through the non-template-strand (NT) channel and travels across the surface of the enzyme.

The template strand, in contrast, follows a path through the active center cleft and exits through the template-strand (T) channel.

The double helix re-forms at –11 in the upstream DNA behind the enzyme.



Two striking structural changes are seen in the enzyme upon isomerization from the closed to the open complex.

First, the pincers at the front of the enzyme clamp down tightly on the downstream DNA.

Second, there is a major shift in the position of the amino-terminal region of σ.

When not bound to DNA, σ region 1.1 lies within the active center cleft of the holoenzyme, blocking the path that, in the open complex, is followed by the template DNA strand.

In the open complex, region 1.1 shifts some 50A˚ and is now found on the outside of the enzyme, allowing the DNA access to the cleft.

Region 1.1 of σ is highly negatively charged (just like DNA). Thus, in the holoenzyme, region 1.1 acts as a molecular mimic of DNA.

The space in the active center cleft, which may be occupied either by region 1.1 or by DNA, is highly positively charged.

Lecture 98

Initial Transcription

RNA polymerase can initiate a new RNA chain on a DNA template and thus does not need a primer.

This requires that the DNA template be brought into the polymerase active site and held stably in a helical conformation.

And that the initiating ribonucleotide be brought into the active site and held stably on the template while the next NTP is presented with correct geometry for the polymerization to take place.

This is particularly difficult because RNA polymerase starts most transcripts with an A, and that ribonucleotide binds the template nucleotide (T) with only two hydrogen bonds.

Thus, the enzyme has to make specific interactions with the DNA template strand, the initiating ribonucleotide, and the second ribonucleotide to hold them all rigidly in the correct orientation to allow chemical attack by the incoming NTP.

This is the reason that most transcripts start with the same nucleotide.

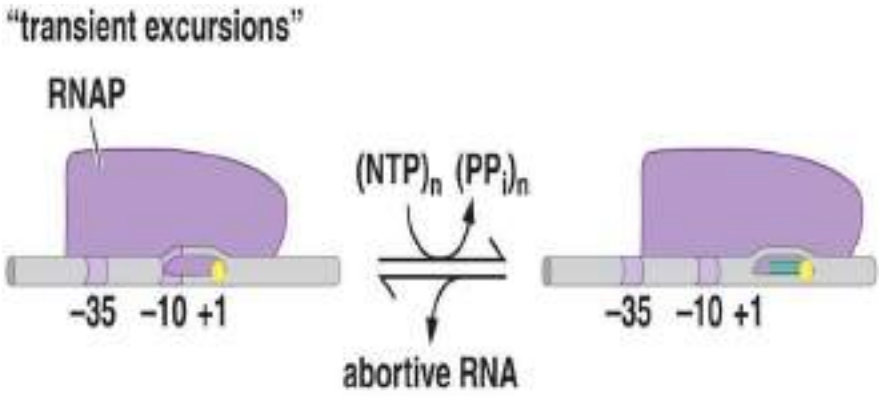
During initial transcription, RNA polymerase produces and releases short RNA transcripts of <10 nucleotides (abortive synthesis) before escaping the promoter, entering the elongation phase, and synthesizing the proper transcript.

It has long been unclear how the enzyme's active site translocates along the DNA template during initial abortive cycles of transcription.

Three general models were proposed to explain this.

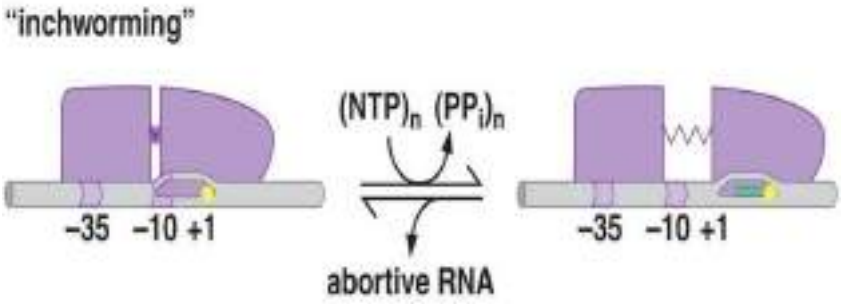
1.The "transient excursion" model proposes transient cycles of forward and reverse translocation of RNA polymerase.

Thus, polymerase is thought to leave the promoter and translocate a short way along the DNA template, synthesizing a short transcript before aborting transcription, releasing the transcript, and returning to its original location on promoter.



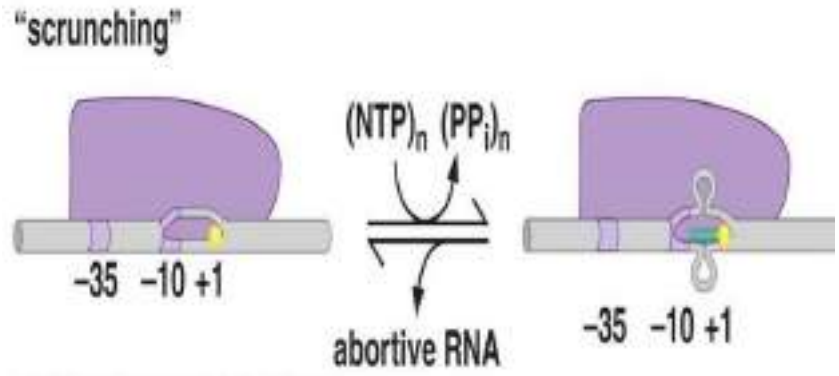
2. "Inchworming" invokes a flexible element within the polymerase that allows a module at the front of the enzyme, containing the active site.

The module moves downstream, synthesizing a short transcript before aborting and retracting to the body of the enzyme still at the promoter.



3. "Scrunching" proposes that DNA downstream from the stationary, promoter-bound, polymerase is unwound and pulled into the enzyme.

The DNA thus accumulated within the enzyme is accommodated as single-stranded bulges.



It is now believed that the third model—scrunching — reflects what actually happens.

The experiments have shown that during initial transcription, the polymerase remains stationary on the promoter, unwinds downstream DNA, and pulls that DNA into itself.

Only the scrunching model is consistent with these results.

Lecture 99

The Elongating Polymerase

The Elongating Polymerase Is a processive machine that synthesizes and proofreads RNA.

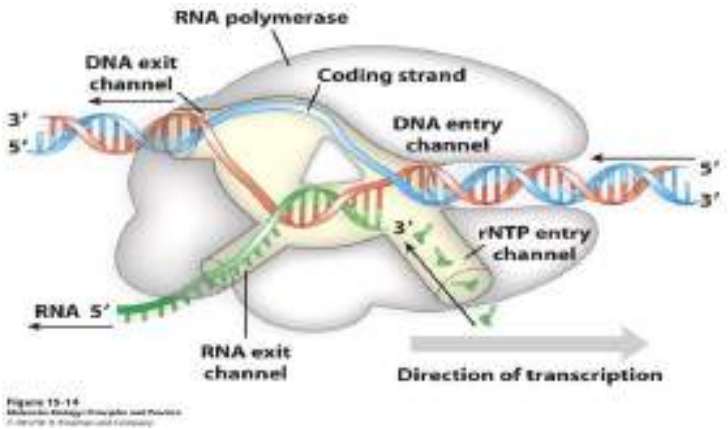
DNA passes through the elongating enzyme in a manner very similar to its passage through the open complex.

Thus, double-stranded DNA enters the front of the enzyme between the pincers.

At the opening of the catalytic cleft, the strands separate to follow different paths through the enzyme.

The strands exit via their respective channels and re-form a double helix behind the elongating polymerase.

Ribonucleotides enter the active site through their defined channel and are added to the growing RNA chain under the guidance of the template DNA strand.



Only 8 or 9 nucleotides of the growing RNA chain remain base-paired to the DNA template at any given time.

The remainder of the RNA chain is peeled off and directed out of the enzyme through the RNA exit channel.

During elongation, the enzyme adds one nucleotide at a time to the growing RNA transcript.

During elongation, polymerase uses a step mechanism: using single-molecule techniques.

It was shown that the enzyme steps forward as a molecular motor, advancing in a single step a distance equivalent to a base pair for every nucleotide it adds to the growing RNA chain.

In addition, the size of the bubble, i.e., the length of DNA that is not double-helical, remains constant throughout elongation.

1 bp is separated ahead of the processing enzyme, 1 bp is formed behind it.

As well as synthesizing the transcript, RNA polymerase performs two proofreading functions on that growing transcript.

The first of these is called pyrophosphorolytic editing.

In this, the enzyme uses its active site, in a simple back-reaction, to catalyze the removal of an incorrectly inserted ribonucleotide, by reincorporation of PPi.

The enzyme can then incorporate another ribonucleotide in its place in the growing RNA chain.

In the second proofreading mechanism, called hydrolytic editing, the polymerase backtracks by one or more nucleotides and cleaves the RNA product, removing the error-containing sequence.

Another group of proteins—the Nus proteins—joins polymerase in the elongation phase and promotes the processes of elongation and termination.

Lecture 100

Termination of Transcription

ii. Rho pulls RNA out of the polymerase, resulting in termination; or

iii. Rho induces a conformational change in polymerase, causing the enzyme to terminate the transcription.

Most recent experiments suggest that the last of the above may be correct and that the conformational change causes the elongating complex to stall, with dissociation following slowly.

Recent studies also have suggested that Rho binds to RNA polymerase throughout the transcription cycle.

Thus, Rho doesn’t reach polymerase by translocating along a nascent, rut-containing transcript like TRCF.

Rather, Rho binds polymerase early in transcription and then at some point also binds the RNA transcript being exuded from that elongating enzyme.

The role of translocation by Rho is thus perhaps to tighten the resulting RNA loop, and when sufficiently tight, polymerase elongation ceases.

How is Rho directed to work on particular RNA transcripts?

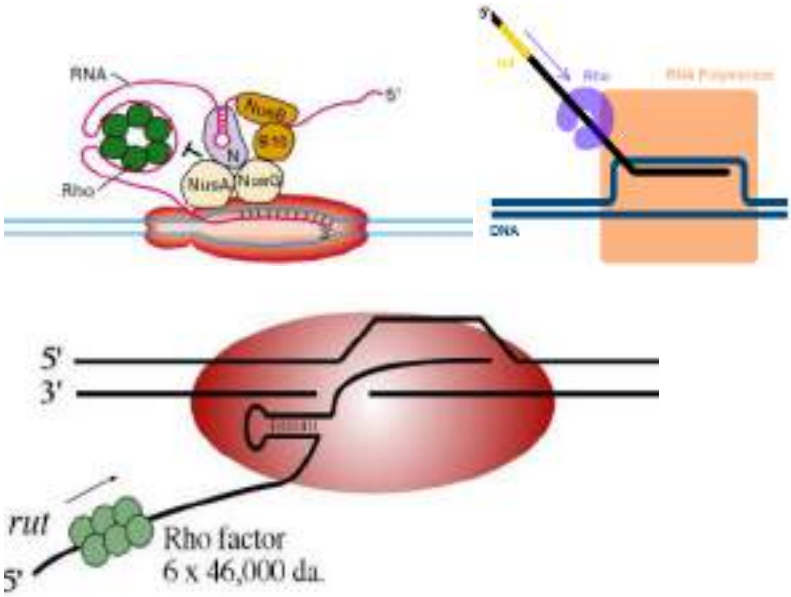
First, there is some specificity in the sites it binds (the rut sites).

These sites consist of stretches of ~40 nucleotides that do not fold into a secondary structure. They are also rich in C residues.

The second level of specificity is that Rho fails to bind any transcript that is being translated.

In bacteria, transcription and translation are tightly coupled—translation initiates on growing RNA transcripts as soon as they start exiting polymerase, while they are still being synthesized.

Thus, Rho typically terminates only those transcripts which are still being transcribed beyond the end of a gene or operon.



Lecture 101

Termination of Transcription (2)

- The termination of transcription is a normal and important function at the ends of gene/s.
- However, an unusual termination may be triggered by damaged DNA or by other unanticipated hindrances.

- The sequences called terminators trigger the elongating polymerase to dissociate from the DNA and release the RNA chain it has made.
- In bacteria, terminators come in two types:-

  1. Rho-Dependant

  2. Rho-Independant
- The first, requires a protein called Rho to induce termination.
- The second causes termination without the involvement of other factors.
- Rho-dependent terminators have rather ill-defined RNA elements called rut sites.
- The work of these sites requires the action of the Rho factor.
- Rho, which is a ring-shaped protein with six identical subunits, binds to single-stranded RNA as it exits the polymerase.
- The protein also has an ATPase activity, and once attached to the transcript, Rho uses the energy derived from ATP hydrolysis to induce termination.
- The precise mechanism of termination remains to be determined, and models include the following:-

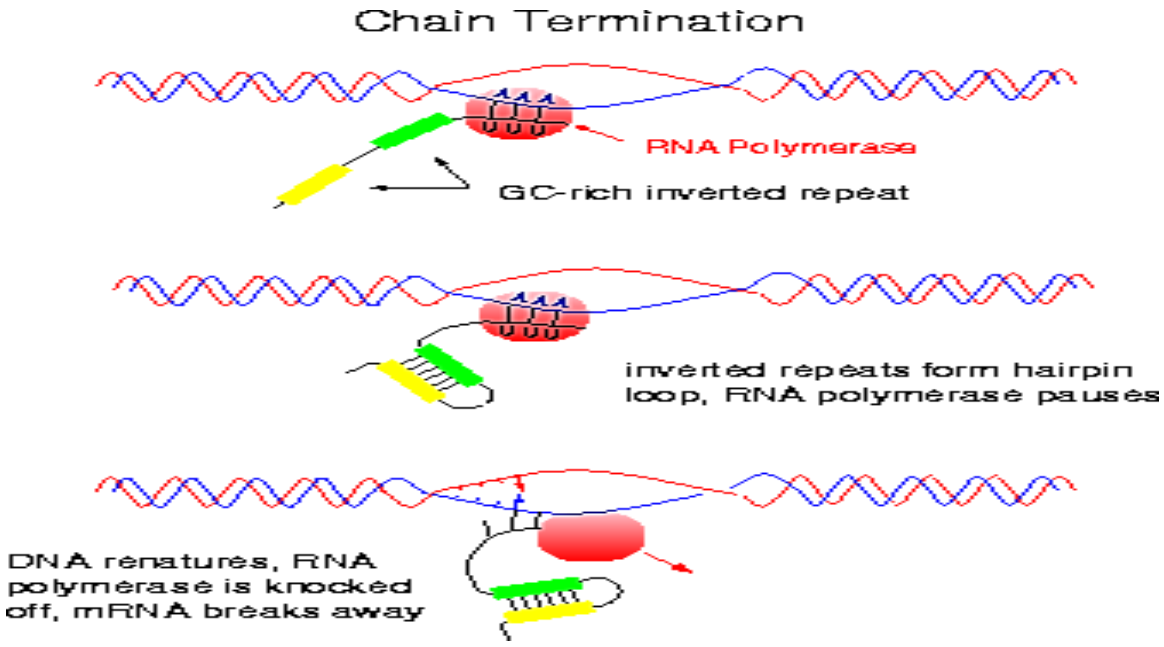
  i. Rho pushes polymerase forward relative to the DNA and RNA, resulting in termination in a manner analogous to termination by the protein TRCF (transcription-repair coupling factor).
- Rho binds double-stranded DNA upstream of the polymerase and translocates along the DNA until it collides the RNA polymerase.

- The collision pushes polymerase forward, causing dissociation of the ternary complex of RNA polymerase, template DNA, and RNA transcript.

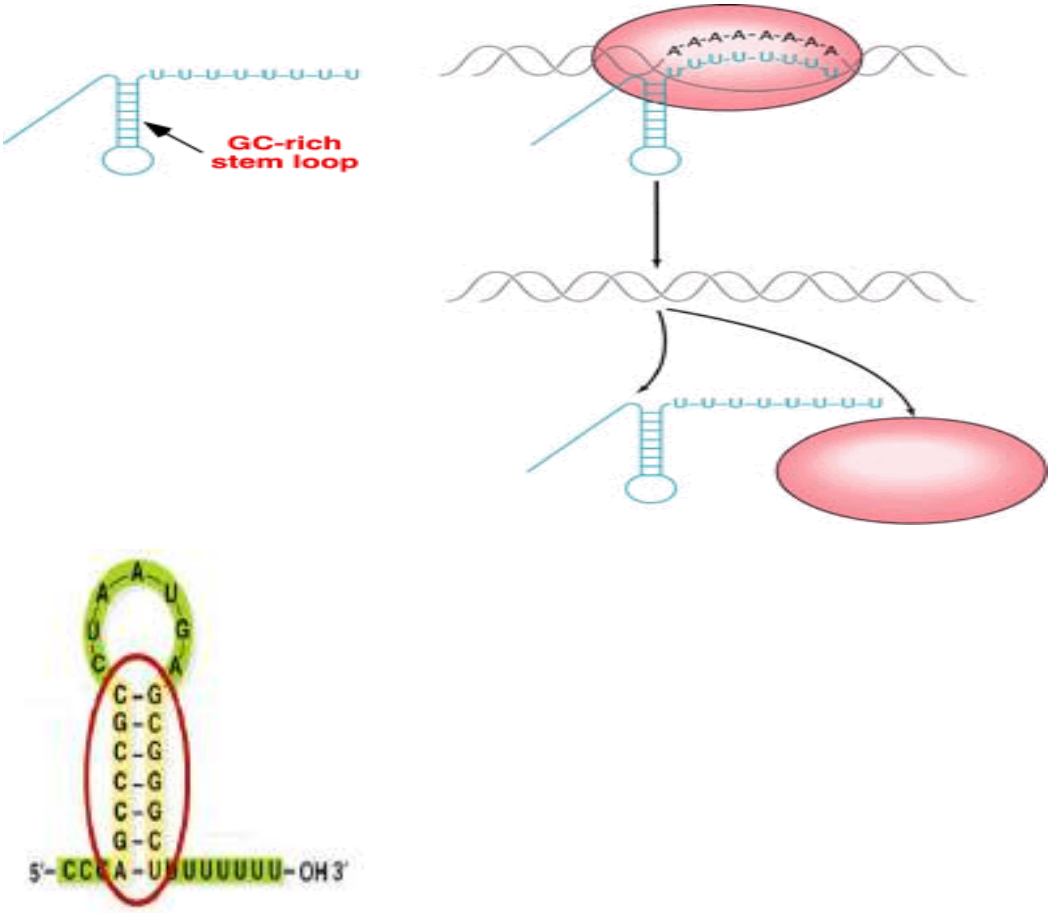
Lecture 102

Rho-independent Terminators

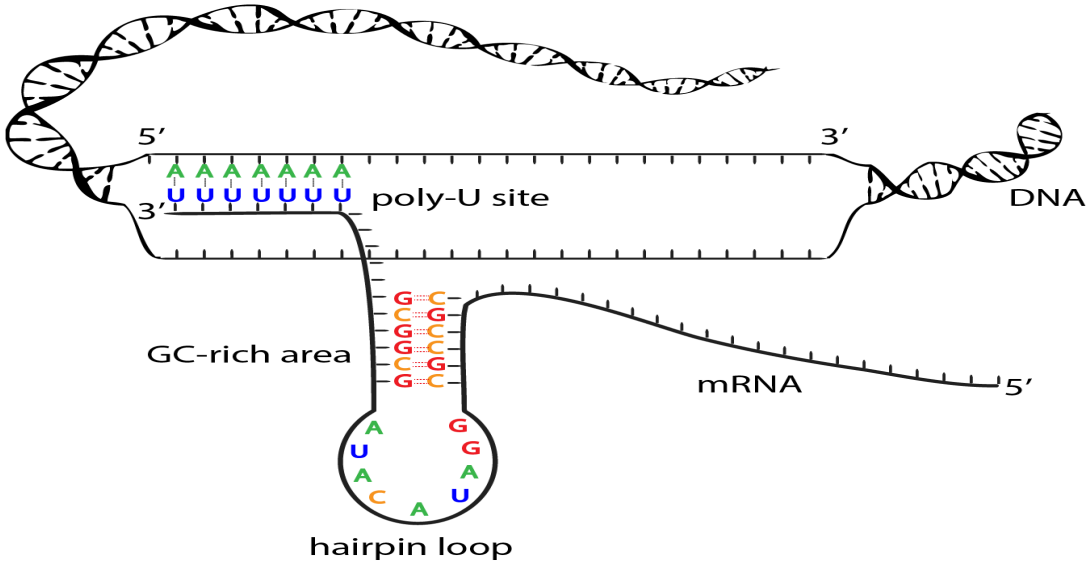
- Rho-independent terminators are also called intrinsic terminators because they need no other factors to work.
- They consist of two sequence elements:-
- A short inverted repeat of 20 nucleotides followed by a stretch of about eight A:T base pairs.



- These elements do not affect the polymerase until they have been transcribed—that is, they function in the RNA rather than in the DNA.
- When polymerase transcribes an inverted repeat sequence, the resulting RNA can form a stem-loop structure often called a “hairpin” by base-pairing with itself.
- Formation of the hairpin causes termination by disrupting the elongation complex.



- The hairpin induces termination by either pushing polymerase forward relative to the DNA and RNA, wresting the transcript from polymerase, or inducing a conformational change in polymerase.
- The hairpin works as an efficient terminator only when it is followed by a stretch of A:U base pairs.
- This is because, under those circumstances, at the time the hairpin forms, the growing RNA chain will be held on the template at the active site by only A:U base pairs.
- Because A:U base pairs are the weakest of all base pairs, they are more easily disrupted by the effects of the stem-loop on the transcribing polymerase, and thus the RNA will more readily dissociate.

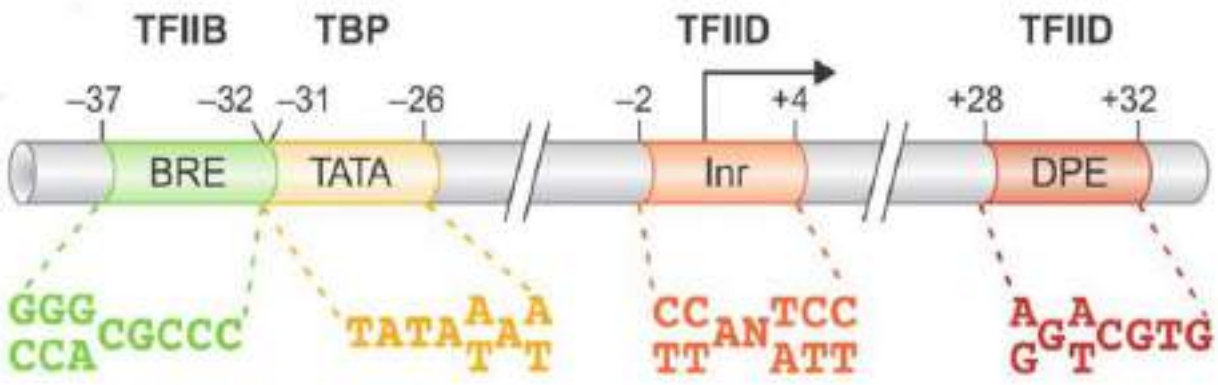


Lecture 103

TRANSCRIPTION IN EUKARYOTES

- Transcription in eukaryotes is undertaken by polymerases closely related to the RNA polymerases found in prokaryotes.
- The process of transcription is identical in both the prokaryotes and eukaryotes. There are, however, differences in the machinery used in each case.
- Bacteria have only one RNA polymerase but all eukaryotes have at least three different ones i.e., Pol I, II, and III; and plants also have a Pol IV and a Pol V.
- In addition, whereas bacteria require only one additional initiation factor (σ), several initiation factors are required for efficient and promoter-specific initiation in eukaryotes.
- In addition, whereas bacteria require only one additional initiation factor (σ), several initiation factors are required for efficient and promoter-specific initiation in eukaryotes.
- In vivo, however, the general transcription factors are not alone sufficient to bind promoter sequences and elicit significant expression.

- Rather, additional factors are required, including DNA-binding regulatory proteins, the so-called Mediator complex, and often chromatin-modifying enzymes.
- The eukaryotic core promotor refers to the minimal set of sequence elements required for accurate transcription initiation by the Pol II machinery.
- A core promoter is typically ~40–60 nucleotides long, extending either upstream or downstream from the transcription start site.
- The elements found in Pol II core promoter include the TFIIB recognition element (BRE), the TATA element (or box), the initiator (Inr), and the downstream promoter elements (known as DPE, DCE, and MTE).



Pol II core promoter

- Typically, a promoter includes some subset of these elements. Thus, for example, promoters typically have either a TATA element or a DPE element, not both.
- Often, a TATA-containing promoter also contains a DCE.
- The Inr is the most common element, found in combination with both TATA and DPEs.
- Typically upstream of the core promoter, there are other sequence elements required for accurate and efficient transcription in vivo.

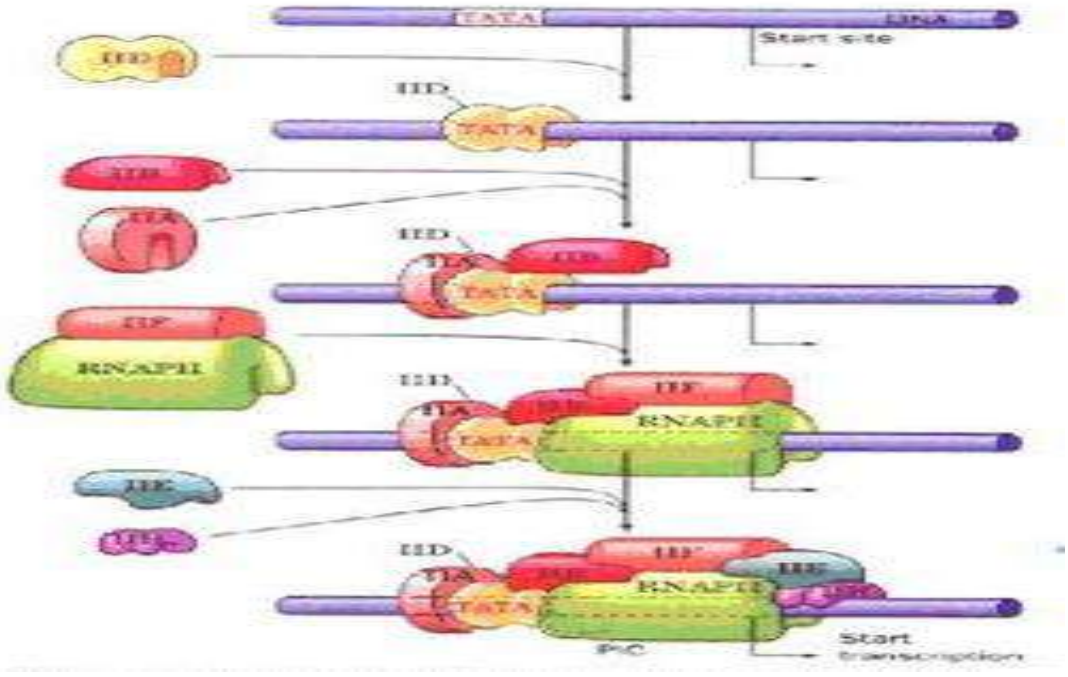
- Together, these elements constitute the regulatory sequences.
- These elements include promoter proximal elements, upstream activator sequences (UASs), enhancers, and a series of other elements called silencers, boundary elements, and insulators.
- All of these DNA elements bind regulatory proteins (activators and repressors), which help or hinder transcription from the core promoter.
- Some of these regulatory sequences can be located many tens or even hundreds of kilobases from the core promoters on which they act.

Lecture 104

Formation of pre-initiation Complex

- The general transcription factors collectively perform the functions performed by σ in bacterial transcription.
- Thus, the general transcription factors help polymerase bind to the promoter and melt the DNA.
- This is comparable to the transition from the closed to the open complex in the bacterial case.
- They also help polymerase escape from the promoter and embark on the elongation phase.
- The complete set of general transcription factors and polymerase, bound together at the promoter and poised for initiation, is called the pre-initiation complex.
- Many Pol II promoters contain a so-called TATA element (some 30 bp upstream of the transcription start site).
- This is where preinitiation complex formation begins.

- The TATA element is recognized by the general transcription factor called TFIID (“TFII” denotes a transcription factor for Pol II).
- Like many of the general transcription factors, TFIID is, in fact, a multi-subunit complex.
- The component of TFIID that binds to the TATA DNA sequence is called TBP (TATA-binding protein).
- The other subunits in this complex are called TAFs, for TBP-associated factors.
- Some TAFs recognize other core promoter elements such as the Inr, DPE, and DCE, although the strongest binding is between TBP and TATA.
- Thus, TFIID is a critical factor in promoter recognition and establishment of pre-initiation complex.



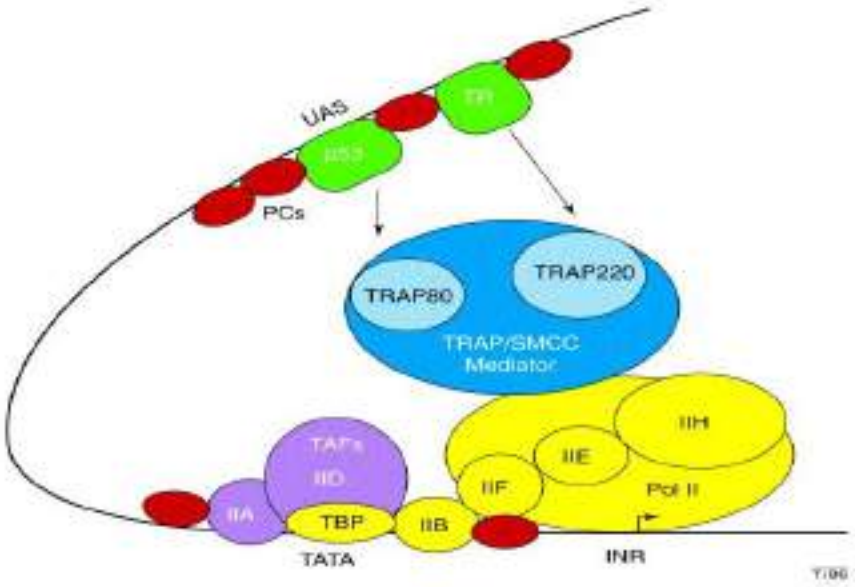
- Upon binding DNA, TBP extensively distorts the TATA sequence.

- The resulting TBP–DNA complex provides a platform to recruit other general transcription factors and polymerase itself to the promoter.
- In vitro, these proteins assemble at the promoter in the following order: TFIIA, TFIIB, TFIIF together with polymerase, and then TFIIE and TFIIH.
- Formation of the preinitiation complex containing these components is followed by promoter melting.
- In contrast to the situation in bacteria, promoter melting in eukaryotes requires hydrolysis of ATP and is mediated by TFIIH.

Lecture 106

General Transcription Factors

- TFIIE and TFIIH
- TFIIE, which consists of two subunits, binds next and has roles in the recruitment and regulation of TFIIH.
- TFIIH controls the ATP-dependent transition of the preinitiation complex to the open complex.
- It is the largest and most complex of the general transcription factors having 10 subunits and a molecular mass comparable to that of the polymerase itself.



- Within TFIIH are two subunits that function as ATPases and another that is a protein kinase, with roles in promoter melting and escape.

- Together with other factors, the ATPase subunits are also involved in nucleotide excision repair.
- How does TFIIH mediate promoter melting?
- It is now believed that a subunit of TFIIH acts as an ATP-driven translocator of double-stranded DNA.
- This subunit binds to DNA downstream from polymerase and feeds double-stranded DNA, with a right-handed threading, into the cleft of the polymerase.
- This action drives the melting of the DNA because the upstream promoter DNA is held in a fixed position by TFIID and the rest of the GTFs.

Lecture 107

General Transcription Factors(2)

- We do not know in detail the functions of all of the other general transcription factors.
- Some of these factors are in fact complexes made up of two or more subunits.
- **TAFs**
- TBP is associated with about 10 TAFs. Two of the TAFs bind DNA elements at the promoter, for example, the initiator element (Inr) and the downstream promoter elements.
- Several of the TAFs have structural homology with histone proteins, and it has been proposed that they might bind DNA in a similar manner, although evidence for such a binding has not been obtained.
- For example, TAF42 and TAF62 from *Drosophila* have been shown to form a structure similar to that of the H3.H4 tetramer of histones.
- Another TAF appears to regulate the binding of TBP to DNA. It does this using an inhibitory flap that binds to the DNA-binding surface of TBP.
- This flap must be displaced for TBP to bind TATA.
- **TFIIB**
- This protein, a single polypeptide chain, enters the preinitiation complex after TBP.
- The crystal structure of the ternary complex of TFIIB–TBP–DNA shows specific TFIIB–TBP and TFIIB–DNA contacts.
- These include base-specific interactions with the major groove upstream (to the BRE) and the minor groove downstream of the TATA element.

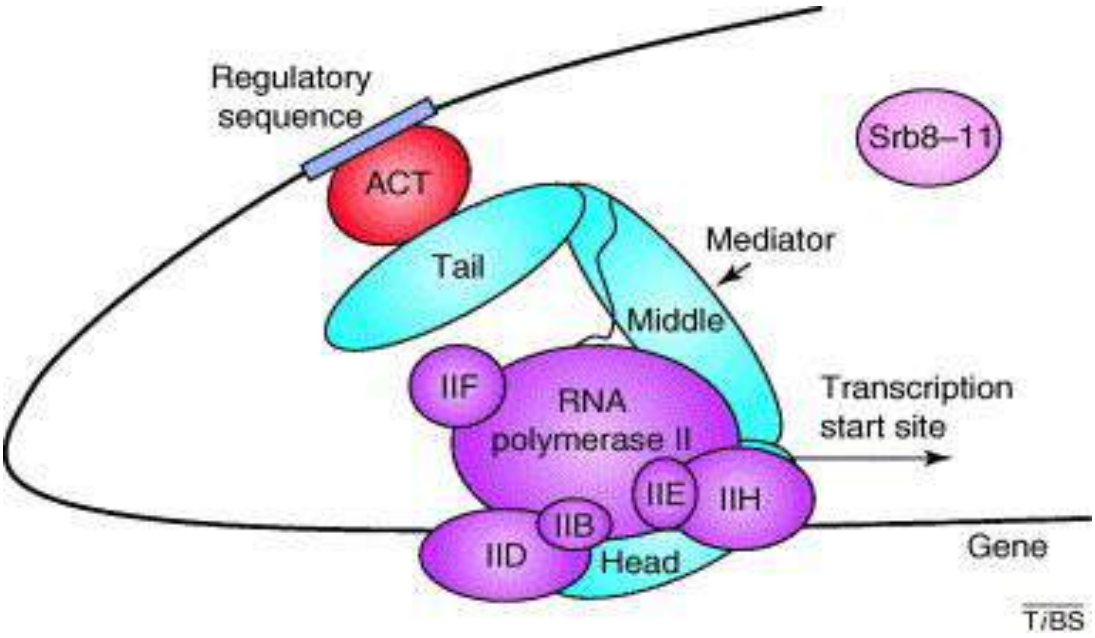
- The asymmetric binding of TFIIB to the TBP–TATA complex accounts for the asymmetry in the rest of the assembly of the preinitiation complex and the unidirectional transcription that results.
- TFIIB also contacts Pol II in the preinitiation complex.
- Thus, this protein appears to bridge the TATA-bound TBP and polymerase.
- Structural studies suggest that segments of TFIIB insert into the RNA-exit channel and active center cleft of Pol II in a manner analogous to the σ region ¾ linker in the bacterial case.
- These regions of TFIIB(called the linker and reader) aid in open complex formation, perhaps by stabilizing the melted DNA until the RNA:DNA hybrid takes over that role.
- **TFIIF**
- This two-subunit factor associates with Pol II and is recruited to the promoter together with that enzyme (and other factors).
- Binding of Pol II–TFIIF stabilizes the DNA–TBP–TFIIB complex and is required before TFIIE and TFIIH are recruited to the preinitiation complex.
- In yeast, this factor includes a third subunit, but the function of the third subunit is not known.

Lecture 108

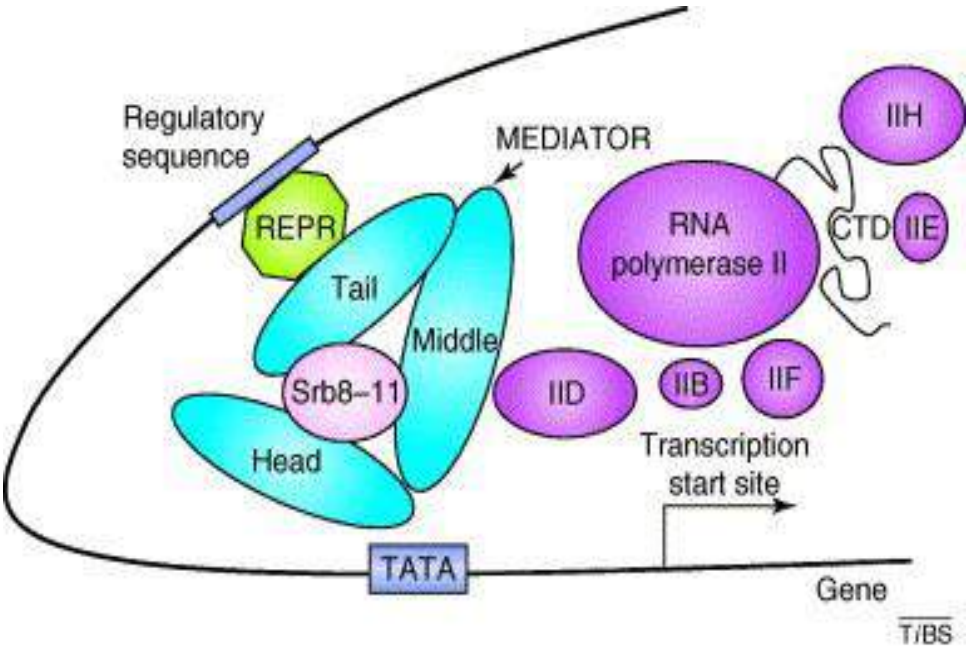
The Mediator Complex

- High regulated levels of transcription in vivo, require transcriptional regulatory proteins, the Mediator complex, and nucleosome modifying enzymes in addition to the above described general transcription factors.

- One reason for these additional requirements is that the DNA template in vivo is packaged into chromatin.
- This condition complicates binding to the promoter of polymerase and its associated factors.
- Transcriptional regulatory proteins called activators help recruit polymerase to the promoter, stabilizing its binding there.
- This recruitment is mediated through interactions between DNA-bound activators, chromatin-modifying and -remodeling factors, and parts of the transcription machinery.
- One such interaction is with the Mediator complex.
- Mediator is associated with the basic transcription machinery, most likely touching the CTD “tail” of the large polymerase subunit through one surface,
- while presenting other surfaces for interaction with DNA-bound activators.
- This explains the need for Mediator to achieve significant transcription in vivo.



- Despite this central role in transcriptional activation, deletion of individual subunits of Mediator often leads to loss of expression of only a small subset of genes, different for each subunit.
- This result likely reflects the fact that different activators are believed to interact with different Mediator subunits to bring polymerase to different genes.
- Mediator also aids initiation by regulating the CTD kinase in TFIIH.
- The need for nucleosome modifiers and remodelers also differs at different promoters or even at the same promoter under different circumstances.
- When and where required, these complexes are also typically recruited by the DNA-bound activators, or sometimes by regulatory RNAs.
- The yeast and human Mediators each include more than 20 subunits, of which seven show significant sequence homology between the two organisms.
- The Mediator from both yeast and humans is organized in modules, each containing a subset of the subunits.
- These modules— called head, middle (or arm), and tail— can be dissociated from one another under certain conditions in vitro.



- Crystal structure of the head module of yeast Mediator reveals that it contains seven subunits (Med17/Srb4, Med11, Med22/Srb6, Med6, Med8, Med18/Srb5, and Med20/Srb2).
- It forms a three-domain structure that binds the transcription complex in such a way as to juxtapose TFIIH and the CTD tail of RNA polymerase, promoting phosphorylation of the latter by the former.

Lecture 109

RNA Elongation and Proofreading

- NusG/SPT5 factors bind to their respective RNA polymerases at the tip of the clamp, overlapping the region contacted by σ region 4 (in bacteria) and TFIIB (in eukaryotes).
- This overlapping and binding raises the interesting possibility that displacing initiation factors may be part of the function of these elongation regulators.

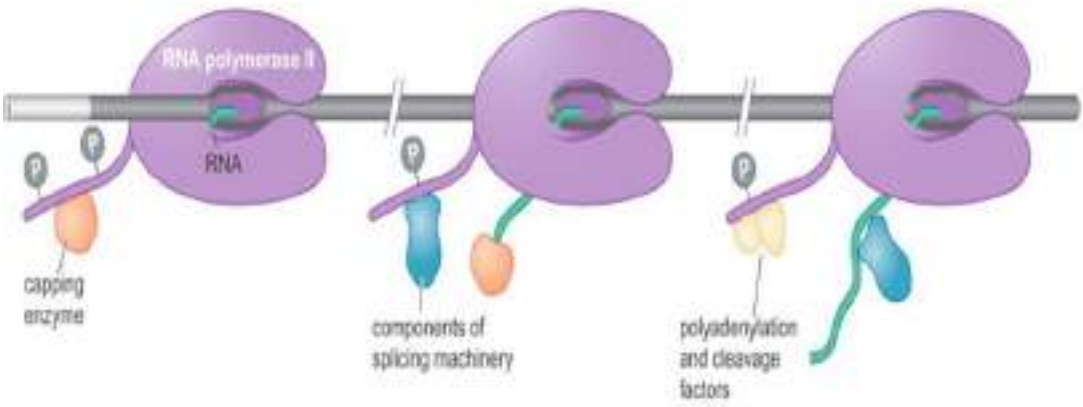
- This also suggests that regulating the rate of elongation is an ancient mechanism of regulating gene expression.
- There are some promoters in higher eukaryotes where the preinitiation complex is recruited effectively, but polymerase remains paused just after initiating transcription.
- Such promoters seem to be associated with genes poised to be expressed either rapidly or in a highly coordinated fashion.
- And their expression is regulated through recruitment by specific activators of the PTEFb kinase, which then releases them from their pause.
- Yet another class of elongation factor is the so-called ELL family.
- These also bind to elongating polymerase and suppress transient pausing by the enzyme.
- The first human ELL protein was originally identified as the product of a gene that undergoes translocations in acute myeloid leukemia.
- Another factor that does not affect initiation, but stimulates elongation, is TFIIS.
- This factor, like ELL, stimulates the overall rate of elongation by limiting the length of time that polymerase pauses when it encounters sequences that would otherwise tend to slow the enzyme's progress.
- It is a feature of polymerase that it does not transcribe through all sequences at a constant rate.
- Rather, it pauses periodically, sometimes for rather long periods, before resuming transcription.

- In the presence of TFIIS, the length of time that polymerase pauses at any given site is reduced.
- TFIIS also contributes to proofreading by polymerase.
- TFIIS stimulates an inherent Rnase activity in polymerase (not part of the active site), allowing an alternative approach to removing misincorporated bases through local limited RNA degradation.
- This feature is comparable to the hydrolytic editing in the bacterial case stimulated by the Gre factors.

Lecture 110

RNA Elongation and Proofreading (2)

- Once polymerase has escaped the promoter and initiated transcription, it shifts into the elongation phase.
- This transition involves the Pol II enzyme shedding most of its initiation factors such as, the general transcription factors and Mediator.
- In their place, another set of factors is recruited. Some of these (such as TFIIS and SPT5) are elongation factors (i.e., factors that stimulate elongation).
- Others are required for RNA processing.
- The enzymes involved in RNA processing are, like several of the initiation factors recruited to the carboxy-terminal (CTD) tail of the large subunit of Pol II



- In this case, however, the factors favor the phosphorylated form of the CTD. Thus, phosphorylation of the CTD leads to an exchange of initiation factors for those factors required for elongation and RNA processing.
- As is evident from the crystal structure of yeast Pol II, the polymerase CTD lies directly adjacent to the channel through which the newly synthesized RNA exits the enzyme.
- As is evident from the crystal structure of yeast Pol II, the polymerase CTD lies directly adjacent to the channel through which the newly synthesized RNA exits the enzyme.
- Together, these features allow the tail to bind several components of the elongation and processing machinery and deliver them to the emerging RNA.
- Various proteins are thought to stimulate elongation by Pol II.
- One of these, the kinase P-TEFb, is recruited to polymerase by transcriptional activators.
- Once bound to Pol II, this protein phosphorylates the serine residue at position 2 of the CTD repeats. That phosphorylation event correlates with elongation.
- In addition, P-TEFb phosphorylates and thereby activates another protein, called SPT5, itself an elongation factor.

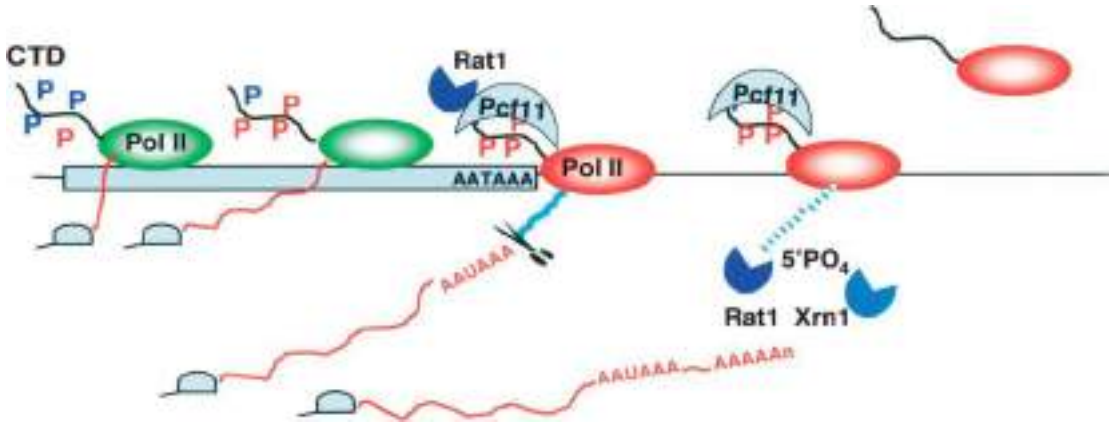
- Finally, TAT-SF1, yet another elongation factor, is recruited by P-TEFb.
- Thus, P-TEFb stimulates elongation in three separate ways.
- SPT5 is comparable to the bacterial elongation factor NusG.
- Indeed, this is the only universally conserved transcription factor across all three kingdoms of life—from bacteria, through Archaea, to eukaryotes.

Lecture 111

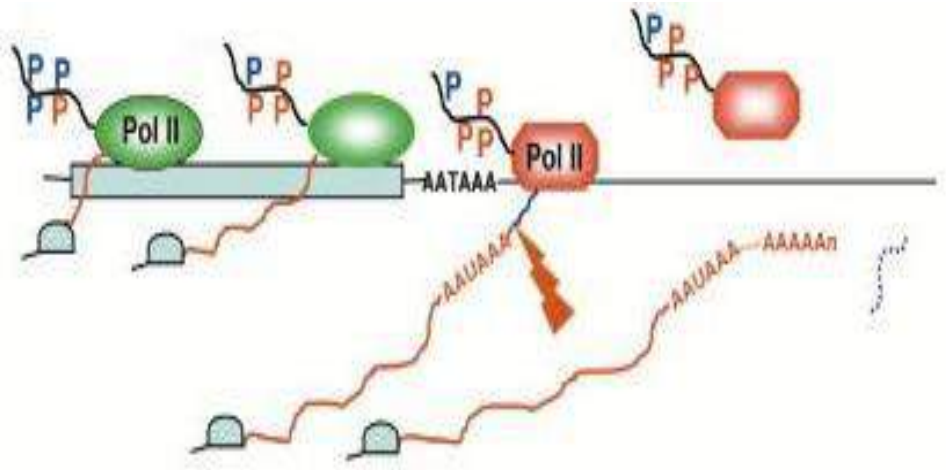
Transcription Termination

- The final RNA processing event, polyadenylation of the 3’ end of the mRNA, is intimately linked with the termination of transcription, although exactly how is still not quite clear.
- Recently, however, an enzyme that degrades the second RNA as it emerges from the polymerase has been identified, and this enzyme may itself trigger termination. This is called the torpedo model of termination.
- The free end of the second RNA is uncapped and thus can be distinguished from genuine transcripts.
- This new RNA is recognized by an RNase called, in yeast, Rat1 (in humans, Xrn2) that is loaded onto the end of the RNA by another protein (Rtt103) that binds the CTD of RNA polymerase.
- The Rat1 enzyme is very processive and quickly degrades the RNA in a 5’-to-3’ direction, until it catches up to the still-transcribing polymerase from which the RNA is being spewed.

- Termination may not require very specific interaction between Rat1 and polymerase and might, in fact, be triggered in a manner rather similar to that of Rho-dependent termination in bacteria.
- The highly processing RNase polymerase either pushes polymerase forward and/or pulls the remains of the nascent RNA transcript from the enzyme.
- It is also possible that other factors are needed in addition to Rat1 to dislodge polymerase as, in vitro, Rat1 is alone insufficient to carry out this function, even after it has degraded the transcript.



- Although the torpedo model for termination is now the favored one, there is an alternative called the allosteric model.
- According to this model, termination depends on a conformational change in the elongating polymerase that reduces the processivity of the enzyme leading to spontaneous termination soon afterward.
- This conformational change would be linked to polyadenylation and could be triggered by the transfer of the 3’-processing enzymes from the CTD tail of polymerase to the RNA.



Lecture 112

Polymerases I and III

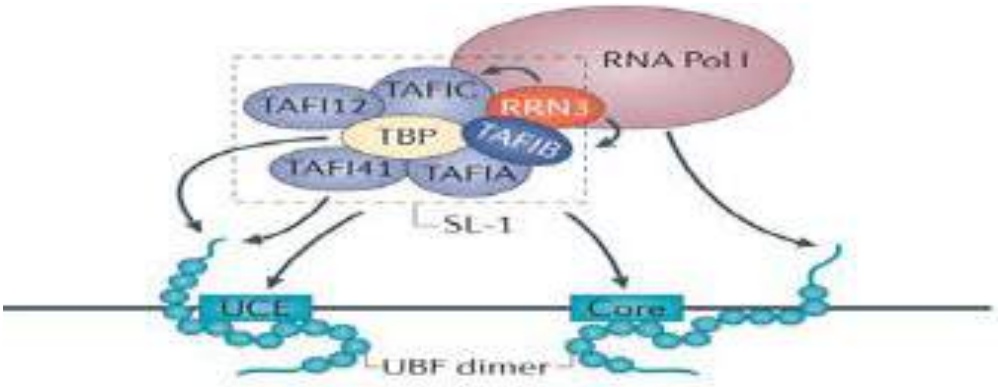
- All eukaryotes have two other RNA polymerases— Pol I and Pol III—in addition to Pol II.
- These enzymes are related to Pol II and even share several subunits but they initiate transcription from distinct promoters and transcribe distinct genes.
- Those genes encode specialized RNAs rather than proteins. Each of these enzymes also works with its own unique set of general transcription factors.
- TBP, however, is universal—it is involved in initiating transcription by Pol I and Pol III, as well as Pol II.
- Although TBP is the only GTF that is used by Pol I and Pol III aswell as by Pol II, it has emerged recently that some of the other GTFs, in fact, have structurally and functionally equivalent components in the other systems.
- Thus, for example, TFIIF seems to have a counterpart in two subunits within Pol I (A49/34.5), and also in Pol II (C37/53).
- Likewise, TFIIE-like subunits are found in Pol I and Pol III enzymes.

- In addition, both these other systems include additional factors comparable to TFIIB: the TAF1B factor in the Pol I system, and the Brf1 subunit of TFIIIB in the case of Pol III.
- Pol I is required for the expression of only one gene, that encoding the rRNA precursor.
- There are many copies of that gene in each cell, and, indeed, it is expressed at far higher levels than any other gene, perhaps explaining why it has its own dedicated polymerase.
- The promoter for the rRNA gene comprises two parts: the core element and the UCE (upstream control element).

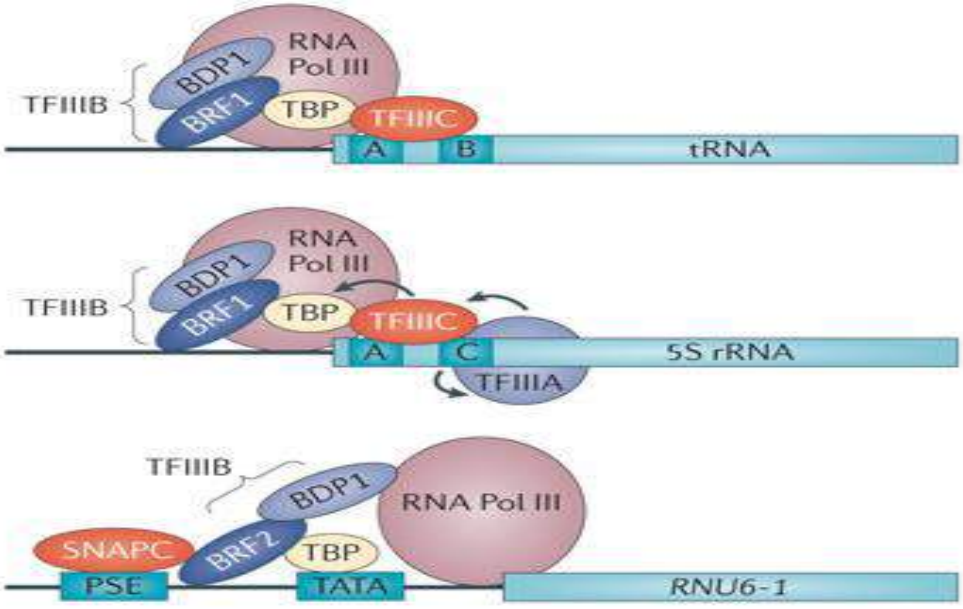
Lecture 113

Polymerases I and III (2)

- The former is located around the start site of transcription, and the latter between 100 and 150 bp upstream (in humans).
- In addition to Pol I, initiation requires two other factors, called SL1 and UBF.
- SL1 comprises TBP and three TAFs specific for Pol I transcription. This complex binds to the core element.



- SL1 binds DNA only in the presence of UBF. This factor binds to UCE, bringing in SL1 and stimulating transcription from the core promoter by recruiting Pol I.
- Pol III promoters come in various forms, and the vast majority have the unusual feature of being located downstream from the transcription start site (i.e., within the coding region of the gene).
- Some Pol III promoters (e.g., those for the tRNA genes) consist of two regions, called Box A and Box B, separated by a short element.
- Others contain Box A and Box C (e.g., the 5S rRNA gene); and still others contain a TATA element like those of Pol II.
- Transcription by Pol III also requires transcription factors in to polymerase.
- In this case, the factors are called TFIIIB and TFIIIC for the tRNA genes and those plus TFIIIA for the 5S rRNA gene.



- The TFIIIC complex binds to the promoter region. This complex recruits TFIIIB to the DNA just upstream of the

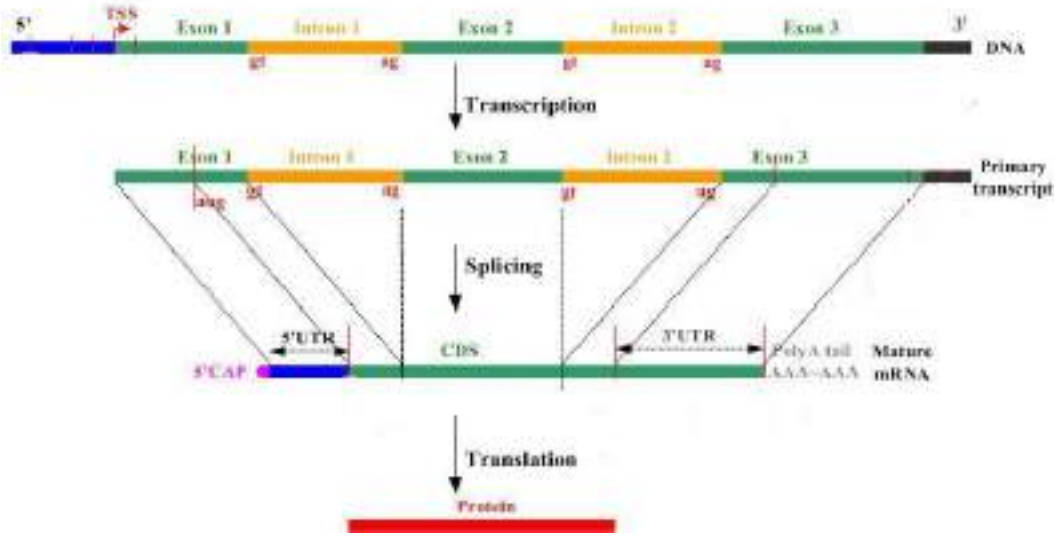
start site, where it, in turn, recruits Pol III to the start site of transcription.

- The enzyme then initiates, presumably displacing TFIIIC from the DNA template as it goes.
- Pol III also uses TBP like other polymerases. In this case, that ubiquitous factor is found within the TFIIIB complex.

Lecture 114

RNA Splicing

- The coding sequence of a gene is a series of three nucleotide codons that specifies the linear sequence of amino acids in its polypeptide product.
- It is generally assumed that the coding sequence is contiguous; i,e., the codon for one amino acid is immediately adjacent to the codon for the next amino acid in the polypeptide chain.
- This is true in the vast majority of cases in bacteria and their phage. But it is rarely so for eukaryotic genes.
- In those cases, the coding sequence is interrupted by stretches of non-coding sequences.
- Many eukaryotic genes are thus mosaics, consisting of blocks of coding sequences separated from each other by blocks of non-coding sequences.
- The coding sequences are called **exons** and the intervening sequences are called **introns**.
- Once transcribed into an RNA transcript, the introns must be removed and the exons joined together to create the mRNA for that gene.



- The number of introns found within a gene varies enormously-from one in the case of most yeast genes (and a few human genes), to 50 in the case of the chicken proα2 collagen gene,to as many as 363 in the case of the Titin gene of humans.
- The sizes of the exons and introns vary as well.
- Indeed, introns are very often much longer than the exons they separate.
- Thus, for example, exons are typically on the order of 150 nucleotides, whereas introns—although they too can be short—can be as long as 800,000 nucleotides (800 kb).
- As another example, the mammalian gene for the enzyme dihydrofolate reductase is more than 31 kb long, and within it are dispersed six exons that correspond to 2 kb of mRNA.
- Thus, in this case, the coding portion of the gene is,10% of its total length.
- Like the uninterrupted genes of prokaryotes, the split genes of eukaryotes are transcribed into a single RNA

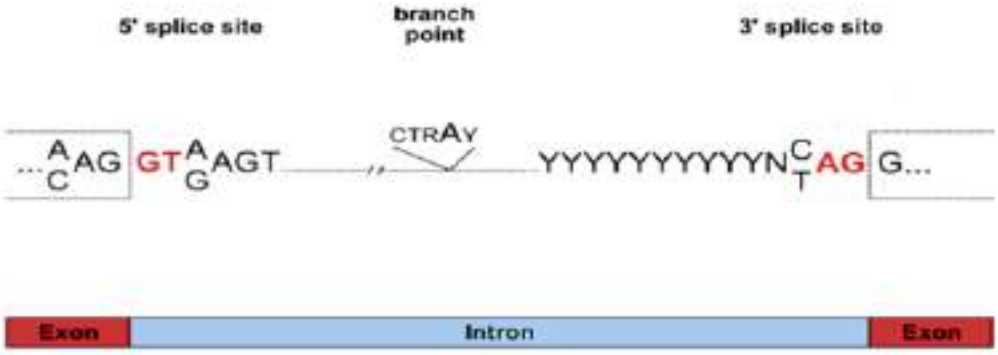
copy of the entire gene - the primary transcript that contains introns as well as exons.

- Because the length and number of introns, the primary transcript (or pre-mRNA) can be very long indeed.
- As already mentioned, the primary transcripts of intron-containing genes must have their introns removed before they can be translated into proteins.
- The process of intron removal is called **RNA Splicing**.
- It converts the pre-mRNA into mature mRNA containing only exons.
- RNA Splicing must occur with great precision to avoid the loss, or addition, of even a single nucleotide at the sites at which the exons are joined.
- The triplet-nucleotide codons of mRNA are translated in a fixed reading frame that is set by the first codon.
- Lack of precision in splicing will change the reading frames of exons.
- Some pre-mRNAs can be spliced in more than one way.
- Thus, mRNAs containing different selections of exons can be generated from a given pre-mRNA.
- Alternative splicing strategy enables a gene to give rise to more than one polypeptide product.
- These alternative products are called isoforms.
- It is estimated that 90% or more of the protein-coding genes in the human genome are spliced in alternative ways to generate more than one isoform.

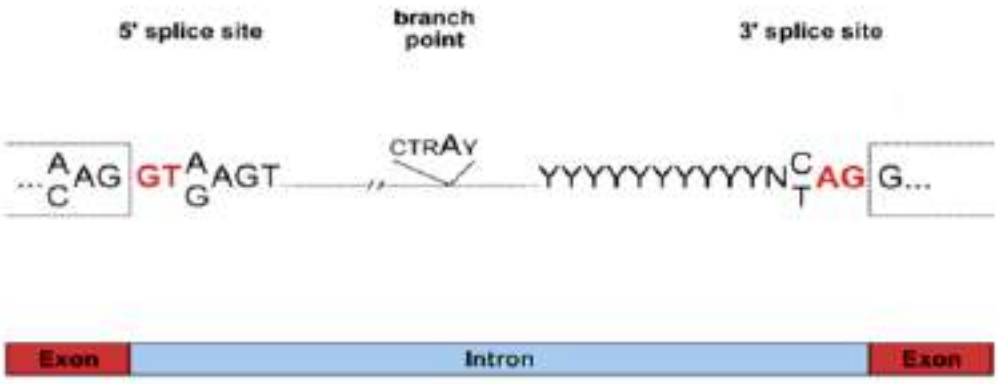
Lecture 115

How splicing site is determined?

- Let us consider the molecular mechanisms of the splicing reaction.
- How are the introns and exons distinguished?
- How are introns removed?
- How are exons join with high precision?
- The borders between introns and exons are marked by specific nucleotide sequences within the pre-mRNAs.
- These sequences delineate where splicing will occur.
- Thus the exon – intron boundary - that is, the boundary at the 5’ end of the intron —is marked by a sequence called the 5’ splice site.
- The intron – exon boundary at the 3’ end of the intron is marked by the 3’ splice site.
- The 5’ and 3’ splice sites were sometimes referred to as the donor and acceptor sites, respectively.



- The figure shows a third sequence necessary for splicing. This is called the branchpoint site (or branchpoint sequence).
- It is found entirely within the intron, usually close to its 3’ end, and is followed by a polypyrimidine tract (Py tract).



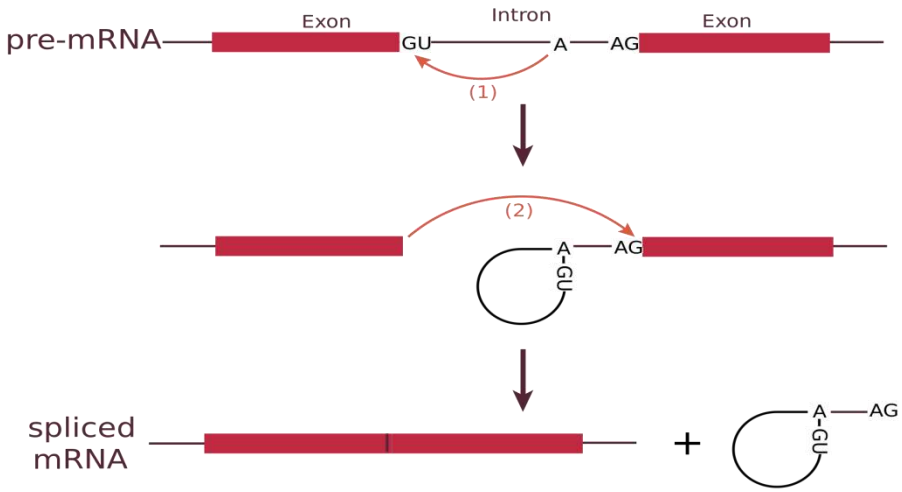
- The most highly conserved sequences are the GU in the 5’ splice site, the AG in the 3’ splice site, and the A at the branch site.
- These highly conserved nucleotide sequences are all found within the intron itself.
- This is perhaps because the sequence of most exons, in contrast to the introns, is constrained by the need to encode the specific amino acids of the protein product.

Lecture 116

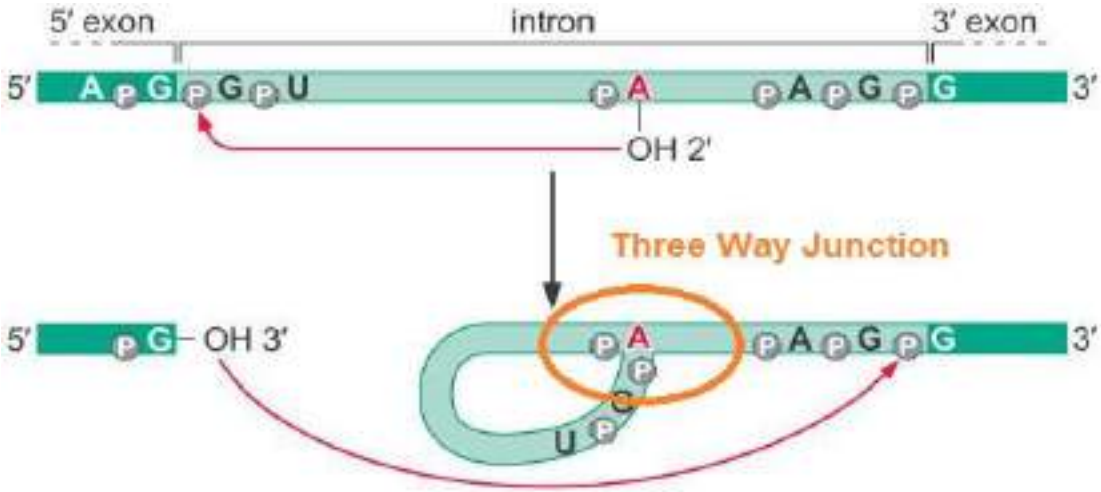
Removal of Introns

- An intron is removed through two successive transesterification reactions in which phosphodiester linkages within the pre-mRNA are broken and new ones are formed.
- The first reaction is triggered by the 2’-OH of the conserved A at the branch site. This group acts as a nucleophile to attack the phosphoryl group of the conserved G in the 5’ splice site.
- As a consequence of this first reaction, the phosphodiester bond between the sugar and the phosphate at the 5’ junction between the intron and the exon is cleaved.

- The freed 5’ end of the intron is joined to the A within the branch site.

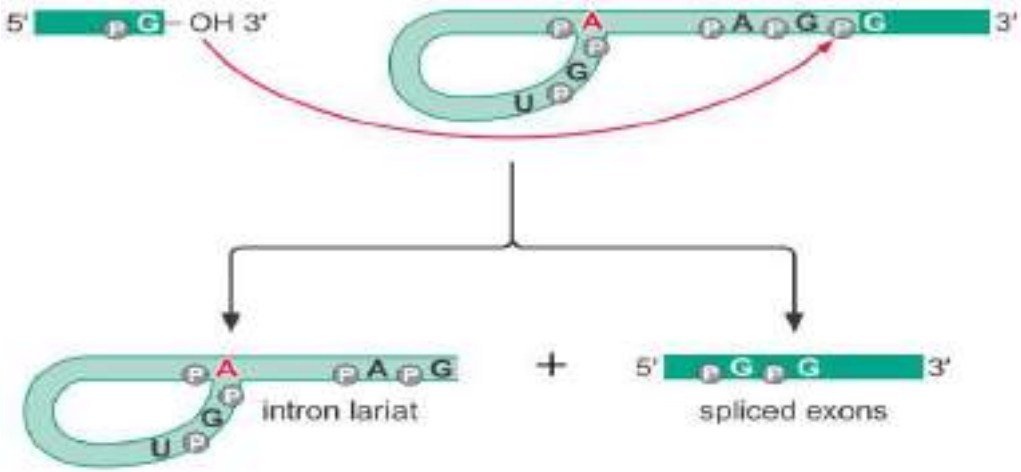


- Thus, in addition to the 5’ and 3’ backbone linkages, a third phosphodiester extends from the 2’-OH of that A to create a three-way junction (hence its description as a branchpoint).



- Note that the 5’ exon is a leaving group in the first transesterification reaction.
- In the second reaction, the 5’ exon reverses its role and becomes a nucleophile that attacks the phosphoryl group at the 3’ splice site.
- This second reaction has two consequences.

- First, and most importantly, it joins the 5’ and 3’ exons; thus, this is the step in which the two coding sequences are actually “spliced” together.
- Second, this same reaction liberates the intron, which serves as a leaving group.
- Because the 5’ end of the intron had been joined to branchpoint A in the first transesterification reaction, the newly liberated intron has the shape of a Lariat.



- In the two reaction steps, there is no net gain in the number of chemical bonds—two phosphodiester bonds are broken, and two new ones are made.
- Because it is just a question of shuffling bonds, no energy input is demanded by the chemistry of this process.
- But a large amount of ATP is consumed during the splicing reaction.
- This energy is required, to properly assemble and operate the splicing machinery.
- Another point regarding the splicing reaction is direction: what ensures that splicing only goes forward—that is, toward the products.

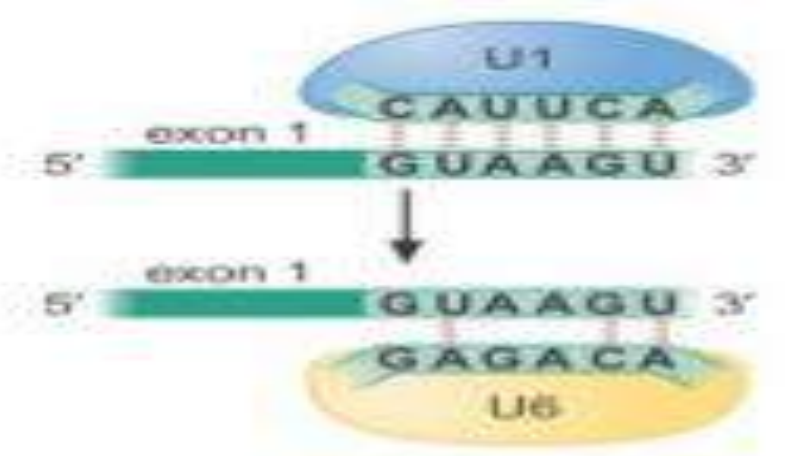
- In principle, the reactions could go in the other direction, and indeed this can be forced to happen under special circumstances.
- But in practice, this does not happen in the cell.

Lecture 117

The Spliceosome

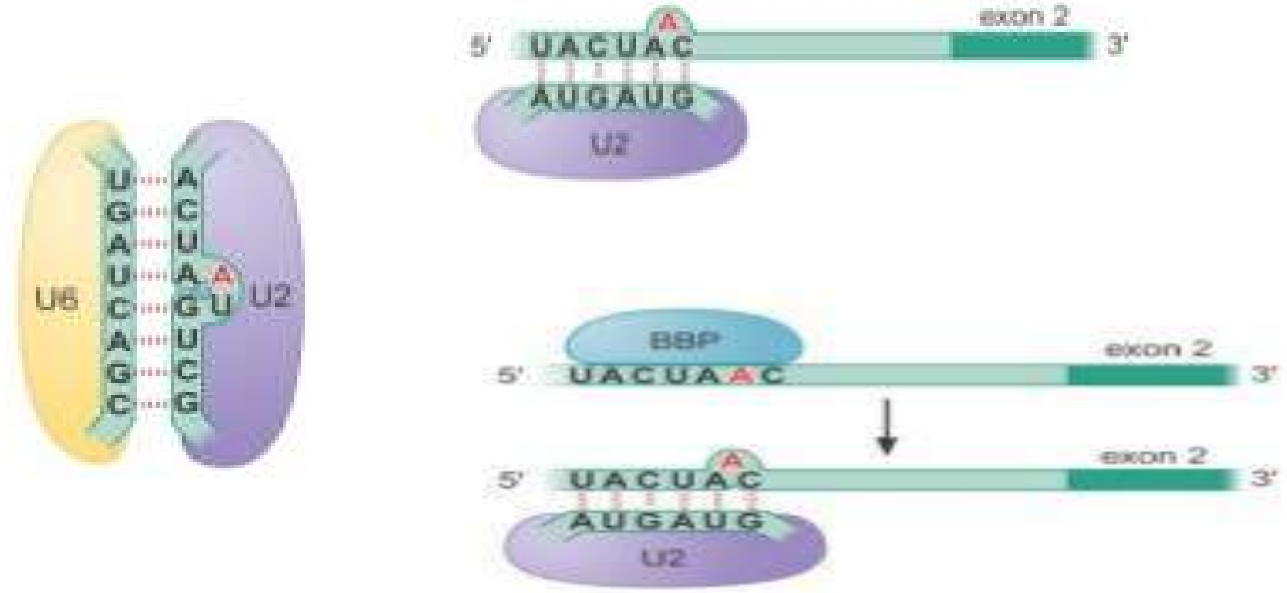
- The transesterification reactions are mediated by a huge molecular “machine” called the Spliceosome.
- This complex comprises about 150 proteins and five RNAs and is similar in size to a ribosome.
- In performing even a single splicing reaction, the spliceosome hydrolyzes several molecules of ATP.
- Strikingly, it is believed that many of the functions of the spliceosome are performed by its RNA components rather than the proteins.
- Thus, RNAs locate the sequence elements at the intron – exon borders and likely participate in catalysis of the splicing reaction itself.
- The five RNAs (U1, U2, U4, U5, and U6) are collectively called small nuclear RNAs (snRNAs). Each of these RNAs is between 100 and 300 nucleotides long in most eukaryotes and is complexed with several proteins.
- These RNA – protein complexes are called small nuclear ribonuclear proteins (snRNPs - pronounced “snurps”).
- The spliceosome is the large complex made up of these snRNPs, but the exact makeup differs at different stages of the splicing reaction.

- Different snRNPs come and go at different times, each performing particular functions in the reaction.
- There are also many proteins within the spliceosome that are not part of the snRNPs, and others besides that are only loosely bound to the spliceosome.
- The snRNPs have three roles in splicing:-
- They recognize the 5’ splice site and the branch site; they bring those sites together as required; and they catalyze the RNA cleavage and joining reactions.
- To perform these functions, RNA–RNA, RNA–protein, and protein–protein interactions are all important.
- Let us consider some of the RNA– RNA interactions.
- These interactions operate within individual snRNPs, between different snRNPs, and between snRNPs and the pre-mRNA.
- The figure below shows the interaction, through complementary base pairing, of the U1 snRNA and the 5’ splice site in the pre-mRNA.
- Subsequently in the reaction, that splice site is recognized by the U6 snRNA.



- In another example, shown below, the branch site is recognized by the U2 snRNA.

- A third example shows an interaction between U2 and U6 snRNAs.
- This brings the 5



- It is these and other similar interactions, and the rearrangements they lead to, that drive the splicing reaction and contribute to its precision.
- Some non-snRNPs are also involved in splicing. One example, U2AF (U2 auxiliary factor), recognizes the polypyrimidine (Py) tract/3’ splice site.
- And then in the initial step of the splicing reaction, it helps another protein, branchpoint-binding protein (BBP), bind to the branch site.
- BBP (also called SF1) is then displaced by the U2 snRNP.
- Other proteins involved in the splicing reaction include RNA-annealing factors, which help load snRNPs onto the mRNA, and DEAD-box helicase proteins.
- The latter use their ATPase activity to dissociate given RNA -RNA interactions, allowing alternative pairs to form

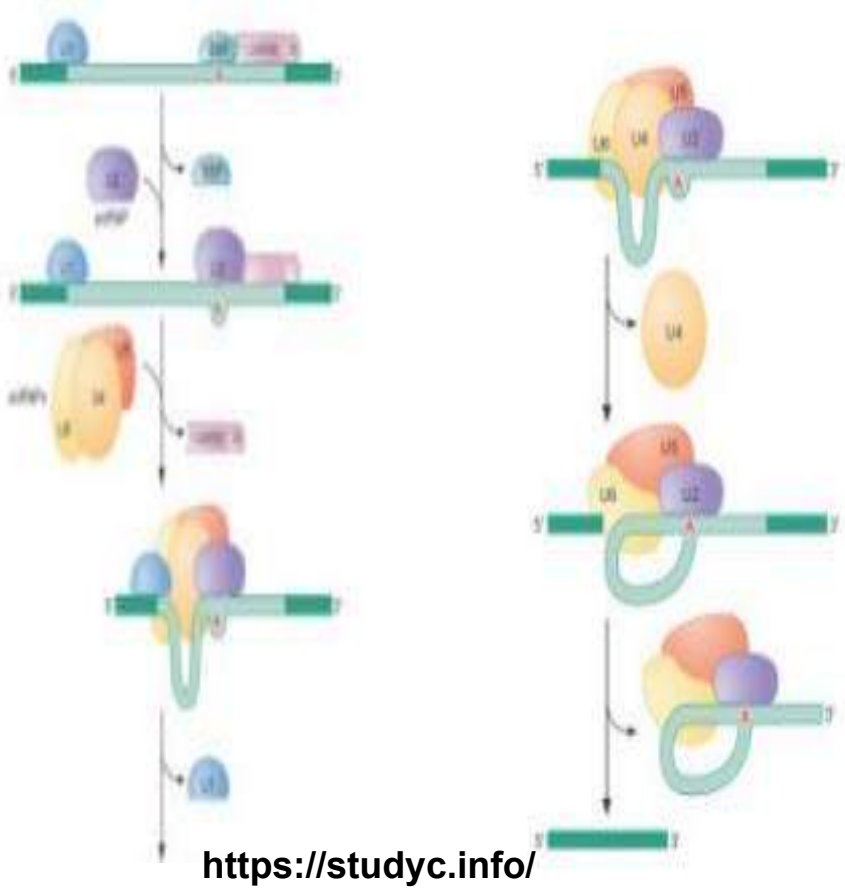
and thereby driving the rearrangements that occur through the splicing reaction.

- They are also required to remove spliced mRNA from the spliceosome and trigger spliceosome disassembly.

Lecture 118

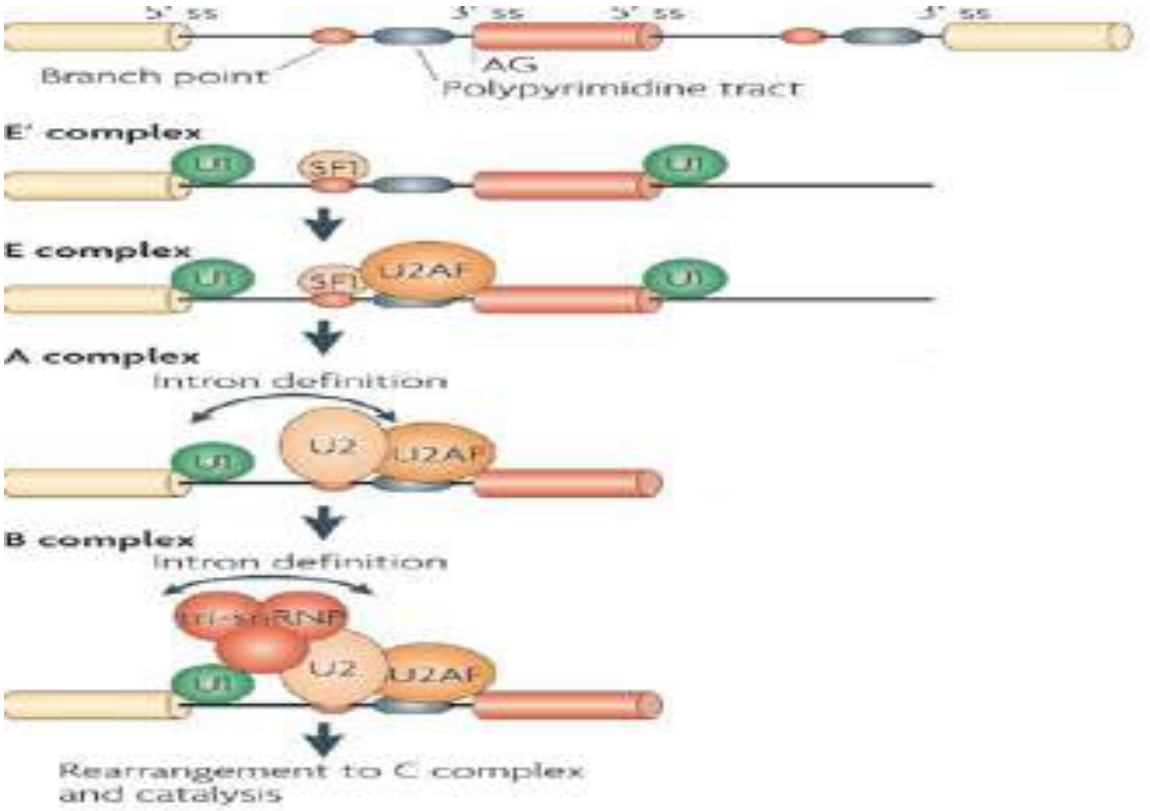
The Splicing Pathway

- In a splicing pathway, a number of the steps may differ slightly in their order or might even reverse.
- But the pathway reveals the series of events undertaken by the spliceosome to drive the splicing reaction in the cell.
- Initially, the 5’ splice site is recognized by the U1 snRNP.
- U2AF is made up of two subunits, the larger of which (65) binds to the Py tract and the smaller (35) binds to the 3’ splice site.
- The former subunit interacts with BBP (SF1) and helps that protein bind to the branch site. This arrangement of proteins and RNA is called the early (E) complex.



- The former subunit interacts with BBP (SF1) and helps that protein bind to the branch site. This arrangement of proteins and RNA is called the early (E) complex.
- U2 snRNP then binds to the branch site, aided by U2AF and displacing BBP (SF1). This arrangement is called the A complex.
- The base pairing between the U2 snRNA and the branch site is such that the branch site A residue is extruded from the resulting stretch of double-helical RNA as a single nucleotide bulge.
- This A residue is thus unpaired and available to react with the 5’ splice site.
- The next step is a rearrangement of the A complex to bring together all three splice sites.
- This is achieved as follows: the U4 and U6 snRNPs, along with the U5 snRNP, join the complex.
- Together, these three snRNPs are called the tri-snRNP particle.
- Within this particle, the U4 and U6 snRNPs are held together by complementary base pairing between their RNA components, and the U5 snRNP is more loosely associated through protein-protein interactions.
- With the entry of the tri-snRNP, the A complex is converted into the B complex.
- In the next step, U1 leaves the complex, and U6 replaces it at the 5’ splice site.

- This requires that the base pairing between the U1 snRNA and the pre-mRNA be broken, allowing the U6 RNA to anneal with the same region.
- Those steps complete the assembly pathway.



- The next rearrangement triggers catalysis and occurs as follows:-
- U4 is released from the complex, allowing U6 to interact with U2 (through the RNA:RNA base pairing).
- This arrangement, called the C complex, produces the active site. That is, the rearrangement brings together within the spliceosome those components that together form the active site.
- The same rearrangement also ensures that the substrate RNA is properly positioned to be acted upon.
- It is striking not only that the active site is primarily formed of RNA, but also that it is only formed at this stage of spliceosome assembly.

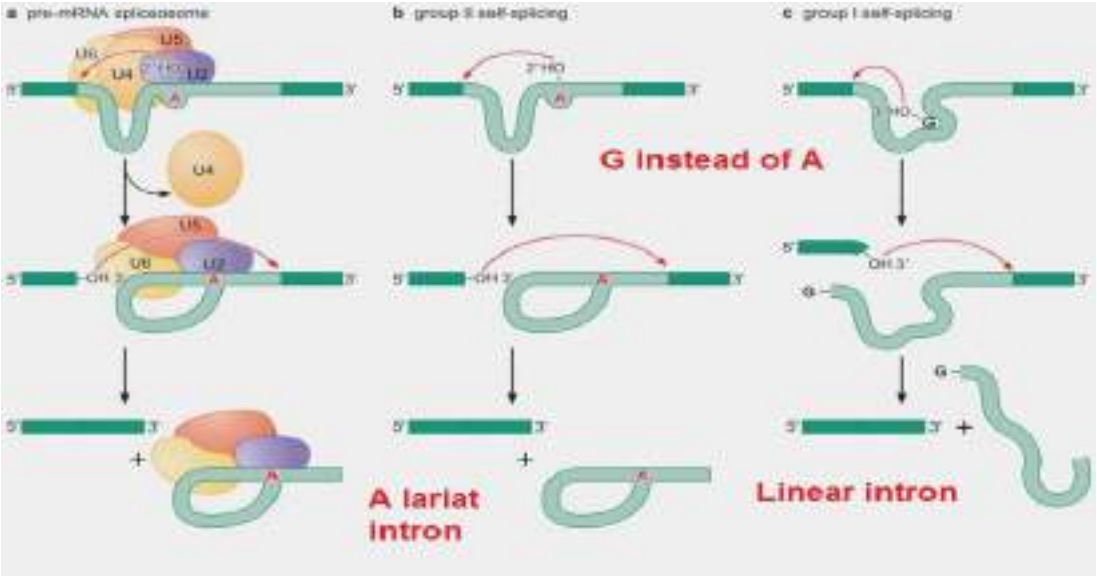
- Presumably, this strategy lessens the chance of aberrant splicing.
- Linking the formation of the active site to the successful completion of earlier steps in spliceosome assembly makes it highly likely that the active site is available only at legitimate splice sites.
- Formation of the active site juxtaposes the 5’ splice site of the pre-mRNA and the branch site, facilitating the first transesterification reaction.
- The second reaction, between the 5’ and 3’ splice sites, is aided by the U5 snRNP, which helps to bring the two exons together.
- The final step involves release of the mRNA product and the snRNPs. The snRNPs are initially still bound to the lariat, but they get recycled after rapid degradation of that piece of RNA.

Lecture 119

Self-Splicing Introns

- Self-Splicing Introns Reveal That RNA Can Catalyze RNA Splicing.
- There are total three classes of splicing found in the cells:-
- Nuclear pre-mRNA
- Group II introns
- Group I introns
- Thus far, we have dealt only with nuclear pre-mRNA splicing, that mediated by the spliceosome found in all eukaryotes.

- The so-called Group I and Group II are self-splicing introns.
- By “self- splicing” we mean that the intron itself folds into a specific conformation within the precursor RNA and catalyzes the chemistry of its own release.
- In terms of a practical definition, “self-splicing” refers to introns that can remove themselves from RNAs in the test tube in the absence of any proteins or other RNA molecules.
- The self splicing introns are grouped into two classes on the basis of their structure and splicing mechanism.
- Strictly speaking, self-splicing introns are not enzymes because they mediate only one round of RNA processing.
- In the case of group II introns, the chemistry of splicing and the RNA intermediates produced are the same as those for nuclear pre-mRNAs.
- For example, the intron uses an A residue within the branch site to attack the phosphodiester bond at the boundary between its 5’ end and the end of the 5’ exon - that is, at the 5’ splice site.
- This reaction produces the branched lariat and is followed by a second reaction in which the newly freed 3’-OH of the exon attacks the 3’ splice site, releasing the intron as a lariat and fusing the 3’ and 5’ exons.



Lecture 120

Translation

- The genetic information contained within the order of nucleotides in messenger RNA (mRNA) is interpreted to generate the linear sequences of amino acids in proteins. This process is known as translation.
- Translation is among the most highly conserved across all organisms and among the most energetically costly for the cell.
- In rapidly growing bacterial cells, up to 80% of the cell's energy and 50% of the cell's dry weight are dedicated to protein synthesis.
- Indeed, the synthesis of a single protein requires the coordinated action of well over 100 proteins and RNAs.
- Unlike the complementarity between the DNA template and the ribonucleotides of the mRNA, the side chains of amino acids have little or no specific affinity for the purine and pyrimidine bases found in RNA.

- For example, the hydrophobic side chains of the amino acids alanine, valine, leucine, and isoleucine cannot form hydrogen bonds with the amino and keto groups of the nucleotide bases.
- Similarly, it is hard to imagine that how the bases of RNA can have unique affinities for the aromatic amino acids phenylalanine, tyrosine, and tryptophan.
- So Francis H. Crick in 1955 proposed that before their incorporation into polypeptides, amino acids must attach to a special adaptor molecule that is capable of directly interacting with and recognizing the coding units of the mRNA.
- Paul C. Zamecnik and Mahlon B. Hoagland (1957) showed that before their incorporation into proteins, amino acids are attached to a class of RNA molecules (representing 15% of all cellular RNA).
- These RNAs are called transfer RNAs (tRNAs) because their attached amino acid is subsequently transferred to the growing polypeptide chain.
- The machinery responsible for translating the language of mRNAs into the language of proteins is composed of four primary components:-
- mRNAs, tRNAs, aminoaceyl-tRNA synthetases and the ribosomes.
- Together, these components accomplish the extraordinary task of translating a code written in a four-base alphabet into a second code written in the language of the 20 amino acids.
- The mRNA provides the information that must be interpreted by the translation machinery and is the template for translation.

- The protein-coding region of the mRNA consists of an ordered series of three nucleotide- long units called codons that specify the order of amino acids.
- The tRNAs provide the physical interface between the amino acids being added to the growing polypeptide chain and the codons in the mRNA.
- Enzymes called aminoacyl-tRNA synthetases couple amino acids to specific tRNAs that recognize the appropriate codon(s).
- The final major player in translation is the ribosome, a remarkable, multimegadalton machine composed of both RNA and protein.
- The ribosome coordinates the correct recognition of the mRNA by each tRNA and catalyzes peptide-bond formation between the growing polypeptide chain and the amino acid attached to the selected tRNA.

Lecture 121

Messenger RNA

The protein-coding region(s) of each mRNA is composed of a contiguous, non-overlapping string of codons called an open reading frame (commonly known as an ORF).

Each ORF specifies a single protein and starts and ends at internal sites within the mRNA. That is, the ends of an ORF are distinct from the ends of the mRNA.

Translation starts at the 5’ end of the ORF and proceeds one codon at a time to the 3’ end. The first and last codons of an ORF are known as the start and stop codons.

In bacteria, the start codon is usually 5’-AUG-3’, but 5’-GUG-3’ and sometimes even 5’-UUG-3’ are also used.

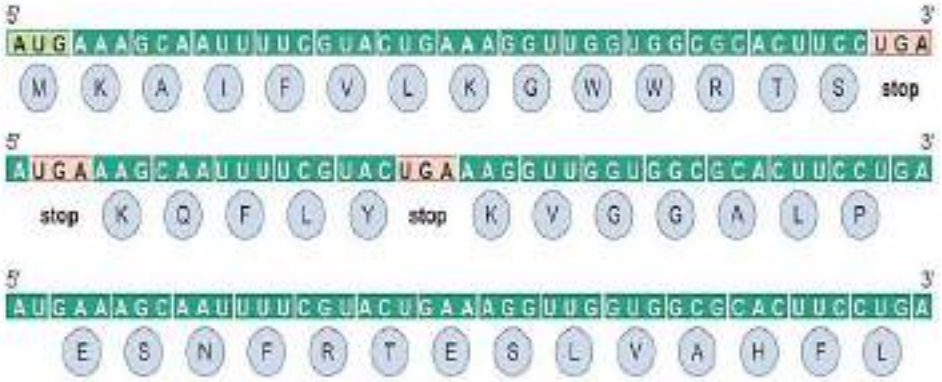
Eukaryotic cells always use 5’-AUG-3’ as the start codon.

The start codon has two important functions.

First, it specifies the first amino acid to be incorporated into the growing polypeptide chain.

Second, it defines the reading frame for all subsequent codons.

Because each codon is immediately adjacent to the next codon, and because codons are three nucleotides long, any stretch of mRNA could be translated in three different reading frames.



Three possible reading frames of the *E. coli trp* leader sequence

Once translation starts, however, the reading frame is determined.

Thus, by setting the location of the first codon, the start codon determines the location of all following codons.

Stop codons, of which there are three (5’-UAG-3’, 5’-UGA-3’, and 5’-UAA-3’), define the end of the ORF and signal termination of polypeptide synthesis.

You can now understand the origin of the term open reading frame. It is a contiguous stretch of codons “read” in a particular frame (as set by the first codon) that is “open” to translation because it lacks a stop codon

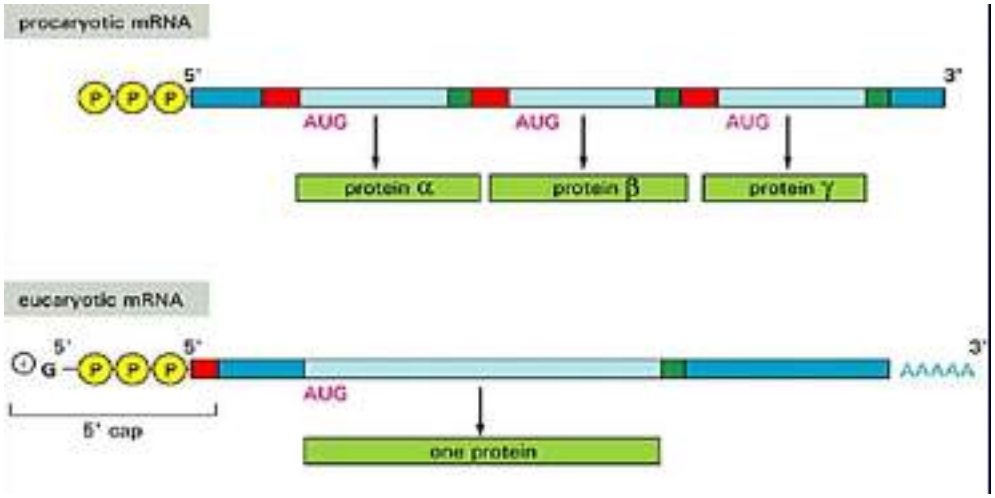
mRNAs contain at least one ORF. The number of ORFs per mRNA is different between eukaryotes and prokaryotes.

Eukaryotic mRNAs almost always contain a single ORF.

In contrast, prokaryotic mRNAs frequently contain two or more ORFs.

mRNAs containing multiple ORFs are known as polycistronic RNAs and those encoding a single ORF are known as monocistronic RNAs.

The polycistronic mRNAs found in bacteria often encode proteins that perform related functions, such as different steps in the biosynthesis of an amino acid or nucleotide.



Lecture 122

Discovery of Messenger RNA

Cells infected with phage T4 provided the ideal system to find the true template for protein synthesis.

Following infection by this virus, cells stop synthesizing E. coli RNA; the only RNA synthesized is transcribed off the T4 DNA.

Most strikingly, not only does T4 RNA have a base composition very similar to T4 DNA, but it does not bind to the ribosomal proteins that normally associate with rRNA to form ribosomes.

Instead, after first attaching to previously existing ribosomes, T4 RNA moves across their surface to bring its bases into positions where they can bind to the appropriate tRNA–amino acid precursors for protein synthesis.

In so acting, T4 RNA orders the amino acids and is thus the long-sought-for RNA template for protein synthesis.

Because it carries the information from DNA to the ribosomal sites of protein synthesis, it is called messenger RNA (mRNA).

The observation of T4 RNA binding to E. coli ribosomes, first made in 1960, was soon followed with evidence for a separate messenger class of RNA within uninfected E. Coli cells.

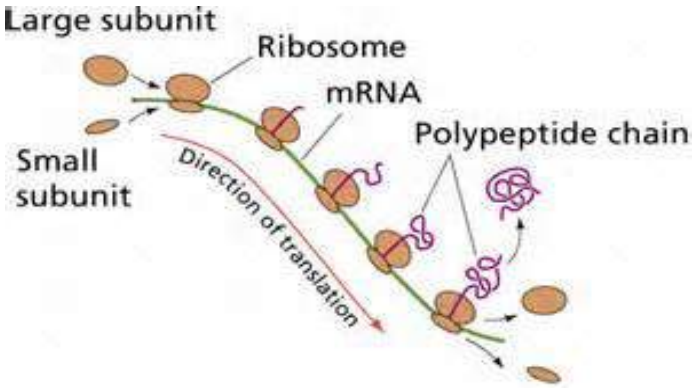
Instead, the rRNA components of ribosomes, together with some 50 different ribosomal proteins that bind to them, serve as the factories for protein synthesis.

They function to bring the tRNA–amino acid precursors into positions where they can read off the information provided by the mRNA templates.

Only a few percent of total cellular RNA is mRNA.

This RNA shows the expected large variations in length and nucleotide composition required to encode the many different proteins found in a given cell.

Hence, it is easy to understand why mRNA was first over looked. Because only a small segment of mRNAis attached at a given moment to a ribosome, a single mRNA molecule can simultaneously be read by several ribosomes.



Lecture 123

Prokaryotic mRNAs

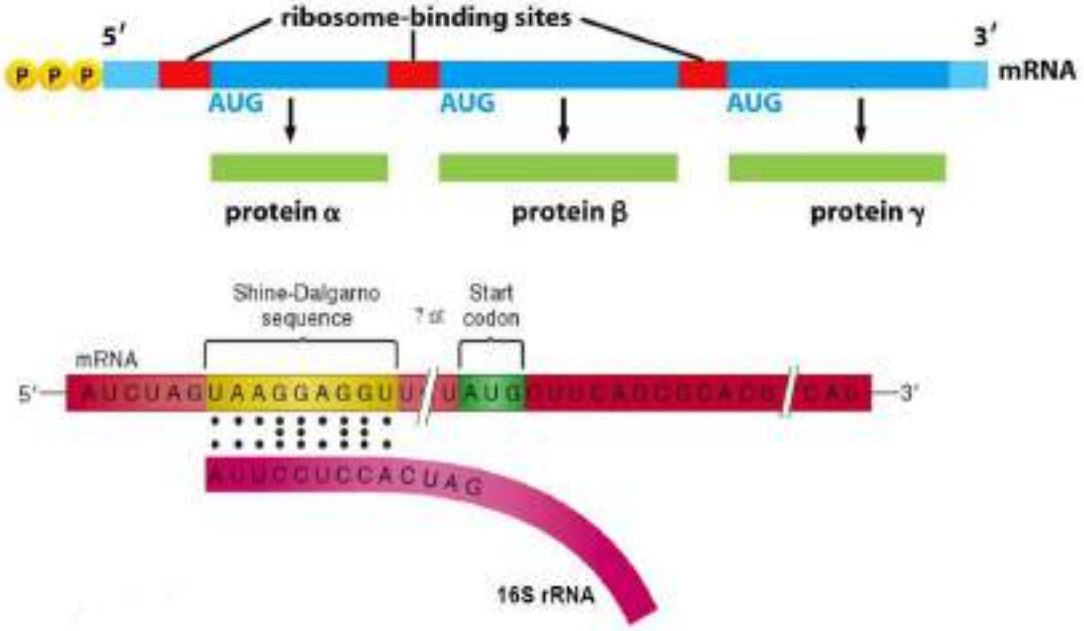
For translation to occur, the ribosome must be recruited to the mRNA.

Prokaryotic mRNAs have a ribosome-binding site that recruits the translational machinery.

To facilitate binding by a ribosome, many prokaryotic ORFs contain a short sequence upstream (on the 5’ side) of the start codon called the ribosome-binding site (RBS).

This element is also referred to as a Shine Őalgarno sequence after the scientists who discovered it by comparing the sequences of multiple mRNAs.

The RBS, typically located 3–9 bp on the 5’ side of the start codon, is complementary to a sequence located near the 3’ end of one of the ribosomal RNA components, the 16S ribosomal RNA (rRNA).



The RBS base-pairs with this RNA, thereby aligning the ribosome with the beginning of the ORF. The core of this region of the 16S rRNA has the sequence 5’-CCUCCU-3’.

Not surprisingly, prokaryotic RBS are most often a subset of the sequence 5’-AGGAGG-3

The extent of complementarity and the spacing between the RBS and the start codon has a strong influence on how actively a particular ORF is translated

High complementarity and proper spacing promote active translation, whereas limited complementarity and/or poor spacing generally support lower levels of translation.

Some prokaryotic ORFs lack a strong RBS but are nonetheless actively translated.

These ORFs are not the first ORF in an mRNA but instead are located just after another ORF in a polycistronic message.

In these cases, the start codon of the downstream ORF often overlaps the 3’ end of the upstream ORF.

Thus, a ribosome that has just completed translating the upstream ORF is positioned to begin translating from the start codon for the downstream ORF.

This phenomenon of linked translation between overlapping ORFs is known as translational coupling.

So in this situation translation of the downstream ORF requires translation of the upstream ORF.

Indeed, with two translationally coupled genes, a mutation that leads to a premature stop codon in the upstream ORF also prevents translation of the downstream ORF.

Lecture 124

Eukaryotic mRNAs

Unlike their prokaryotic counterparts, eukaryoticmRNAs recruit ribosomes using a specific chemical modification called the 5’ cap, which is located at the extreme 5’ end of the mRNA.

The 5’ cap is a methylated guanine nucleotide that is joined to the 5’ end of the mRNA via an unusual 5’-to-5’ linkage.

Created in three steps, the guanine nucleotide of the 5’ cap is connected to the 5’ end of the mRNA through three phosphate groups.

The resulting 5’ cap is required to recruit the ribosome to the mRNA. Once bound to the mRNA, the ribosome moves in a 5’ 3’ direction until it encounters a 5’-AUG-3’ start codon, a process called scanning.

Two other features of eukaryotic mRNAs stimulate translation. One feature is the presence, in some mRNAs, of a purine three bases upstream of the start codon and a guanine immediately downstream (5’-G/ANNAUGG-3’).

This sequence was originally identified by Marilyn Kozak and is referred to as the Kozak sequence. Many eukaryotic mRNA slack these bases, but their presence increases the efficiency of translation.

In contrast to the situation in prokaryotes, these bases are thought to interact with the initiator tRNA, not with an RNA component of the ribosome.

A second feature that contributes to efficient translation is the presence of a poly-A tail at the extreme 3’ end of the mRNA.

This tail is added enzymatically by the enzyme poly-A polymerase.

Despite its location at the 3’ end of the mRNA, the poly-A tail enhances the level of translation of them RNA by enhancing the recruitment of key translation initiation factors.

Importantly, in addition to their roles in translation, these 5’- and 3’-end modifications also protect eukaryotic mRNAs from rapid degradation.

Lecture 125

Transfer RNA

The heart of protein synthesis is the “translation” of nucleotide sequence information (in the form of codons) into amino acids.

This is accomplished by tRNA molecules, which act as adaptors between codons and the amino acids they specify.

There are many types of tRNA molecules, but each is attached to a specific amino acid, and each recognizes a particular codon, or codons, in the mRNA (most tRNAs recognize more than one codon).

tRNA molecules are between 75 and 95 ribonucleotides in length.

Although the exact sequence varies, all tRNAs have certain features in common.

First, all tRNAs end at the 3’ terminus with the sequence 5’-CCA-3’.

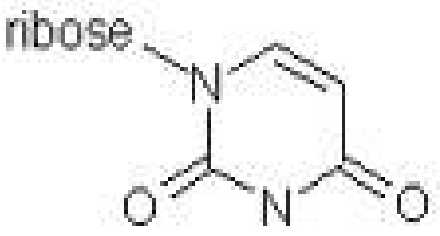
Consistent with this absolute conservation, the 3’ end of this sequence is the site that is attached to the cognate amino acid.

A second striking aspect of tRNAs is the presence of several unusual bases in their primary structure.

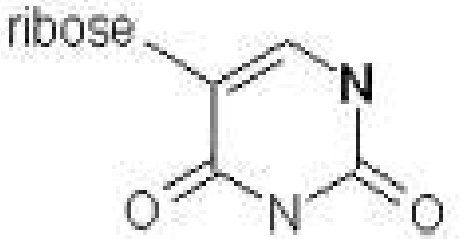
These unusual features are created post- transcriptionally by enzymatic modification of normal bases in the polynucleotide chain.

For example, pseudouridine (U) is derived from uridine by an isomerization in which the site of attachment of the uracil base to the ribose is switched from the nitrogen at ring position 1 to the carbon at ring position 5.

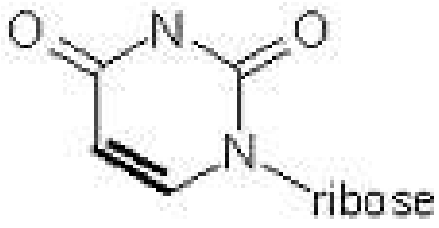
Likewise, dihydrouridine (D) is derived from uridine by enzymatic reduction of the double bond between the carbons at positions 5 and 6.



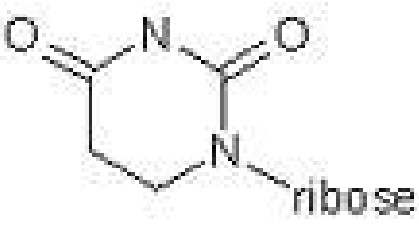
uridine



Pseudouridine



uridine



dihydrouridine

Other unusual bases found in tRNA include hypoxanthine, thymine, and methylguanine.

These modified bases are not essential for tRNA function, but cells lacking these modified bases show reduced rates of growth.

This observation suggests that the modified bases lead to improved tRNA

function.

For example, hypoxanthine plays an important role in the process of codon recognition by certain tRNAs.

Lecture 126

Discovery of Transfer RNA

The discovery of how proteins are synthesized required the development of cell-free extracts capable of making proteins from amino acid precursors as directed by added RNA molecules.

These were first effectively developed in 1953 by Paul C. Zamecnik and his collaborators. Key to their success were the recently available radioactively tagged amino acids.

They used these labelled amino acids to mark the trace amounts of newly synthesized proteins.

Early on, the cellular site of protein synthesis was pinpointed to be the ribosomes.

Several years later, Zamecnik, with his collaborator Mahlon B. Hoagland, made an important discovery that prior to their incorporation into proteins, amino acids are first attached to what we now call transfer RNA (tRNA) molecules.

Transfer RNA accounts for some 10% of all cellular RNA.

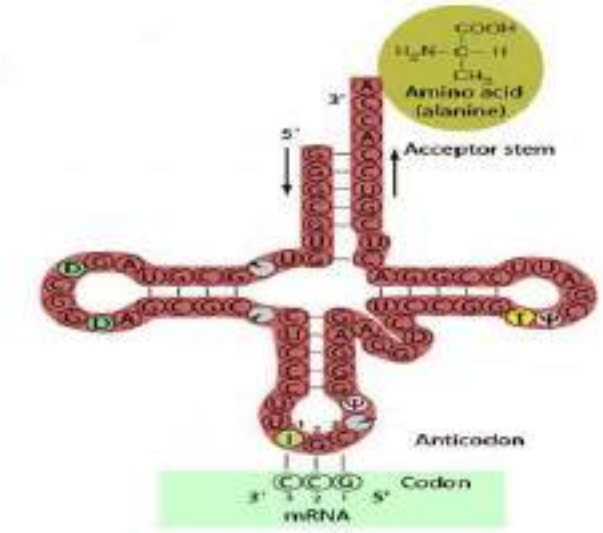
To nearly everyone except Crick, this discovery was totally unexpected.

He had, of course, previously speculated that his proposed “adaptors” might be short RNA chains,

because their bases would be able to base-pair and “read” the appropriate groups on the RNA molecules that served as the templates for protein synthesis.

The transfer RNA molecules of Zamecnik and Hoagland are in fact the adaptor molecules postulated by Crick.

Each transfer RNA contains a sequence of adjacent bases (the anticodon) that bind specifically during protein synthesis to successive groups of bases (codons) along the RNA template.



Lecture 127

Secondary Structure of tRNA

RNA molecules typically contain regions of self complementarity that enable them to form limited stretches of double helix that are held together by base pairing.

tRNA molecules show a characteristic and highly conserved pattern of single-stranded and double stranded regions (secondary structure) that can be illustrated as a cloverleaf.

The principal features of the tRNA cloverleaf are an acceptor stem, three stem-loops (referred to as the □U loop, the D loop, and the anticodon loop), and a fourth variable loop.

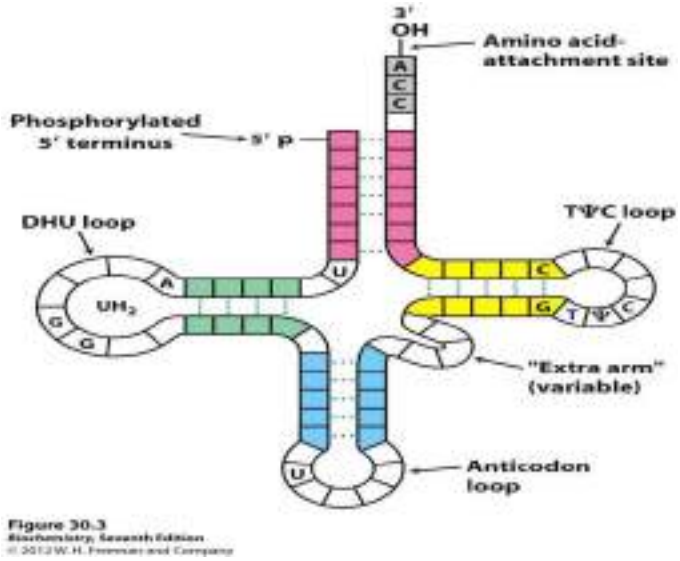


Figure 30.3

The Acceptor Stem:

It is so-named because it is the site of attachment of the amino acid, is formed by pairing between the 5’ and 3’ ends of the tRNA molecule.

The □U Loop:

It is is so-named because of the characteristic presence of the unusual base □U in the loop. The modified base is often found within the sequence 5’-TCUCG-3’.

The D Loop:

It takes its name from the characteristic presence of dihydrouridines in the loop.

The Anticodon Loop:

As its name implies, contains the anticodon, a three-nucleotide-long sequence that is responsible for recognizing the codon by base pairing with the mRNA.

The Variable Loop:

It sits between the anticodon loop and the □U loop and, as its name implies, varies in size from 3 to 21 bases.

Lecture 128

Attachment of Amino Acids to tRNA

tRNA molecules to which an amino acid is attached are said to be charged, and tRNAs that lack an amino acid are said to be uncharged.

Charging requires an acyl linkage between the carboxyl group of the amino acid and the 2’- or 3’-hydroxyl group of the adenosine nucleotide that protrudes from the acceptor stem at the 3’ end of the tRNA.

This acyl linkage is a high-energy bond because its hydrolysis results in a large change in free energy.

This is significant for protein synthesis: the energy released when this acyl bond is broken is coupled to the formation of the peptide bonds that link amino acids to each other in polypeptide chains.

All aminoacyl-tRNA synthetases attach an amino acid to a tRNA in two enzymatic steps:

Adenylation

tRNA charging

Step one is **adenylation** in which the amino acid reacts with ATP to become adenylylated with the concomitant release of pyrophosphate.

Adenylylation refers to transfer of AMP, as opposed to adenylation, which would indicate the transfer of adenine.

The principal driving force for the adenylylation reaction is the subsequent hydrolysis of pyrophosphate by pyrophosphatase.

As a result of adenylylation, the amino acid is attached to adenylic acid via a high-energy ester bond in which the carbonyl group of the amino acid is joined to the phosphoryl group of AMP.

Step two is tRNA Charging in which the adenylylated amino acid, which remains tightly bound to the synthetase, reacts with tRNA.

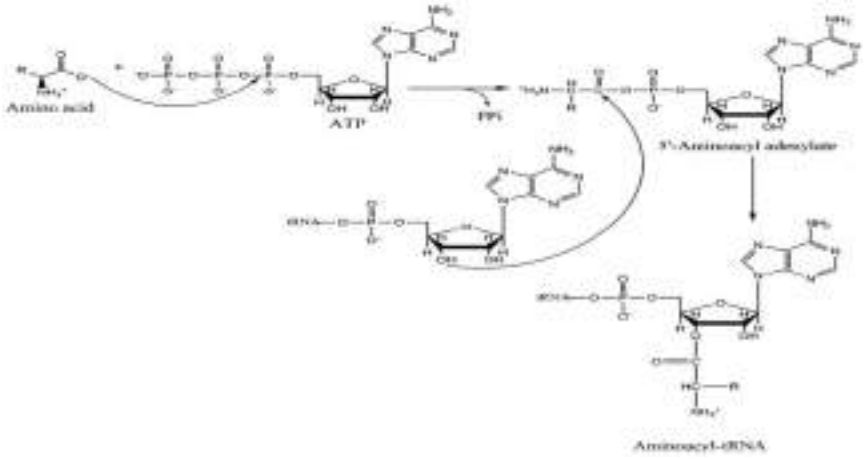
This reaction results in the transfer of the amino acid to the 3’ end of the tRNA via the 2’- or 3’-hydroxyl and the release of AMP.

There are two classes of tRNA synthetases:

Class I enzymes attach the amino acid to the 20-OH of the tRNA and are generally monomeric.

Class II enzymes attach the amino acid to the 30-OH of the tRNA and are typically dimeric or tetrameric.

Although the initial coupling between the tRNA and the amino acid is different, once released from the synthetase, the amino acid rapidly equilibrates between attachment at the 3’-OH and the 2’-OH.



Each of the 20 amino acids is attached to the appropriate tRNA by a single, dedicated tRNA synthetase.

Because most amino acids are specified by more than one codon, it is not uncommon for one synthetase to recognize and charge more than one tRNA (known as isoaccepting tRNAs).

Nevertheless, the same tRNA synthetase is responsible for charging all tRNAs for a particular amino acid.

Thus, one and only one tRNA synthetase attaches each amino acid to all of the appropriate tRNAs.

Lecture 129

The Ribosomes

The ribosome is the macromolecular machine that directs the synthesis of proteins.

The ribosome is larger and more complex than the minimal machinery required for DNA or RNA synthesis.

The machinery for polymerizing amino acids is composed of at least three RNA molecules and more than 50 different proteins, with an overall molecular mass of ?2.5 MDa.

Compared with the speed of DNA replication i.e., 200 –1000 nucleotides per second; translation takes place at a rate of only two to 20 amino acids per second.

In prokaryotes, the transcription machinery and the translation machinery are located in the same compartment. Thus, the ribosome can commence translation of the mRNA as it emerges from the RNA polymerase.

This situation allows the ribosome to proceed in tandem with the RNA polymerase as it elongates the transcript.

Recall that the 5’ end of an RNA is synthesized first, and thus the ribosome, which begins translation at the 5’ end of the mRNA, can start translating a nascent transcript as soon as it emerges from the RNA polymerase.

Lecture 130

Formation of Peptide Bonds

Each new amino acid is added to the carboxyl terminus of the growing polypeptide chain (often referred to as synthesis in the amino- to carboxy-terminal direction).

The ribosome catalyzes a single chemical reaction —the formation of a peptide bond.

This reaction occurs between the amino acid residue at the carboxy-terminal end of the growing polypeptide and the incoming amino acid to be added to the chain.

Both the growing chain and the incoming amino acid are attached to tRNAs; as a result, during peptide-bond formation, the growing polypeptide is continuously attached to a tRNA.

The actual substrates for each round of amino acid addition are two charged species of tRNAs —an aminoacyl-tRNA and a peptidyl-tRNA.

As you know the aminoacyl-tRNA is attached at its 3’ end to the carboxyl group of the amino acid. The peptidyl-tRNA is attached in exactly the same manner (at its 3’ end) to the carboxyl terminus of the growing polypeptide chain.

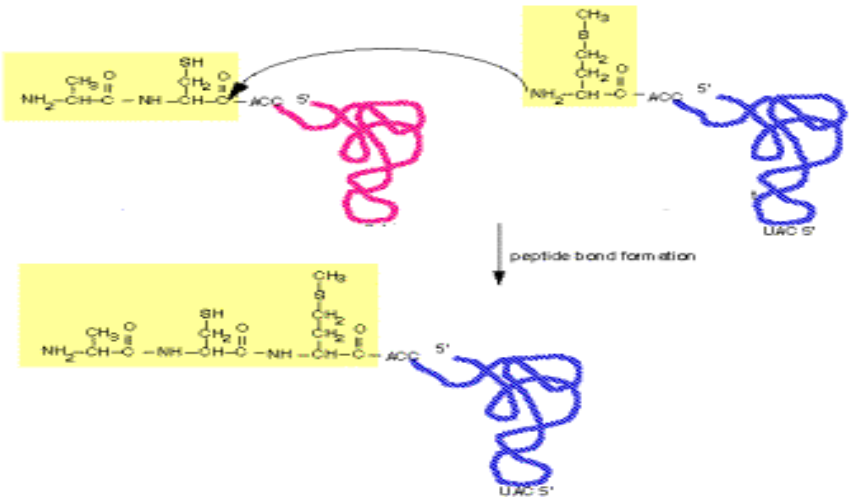
The bond between the aminoacyl-tRNA and the amino acid is not broken during the formation of the next peptide bond.

Instead, the bond between the peptidyl-tRNA and the growing polypeptide chain is broken as the growing chain is attached to the amino group of the amino acid attached to the aminoacyl-tRNA to form a new peptide bond.

To catalyze peptide-bond formation, the 3’ ends of these two tRNAs are brought into close proximity by the ribosome.

The resulting tRNA positioning allows the amino group of the amino acid attached to aminoacyl-tRNA to attack the carbonyl group of the most carboxy-terminal amino acid attached to the peptidyl-tRNA.

The result of this nucleophilic attack is the formation of a new peptide bond between the amino acids attached to the tRNAs and the release of the polypeptide chain from the peptidyl tRNA.



There are two consequences of this method of polypeptide synthesis. First, this mechanism of peptide-bond formation requires that the amino terminus of the protein be synthesized before the carboxyl terminus.

Second, the growing polypeptide chain is transferred from the peptidyl-tRNA to the aminoacyl-tRNA. For this reason, the reaction to form a new peptide bondis called the peptidyl transferase reaction.

Interestingly, peptide-bond formation takes place without the simultaneous hydrolysis of a nucleoside triphosphate.

This is because peptide-bond formation is driven by breaking the high-energy acyl bond that joins the growing polypeptide chain to the tRNA.

Recall that this bond was created during the tRNA synthetase – catalyzed reaction that is responsible for charging tRNA.

And the charging reaction involves the hydrolysis of a molecule of ATP.

Thus, the energy for peptide-bond formation originates from the molecule of ATP that was hydrolyzed during the tRNA charging reaction.

Lecture 131

Binding Sites on Ribosomes for tRNA

The ribosome is composed of two subassemblies of RNA and protein known as the large and small subunits.

The large subunit contains the peptidyl transferase center, which is responsible for the formation of peptide bonds.

The small subunit contains the decoding center in which charged tRNAs read or "decode" the codon units of the mRNA.

Both the decoding center and the peptidyl transferase center are buried within the intact ribosome.

Yet, mRNA must be threaded through the decoding center during translation, and the nascent polypeptide chain must escape from the peptidyl transferase center.

How do these polymers enter and exit the ribosome?

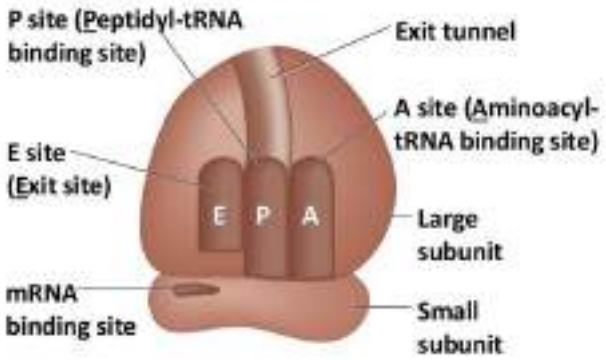
The answer is provided by the structure of the ribosome, which reveals that there are "tunnels" in and out of the ribosome.

To perform the peptidyl transferase reaction, the ribosome must be able to bind at least two tRNAs simultaneously.

In fact, the ribosome contains three tRNA-binding sites, called the A-, P-, and E-sites.

The A-site is the binding site for the aminoacylated-tRNA, the P-site is the binding site for the peptidyl-tRNA, and

The E-site is the binding site for the tRNA that is released after the growing polypeptide chain has been transferred to the aminoacyl-tRNA (E is for “exiting”).



Each tRNA binding site is formed at the interface between the large and the small subunits of the ribosome.

In this way, the bound tRNAs can span the distance between the peptidyl transferase center in the large subunit and the decoding center in the small subunit.

The 3' ends of the tRNAs that are coupled to the amino acid or to the growing peptide chain are adjacent to the large subunit.

The anticodon loops of the bound tRNAs are located adjacent to the small subunit.

Lecture 132

Initiation of Translation

For translation to be successfully initiated, three events must occur:-

i) the ribosome must be recruited to the mRNA.

ii) a charged tRNA must be placed into the P-site of the ribosome.

iii) the ribosome must be precisely positioned over the start codon.

The correct positioning of the ribosome over the start codon is critical because this establishes the reading frame for the translation of the mRNA.

In prokaryotes, the assembly of the ribosome on an mRNA occurs one subunit at a time. The small subunit associates with the mRNA first.

In prokaryotes, the association of the small subunit with the mRNA is mediated by base-pairing interactions between the RBS and the 16S rRNA.

For ideally positioned RBSs, the small subunit is positioned on the mRNA such that the start codon will be in the P-site when the large subunit joins the complex.

The large subunit joins its partner only at the very end of the initiation process, just before the formation of the first peptide bond.

Thus, many of the key events of translation initiation occur in the absence of the full ribosome.

Translation initiation is the only time a tRNA binds to the P-site without previously occupying the A-site. This event requires a special tRNA known as the initiator tRNA.

The initiator tRNA base-pairs with the start codon (AUG or GUG). AUG and GUG have a different meaning when they occur within an ORF, where they are read by tRNAs for methionine and valine, respectively.

Although the initiator tRNA is first charged with a methionine, a formyl group is rapidly added to the methionine amino group by a separate enzyme (Met-tRNA transformylase).

Thus rather than valine or methionine, the initiator tRNA is coupled to N-formyl methionine. The charged initiator tRNA is referred to as fMet-tRNAfMet.

Because N-formyl methionine is the first amino acid to be incorporated into a polypeptide chain, one might think that all prokaryotic proteins have a formyl group at their amino termini.

This is not the case, however, because an enzyme known as a deformylase removes the formyl group from the amino terminus during or after the synthesis of the polypeptide chain.

In fact, many mature prokaryotic proteins do not even start with a methionine; aminopeptidases often remove the amino-terminal methionine as well as one or two additional amino acids.

Lecture 133

The Initiation Factors

The initiation of prokaryotic translation commences with the small subunit and is catalyzed by three translation initiation factors called IF1, IF2, and IF3.

Each factor facilitates a key step in the initiation process.

IF1:

It prevents tRNAs from binding to the portion of the small subunit that will become part of the A-site.

IF2:

It is a GTPase that interacts with three key components of the initiation machinery: the small subunit, IF1, and the charged initiator tRNA (fMet-tRNA fMet).

By interacting with these components, IF2 facilitates the association of fMet-tRNA fMet with the small subunit and prevents other charged tRNAs from associating with the small subunit.

IF3:

It binds to the small subunit and blocks it from re-associating with a large subunit. Because initiation requires a free small subunit, the binding of IF3 is critical for a new cycle of translation.

IF3 becomes associated with the small subunit at the end of a previous round of translation when it helps to dissociate the 70S ribosome into its large and small subunits.

Each of the initiation factors binds at, or near, one of the three tRNA binding sites on the small subunit.

Consistent with its role in blocking the binding of charged tRNAs to the A-site, IF1 binds directly to the portion of the small subunit that will become the A-site.

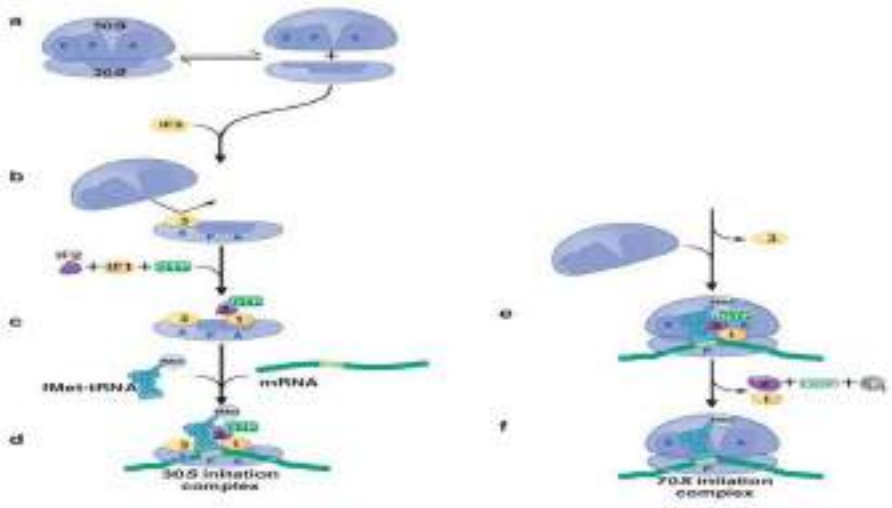
IF2 binds to IF1 and reaches over the A-site into the P-site to contact the fMet - tRNA fMet.

Finally, IF3 occupies the part of the small subunit that will become the E-site.

Thus, of the three potential tRNA-binding sites on the small subunit, only the P-site is capable of binding a tRNA in the presence of the initiation factors.

With all three initiation factors bound, the small subunit is prepared to bind to the mRNA and the initiator tRNA .

These two RNAs can bind in either order and independently of each other.



Binding fMet-tRNA fMet to the small subunit is facilitated by its interactions with IF2 bound to GTP and base pairing between the anticodon and the start codon of the mRNA.

Similarly, base pairing between the fMet-tRNA fMet and the mRNA serves to position the start codon in the P-site.

The last step of initiation involves the association of the large subunit to create the 70S initiation complex.

When the start codon and fMet-tRNAfMet base-pair, the small subunit undergoes a change in conformation.

This altered conformation results in the release of IF3.

In the absence of IF3, the large subunit is free to bind to the small subunit with its cargo of IF1, IF2, mRNA, and fMet-tRNAfMet.

In particular, IF2 acts as an initial docking site of the large subunit, and this interaction subsequently stimulates the GTPase activity of IF2.GTP.

IF2 bound to GDP has reduced affinity for the ribosome and the initiator tRNA, leading to the release of IF2.GDP as well as IF1 from the ribosome.

Thus, the net result of initiation is the formation of an intact (70S) ribosome assembled at the start site of the mRNA with fMet-tRNAfMet in the P-site and an empty A-site.

The ribosome – mRNA complex is now poised to accept a charged tRNA into the A-site and commence polypeptide synthesis.

Lecture 134

Translation Elongation

Once the ribosome is assembled with the charged initiator tRNA in the P site, polypeptide synthesis can begin.

There are three key events that must occur for the correct addition of each amino acid.

First, the correct aminoacyl-tRNA is loaded into the A site of the ribosome as dictated by the A-site codon.

Second, a peptide bond is formed between the aminoacyl-tRNA in the A site and the peptide chain that is attached to the peptidyl-tRNA in the P site.

This peptidyl transferase reaction results in the transfer of the growing polypeptide from the tRNA in the P site to the amino acid moiety of the charged tRNA in the A site.

Third, the resulting peptidyl-tRNA in the A site and its associated codon must be translocated to the P site so that the ribosome is poised for another cycle of codon recognition and peptide bond formation.

As with the original positioning of the mRNA, this shift must occur precisely to maintain the correct reading frame of the message.

Two auxiliary proteins known as elongation factors control these events.

Both of these factors use the energy of GTP binding and hydrolysis to enhance the rate and accuracy of ribosome function.

Unlike the initiation of translation, the mechanism of elongation is highly conserved between prokaryotic and eukaryotic cells.

Aminoacyl-tRNAs do not bind to the ribosome on their own. Instead, they are "escorted " to the ribosome by the elongation factor EF-Tu.

Once a tRNA is aminocylated, EF-Tu binds to the tRNA's 3 ' end, masking the coupled amino acid. This interaction prevents the bound aminoacyl-tRNA from participating in peptide bond formation until it is released from EF-Tu.

Like the initiation factor IF2, the elongation factor EF-Tu binds and hydrolyzes GTP and the type of guanine nucleotide bound governs its function.

EF-Tu can only bind to an aminoacyl-tRNA when it is associated with GTP. EF-Tu bound to GDP, or lacking any bound nucleotide, shows little affinity for aminoacyl-tRNAs.

Thus, when EF-Tu hydrolyzes its bound GTP, any associated aminoacyl-tRNA is released.

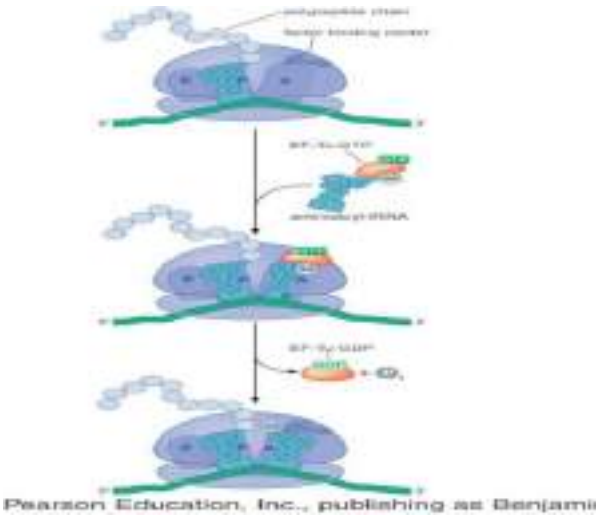
The trigger that activates the EF-Tu GTPase is the same domain on the large subunit of the ribosome that activates the IF2 GTPase when the large subunit joins the initiation complex.

This domain is known as the factor binding center.

EF-Tu only interacts with the factor binding center after the tRNA is loaded into the A site and a correct codon-anticodon match is made.

At this point, EF-Tu hydrolyzes its bound GTP and is released from the ribosome.

The control of GTP hydrolysis by EF-Tu is critical to the specificity of translation.



The error rate of translation is between 10-3 to 10-4.

The ultimate basis for the selection of the correct aminoacyl-tRNA is the base pairing between the charged tRNA and the codon displayed in the A site of the ribosome.

However, in some cases, the base pairing in the anticodon-codon interaction may be mismatched, yet the ribosome rarely allows such mismatched aminoacyl-tRNAs to continue in the translation process.

Lecture 135

The Ribosome Is a Ribozyme

Once the correctly charged tRNA has been placed in the A site and has rotated into the peptidyl transferase center, peptide bond formation takes place.

This reaction is catalyzed by RNA, specifically the 23 S rRNA component of the large subunit.

Early evidence for this came from experiments in which it was shown that a large subunit that had been largely stripped of its proteins was still able to carry out peptide bond formation.

Proof that the peptidyl transferase is entirely composed of RNA has come from the high-resolution, three-dimensional structure of the ribosome, which reveals that no amino acid is located closer than 18 Aº from the active site.

Because catalysis requires distances in the 1 - 3 Aº range, it is clear that the peptidyl transferase center is a ribozyme. That is an enzyme composed of RNA.

How does the 23 S rRNA catalyze peptide bond formation?

The exact mechanism remains to be determined, but some answers to this question are beginning to emerge.

First, base-pairing between the 23 S rRNA and the CCA ends of the tRNAs in the A and the P sites help to position the alpha-amino group of the aminoacyl-tRNA to attack the carbonyl group of the growing polypeptide attached to the peptidyl-tRNA.

These interactions are also likely to stabilize the aminoacyl-tRNA after accommodation.

Because close proximity of substrates is rarely sufficient to generate high levels of catalysis, it is hypothesized that other elements of the ribosomal RNA change the chemical environment of the peptidyl transferase active site.

For example, it has been proposed that nucleotides in the peptidyl transferase center accept a hydrogen from the alpha amino group of the aminoacyl-tRNA, making the associated nitrogen a stronger nucleophile.

This is a common mechanism used by many proteins to stimulate nucleophilic attack of carbonyl groups.

Lecture 136

Translocation in the Large Subunit

Once the peptidyl transferase reaction has occurred, the tRNA in the P-site is deacetylated (no longer attached to an amino acid), and the growing polypeptide chain is linked to the tRNA in the A-site.

For a new round of peptide chain elongation to occur, the P-site tRNA must move to the E-site and the A-site tRNA must move to the P-site.

At the same time, the mRNA must move by three nucleotides to expose the next codon.

These movements are coordinated within the ribosome and are collectively referred to as translocation.

The initial steps of translocation are coupled to the peptidyl transferase reaction.

Once the growing peptide chain has been transferred to the A-site tRNA, the A- and P-site tRNAs have a preference to occupy new positions in the large subunit.

The 3’ end of the A-site tRNA is bound to the growing polypeptide chain and prefers to bind in the P-site of the large subunit.

The now deacetylated P-site tRNA is no longer attached to the growing polypeptide chain and prefers to bind in the E-site of the large subunit.

In contrast, at this time, the anticodons of these tRNAs remain in their initial location in the small subunit bound to the mRNA.

Thus, translocation is initiated in the large subunit before the small subunit, and the tRNAs are said to be in “hybrid states.”

Their 3’ ends have shifted into a new location, but their anticodon ends are still in their pre–peptidyl transfer position.

Importantly, this change is associated with a counter clockwise rotation of the small subunit relative to the large subunit facilitating interaction of the tRNAs with distinct tRNA-binding sites in the different subunits.

The completion of translocation requires the action of a second elongation factor called EF-G.

Initial binding of EF-G to the ribosome occurs when associated with GTP.

After the peptidyl transferase reaction, EF-G–GTP binds to and stabilizes the ribosome in the rotated, hybrid state.

When EF-G–GTP binds, it contacts the factor-binding center of the large subunit, which stimulates GTP hydrolysis.

GTP hydrolysis changes the conformation of EF-G with two consequences.

First, interactions between EF-G–GDP and the ribosome are thought to "unlock" the ribosome.

Structural studies reveal that there are "gates" that separate the A-, P-, and E-sites and EF-G–GDP is said to unlock the ribosome by opening these gates.

Second, the changed EF-G–GDP conformation binds to the A-site of the decoding center.

This interaction competes with the tRNA for binding to the A-site of the decoding center.

Because the ribosome is unlocked, the formerly A-site tRNA can move into the P-site, allowing EF-G–GDP to bind the A-site.

Completion of translocation is accompanied by a clockwise rotation of the small subunit back to its starting position.

The resulting ribosome structure has dramatically reduced affinity for EF-G–GDP.

Release of EF-G results in the return of the ribosome to a "locked" state in which the tRNAs and mRNA are once again tightly associated with the small subunit decoding center and the gates between the A-, P- and E-sites are closed.

Together, these events result in the translocation of the A-site tRNA into the P-site, the P-site tRNA into the E-site, and the movement of the mRNA by exactly 3 bp.

The ribosome is now ready for a new cycle of amino acid addition to begin.

Lecture 137

Termination of Translation

The ribosome's cycle of aminoacyl-tRNA binding, peptide-bond formation, and translocation continues until one of the three stop codons enters the A-site.

It was initially postulated that there would be one or more chain terminating tRNAs that would recognize these codons.

However, this is not the case.

Instead, stop codons are recognized by proteins called release factors (RFs) that activate the hydrolysis of the polypeptide from the peptidyl-tRNA.

There are two classes of release factors.

Class I release factors recognize the stop codons and trigger hydrolysis of the peptide chain from the tRNA in the P-site.

Prokaryotes have two class I release factors called RF1 and RF2.

RF1 recognizes the stop codon UAG and RF2 recognizes the stop codon UGA.

The third stop codon, UAA, is recognized by both RF1 and RF2.

In eukaryotic cells, there is a single class I release factor called eRF1 that recognizes all three stop codons.

Class II release factors stimulate the dissociation of the class I factors from the ribosome after release of the polypeptide chain.

Prokaryotes and eukaryotes have only one class II factor called RF3 and eRF3, respectively.

Like EF-G, IF2, and EF-Tu, class II release factors are regulated by GTP binding and hydrolysis.

How do release factors recognize stop codons?

Because release factors are composed entirely of protein, protein–RNA interaction must mediate stop codon recognition.

Experiments in which short coding regions were genetically swapped between RF1 and RF2 (having different stop-codon specificity) identified a three-amino-acid sequence that is critical for release factor specificity.

Exchange of these three amino acids between RF1 and RF2 swaps the stop-codon specificity of the two complexes.

For this reason, this three-amino-acid sequence is called a peptide anticodon and must interact with and recognize stop codons.

A 3D structure of RF1 bound to the ribosome confirms that RF1 binds to the A-site of the ribosome.

In this structure, the peptide anticodon is located very near the anticodon, but it is likely that there are additional protein regions that contribute to codon recognition.

A region of class I release factors that stimulates polypeptide release has also been identified.

All class I factors share a conserved three-amino-acid sequence (glycine, glycine, glutamine) that is essential for polypeptide release.

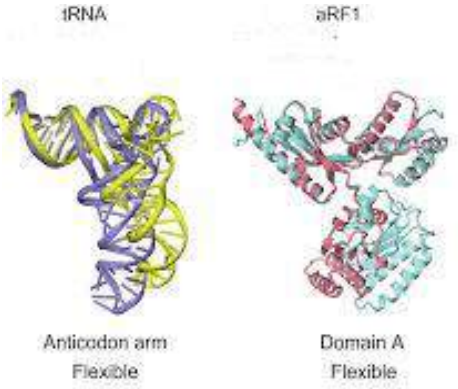
Moreover, the structure of RF1 bound to the ribosome confirms that the GGQ motif is located in close proximity to the peptidyl transferase center.

It remains unclear whether the GGQ motif is directly involved in the release of polypeptide from the peptidyl-tRNA or it induces a change in the peptidyl transferase center that allows the center itself to catalyze hydrolysis.

Studies of the conserved bases found adjacent to the CCA ends in the peptidyl transferase center indicate that several of these residues are required for peptide hydrolysis.

Indeed, these bases appear to play a more important role in peptide release than they do in peptide-bond formation.

Together, these studies have led to the hypothesis that class I release factors functionally mimic a tRNA, having a peptide anticodon that interacts with the stop codon and a GGQ motif that reaches into the peptidyl transferase center.



Once the class I release factor has triggered the hydrolysis of the peptidyl tRNA linkage, it must be removed from the ribosome.

This step is stimulated by the class II release factor, RF3.

RF3 is a GTP-binding protein but, unlike the other GTP-binding proteins involved in translation, this factor has a higher affinity for GDP than GTP.

Thus, free RF3 is predominantly in the GDP-bound form.

RF3-GDP binds to the ribosome in a manner that depends on the presence of a class I release factor.

After the class I release factor stimulates polypeptide release, a change in the conformation of the ribosome and the class I release factor stimulates RF3 to exchange its bound GDP for a GTP.

The binding of GTP to RF3 leads to the formation of a high-affinity interaction with the ribosome that favors the rotated hybrid state.

This change in conformation displaces the class I factor from the ribosome.

These changes also allow RF3 to associate with the factor-binding center of the large subunit. As with other GTP-binding proteins involved in translation, this interaction stimulates the hydrolysis of GTP.

In the absence of a bound class I factor, the resulting RF3.GDP has a low affinity for the ribosome and is released.

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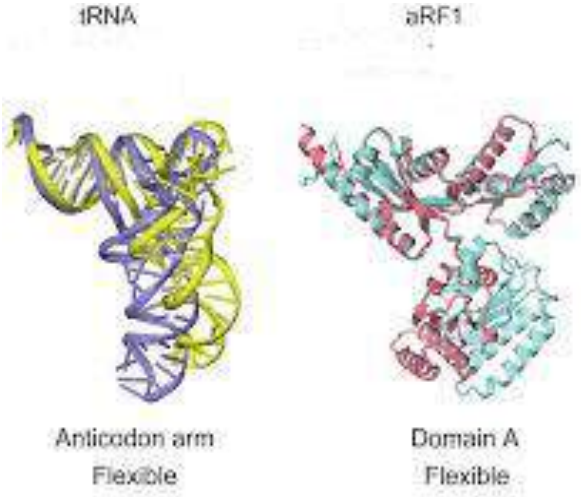

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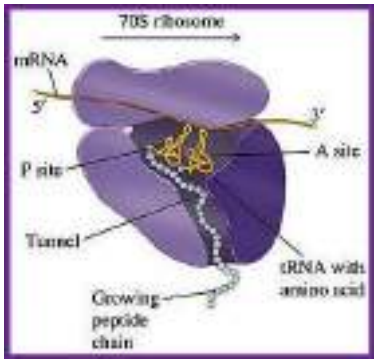
affinity for the ribosome and is released.

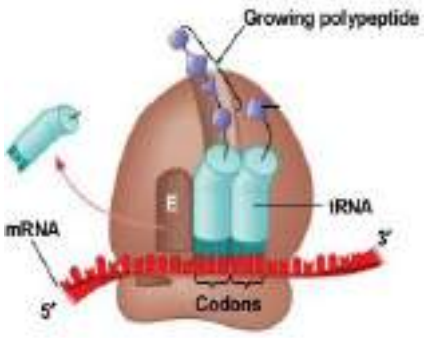
Lecture 139

Nascent polypeptide processing & folding

The nascent protein passes through a peptide exit tunnel that extends from the peptide transferase centre to the ribosome surface.

The exit tunnel in the bacterial ribosome is about 80-100 Aº long and about 10 Aº in its diameter at its narrowest point but widens to about twice that diameter at the rim of the exit pore.





The exit tunnel can accommodate an □ helix with about 60 residues or an extended peptide with about half that number of residues.

The space within the exit tunnel may permit the nascent polypeptide chain to assume an □-helical conformation but is too narrow to permit more extensive folding.

As the nascent peptide chain emerges from the tunnel, it interacts with enzymes that catalyze co-translational modifications, chaperones that assist in folding and prevent mis-folding,and the signal recognition particle that facilitates transport across the cell membrane.

Peptide deformylase and methionine aminopeptidase bind at the rim of the bacterial ribosome's exit pore.

The deformylase cleaves the N-terminal formyl group from the nascent polypeptide as it emerges from the exit tunnel.

Then the aminopeptidase recognizes about 60% of the different nascent polypeptides and removes their N-terminal methionine.

Nascent eukaryotic polypeptides do not have an N-terminal formyl group but do begin with methionine.

Ribosome-bound methionine aminopeptidases remove the N-terminal methionine.

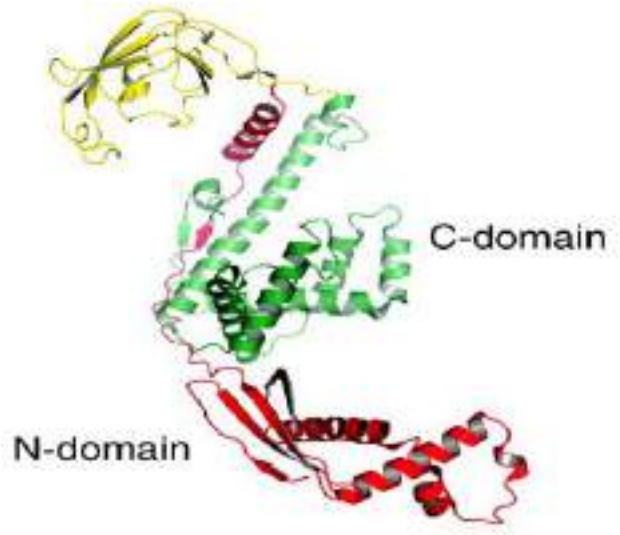
In bacteria, co-translational protein folding is assisted by a 48 kDa chaperone called the trigger factor, which binds at the bacterial ribosome's exit pore. The trigger factor protein transiently associates with the L23 protein on the 50S subunit.

Its residence time on the ribosome depends on whether the ribosome has a nascent protein in the exit tunnel.

If a nascent protein is not present, the average residence time is about 11 – 15 seconds but this time increases several –fold when a nascent protein is present.

The trigger factor contains three domains that arrange to form a characteristic elongated dragon-shaped structure.

The N-terminal domain forms the tail that binds to the 50S subunit.



The middle domain which forms the dragon's head has peptidyl prolyl cis/tran isomerase activity that is not essential for the trigger factor's chaperone's function.

The C-terminal domain the central body of the dragon and is responsible for the trigger factor's chaperone activity.

The trigger factor binds to the hydrophobic patches as they emerge from the ribosome and sometimes remain associated with the segment even after polypeptide chain completion.

Moreover, a single nascent polypeptide chain or free polypeptide may have two or more trigger factors associated with it.

A chaperone also associates with the large subunit of the eukaryotic ribosome.

This chaperone consists of three different subunits that differ in both sequence and structure from the trigger factor.

In yeast, deletion of any single subunit results in slow growth and cold sensitivity.

Protein folding is very important for the normal functioning of the cells.

The accumulation of toxic proteins, which result from misfolding may lead to several neurological disorders including Alzheimer's, Huntington's and Parkinson's diseases.

Lecture 140

Nascent polypeptide processing & folding

Lecture 141

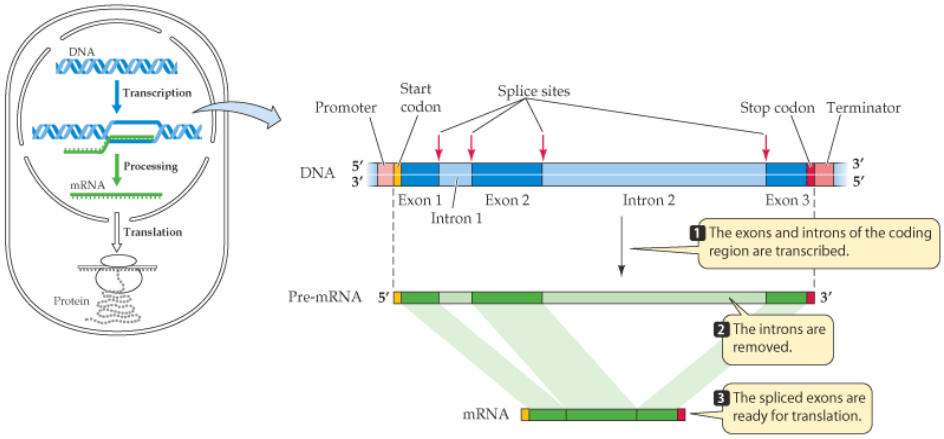
RNA Processing

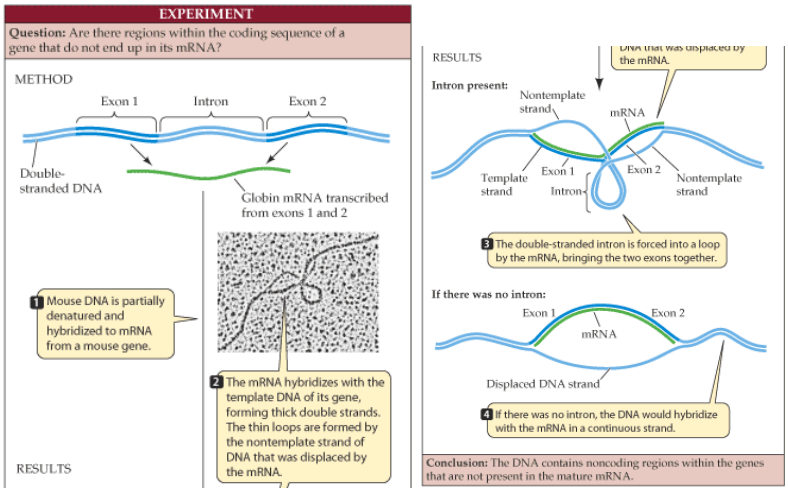
pre-mRNA (freshly transcribed) is processed to make it functional.

1-Introns are removed during RNA maturation

2) The transcribed pre-mRNA is altered by the addition of a G cap (modified GTP) at the 5’ end to protect RNA and facilitate translation.

3) After the last codon a poly A tail (100-300) is added at the 3’ end for export and protection



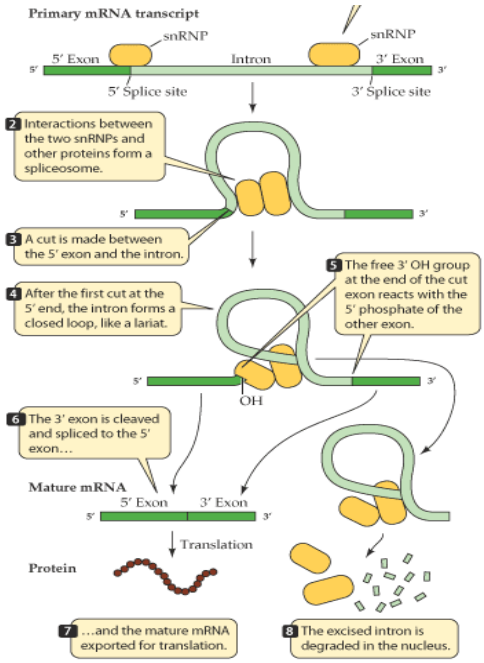


The Mechanism

snRNPs bind Intron-exon bounderies consensus sequences

RNA of U1 has complementary bases that bind CS at 5’ exon-intron boundery.

RNA of U2 has complementary bases that bind CS near 3’ intron-exon boundery.



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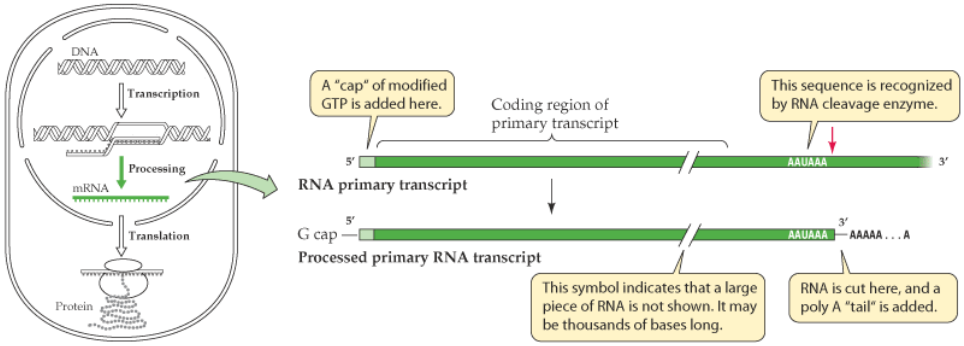


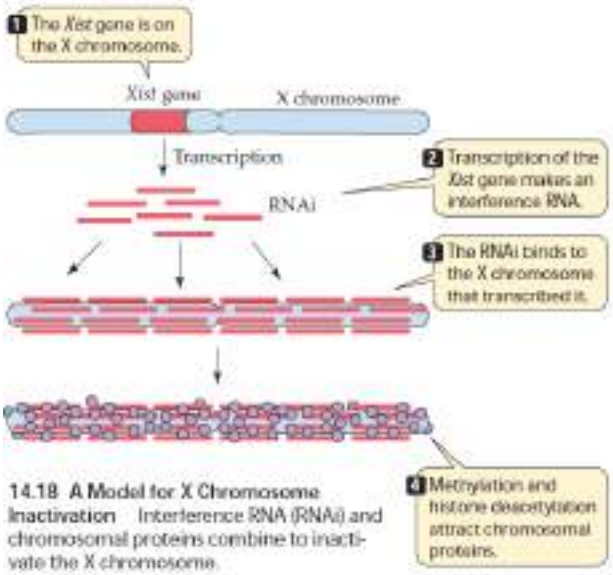
The Mechanism

X chromosomes have a special gene Xist (X inactivation-specific transcript).

RNA transcribed from it does not leave nucleus.

It bind to the X chromosome from which it is transcribed, and this leads to a spreading of inactivation along the chromosome.

The active X makes interference RNA (RNAi) anti-Xist gene, appropriately called Tsix. This gene codes for an RNAi that binds by complementary base pairing to Xist RNA at the active X chromosome.



14.18 A Model for X Chromosome Inactivation Interference RNA (RNAi) and chromosomal proteins combine to inactivate the X chromosome.

Lecture 142

Establishing the Genetic Code

Given the existence of 20 amino acids but only four bases, groups of several nucleotides must somehow specify a given amino acid.

Groups of two nucleotides, however, would specify only 16 (4x 4) amino acids.

So from 1954, most attention was given to how triplets (groups of three) might work, even though they obviously would provide more permutations (4 x 4 x 4) than needed if each amino acid was specified by only a single triplet.

It was assumed that successive groups of nucleotides along a DNA chain code for successive amino acids along a given polypeptide chain.An elegant mutational analysis on bacterial proteins, carried out in the early 1960s by Charles Yanofsky and Sydney Brenner, showed that co-linearity does in fact exist.

Equally important were the genetic analyses by Brenner and Crick, which in 1961 first established that groups of three nucleotides are used to specify individual amino acids.

But which specific groups of three bases (codons) determine which specific amino acids could only be learned by biochemical analysis.

The major breakthrough came when Marshall Nirenberg and Heinrich Matthaei, then working together, observed in 1961 that the addition of the synthetic polynucleotide poly U (UUUUU . . .)

to a cell-free system capable of making proteins leads to the synthesis of polypeptide chains containing only the amino acid phenylalanine.

The nucleotide groups UUU thus must specify phenylalanine.Use of increasingly more complex polynucleotides as synthetic messenger RNAs rapidly led to the identification of more and more codons.

Particularly important in completing the code was the use of polynucleotides like AGUAGU, put together by organic chemist Har Gobind Khorana.

In this way, more specific sets of codons were tested.

Completion of the code in 1966 revealed that 61 out of the 64 possible permuted groups corresponded to amino acids, with most amino acids being encoded by more than one nucleotide triplet.

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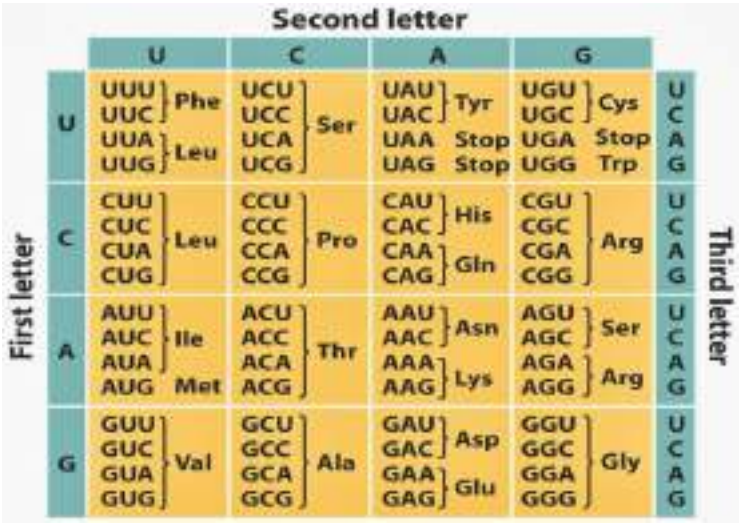


Lecture 143

DNA MUTATIONS

DNA mutation can be defined as a permanent transmissible change in the genetic material (DNA/RNA).

In other words, it is a permanent change in the nucleotide sequence of the genome of an organism.

DNA can be easily damaged even under normal physiological conditions.

Many different kinds of chemical and physical agents can damage DNA.

Some of these agents are endogenous which are produced inside the cells as a result of normal metabolic pathways.

While some others are exogenous agents which come from the surrounding environment.

On one hand, DNA stability is required to ensure that the genetic information may pass accurately from one generation to the next

It is also required for the correct functioning of thousands of genes.

On the other hand the genetic variation is needed to drive evolution.

If this variation would be lacking, the new species, including humans, would have not arisen.

So the life and biodiversity depend on a happy balance between DNA damage (mutation) and its repair.

Lecture 144

NATURE OF MUTATIONS

DNA mutations may be very simple (single base change) or very complex and may include several thousands of nucleotides.

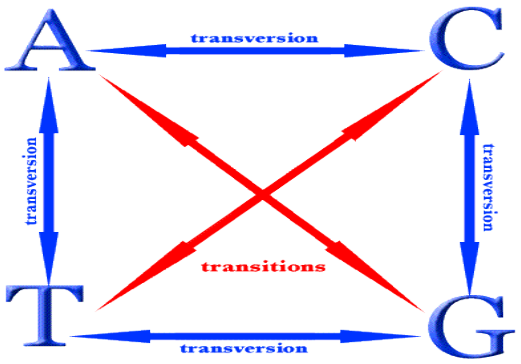
The simplest mutations are switches of one base for another. There are two kinds of such mutations which include:-

Transitions

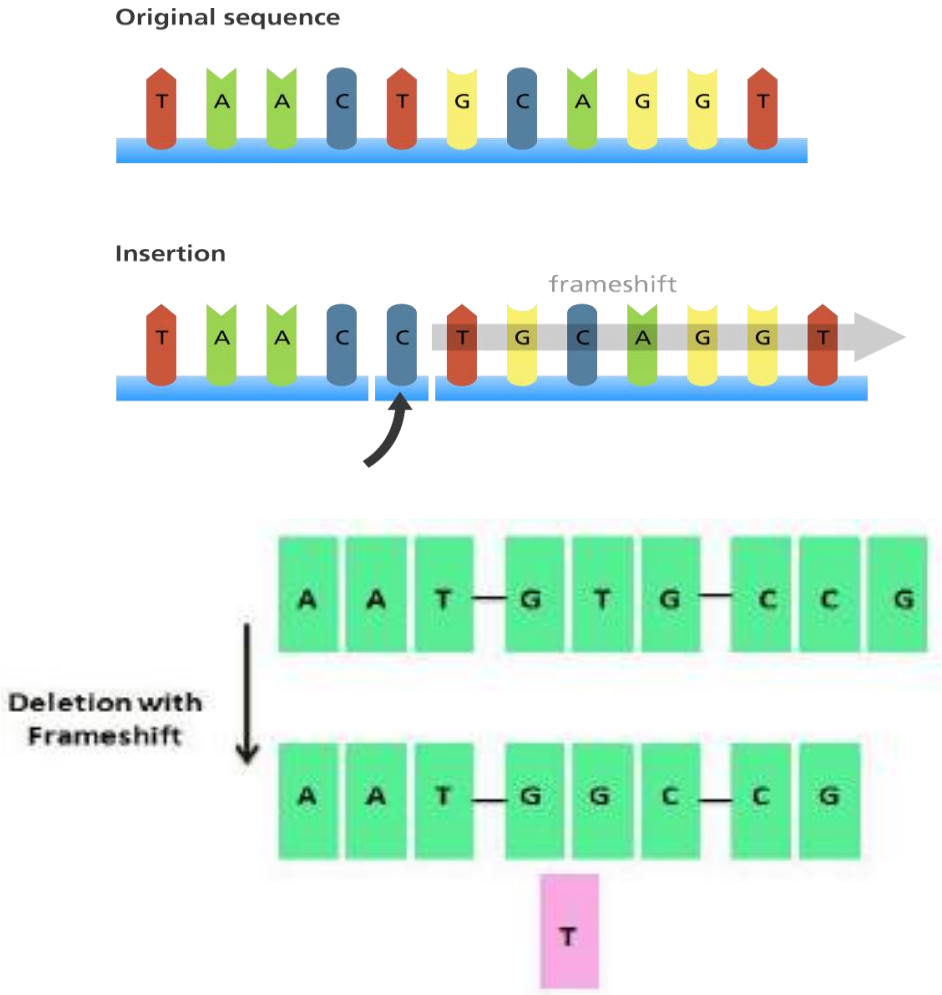
Transversions

Transitions are pyrimidine-to-pyrimidine and purine-to-purine substitutions, such as thymine (T) to cytosine (C) and adenine (A) to guanine (G).

Transversions are pyrimidine-to-purine and purine-to-pyrimidine substitutions, such as T to G or A and A to C or T.



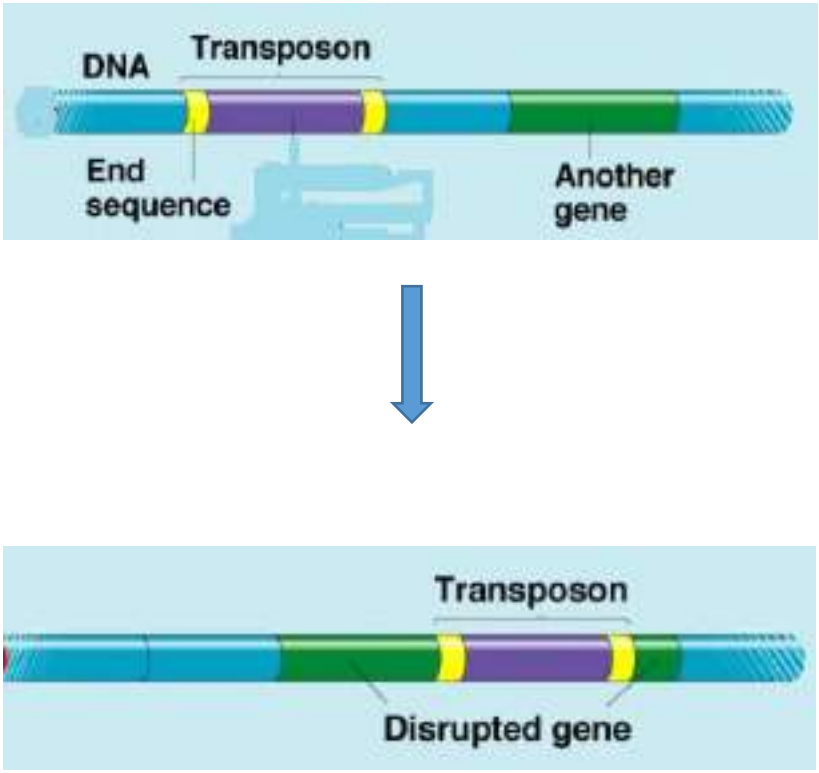
Other simple mutations are insertions or deletions of a nucleotide or a small number of nucleotides.



All such mutations that alter a single nucleotide are called point mutations.

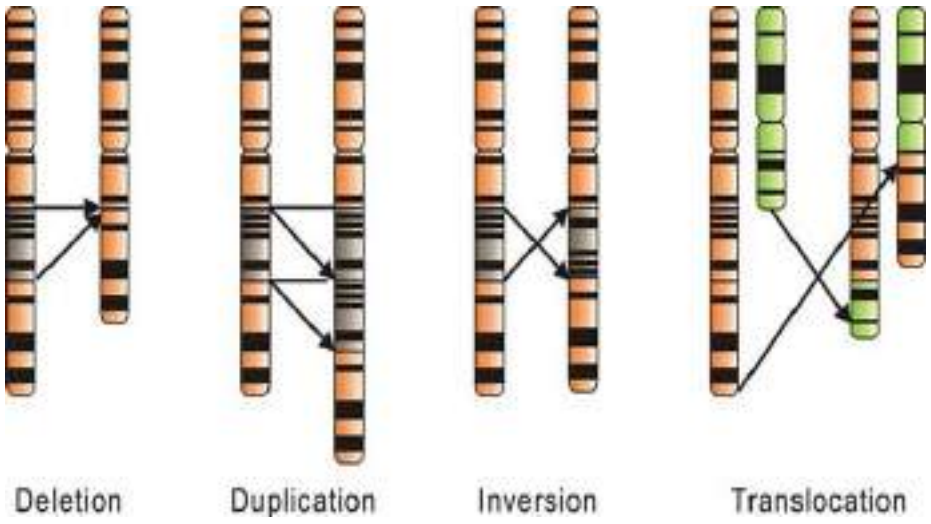
Other kinds of mutations cause more drastic changes in DNA, such as extensive insertions and deletions and gross rearrangements of chromosome structure.

Such changes might be caused, for example, by the insertion of a transposon, which typically places many thousands of nucleotides of foreign DNA in the coding or regulatory sequences of a gene.



Another type of mutations which are more drastic occur at chromosomal levels.

These are changes in appearance of the individual chromosomes through mutation-induced rearrangements.


Deletion
Duplication
Inversion
Translocation
