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Cell and Molecular Bioligy

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ABSTRACT

Cell biology has become increasingly important in every aspect of life sciences. Many of the advancements in contemporary science are the result a better understanding of cellular components and their functions. An understanding of cell biology is an asset in thr work place and more valuable than one may think. Cells have the ability to grow, reproduce, process information, respond to stimuli and carry out various biochemical reactions. The life of a cell depends upon thousands of chemical reactions and interactions coordinated with genetic instruction of cells and its environment. The molecules which form the basis of life provide scientists with a more atomostic and predictable tool for study. Working with whole cell or organism can be unpredictable, with the outcome of expriments relying on the interaction of thousands of molecular pathways and external factors. Molecular biology allocates scientists with a toolkit with which they may 'tinker' with the way life works. They may use them to determine the function of single genes or proteins, and find out the consequences of gene or protein alteration.

INTRODUCTION

the ability of individual cells is to grow, reproduce, execute information, respond to various stimuli, and carry out stunning attire of biochemical reaction. Alike us, other multicellular organism contain billion and trillion of cells organized into complex structure, but many organisms consist of single cell. Cell, smallest single unit of life, forms basis of all living things. the life of a cell depends upon thousands of chemical reaction and interaction coordinated with genetic instruction of cell and its environment. cell biology, starts at the microscopic level, with molecules and cells and their function from the sub

cellular processes which keep them functioning to interact with each other. Whilst molecular biology concentrates largly on nucleic acid and proteins, the molecules of life are used by the cell to survive, reproduce and how these molecules carry out normal functioning of cell. Molecular biology explores cells, their characteristics, parts, and chemical processes, and pays special attention to how molecules control a cell's activities and growth. Deoxyribonucleic acid (DNA) is the molecule which carries the genetic instructions for almost all living systems except some viruses. Its unique chemistry not only allows this information to be copied and passed on to an organism's descendents, it also allows scientists opportunities to investigate and manipulate an organisms at a molecular level. study of cell biology in conjunction with molecular biology pays special attention to how molecules control a cell's activities and growth and when and why certain genes are switched "on" or "off". An understanding of each of the factors has granted us a deeper understanding of life processes, how living things work and use this knowledge to ecplore molecular machinery of life.

THE CELL: AN OVERVIEW

THE FIRST CELL

The Earth was formed at least 4.5 billion years ago and it took approximately 750 years to get cooled sufficiently before the first life emerged. Before 1920s it was matter of speculation that how first cell came into existence and how life originated, since there was no confirmatory evidence. The primitive Earth's atmosphere was reducing as it contained no or little free oxygen. Atoms joined slowly to form simple organic molecule, which in turns joined to form macromolecules. In 1950s, Stanley Miller using H_2, CH_4, NH_3 and water vapor in electric discharge chamber synthesized the organic molecules, amino acids, polypeptides (Fig. 1), which provided the basic unit from which the first living cell came into existence.

In early 1980s, Sid Altman and Tom Cech succeeded in polymerizing nucleotides in laboratory and demonstrated RNA dependent RNA synthesis by some catalytic activity of RNA *i.e.*, RNA enzymes, believing RNA to be first genetic material. Self replicating RNA encapsulated into phospholipid membrance was assumed to be the first cell (Mills *et al*., 1967).

After the development of optical lenses, in seventeenth century, Robert Hooke (1665) axamined thin slices of cork with a self built microscope. he observed a box like compartment appeared as honey comb like structure and used the term 'cell' (L. cella: hollow sphere) for the first time.

In the late seventeenth century (1674) Antony van Leeuwenhoek saw the living cell and named 'animalcules' using simple magnifying glass with 250-300 folds magnification. Several discoveries were made to know the notion of the cell and their products to prove cell as basic structural and functional unit of life.

Matthias Jacob Schleiden (1838), a German botanist, concluded that basic unit of plant tissue is cell and it was Theodor Schwann (1839), a German zoologist described similarity between plant and animal tissues ater he saw the animal cartilage. Robert Brown (1831), English botanist found that every cell contained a round structure called nucleus, derived from Latin word 'kernal'.

Rudolf Virchow (1855), a German physiologist, expanded cell theory by famous Latin aphorism, *'Omnis cellula e cellula'* which became the tenet of modern cell theory meaning all cells arise from pre-existing cell.

Every cell has its molecular as well as chemical basis of life having an organized coordination. Messages carried from cell to cell and/or further to organs are controlled be molecules.

Prokaryotic and Eukaryotic Cell

R. H. Whittaker (1969) postulated five kingdom classification *viz.*, monera, protista, fungi, plantae and animalia. Monerans include bacteria, cyanobacteria, mycoplasmas and rickketsias. present day prokaryotes include two kinds of bacteria: the archaebacteria (archae or archaeans) and eubacteria (true bacteria).

Only monerans includes prokaryotic cell (Fig. 2), whereas rest all four kingdom comprises of eukaryotic cell (Fig. 3). Based on homologies in prokaryotic and eukaryotic molecular organization and function, it is believed that eukaryotes originated from prokaryotes. Table 1 compares structural and molecular organization of prokaryotes and eukaryotes.

Table 1: Differences between prokaryotic and eukaryotic cell

prokaryotic cell	eukaryotic cell
1. The cell size is usually small (0.1-10 µm)	The cell size is comparatively large (10-100 µm).
2. Nuclear envelop and hence organized nucleus is absent.	Organized nucleus with nuclear envelop is present.
3. Flagellum is single stranded, if present.	Flagellum is 11 stranded, if present.
4. cell wall, if present, possesses muramic acid.	Cell wall, if present without muramic acid.
5. DNA is without histone protein *i.e.* naked	DNA is associated with histone protein.
6. Divide usually by amitosis.	Divide usually by mitosis or meiosis.
7. site of transcription and translation is cytoplasm.	Site of transcription is nucleus whereas translation takes place in cytoplasm.
8. cell bound organelles (e.g., mitochondria, chloroplast, endoplasmic reticulum, lysosomes) are absent.	Cell bound organelles are present.
9. Ribosomes are of 70S type (50S+30S).	Ribosomes are of 80S type (60S + 40S) except in mitochondria and chloroplast in which it is 70S type.
10. Thylakoid, if present, lie in the cytoplasm.	Thylakoid, if present, are enclosed in the chloroplast.
11. Respiratory enzymes are present on infolding of plasma membrance *i.e.* mesosome.	Respiratory enzymes are present in mitochondria and cytoplasm.

CHEMICAL FOUNDATION OF THE CELL

Cell properties are complex and diverse having capability of self replication as well as performing specialized function in multicellular organism. Modern cell biology seeks the complete knowledge of thousands of chemical interactions and events taking place in life of the cell. Cellular activities can be predicated by the chemical composition and properties of the molecule participating in chemical and physical reaction of the cell.

Water, Salts, Ions, and Trace Elements

The first life appeared in water, the most abundant component of cells and organisms, accounting for 70-80% of the total cell mass. The critical property of water being a polar molecule plays an indispensable role as universal solvent. Water molecule with H-O-H bond angle of 104.5° forms hydrogen bond with its molecule or other polar molecule and interact with other charged ions also. Biomolecules such as sugars are readily soluble in water (hydrophilic). In contrast, fat (triglyceride) are poorly soluble in aqueous environment (hydrophobic). Phospholipids molecules shows amphipathic nature due to presence of both hydrophobic as well as hydrophilic molecule. Water molecule has high cohesiveness responsible for high surface tension of water, high boiling point, high specific heat which provides temperature stabilizaition to prevent unusual change in the cell, high heat of vaporization makes water an excellent coolant. Water ionizes into hydroxide anion (OH^-) and a hydrogen ion (H^+), is an another important property. Dissociated salts in the form of cations (*e.g.*,Na^+ and K^+) and anions (*e.g.* Cl^-) plays a key role in maintaining equilibrium and osmotic pressure.

some inorganic ions such as magnesium act as cofactor for many enzymes, inorganic phosphate forms adenosine tri phosphate (ATP), calcium ion is found in circulating blood and tissue fluids. Phosphate mainly contributes in buffering pH of body fluids and blood. Certain mineral salts such as iron are an important

constituent of haemoglobin, cytochromes and peroxidases. Normal cellular activities also require managanese, copper, cobalt, selenium, nickel, molybdenum and zinc in trace amounts.

Molecular Interactions

two kinds of interaction play key role in establishment of a biological system. A high energy stable covalent bond which typically have greater thermal energy at room temperature as well as at body temperature. Non covalent interactions include: ionic bonds, hydrogen bonds, van der Waals attraction and hydrophobic force.

Ionic bonds: Purely electrostatic, results from attractions between cation and anion as in formation of NaCl molecule.

Hydrogen bond: Electropositive hydrogen bond atom is partially shared to electronegative donor as in water molecule.

van der Waals attractions: A weak, non specific interaction generated due to attraction between two atoms.

Hydrophobic force: Non polar molecule when brought together in water, a non specific force came into play which reduces their contact, called hydrophobic force.

Building Blocks of Cell

There are three major macromolecules in the cell such as protein, nucleis acid and polysaccharides. Proteins are polymer of amino acids having ten to several thousands degree of polymerization joined together by peptide bond. Nucleic acid is the linear polymer of nucleotide containing hundred to millions of nucleotides joined together by phosphodiester bond, whereas polysaccharides are the linear and/or branched polymer of monosaccharides joined together by glycosidic bonds.

Amino Acids

Amino acids are considered as building blocks of protein, consisting of a central asymmetric carbon atom bonded to four different side chains called R group (except Glycine). Four different chemical groups are; an amino ($-NH_2$) group, a carboxyl group ($-COOH$), a hydrogen (H) atom with a variable side chain. Leucine, lysin, serine and glutamic acid are the most abundant constituting about 32 percent of the total amino acids of a typical protein. Cysteine, methionine and tryptophan are only 5% of the protein, despite the fact that the amount of amino acid vary from protein to protein.

Nucleotides

Nucleotides are the monomer which forms DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). nucleotides consist of a phosphate gtoup, a five carbon sugar (ribose or deoxyribose) that in turns linked to nitrogenous base. There are two kinds of nitrogen bases are present, purines and pyrimidines. the base adenin (A), guanine (G) are purines whereas cytosine (C), thymine (T) and uracil (U) falls under pyrimidine (Fig. 4). In nucleotide, C'_1 atom of sugar is attached by N_1 position of pyrimidine and N_9 position of purine.

Sugars

The building block of polysaccharide is simple sugar which have the general formula of $(CH_2O)_n$. Sugars may exist both in ring as well as open chain structure. In open chain structure it contain a number of hydroxyl group and either an aldehyde ($-CHO$) or ketone ($>C=O$) group. D-glucose ($C_6H_{12}O_6$), a monosaccharide, the biologically important external source of energy. Disaccharides (maltos, lactose, sucrose etc.) consist of two monosaccharides, simplest among polysaccharide. Simple polysaccharides are composed of glucose monomer only and complex have ten to thousands of monosaccharides. Starch is a branched storage polymer of carbohydrate of animal cell.

Fatty Acids

fatty acids, an important source of energy is stored as triglycerides in adipose tissues and can be broken down into usable energy. It contains hydrocarbon chain with hydroxyl (−COOH) group.Even nember of carbon atoms (usually 14, 16, 18 and 20) is predominant in cells, nearly insoluble in water due to presence of hydrophobic chain. Saturated fatty acidsare those which is without carbon-carbon double bond, unsaturated have at least one carbon-carbon double bond and fatty acids with more than one carbon-carbon double bond are referred as polysaturated (Table 2). Phospholipi, an important constituent of cell membrance generally consists of fatty acid and glycerol. Each fatty acid has a hydrophobic two chains of fatty acids joined by an ester bond and a hydrophilic head with phosphate group.

Table 2: Saturated and unsaturated fatty acids

Common name	**No ofcarbon atoms/ No of double bonds**	**Formula**
Saturated fatty acid		
Myristic	14.0	$CH_3(CH_2)_{12}COOH$
Palmitic	16.0	$CH_3(CH_2)_{14}COOH$
Stearic	18.0	$CH_3(CH_2)_{16}COOH$
Unsaturated fatty acid		
Oleic	18.1	$CH_3(CH_2)_7CH=CH(CH_2)_7$ COOH
Linoleic	18.2	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7$ COOH
Arachidonic	20.4	$CH_3(CH_2)_4(CH=CHCH2)_3CH=CH(CH_2)_3$ COOH

PROTEIN STRUCTURE AND FUNCTION

Proteins, the ubiquitous working molecule of the cell, play most of the predominant role in biological system. Proteins are polymer of amino acid joined together by peptide bond. Since 20 common amino acids (Table 3) are typically present in cell and are involved in formation of stable and functional

protein. Some proteins consist of single polypeptide that occurs spontaneously, called monomeric proteins. Those amino acids consist of more than one polypeptide called multimeric protein.

There are four hierarchical levels of organization of protein: the primary, secondary, tertiary and quaternary structure (Fig. 5). Amino acid sequence is considered as primary structure of peotein, secondary structure involves stretch of polypeptide chain that forms α-helix and β-pleated sheets. Tertiary structure is a three dimensional structure of a polypeptide chain, whereas when two or more than two polypeptide chains form a single multimeric protein, it is said to be quaternary structure of protein. knowledge of the chemical properties of the common amino acids is central to an understanding og biochemistry involve in cell metabolism. Polarity is defined as the tendency to interact with water at biological pH (~ pH 7.0); it variea widely, form non polar and hydrophobic to highly polar and hydrophilic.

Over all shape of polypeptide chain varies form protein to protein and depends upon the proportion and sequence of amino acids in the protein. Broadly, protein is divided into two groups: globular protein and fibrous protein. Fibrous proteins are highly ordered three dimensional structures having extensive either α-helix or β-sheets. If the polypeptide chains are folded into compact structure rather than extended filament, protein is called globular protein. There are many such proteins involved in cellular structure, often folded on one another to give a tertiary structure made up of helices and sheets.

Table 3: Amino acids, their abbreviations and polarity

Amino acid	Three letter / one letter abbreviation
Alanine	Ala / A
Arginine	Arg / R
Aspargine	Asn / N
Aspartate	Asp / D
Cysteine	Cys / C
Glutamate	Glu / E
Glutamine	Gln / Q
Glycine	Gly / G
Histidine	His / H
Isoleucine	Ile / I
Leucine	Leu / L
Leysine	Lys / K
Methionine	Met / M
Phenylalanine	Phe / F
Proline	Pro / P
Serine	Ser / S
Threonine	Thr / T
Tryptophan	Trp / W
Tyrosine	Tyr / Y
Valine	Val / V

GENE TO PROTEIN: BASIC MOLECULAR MECHANISM

DNA is the store house of the cell library containing information to build a cell. the stored information is inherited from generation to generation, assuring continuity of the species.

Before 1940s it was belived that genes were made up of protein rather than DNA.

As we know that proteins are constructed from 20 amino acids that can be rearranged in a vast number of combination, of which only some combination from stable protein, rest all are degraded rapidly. Modern era of molecular biology initiated with the discovery of double helical structure of DNA (Fig. 6) by James D. Watson and Francis H. C. Crick (1953). DNA consisting of two intertwined polynucleotide strands with a right handed helix (except in Z-DNA). The most common DNA (B-DNA) has diameter of 20 Å (2 nm) and contains ten nucleotide pair per turn and advances 3.4 Å (0.34 nm) per nucleotide pair. The two antiparallel polynucleotide chains of DNA are complementary to each other. A lies opposite to T and C lies opposite to G and *vise versa*. It means base sequence of one chain determines the base sequence of opposite strand. Successive nucleotide are joined together by phosphodiester bonds that join the 5′ carbon of one nucleotide to 3′ nucleotide of next nucleotide, the chain is said to have 5′-3′ orientation and the opposing strand exhibits 3′-5′ orientation.

DNA and It's Supercoiling

DNA double helix when twisted upon itself, it forms supercoiled DNA which was identified first in small viruses. All bacteria, many viruses, mitochondria and chloroplast have circular DNA. Each of the two stands in the circular DNA molecule forms closed structure without any free end. Supercoiling also occurs in linear DNA molecule when packed into chromosome resulting into more compact structure. The inter conversion between relaxed and supercoiled forms of DNA is catalyzed by an enzyme called topoisomerase.

Renaturation and Denaturation of DNA

DNA double helix is stabilized by relatively non covalent weak bond called hydrogen bond which can be readily separated. In a solution of DNA, thermal energy breaks hydrogen bond between the bases and other forces which helps in stabilizing DNA, it is said to be danatured. The denaturation or melting of

DNA can be revealed by measuring the absorbance under ultraviolet (UV) light at 260 nm. As DNA denatures the absorbance 260 nm increases. The reverse process, which establishes a double helix from denatured DNA is called DNA renaturation. Melting temperature (Tm), is defined as the temperature at which one half of the absorbance change has been achieved. More the GC content of the DNA helix, more will be the Tm, as GC base pairs held together by three hydrogen by three hydrogen bond whereas AT base pair having only two hydrogen bond, requires more energy for breaking of hydrogen bond.

The Organizations of DNA

Genome is the sum total of all the genes of an organism, consist of DNA (except some RNA viruses) that contain copy of genetic information of that organism or virus. Eukaryotic cells have a nuclear genome and a mitochondrial genome. In case of plants and algae it has a chloroplast genome also. Size of the genome is expressed as total number of base pairs (bp) and it increase in complexity of the organism.

DNA Packaging

Incredible amount of DNA in found is prokaryotes as well as in eukaryotes. A typical E. coli cell measuring about 1μm in diameter and 2 μm in length accommodates a circular DNA molecule af 1600 An adult human body contains about 10^{14} cells and thus a total DNA length of 2×10^{11} km. A modest human cell contains DNA wrap around the cell to provide 15,000 times compaction.

The Structure of Chromosome

chromosome, a nucleic acid molecule which is the repository molecule of genetic information in a virus, bacterium, a eukaryotic cell or all organelle such as chloroplast and mitochondrial. The bacterial chromosome is a circular naked DNA molecule, localized in special region of the cell known as nucleoid. The chromosomal material called chromatin, randomly dispersed in certain part of nucleus. Chromatin consist of fibers containing protein and DNA along with

little amount of RNA. In eukaryotes, DNA in the chromatin is tightly bound to a basic protein known as histone (Fig. 7). Histones are divided into five type (H1, H2A, H2B, H3 and H4) and chromatin protein are clustered along DNA molecule in a regular pattern that repeats at interval of roughly 200 bp. Histone packages and orders the DNA in structural unit known as nucleosome. Each nucleosome core particle contains a double stranded 146 bp long DNA fragment bound to histone octamerr and 55 bp long double stranded linked DNA associated with histone H1. Isolated chromatin fibers appears as 'beads on string' measures about 11 nm dimeter but the chromatin of intact cell often forms a thicker fiber of 30 nm thickness called 30 nm chromatin fiber.

RNA: Its Function in Gene Expression

RNA is similar to DNA in chemical composition; the ribose sugar of RNA has an additional hydroxyl group 2' position and thymine of DNA is replaced by uracil in RNA. Despite being single stranded, RNA molecule often exhibits double helical structure due to its ability to fold back on themselves to form base paired segment between short stretches of complimentary sequences. RNA can also from a three dimensional structure to carry out the task of genetic information, carry the information coded in DNA converted to protein.

The Central Dogma of Molecular Biology

In 1956, Francis H. C. Crick, referred the path of flow of genetic information as the central dogma (Fig. 8).

After the discovery of DNA in 1953, subsequent elucidation of how DNA directs synthesis of RNA which then direct synthesis of protein. DNA acts as a template for its own replication which assures its genetic continuity from generation to generation. The information contained in DNA arranged in heredity unit called gene, responsible for a particular trait of an organism. The information coded in DNA is copied into mRNA, process known as transcription, which has distinct role in protein synthesis. The accurate and

stepwise assembly of amino acid into protein occurs by translation of mRNA molecule.

CELL ORGANIZATION AND BIOCHEMISTRY

Biomembranes and Cell Architecture

Cell membrane is one of the structural feature that determines the boundary of cell along with internal compartmentalization which serves as permeability barrier. There are many synthetic and metabolic events carried out in cell such as: replication of DNA, the synthesis of protein followed by production of chemical energy by oxidation of glucose.

Membrane as Permeability Barrier

The interior of the cell must be separated from surrounding environment. Membrane serves this purpose well because the hydrophobic interior of the membrane is an effective permeability barrier of hydrophilic molecules and ions. As a whole, plasma or cell membrane serve as this barrier by surrounding the cell and regulates the passage of materials both into and out of the cell.

Membrane and Its Function

Membranes have particular function associated with them because many important molecules and structures are directly associated with them. There are many distinct enzymes present in / or the membrane of organelles *vis*., mitochondria, chloroplast, endoplasmic reticulum (ER), Golgi complex, lysosome and peroxisome. The function of membrane protein is to transport substances into and out of cell and cell organelles. Intracellular vesicles facilitate the transport of large molecule as large as protein into the cell (endocytosis) or out of the cell (exocytosis).

Membrane Lipids and Proteins

Phospholipid the fundamental building block of the cell membrane which are amphipathic molecules having two hydrophobic fatty acid chain linked to phosphate containing hydrophobic head group. Phospholipid spontaneously

forms bilayer in aqueous solution as their fatty acid tails are poorly soluble in water. Hydrophobic tail buried in interior of the membrane and the polar head groups are exposed on both sides, in contact with the water. Most of the cell membrane contains about 50% of the lipids; however it varies in different types of membrane of mitochondria contains about 70% af the protein, reflecting the abundance of protein for electron transport and oxidative phosphorylation. In addition to the phospholipid, animal cell also contain glycolipids and cholesterol constituting about 40% of the total membrane lipid molecule. An important feature of lipid bilayer is that they behave as two dimensional fluid in which lipid and protein molecule are free to rotate and can move in lateral direction also.

Singer and Nicolson Model of Plasma Membrane

S. Jonathan Singer and Garth Nicolson (1972) proposed a model for membrane organization called fluid mosaic model in which proteins are inserted into lipid bilayer (Fig. 9). These proteins are divided into integral membrane protein as they are embedded directly in the lipid bilayer and peripheral membrane protein, which are not associated with the membrane but they are indirectly associated with membrane. Integral proteins are transmembrane protein, spans the lipid bilayer with exposed protein on both the sides of the membrane. transmembrane proteins are amphipathic like phospholipid with exposed hydrophilic portion on both sides of the aqueous environment.

Eukaryotic Cell Organell

A typical eukaryotic cell consists of four major structural features: plasma membrane or cell membrane to define its boundary and retains its contents, a nucleus as a main repository of DNA, membrane bound organelles for localization of various cellular functions, and the cytosol interlaced by a cytoskeleton of tubules and filaments (Fig. 3). In addition to that plant cell have

a rigid cell wall outside the plasma membrane whereas. animal cell do nat have a cell wall.

Nucleeus: The Information Center of the Cell

The presence of nucleus is the main feature to distinguish between prokaryotic and eukaryotic cell. Nucleus serves as a control system of the cell as well as repository of genetic information. It is surrounded by a system of two concentric inner and outer membranes. The outer nuclear membrane is continuous with endoplasmic reticulum (ER) and has ribosome bound to its cytosolic surface. In contrast, inner membrane carries protein that is specific to the nucleus which binds to nuclear lamina, a fibrous meshwork that provides structural support to the nucleus.

The small channels through which small molecules, ions, proteins and RNAs can travel between the nucleus and cytoplasm is called nuclear pore complex. Depending on their size, small molecules of less than 20 - 40 kDa pass freely through the pore in either direction whereas, large molecules such as proteins and RNAs pass through the central pore (approximately 10 - 40 nm) of the nuclear membrane complex by an active process in specific direction.

Nucleolus, the most prominent nuclear body, present in the nucleus which is responsible for the synthesis and assembly of most of the RNA and protein components. It is associated with the specific region of the particular chromosome that contain gene for rRNA. to meet the need for transcription of large number of rRNA molecules, all cells contain multiple copies of rRNA genes.

The nucleus

The most prominent structure of the cell serves as the information centre for the cell. It is surrounded by two membranes, inner and outer. Nuclear pore also provides passage to ribosome, mRNA molecule, chromosomal protein and enzymes needed for the activities of nucleus. Nucleolus is also present in the

nucleus, responsible for synthesis and assembly of most of the protein compartments and RNA which makes up the ribosome. In growing cell, the nucleus is metabolically active, involved in DNA replication and synthesizing rRNA, tRNA and mRNA. mRNA binds to specific protein to form ribonucleoprotein within the nucleus. Most of the cell's rRNA is synthesized in the nucleolus. In the electron microscope, a dark area closely associated with nuclear membrane containing concentrated DNA is called heterochromatin.

The Mitochondria

Mitochondria, a large and prominent organelle surrounded by a double membrane system, involved in the oxidation of sugars and other cellular fuel molecules. The purpose behind these oxidative events is to extract energy from food and conserve as much of it in the formof ATP (adenosine triphosphate). Most of the enzymes and intermediate of various cellular processes such az tricarboxylic acid (TCA) cycle, fat oxidation and ATP generation are present inside the mitochondrial matrix. Most of the intermediates of electron transport chain are located onnthe inner folding of the mitochondrial membrane, cristae. The number and location of the mitochondrial within a cell can be related directly to their role in that cell. Mitochondria contain their own genetic system, which is separate and distinct from the nuclear genome of the cell, The genome of mitochondria is usually circular DNA molecules like those of bacteria, which is present in multiple copies per organelle. Like DNA of nuclear genome, mitochondrial DNA can be altered by mutation, which is frequently deleterious of the organelle. Most of the mitochondrial genome do nat encodes for the portein required for mitochondrial DNA replication, its transcription and translations. Instead, the gene that encodes portein required for the replication and expression of mitochondrial DNA are contained n the nucleus. Approximately 1000 proteins encoded by nuclear gene (mora than 90% of the mitochondrial protein) are synchronized on free cytosolic ribosome and important into mitochondria as complete polypeptide chain.

The Chloroplast

Chloroplast, the organelle responsible for photosynthesis, is in many aspects similar to mitochondria. Like mitochondria, chloroplasts are surrounded by both inner and outer membrane. In addition to outer and inner membrane, chloroplast have third internal membrane system called thylakoid membrane system. The thylakoid membrane forms a network of flattened disc called thylakoid which is frequently arranged in stacks called as grana which are three membrane structures making the internal structure of the chloroplast more complex than mitochondria. These three membrane divide chloroplast into three distinct external compartments: the internal space between the two membrane of the chloroplast envelop, the stroma which lies inside the envelope but outside the thylakoid membrane and the thylakoid lumen. There is similarity between chloroplast and mitochondrial membrane, as expected both organelles are involved in chemiosmotic generation of ATP. The outer membrane of chloroplast, like that of mitochondria contains porins protein which makes it freely permeable to small molecules. In contrast the inner membrane is impermeable to ions and metabolites, which are able to enter the chloroplast only via specific membrane transporter. The chloroplast stroma is also equivalent in junction to the mitochondrial matrix: it contains the chloroplast genetic system and a variety of metabolic enzymes, including those responsible for the critical conversion of CO_2 to carbohydrate during photosynthesis. Although known primarily for their role in photosynthesis, chloroplasts are involved in variety of other processes as well. An important example involves the reduction of nitrogen from the oxidation level of nitrate that plant obtains from the soil to oxidation level of ammonia, the form required for protein synthesis.

Endoplasmic Reticulum

Eukaryotic cell has network of membrane enclosed tubules and sacs (cisternae) that extends from nuclear membrane throughout the cytoplasm. The endoplasmic reticulum (ER) consists of tubular membrane and flattened sac that appears to be interconnected. The ER is the continuous with the outer membrane of the nuclear envelop which may either be rough or smooth. The rough ER actively participates in the protein synthesis and most of them are transported into or across the membrane as they are synthesized. Not all proteins are synthesized on rough ER,there are many proteins synthesized on ribosome that are not bound to the ER but are instead found free in cytoplasm. We can say in general, secretory proteins and membrane bound proteins are made by ribosome on the rough ER, whereas protein intended for use within the cytoplasm are made on free ribosome. On the contrary, smooth ER has no role in the protein synthesis; it plays an important role in the lipid and steroid synthesis. It is also responsible for the inactivation and detoxification of drugs and other toxic or harmful compounds for the cell.

Golgi Complex

Golgi complex or Golgi apparatus functions as a factory in which peotein received from the ER are further processed and sorted for transport. Initial step in the process of glycosylation i.e., addition of short chain of carbohydrates takes place within the lumen of the rough ER but the process usually completes in the Golgi complex. It is the processing station, with vesicles both fusing with it and arising from it. Everything that goes into it comes out in a modified and packaged form for export from the cell. In addition, most glycolipids and sphingomyelin are synthesized within the Golgi body. Complex polysaccharide in the cell wall of plant is also synthesized by Golgi complex. It is also involved in the formation of primary lysosome.

Secretory Vesicles

Secretory proteins and other substances after processing from Golgi complex are packaged into secretory vesicles. The cells of pancreas contain many vesicles because it is responsible for synthesis of many digestive enzymes. These enzymes are synthesized onnthe rough ER, packaged by Golgi apparatus and then released via Secretory Vesicles. The vesicles then fuses with the plasma membrane and discharges their content to the exterior of the cell by the process known as exocytosis.

Lysosome

The lysosome is a single membrane bound cell organelle used by the cell for storing hydrolases. Hydrolysas are capable of digesting specific biological molecule such as proteins, carbohydrates or fat. Lysosomal enzymes are somewhat similar to secretory proteins in their synthesis and packaging as they are on rough ER and then transported to Golgi apparatus. About 50 lysosomal hydrolases are known which are able to digest most of the biological substances. Lysosome has been found in plants, animals and protozoa, whereas it is absent in bacteria. The enzymes of the lysosome arw not readily available as they are enclosed by the membrane. The most remarkable property of the lysosome is its polymorphism especially the size of particle and irregularities of its internal structure. It is believed that the polymorphic nature of the lysosome is the result of association of primary lysosome with the different materials that are phagocytosed by the cell. There are four types of lysosomes present; one primary and the other may be grouped together as secondary lysosome. The primary lysosome is small bodies whose enzymatic content is synthesized by the ribosome and accumulated by ER from there they penetrate into the Golgi region. Secondary lysosome includes: heterophagosome, residual bodies and autophagic vacuole. The heterophagosome or digestive vacuole is formed as a result of phagocystosis or pinocytosis of the foreign material by the cell. If the

digestion is incomplete it forms residual bodies and are eliminated by defecation in amoeba and some other protozoan. The autophagic vacuole or autophagoaome are present in normal cell in which lysosome contains a part of the cell in the process of digestion. The part of the cell may be a mitochondrion or a portion of the ER.

Peroxisome

peroxisome is small, single membrane enclosed organelle. Peroxiome has been found in both plant and animal cells, as well as in fungi, protozoa and algea. The peroxisome resembles lysosome in size and involved in formation and decomposition of hydrogen peroxide (H_2O_2) and also involved in the β-oxidation of fatty acids. In animal cells, peroxisome is found in most cell type but is especially prominent in liver and kidney cells. Specialized peroxisome, called glyoxysomes in plant cell plays a key role in conversation of stored fat into carbohydrates. In photosynthetic tissue, leaf peroxisome are prominent because of their role in photorespiration, which serves to metabolize a side product formed during photosynthesis. Peroxisome do not have their own proteins however Peroxin are synthesized from the nuclear genome which plays an important role in Peroxisome assembly.

Vacuole

Cell also contains variety of other membrane bound organelle called vacuoles. In yeast and plant cells which lacks lysosome, proteins are transported from the Golgi apparatus to an additional destination i.e. lysosome. In animals vacuoles are frequently used for temporary storage of water, ions and nutrients and their transport. Some protozoa take up food particles and/or other materials from their their environment by a process called phagocytosis (cell eating). Phagocytosis, a form of endocytosis involved in inpocketing of the plasma membrane around the substance followed by pinching off process that internalize the membrane bounded particle as a vacuole. The vacuole has a high

concentration of solute and is surrounded by differentially permeable membrane called the tonoplast. A variety of the vacuolar membrane allow plant cell to accumulate and store water, ions and nutrients within vacuoles. In contrast to lysosomal targeting, proteins are directed to vacuoles by short peptide sequences instead of carbohydrate markers.

Ribosome

Ribosome is a spheroid tiny structure found in both prokaryotic and eukaryotic cells. Prokaryotic ribosome are smaller and sediment at 70S (subunit of 30S and 50S) whereas eukaryotes sediments at 80S (subunit of 40S and 60S). It provides a scaffold for the ordered interaction of all the molecules involved in synthesis of protein. During the protein synthesis several ribosomes become attached to one mRNA molecule to form a polysomes or polyribosomes. The gap between two subunit of ribosomes provides the passage for mRNA and helps in the formation of polysomes.

Ribosomal RNA (rRNA)

The major constituent of ribosomes is RNA and protein present in almost in equal amount. rRNA generally represents more than 80% of the RNA present in cells. Prokaryotic ribosome contains three RNA molecules: 16S rRNA in the small subunit and 23S and 5S in the large subunit. In eukaryotes, it has four subunits: 18S in the smaller subunit and 28S, 5.8S and 5S in the large subunit. rRNA often forms a “hairpin loops” due to complimentary base pairing in the same rRNA molecule. In addition to maintaining ribosome structure, rRNA also participates in protein synthesis by virtue of its base pairing properties. Most prokaryotic 16S rRNA has a sequence complimentary to ribosome binding site, called Shine-Dalgarno sequence.

cytoskeleton

cytoskeleton, in eukaryotic cell provides the internal framework for its distinct shape and high level of internal organizations. Theree major structural elements

of the cytoskeleton are microtubules, microfilaments and intermediate filaments. Microtubules are the largest structural

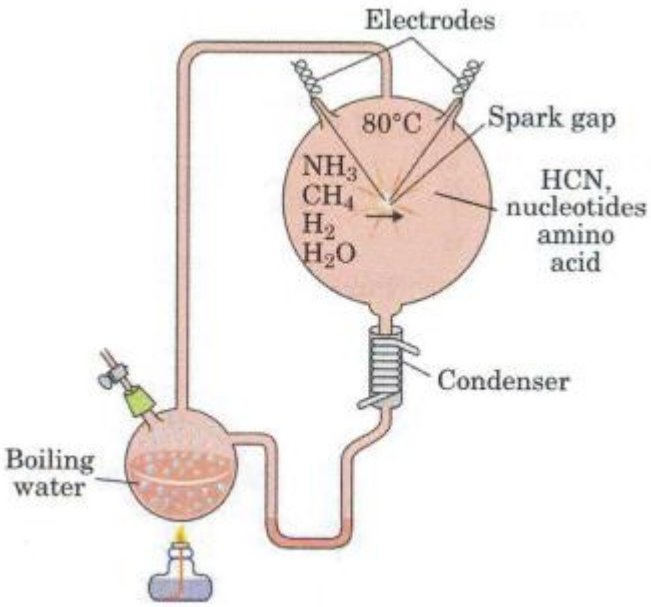

Fig. 1: spark discharge apparatus used by Miller and Urey (1953) expriments demonstrating abiotic formation of organic compound under primitive atmosphere condition.

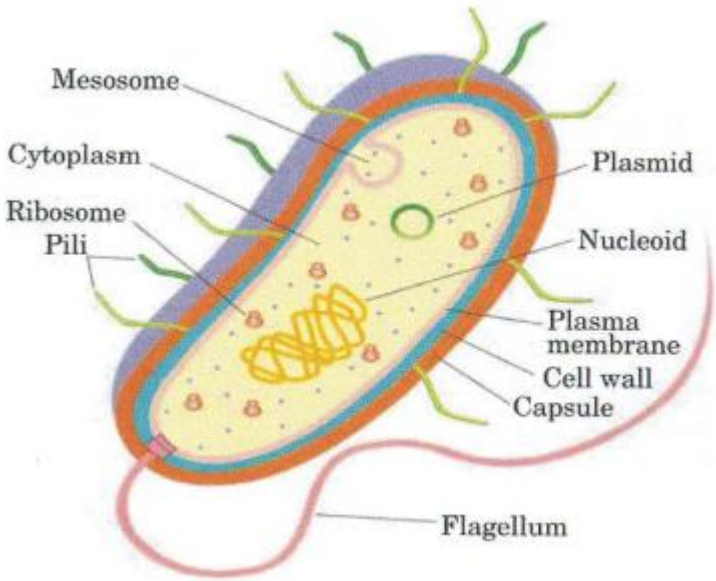

Fig. 2: schematic diagram of a typical prokaryotic cell.

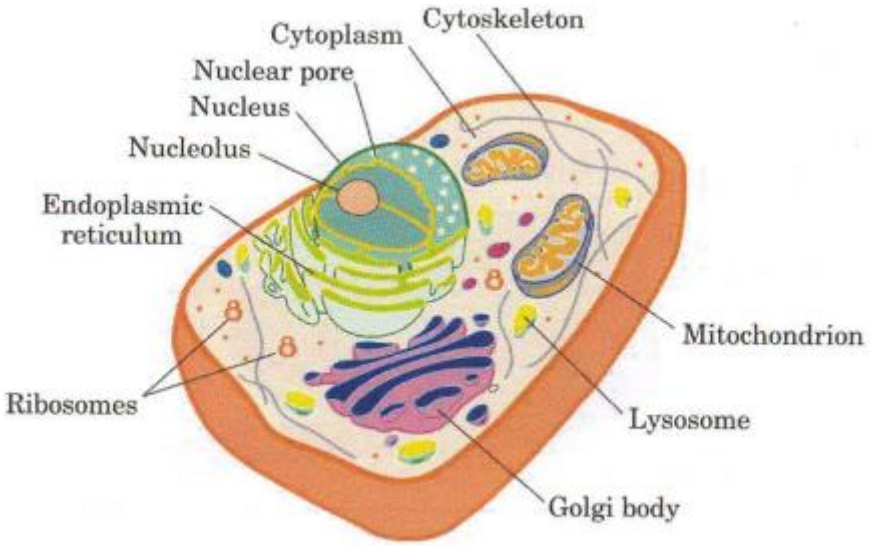

Fig. 3: schematic diagram of a typical eukaryotic cell.

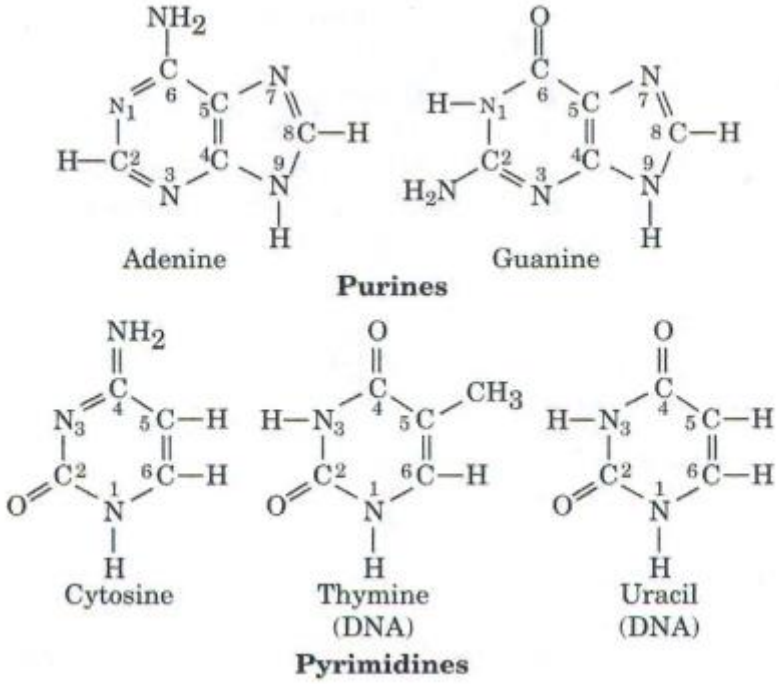

Fig. 4: Major purines and pyrimidines of nucleic acids.

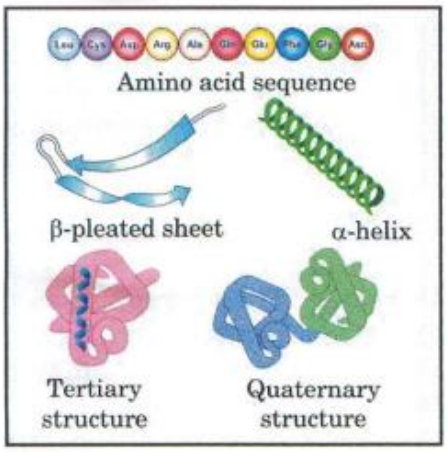

Fig. 5: Foue levele of protein organizational structure, a. Primary structure, b. Secondary structure, c. tertiary structure. d. Quaternary structure.

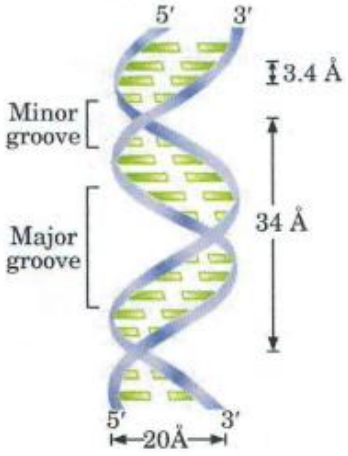

Fig. 6: Double helical model of DNA

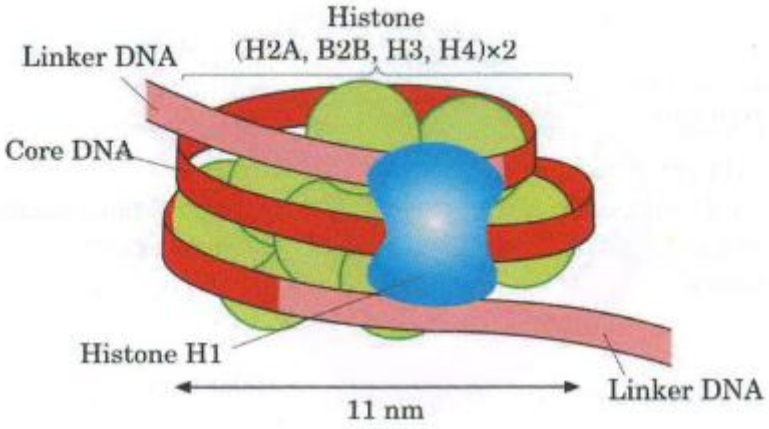

Fig. 7: schematic diagram of nucleosome consisting of DNA double helix wound around a histone octamer.

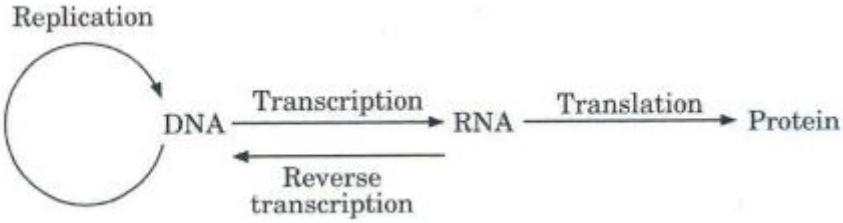

Fig. 8: Central dogma of molecular biology

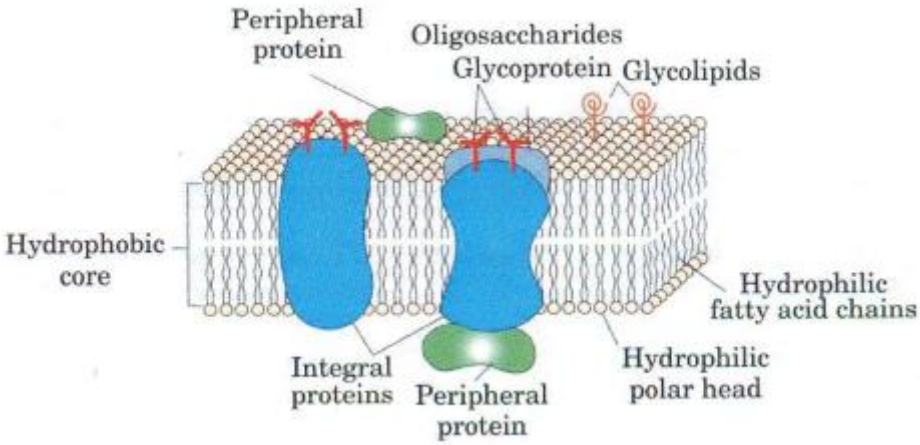

Fig. 9: Fluid mosaic model of plasma membrane shows integral proteins floating in a lipid bilayer.

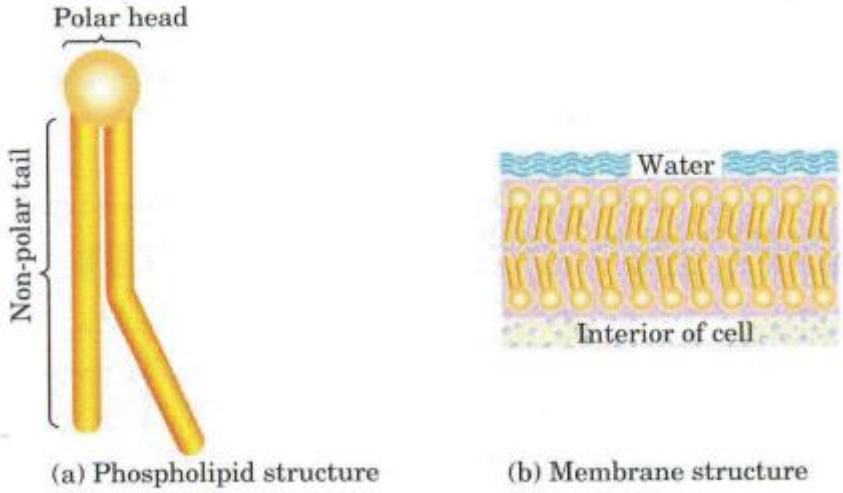

Fig. 10: Structure of (a) phospholipid and (b) membrane structure

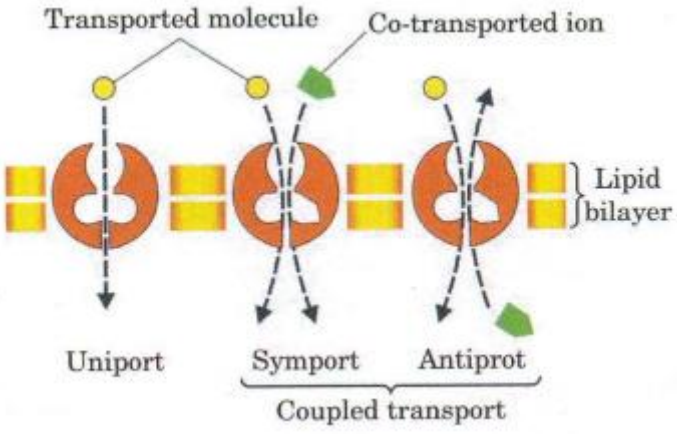

Fig. 11: There types of carrier mediated transport. The schematic shows carrier protein functioning as uniport, symport and antiport.

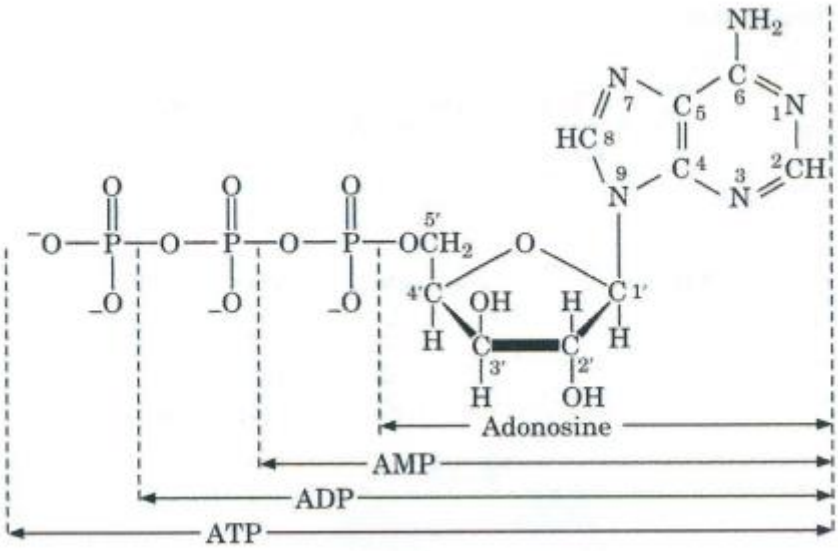

Fig. 12: Structure of adenosine, adenosine mono phosphate (ATP), adenosine di phosphate (ADP) and adenosine tri phosphate (ATP).

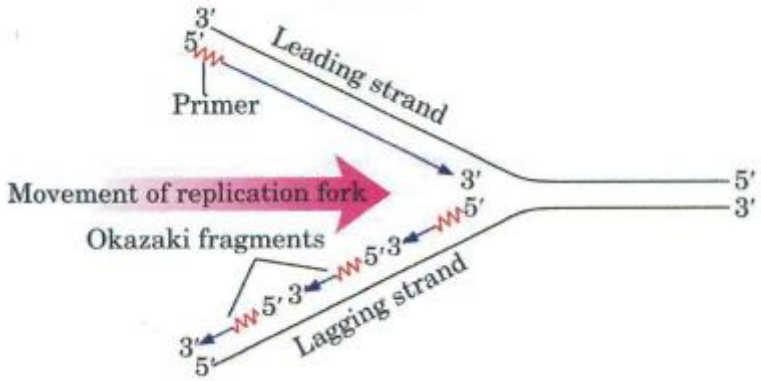

Fig. 13: Synthesis of DNA at replication fork. /the leading strand is synthesized continuously but the lagging strand synthesized as Okazaki fragments.

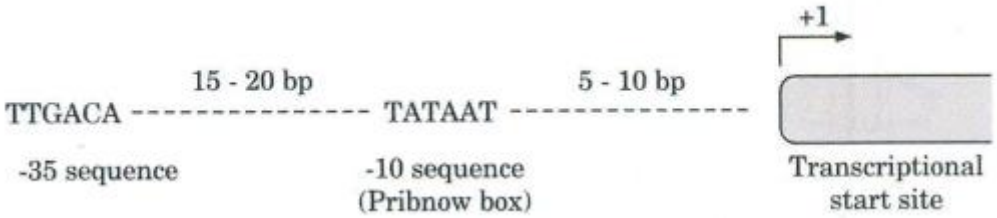

Fig. 14: Prokaryotic promoter showing - 10 sequence, -35 sequence and transcriptional start site.

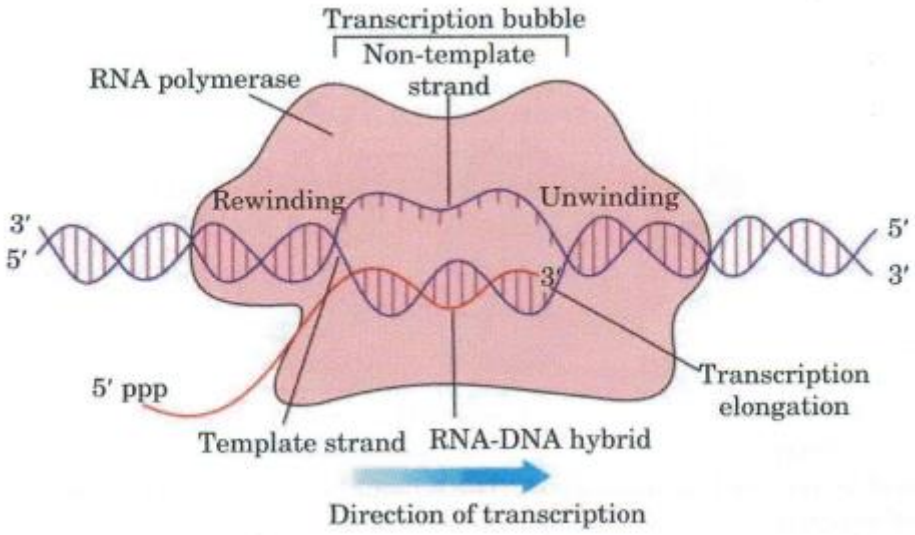

Fig. 15: a transcription bubble showing a transient DNA - RNA hybrid.

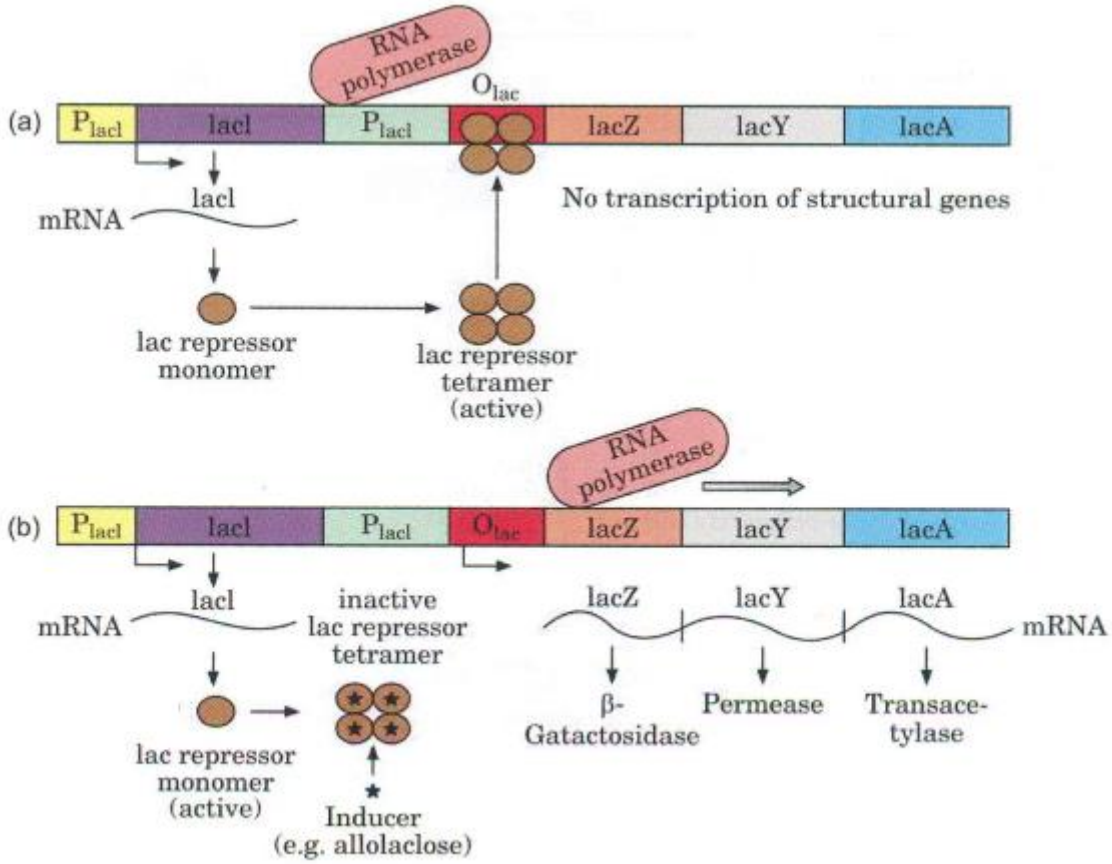

Fig. 16: (a) repression of transcription by *lac* repressor in the absence of inducer, (b) inactivation of *lac* repressor and transcription of structural gene.

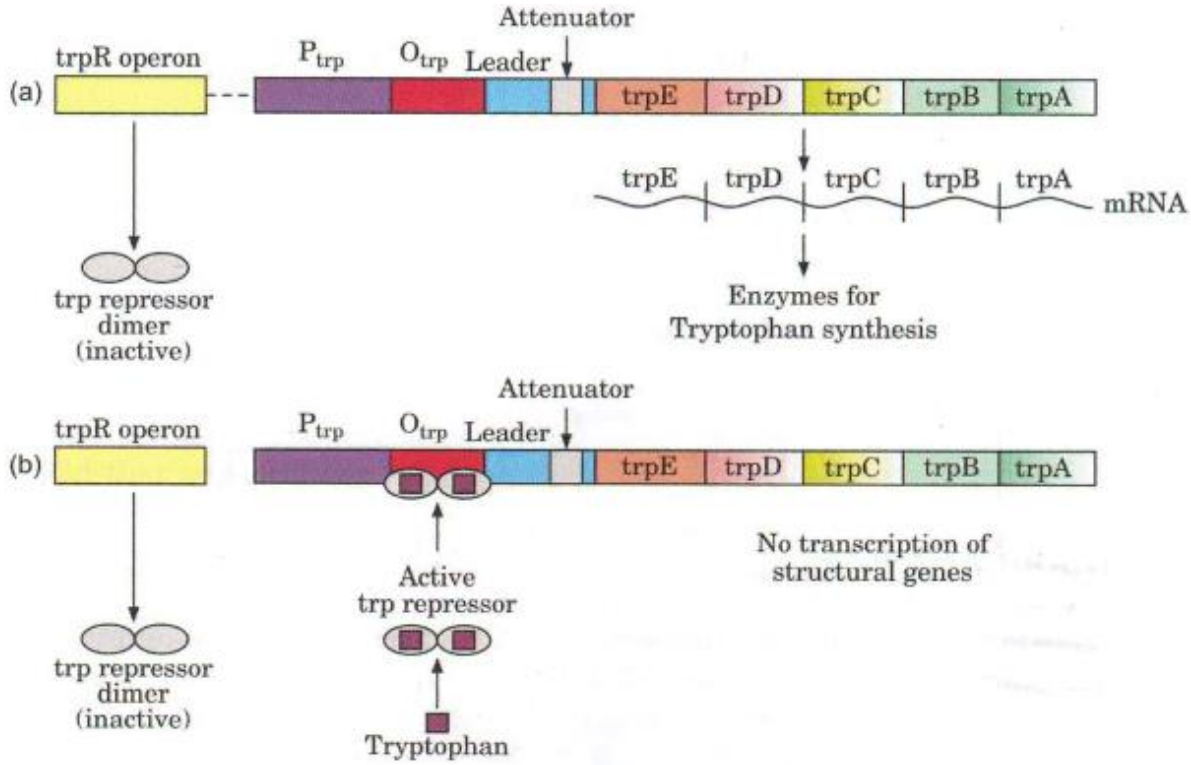

Fig. 17: Regulation of *trp* operon, a. transcription in the absence of tryptophan b. no transcription in the presence of tryptophan.

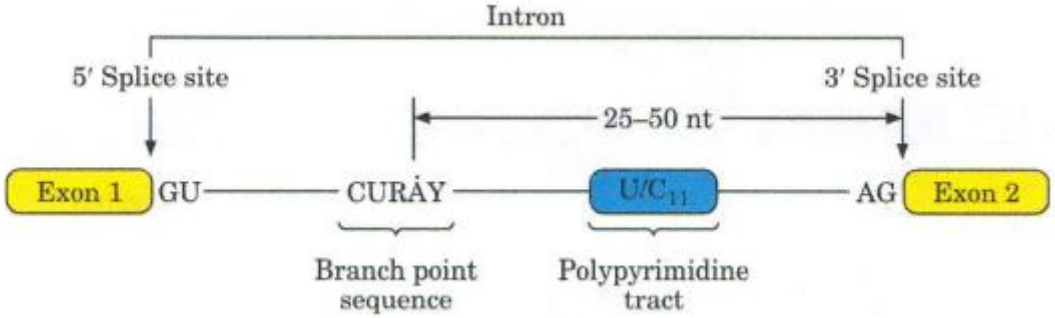

Fig. 18: conserved sequence of RNA splicing.

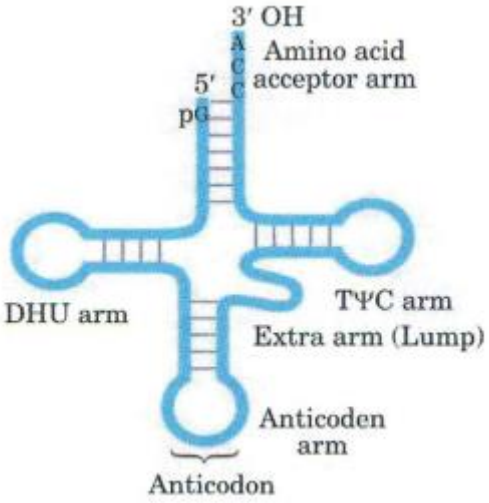

Fig. 19: Clover leaf model of tRNA

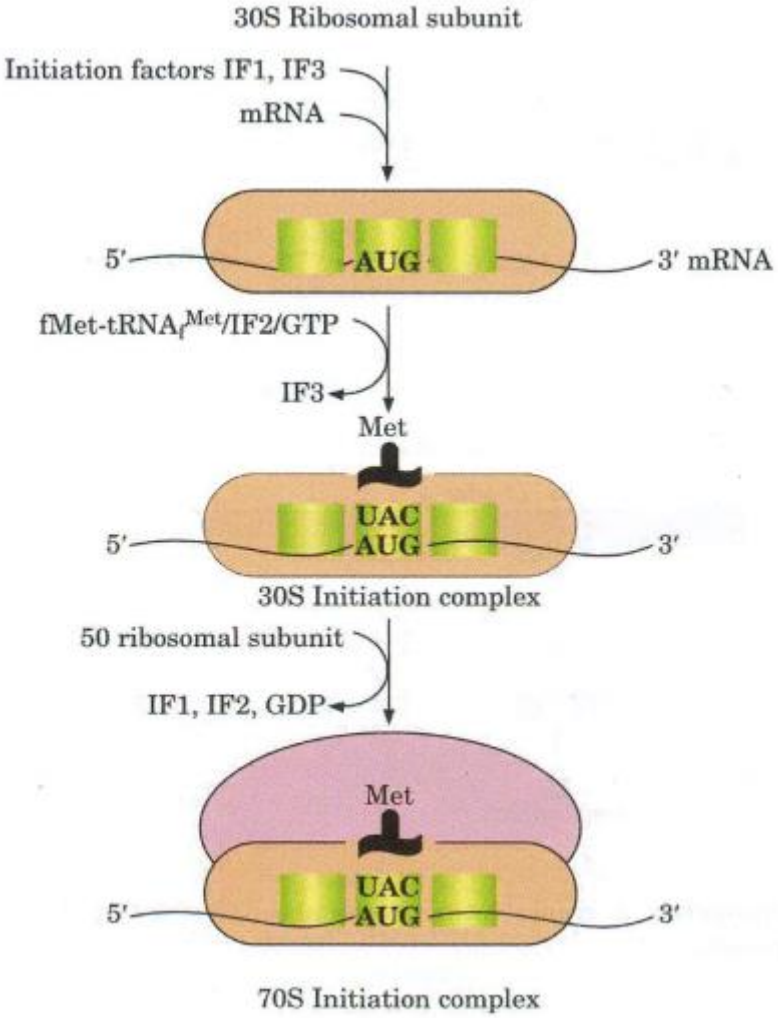

Fig. 20: Initiation of protein synthesis in prokaryotic cell.

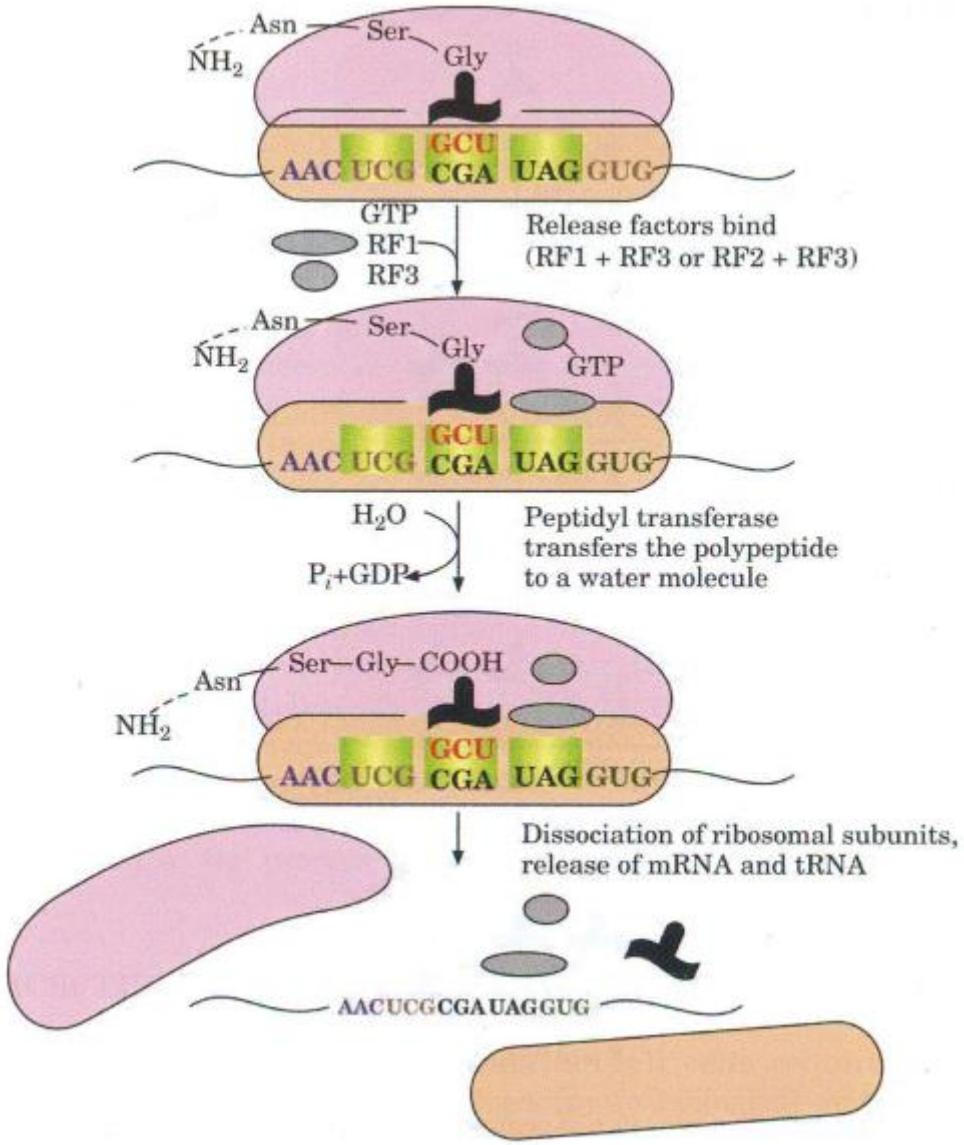

Fig. 21: Elongation of protein synthesis in prokaryote.

elements of the cytoskeleton. A well known microtubular based structure of the cell is axoneme of the cilia and flagella. It is involved in the organization of cytoplasm, overall shape of the cell, formation of spindle fibres that separates chromosome prior to the cell division. Microfilaments are involved in a variety of other cellular phenomenon, involved in connection with the plasma membrane influencing locomotion, amoeboid movement and cytoplasmic streaming. It is also involved in development and maintenance of cell shape. Intermediate filament is the most stable and least soluble constituent of the

cytoskeleton. It is believed that intermediate filaments act as scaffold that supports the entire cytoskeleton.

TRANSPORT OF IONS AND SMALL MOLECULES ACROSS MEMBRANE

Plasma membrane, a selective permeable membrane serves as barrier between cell and the extracellular environment. Essential molecules such as ions, glucose, amino acids and lipids enter the cell whereas metabolic intermediates remain innthe cell. Phopholipid bilayer (Fig. 10), the basic structural unit of biomembrane is essentially impermeable to most of the water soluble molecules, ions and waters itself.

Transport by Passive or Simple Diffusion

The simplest mechanism by which molecules can cross the plasma membrane is passive diffusion. No metabolic energy is spent because it moves from a higher concentration to lower

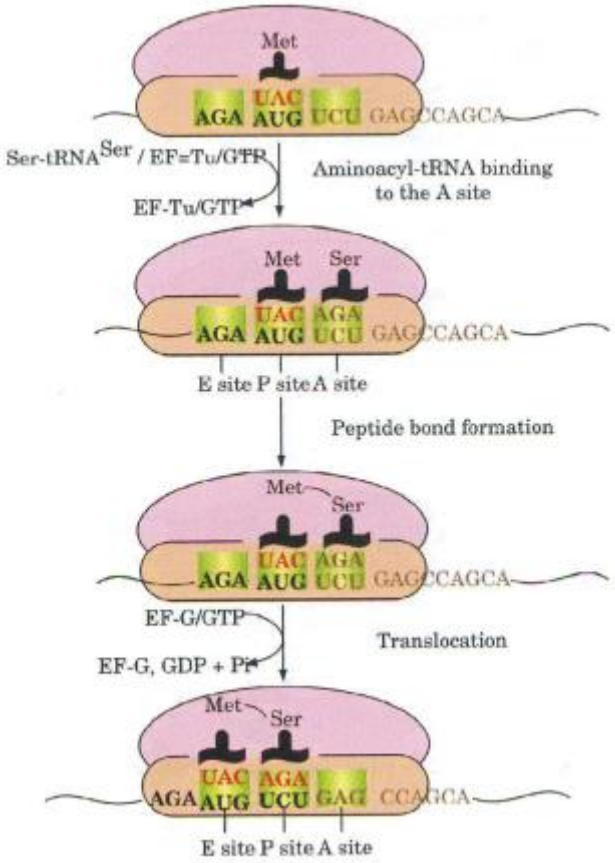

Fig. 22: Termination of protein synthesis in prokaryotic cell

concentration. During passive diffusion, a molecule simply dissolved in the phospholipid bilayer, diffuse across it and then finally dissolves in the aqueous solution at the other side of the membrane. The direction of the transport is simply determined by the concentration gradient without involvement of any membrane protein. Thus, we can say it a non selective process by which any molecule able to dissolve in the phospholipid bilayer is able to cross the plasma membrane and equilibrate between outside and inside. Larger uncharged polar molecules such as glucose are unable to cross the plasma membrane. Instead, the passage of these molecules across the membrane requires the activity of specific transport and channel proteins, which therefore control the traffic of most of the biological molecules in and out of the cell.

Transport by Facilitated Diffusion

Facilitated diffusion involves the movement of molecules in the direction determined by their relative concentration inside and outside the cell. It differs from the passive diffusion in that the transport molecules do not dissolve in the phospholipid bilayer. Instead, their passage is mediated by protein that enables the transport molecule to cross the membrane without directly interacting with its hydrophobic interior. Carrier proteins and channels proteins are involved in facilitated diffusion. Carrier proteins bind specific molecule to be transported from one side of the membrane, undergo conformational changes that allow the molecules to pass through the membrane. Sugars, amino acids,nucleosides cross the plasma membrane of most cell with the help of carrier protein. In contrast, channel protein forms open pores through the membrane; allow the free diffusion of any molecule of the suitable charge and size. The best characterized channel proteins are ion channels which mediate the passage of ion across the plasma membrane. Ion channels are highly selective because narrow pores in the channel restrict the passage of ions across the membrane.

Active Transport

In active transport, energy provided by a coupled reaction such as hydrolysis of ATP which is used to drive the uphill transport of molecules in the energetically unfavorable direction. In the transmission of never impulse, ions channels play an important role as flow of ions across the membrane channel is dependent upon the establishment of the ion gradient of Na^+ and K^+ All cell including muscles and nerve contain ion pumps that use energy derived from ATP hydrolysis to actively transport ion across the plasma membrane. The concentration of Na^+ is approximately 10 times higher than inside the cell, whereas concentration of K^+ is higher inside than out. These ion gradients are maintained by the Na^+ - K^+ pump (Na^+ - K^+ ATPase) which use energy derived

from ATP hydrolysis to transport Na^+ and K^+ against their electrochemical gradient.

Unlike simple diffusion, the facilitated diffusion of a molecule across a biological membrane move in a single direction called uniport (e.g., erythrocyte glucose transport), dependent on specific integral membrane proteins, often called uniporters (Fig. 11). Whereas, in an iondriven transport, if both the molecule and the ion mobe in the same direction, it is termed symport, and the protein involved in the process is called a symporter (*e.g.*, Na^+ / glucose transporter); of the molecule and the ion move in the opposite direction, it is termed antiport,and the protein involved in the process is called an antiporter (*e.g.,* erythrocyte band 3 anion transporter).

BIOENERGETICS: THE FLOW OF ENERGY IN THE CELL

In addition to molecules, enzymes and information, all cells require ATP (adenosine tri phosphate) for capturing and transferring free energy in biological system. In absence of the catalyst, most of the chemical reaction taking place in the cell would occur too slowly to maintain life processes. In this regard, mitochondria play a vital role in the generation of metabolic energy derived from fat, carbohydrate and proteins which is converted to ATP by the process of oxidative phosphorylation.

Metabolic Pathway

The sum total af all the chemical reactions taking place in a cell is collectively called metabolism. The overall metabolism of cell consists, in turn, of many specific metabolic pathway, each of which accomplishes a particular task.

The metabolic pathway are of two different types: anabolic and catabolic. The pathways that synthesize cellular components are called anabolic pathways whereas those involved in the breakdown of cellular constituent are called catabolic pathways. Anabolic pathways usually involve a substantial increase in molecular order therefore decrease in entropy hence energy requiring

(endergonic). By contrast, catabolic pathways are energy liberating (catabolic), in part because they involve a decrease in molecular order hence decrease in entropy.

ATP

The anabolic reaction of the cell is responsible for growth and repair of life processes, whereas catabolic reactions release the energy needed to drive the anabolic reactions and to carry out other kinds od cellular work. The most commonly used energy intermediates in most of cell is ATP (Fig. 12) which is the 'energy currency' of the cell. Hydrolysis of ATP to ADP and Pi is exergonic with a standard free energy Change if - 7.3 kcal / mol. The reverse reaction is endergonic where ATP is synthesized from ADP and Pi with the loss of a water molecule by condensation with a free energy Change of +7.3 kcal / mol. All chemical bonds require energy to be broken and release energy when they form. The energy is therefore a characteristic of a reaction in which the molecule is involved and not of particular bond with the molecule.

In aerobic oxidaation fatty acids and sugars (principally glucose) are metabolized to carbon dioxide and water and releases energy. In animal and non photosynthetic cells energy is generated by this process. The initial step of oxidation involves the process called glycolysis occur in cytosol of both prokaryote and eukaryote and do not require oxygen. The final steps that generates more ATP requires oxygen. In eukaryotes final steps takes place in mitochondria whereas in prokaryotes cell bound organelles are absent hence it takes place in plasma membrane. The final stages of fatty acids metabolism occur some times in mitochondria however, most of the eukaryotic cell fatty acids metabolizes to carbon dioxide and water in peroxisome. In photosynthesis, light energy is converted to chemical energy of phosphoanhyride of ATP and stored in the chemical bonds of carbohydrate mainly sucrose and starch.

Molecular Biology Of Cell

Genes and chromosomes

Genetic material is the store house of all genetic information which controls the inheritance pattern from one generation to the next as well as expresses its effect through the formation and functioning of trait. The fundamental unit of all living organism is gene which is biochemically a segment of DNA or RNA in some viruses. The modern biochemical definition of gene is the entire DNA that encodes either an RNA or polypeptide chain with structural or catalytic function. In molecular terms, gene is the nucleotide sequence which is necessary for synthesis of a functional RNA and then polypeptide.
Organism's basic complement of its genetic material is genome. Most plants and animals contain two copies of their genome and they are called diploid. In contrast, most of the fungi, algae and bacteria contain single copy of the genome, haploid. The nucleic acid molecule is the repository of organism genetic information that is organized into chromosome. Each chromosome in the genome carries a different set of genes; in diploid cell its component genes are present twice. In somatic cell of human there are two sets of 23 chromosomes, for a total of 46 chromosomes.

Nucleotides

A nucleotide is a phosphate ester of nucleoside in which phosphate group is joined to a nucleoside at the hydroxyl group attached to C-5 of the sugar. The sugar present in the nucleoside is deoxyribose and hence is called deoxynucleotide. Deoxynucleotides may have single, two and three phosphate group, which are called deoxynucleoside 5' monophosphates (dNMPs), deoxynucleoside 5' diphosphates (dNDPs) and deoxynucleoside 5' triphosphates (dNTPs), respectively. dNTPs such as deoxyadenosine 5' triphosphate (dATP), deoxyguanosine 5' triphosphate (dGTP), deoxcytidine 5' triphosphate (dCTP)

and deoxythymidine 5' triphosphate (dTTP) are the precursor for DNA synthesis.

Different nucleotides in DNA are joined together by covalent bonding between phosphate and sugars to form a long polymer. For a nucleotide, the phosphate attached to the hydroxyl group at 5' position of the sugar is in turn bonded to the hydroxyl group on the carbon of the sugar of the next nucleotide. The bond between the two deoxynucleotide is a 3', 5' phosphodiester bond, since each phosphate-hydroxyl bond is an ester bond. The first nucleotide has a 5' phosphate not bonded to any other nucleotide and the last nucleotide has a free 3' hydroxyl, it has 5' end and 3' end and hence polarity. Different genes have different sequence of the four nucleotide (A, G, T and C) and code for different biological message.

Double Helical Model of DNA

Watson and Crick (1953) described the three dimensional structure of DNA (Fig. 6) starting from X-ray diffraction photographs taken by two physicists Franklin and Wilkins. They deducted that DNA is composed of two strand wound around each other. The two strands run in opposite directions, one strands is 3' - 5' hence organized in antiparallel arrangement. The bases of two strands form hydrogen bond to each other; A pairs with T and G pairs with C and which is called complimentary base pairing. The biologically important form of DNA is B DNA which is found in most living systems. There are certain other forms of DNA such as A, C and Z-DNA having different characteristics (Table 4).

Table 4: Important feature of different forms of DAN.

Helix type	Base pair per turn	Rotation per bp	Vertical rise per bp	Helical diameter
A	11	+32.7° (right handed)	2.56 Å	23 Å
B	10	+36.0° (right handed)	3.38 Å	19 Å
C	9.33	+38.6° (right handed)	3.32 Å	19 Å
Z	12	-30.0° (left handed)	3.71 Å	18 Å

Chromosome and Chromatin

'Chromosome' term is used to refer to a nucleic acid molecule which is repository of genetic information in eukaryotic cell, a bacterium, a virus or an organelle.

Prokaryotic and Eukaryotic Chromosome

The DNA of bacterial cell (*e.g.*, E. coli) is a circular double stranded molecule which may be referred as bacterial chromosome or nucleoid or incipient nucleus. The circular DNA is packed into region of cell called nucleoid or incipient nucleus or pro chromosome where it is organized into many loops that are bound to a central protein scaffold attached to the cell membrane. The DNA supercoiled, that is, it is twisted upon itself and complexed with non - histone proteins (NHP).

Eukaryotic cells contain double helical DNA associated with histone proteins. This DNA - protein complex is called chromatin. In addition to the nucleus, eukaryotic organelles (mitochondria and chloroplast) also contain DNA but it is double stranded and circular. In nucleus, the length of the packaged DNA molecule varies. In yeast cells it is 2.6 times more DNA than E. coli whereas human cells have almost 700 times as much DNA as in E.coli. Most of the human cells are diploid and each cell contains a total of 2 m of DNA.

Chromatin structure

In non dividing eukaryotic cell, chromatin is amorphous and appears to be randomly dispersed in the certain parts of nucleus. It consists of fibers containing protein and DNA in approximately equal masses with a very small amount of RNA. The DNA in the chromatin is tightly associated with protein called histones.

Nucleosomes

The binding of chromosomal DNA to histone is tightly associated with protein called histones, which package and order the DNA into structural units called

nucleosomes. In chromosomes, the overall ratio of DNA to histone on weight basis is approximately equal to 1:1. Each nucleosome molecule contains eight histone molecules: two copies each of H2A, H2B, H3 and H4 and a H1 which binds to linker DNA connecting two nucleosome unit (Fig. 7). The DNA is wound round the histone octamer in about 1.8 turns of the left handed DNA. The overall packing ratio is about 7 that is the DNA length is shortened about seven folds by winding around nucleosome. The compaction in a chromosome is greater than 10, 000 folds. Nucleosome chromosome appears to be organized into a 30 nm fiber, if isolated very carefully by gentle method.

DNA Replication in Bacteria

The synthesis of DNA molecule can be divided into three main steps: initiation, elongation and termination.

Initiation

The E. coli origin of replication (*OriC*) consists of about 245 bp and it bears highly conserved DNA sequence for bacterial replication origin. The main component of DNA initiation is the DnaA protein which binds to the four 9 bp repeats and then recognizes the three 13 bp repeats and successively dentures it.The whole process requires ATPand the bacterial histone like protein which results into unwinding of DNA bi - directionally and creating two potential replication forks.

Elongation

The elongation phase of replication includes related operations: leading and lagging strand synthesis. The DNA is unwound to release topological stress by topoisomerase and stabilized by single strand binding (SSB) protein. DNA strand cannot start DNA synthesis without a primer, it requires a RNA polymerase called primase. The RNA primer made by primase is then extended by DNA polymerase III, the main polymerizing enzyme. E. coli contains two other DNA polymerase, DNA polymerase II and DNA polymerase I . DNA

polymerase I catalyzes the stepwise addition of deoxyribonucleotides to the 3'-OH end of a DNA chain:

$$(DNA)_{\text{n residue}} + dNTP \rightarrow (DNA)_{n+1} + PPi$$

It requires DNA template, a primer with a free 3' - OH, all four dNTPs (dATP, dGTP, dCTP, dGTP) and Mg^{2+} ions.

DNA polymerase is a template directed enzyme that recognizes the next nucleotide on the DNA template and then adds a complementary nucleotide to the 3' - OH of the primer, creating a 3' 5' polymerase, 3' - 5' exonuclease 5' - 3' exonuclease.

DNA polymerase II and DNA polymerase III catalyze the synthesis of DNA from deoxynucleotidyl 5' - tri phosphate which synthesizes DNA in 5' - 3' direction and have 3' - 5' exonuclease activity.

After the opening of replication fork (Fig. 13), both the DNA strand serves as template for synthesis of new DNA strand. As a result of replication, two daughter strand is formed each of which has one parent strand and one newly synthesized. Thus, replication is called semi conservative. Double strand DNA is anti-parallel; one strand runs in 5' - 3' direction and the complementary strand runs in 3' - 5' direction. The new DNA made in continuous piece in correct 5' - 3' direction is called leading strand. On the other hand, DNA polymerase synthesizes short pieces of new DNA (~1000 nucleotide long) in the 5' - 3' direction and then joins the pieces together by DNA ligase. These small fragments are called Okazaki fragment, named after their discoverer.

Termination

Two replication fork of the circular chromosome of E. coli meet a terminus region containing many copies of sequence called Ter (terminus). The Ter sequence binds with a protein Tus (terminal utilization substance) and then Ter - Tus complex arrest the replication fork. The new DNA strand formed by this discontinuous method results into lagging strand of DNA. After the completion

of replication, the origin of replication is sequestered in new replication factories at the centre of the cell and the entire process is repeated.

DNA Replication in Eukaryote

The eukaryotic DNA molecule is comparatively larger than those of bacterial DNA and organizes into complex nucleoprotein complex called chromatin. The essential features in the DNA replication are similar to that of prokaryotic replication with some interesting variations. Origin of replication in eukaryotes is called autonomously replicating sequences (ARS) or replicators. Initiation of replication requires origin recognition complex (ORC), which binds to several sequence within the replicator. Multiplication of chromosomal DNA occurs in the S-phase of cell cycle which requires many origins and proceeds bi-directionally from each origin. At each origin, replication bubble forms consisting of two replication forks moving in opposite direction. The DNA replicated under the control of a single origin is called replicon. The synthesis of DNA proceeds until the replication bubble merges together. Like bacteria, eukaryotes have also many types of DNA polymerases such as α, β, γ, δ and ε. The replication of nuclear chromosome requires α and δ subunits. The DNA polymerase α is multi subunit enzyme with similar structure and properties in all eukaryotic cells. DNA polymerase α carries a primase subunit, responsible for the synthesis of short primers and these primers are extended by the multi subunit DNA polymerase δ. It is believed that the 3' - 5' proof reading exonuclease activity and synthesis of both lagging and leading strand is also carried out by DNA polymerase δ. DNA polymerase β and ε are involved in DNA repair. All these DNA polymerases are located in the nucleus except DNA polymerase γ, which is located in mitochondria. The basic strategy of replication of double stranded chromosomal DNA is similar to that of bacterial DNA replication. However, in eukaryotes, Okazaki fragment moves at one tenth slower rate than that of prokaryote.

Telomere Replication

The synthesis of replication in eukaryotic chromosome involves the synthesis of special structure called telomere. The normal mechanism of replication of linear DNA molecule in eukaryotes has at the end of lagging strand is not replicated. This creates a gap at the end of chromosome resulting into shortening of double stranded replicated protein. The solution of this particular problem lies in the replication of chromosomal ends, telomere by enzyme called telomerase. Each telomere contains many copies of repeated G rich sequence complimentary to a short RNA molecule. This sequence is usually in a form od 5' (T_xG_x) in one strand and 3' (C_yA_x) in the complementary stand where x and y are typically in the range of 1 to 4.

RNA Metabolism

Gene expression generally involves production of an RNA molecule transcribed from a DNA template. RNA is the only macromolecule known to have role in storage as well as transmission of information which depicts its role as essential chemical ntermediates in the development of life on earth. All RNA molecules (except RNA genome of certain viruses), derived from information coded in DNA. During transcription, a number of enzymes convert the genetic information coded in double stranded DNA into RNA with a base sequence complementary to one of the DNA stra.Among three major kinds of RNA produced, messenger RNA (mRNA) encode the amino acid sequence of one or more polypeptide. Transfer RNA (tRNA) reads the information encoded in the mRNA and transfer the amino acid to a growing chain of polypeptide during protein synthesis. The constituent of ribosomes, ribosomal RNA (rRNA) is involved the synthesis of protein.

Transcription in Prokaryotes

transcription in E. coli requires RNA polymerase, a DNA dependent RNA polymerase for transcription and takes place in three stages; Initiation, elongation and termination.

Initiation

RNA polymerases recognize a specific site on the DNA upstream to the site of initiation of transcription called promoter site and then unwinds the DNA locally. In addition to the DNA template, RNA polymerase requires all four ribonucleotide 5' - triphosphates (ATP,GTP,UTP and CTP) as precursor of the nucleotide unit of RNA. In prokaryotes, all genes are transcribed by a single large RNA polymerase with a subunit structure ααββ'ωσ. The holoenzyme is needed to start transcription since the σ subunit is essential of recognition of promoter and binds at special site of the DNA. In E. coli, the most common subunit is σ^{70} (Mr 70,000). In E. coli, RNA polymerase holoenzyme binds within the region stretching form 30 - 70 bp before the transcription start site. The promoter region thus extends between -70 and +30. Within the promoter two highly conserved 6 bp sequence, called consensus sequence is present. The consensus sequence at the -10 region is 5' - TATAAT - 3', known as Pribnow box by the name of discoverer Pribnow (Fig. 14). It is important recognition site that interacts with the ó subunit of RNA polymerase. Sequence at the -35 region is 5' - TTGACA - 3' and is important in DNA unwinding during transcription initiation. A third AT rich recognition element called UP (upstream promoter) element found in the region of -40 and -60 in the promoter of certain highly expressed genes. The UP element is bound by the subunit of the RNA polymerase. RNA polymerase does not need any primer to begin the process of transcription.

Elongation

Afted initiation of transcription, σ subunit is released from transcriptional complex and leave the core enzyme (ααββ'ω), responsible for elongation of

RNA transcript. The first nucleotide in the RNA transcript is usually pppG.The RNA polymerase then synthesizes RNA in the 5' - 3' direction using four ribonucleoside 5' phosphate.The growing RNA chain (3' - OH end) attacks phosphate group of the incoming ribonucleoside 5' triphosphates to form 3', 5' phosphodiester bond. RNA polymerase, DNA template and RNA transcript forms the ternary complex. The RNA transcript forms a transcript RNA - DNA hybrid at the unwound region of DNA called transcription bubble (Fig. 15). The DNA is unwound ahead of transcription bubble and after the passing of transcription complex, the DNA rewinds.

Termination

Escherichia coli posses two main principle mechanisms of transcription. One requires a protein factor ρ (rho) and the other does not require any other protein factor other than RNA polymerase. Rho - dependent terminator lacks the sequence of repeated A residue in the template strand but includes a rho - utilization element (a CA rich sequence). During the termination process, rho subunit having ATP - dependent RNA - DNA helicase activity promotes translocation of protein along the RNA and ATP is hydrolyzed by rho protein. DNA sequence where RNA polymerase terminates without rho factor is called rho - independent termination sites. These sites have series of T residues preceding GC rich self complimentary region. When a self complimentary region of growing RNA chain is synthesized, a hairpin structure is formed to stop transcription.

Regulation of Gene Expression in Prokaryotes

The lac operon

Out of 4,000 genes of E. coli, only a fractio2is expressed in a cell at any given time. Bacteria have a simple general mechanism for coordinating the regulation of genes. Genes cluster and promoter along with additional sequence that function together in regulation are called as operon.

In the *lac* operon (Fig. 16), the structural genes are *lac* Z, *lac* Y, *lac* A genes. lac Z genes encoding β - galactosidase which hydrolyses lactose to glucose and galactose, the permease (also known as lactose permease) transport lactose into the cell across the cell membrane. *lac* A gene encodes transacetylase 9also known as thiogalactoside transacetylase), the role of which is not clearly known. Under normal conditions, E. coli cells produces very little amount of these proteins but when lactose is available; it causes a large increase in amount of each enzyme. Thus each enzyme is an inducible enzyme and the process is called induction. Before induction, few molecule of β - galactosidase converts lactose to allolactose which turns on the function of all the three genes in the transcription and act as inducer. Another good example of inducer which is not metabolized by E. coli is isopropyl β - D - 1 -thiogalactoside (IPTG) and hence it is used in the experimental studies of induction in laboratories.
lac operon was proposed by Jacob and Monod (1961) for regulation of transcription. *lac* operon possesses three elements: structural genes, an operator sites and regulator gene. structural gene encodes the protein to be regulated, operator site is a DNA sequence that regulates transcription of the structural gene and regulator gene encodes a protein that recognizes the operator sequences.

lac repressor is a protein encoded by the *lac* I gene which has its own promoter ($P_{lac\ I}$, Four identical Lac repressor monomer come together to form tetramer protein which bind to the lac operator site, O_{lac}. In the absence of an inducer (allolactose or IPTG) the lac I gene is transcribed ant the resulting repressor protein bound to the operator site of the lac operon, O_{lac} and prevents transcription of the *lac* Z, *lac* Y and *lac* A genes. During induction by inducers, it binds to the tetramer repressor protein causing change in conformation of the repressor that greatly reduces its affinity to the lac operator site. In absence of inducer, the lac repressor rapidly binds to the lac operator site and transportation

is inhibited almost immediately. The *lac* ZYA RNA transcript is too unstable and so degrades quickly such that further synthesis of the β - galactosidase, permease and transacetylase.

The *lac* operon undergoes positive regulation. The other factor in addition to lactose affects the expression of lac gene in a complex internal bacterium environment. A regulatory mechanism known a catabolism repression restrict expression of gene required for catabolism of lactose, arabinose and other sugars in the presence of glucose; even in the presence of secondary sugars. The effect of glucose is mediated by a co - activator cAMP and an activator protein known as cAMP receptor protein (CRP) also called catabolic activator protein (CAP). The CAP is a dimer protein, cannot bind to the Lac promoter just upstream from the binding site for the RNA polymerase. It increases the binding of RNA polymerase and so stimulates transcription of the lac protein. The binding od CRP proteins to the Lac promoter depends upon the carbon source available to the bacterium. when glucose is present, E. coli does not need to use lactose as a carbon source and so the *lac* operon does not need to be active. Glucose inhibits adenylate adenylate cyclase which synthesizes cAMP from ATP. Thus, when glucose is present, the intracellular level of cAMP falls, so CRP cannot bind to the lac operon and hence the *lac* operon is only weak active even in the presence of lactose. In the absence of lactose, the adenylate cyclase in not inhibited, the level of intracellular rises and binds to CRP. Therefore, in absence of glucose and presence of lactose, the CRP - cAMP complex stimulates transcription of the *lac* operon and allows the lactose to be used as an alternative carbon source. When lactose is present, the lac repressor of course ensures that the *lac* operon remains inactive.

The overall coordinated control ensure that the *lac* Z, *lac* Y and *lac* A genes are transcribed strongly only if glucose is absent and lactose is present. A good example of negative control or negative regulation og gene expression is the *lac*

operon, as it prevents the transcription of structural genes. Positive regulation or gene control of gene expression is when the regulatory proteins binds to the DNA and increases its transcription rate, hence called activator. The CAP / CRP involved in regulation of *lac* operon which is a good example of an activator. Therefore, the *lac* operon is subject to both negative as well as positive control.

The trp operon

The E. coli trypyophan (*trp*) operon (Fig . 17) includes five structural genes encoding to convert chorismite to tryptophan. The *try* operator region partially overlaps the *trp* promoter (P*trp*) and it is regulated when tryptophan is in the short supply in the cell. When tryptophan is present, a *trp* repressor protein encodes a unique dimer protein *trp* R. However, this is inactive and so is unable to bind to the *trp* operator and the gene responsible for *trp* operator is transcribed. In the presence of tryptophan, enzyme responsible for tryptophan biosynthesis are not required and hence expression of these genes are turned off. This is achieved by the binding of tryptophan to repressor, results in its activation and binding to operator and stops transcription of the structural genes. Here, tryptophan acts as co - repressor, prevents transcription and hence acts as negative control.

Another mechanism, known as attenuation is also used to control expression of the *trp* operon. The 5' end of polycistronic mRNA transcribed from *trp* operon has a leader sequence upstream of the coding region of *trp* E structural gene, encodes leader peptide (14 amino acid long) containing two tryptophan residue. Fine tune expression of the trp operon is done by leader sequence by forming a variety of hair - pin or steam loop structure. When plenty of tryptophan is present, ribosome binds to the *trp* polycistronic mRNA that is being transcribed and begins to translate the leader sequence, stem loop forms, resulting into termination of transcription. Therefore, in presence of tryptophan, further transcription of *trp* operon is prevented.

Transcription in Eukaryotes

In prokaryotes, all the RNA is synthesized bu single RNA polymerase whereas eukaryptic cells have three RNA polymerase responsible for the transcription of the different types of RNA.

RNA polymerase I is located in the nucleolus and transcribe the 28S, 18S and 5.8S rRNA genes. In the nucleoplasm, RNA polymerase II is present, responsible for transcription of protein coding genes to yield pre - mRNA and also genes encoding small nucler RNA (SnRNAs) involved in mRNA processing. RNA polymerase III is also located in the nucleoplasm and it transcribes the genes for tRNA, 5S rRNA, U6 Sn RNA and 7S RNA associated with signal recognition particle (SRP). The basic mechanism of RNA synthesis is almost same in prokaryote as well as in eukaryote and these are as follows:

- The initiation of RNA synthesis by RNA polymerase is directed by the presence of promoter site towards the 5' side of the transcriptional start site.
- The process of RNA synthesis does not require primer.
- Synthesis of RNA occurs in 5’3' direction with the RNA polymerase catalyzing in nucleophilic attack by 3' OH og growing RNA chain.

Transcription of Protein Coding Gene in Eukaryotes

Majority of the protein coding genes in eukaryotes are discontinuous. Exones, the coding sequence of the gene are interruoted by non coding section of DNA called introns. The number of intron in a protein coding gene varies and its range varies frome 80 bp to 10,000 bp. After processing of the primary transcript, per - mRNA molecule, it yields mature mRNA for translation. The pre - mRNA receives a 5' cap and a tail of 250 - 300 bp A residue, called poly (A) tail. Like transcription of prokaryotes, eukaryotic transcription also occurs in three steps: initiation, elongation and termination.

Initiation

Highly conserved sequence is located 25 - 35 bp upstream to the transcription start site in most of the promoter site of RNA polymerase II. TATA box with consensus TATA (A / T) A (A / T) is located on the upstream at position about - 25. The sequence near TATA box is essential for recognition of RNA polymerase as well as it influence the efficiency of initiation. Some eukaryotic protein coding gene lacks a TATA box and has initiator element instead near transcriptional start site. RNA polymerase II requires additional proteins called transcriptional factors, in order to start initiation of transcription. The first component binds to the pre initiation.

Complex at the TATA box of a typical polymerase II promoter, TATA - binding protein (TBP). The complex includes many transcriptional factors vis., TFIIB, TFIIE, TFIIF, TFIIH; TFIID, TFIIA and TFIIJ. In eukaryotes, the best co - activator is the TFIID, a large comp;ex includes ten or more TBP associated factors (TAFs). As soon as TFIID complex has bound, TFIIA binds and stabilizes the TFIID - TATA box interaction. TFIIB then binds to TFIID as well as RNA polymerase II to acts as bridging protein. The binding of TFIIF takes place followed by binding of TFIIE, H and J. The final protein complex is formed; called transcription initiation complex is now ready to transcribe the gene.

Elongation

The primary transcript is formed by the RNA molecule made from protein coding gene by RNA polymerase. TFIIF remains associated with the RNA polymerase II throughout entire elongation process. During the process of elongation, the activity of polymerase is greatly enhanced due to elongation factors.

Termination

Once the RNA transcript is ready. transcription is terminated and RNA polymerase is recycled by dephosphorylation and becomes ready to initiate other transcript.

Inhibition of DNA Dependent RNA Polymerase

***Actinomycin D*:** An antibiotic responsible for inhibiting elongation of RNA strand by RNA polymerase in both bacteria and eukaryote.

***Rifampicin*:** It inhibits the bacterial RNA synthesis by binding to the β subunit of the RNA polymerase, result in inhibition of transcription.

***α - amamitin*:** Disrupts the RNA formation in animals by blocking RNA polymerase II (in higher concentration it also blocks RNA polymerase III).

Processing of RNA

Some of the RNA molecule of bacteria and almost all RNA molecule of eukaryote are processed to some extent after their synthesis. The molecule involved in these reactions is RNA rather than pretein, discovery of ribozyme is one the key revolution. Processing of the primary transcript, a newly synthesized RNA molecule is most extensive in eukaryotic mRNAs and in tRNA of both eukaryote and bacteria.

In eukaryotes, the product of transcription is pre - mRNA which requires processing to produces a functional mRNA. The addition of 5' cap, a residue of 7 - methyl guaosine linked to 5' terminal residue of the mRNA. The 5' cap protects mRNA from ribonuclease action and also helps in binding of mRNA to ribosome to initiate translation. To achieve capping, the terminal 5' capping, terminal 5' phosphate is first is removed by phosphatase first and guanosyl transferase catalyzes a reaction whereby the resulting diphosphate 5' end attacks the α - phosphorous atom of the a GTP molecule to add a G residue in an typical 5’5' triphosphate link. the methylation of G residue is done at N - 7 position in which S - adenosyl methionine acts as methyl donor.

The polyadenylation of 200 - 250 A residues at the 3' end of the eukaryotic pre - mRNA form poly (A) tail. Polyadenylation reaction requires a typical polydenylation sequence (5' - AAUAAA - 3') located near the 3' end of the pre - mRNA followed by a sequence 5' - YA - 3' (where Y is a pyrimidine). The poly (A) tail helps 3' end of the mature final transcript in stabilizing the mRNA by protecting it form ribonuclease.

RNA Splicing

Splicing is the removal of intron sequence and joining the ends of nearby exons. Introns start with the sequence GU at the 3' splice site (3' exon - intron boundary) and ends with a sequence AG (Fig. 18). A conserved stretch of about 11 pyrimidine residue, lies upstream of the AG at the 3' splice site and a signal branch point sequence located about 20 - 5 - nucleotide upstream of the splice site is present. In vertebrates and yeast, this sequence is 5' - CURAY - 3' and 5' - UACUAAC - 3', respectively (where R is used for purine and Y is used for pyrimidine).

In the first step of splicing, a ribose 2' or 2' hydroxyl group makes a nucleophilic attack on the phosphorous making new phosphodiester bond at each place at the expense of the old. Among different group of introns splicing, group I splicing region requires a guanine nucleoside or nucleotide cofactor and the 3' hydroxyl group of guanosine is used as nucelophile in the first step of splicing pathway. The guanosine 3' hydroxyl group forms a normal 3', 5' - phosphodiester bond with 5' end of intron. The e' - OH of the exon that is displaced in the first step then acts as nucleophile in a similar kind of reaction at the 3' end of the intron, As a result, precise excision of the intron and ligation of exon takes place.

The reaction pattern is similar with group II pattern except for the nucleophile in the first step which is 2' - OH of an A residue within the intron. A branch intermediate is formed as lariat structure. Most of the introns are not self splicing and these are not designated as any group number. However, self

splicing of intron was reported with group I rRNA intron of *Tetrahymena thermophila*, a ciliated protozoan by cech *et al*. (1986).

Group III introns are found in primary transcript of mRNA, called spliceosomal introns because removal of introns is catalyzed by spliceosome, a large protein complex. Within the spliceosome, the intron undergoes splicing by the same lariat - forming mechanism as the group II introns. These spliceosomes are made up of RNA - protein complex, small nuclear ribonucleoproteins (snRNAs often pronounced as “snurp”).

The forth group of introns group IV, found in certain tRNAs, differs from group I and group II introns in that the splicing reaction requires ATP and endonuclease. The endonucleases involve in the cleavage of phosphodiester bond at both ends of introns and the two end of exons are joined by DNA ligase.

Ribosomal RNA

There are seven ribosomal RNA (rRNA) transcription units scattered throughout the genome, each of which contain single copy of each of the 23S, 16S and 5S rRNA gene and one of the fourcopies of various tRNA gene. Pre - rRNA transcript is transcribed by single prokaryotic RNA polymerase and it is then cleaved by RNA polymerase II to yeild precursor of the 23S, 16S and 5S rRNAs. Further, these precursors are then trimmed at 5' and 3' end to generate a mature rRNA.

In eukaryotes, the genes coded for 28S, 18S and 5.8S rRNA are typically clustered together and tandemly repeated. The rRNA transcription units are transcribed by RNA polymerase I in nucleolus. Whereas, 5S rRNA genes are transcribed by RNA polymerase III.

Transfer RNA

Transfer RNA (tRNA) molecule play an important role in protein synthesis by establishing covalent bonding to a specific amino acid to form aminoacyl - tRNA molecule, which recognizes the corresponding codon in the mRNA. The

tRNA molecule exists in a clover leaf secondary structure (Figure 19) by establishing internal base pairing. There are four stem loops called arm along with a variable arm or optional arm present in some RNA. The anticodon arm contains the three nucleotide of the anticodon in its loop which forms complimentary codon in the mRNA molecule during the process of translation. The DHU (dihydrouridine) arm contains an unusual nucleotide dihydrouridine (D). The TΨC arm, which contains ribothymidine (T) and pseudouridine (Ψ). In E.coli, tRNA is transcribed by single RNA polymerase and then primary RNA transcript is processed through cleavage and trimming reaction at 5' and 3' end of precursor tRNA involves endonuclease (RNase E, F and P) and exonuclease (RNase D) to form a mature tRNA molecule.

In eukaryotes, the mRNA is transcribed by RNA polymerase III and it requires transcriptional factor III C (TFIIIC) as well as transcriptional factor (TEIIIB). Pre - tRNA molecule after synthesis folds up into stem loop structure and non tRNA molecule is cleaved from the 5' and 3' end by ribonuclease.

The Genetic Code

Marshal Nirenberg and Heinrich Matthaei (1961) succeeded to synthesize first triplet genetic codon UUU which encodes phenylalanine and the Indian legend Har Gobind Khorana (1979) was the first scientist to synthesize oligonucleotides, also contributed in synthesizing two complimentary deaxyribonucleotide by organic chemical method. In prokaryotes as well as eukaryotes, the DNA sequence of a single gene is collinear with amino acid sequence of the coded polypeptide. Clearly the relationship between the nucleotide and the sequence of amino acid is called the genetic code and it is read in the group of three (triplet) called codon. Each codon is specific for a particular amino acid, however, there are termination codons or stop codons which do not code for any amino acid and these are UAG, UAA and UGA.

All prokaryotic polypeptide starts with a modified methionine (N - formyl methionine) whereas in eukaryotic polypeptide chain starts with methionine. The codon AUG also function as start codon and codes methionine.

A striking feature of the genetic codon is its degeneracy; it means that an amino acid may be specified by more than one codon. Only methionine and tryptophan have single codon whereas three amino acids (Leucine, Serine, Arginine) have six codon and isoleucine has three codon, five amino acids have four and nine amino acids have two codons (Nirenberg, 2004) as detailed in Table 5.

		Second letter of codon			
		U	***C***	***A***	***G***
First letter of codon (5')	U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys
		UUC Phe	UCC Ser	UAC Tyr	UGC Cys
		UUA Leu	UCA Ser	**UAA Stop**	**UGA Stop**
		UUG Leu	UCG Ser	**UAG Stop**	UGG Trp
	C	CUU Leu	CCU Pro	CAU His	CGU Arg
		CUC Leu	CCC Pro	CAC His	CGC Arg
		CUA Leu	CCA Pro	CAA Gln	CGA Arg
		CUG Leu	CCG Pro	CAG Gln	CGG Arg
	A	AUU Ile	ACU Thr	AAU Asn	AGU Ser
		AUC Ile	ACU Thr	AAC Asn	AGC Ser
		AUA Ile	ACA Thr	AAA Lys	AGA Arg
		AUG Met Start	ACG Thr	AAG Lys	AGG Arg
	G	GUU Val	GCU Ala	GUA Asp	GGU Gly
		GUC Val	GCC Ala	GAC Asp	GGC Gly
		GUA Val	GCA Ala	GAA Glu	GGA Gly
		GUG Val	GCG Ala	GAG Glu	GGG Gly

It was believed that the genetic code is 'universal', means all living organism used the same code. There is a difference in few cases such as in case of

mitochondria AUA code for Met not for Ile. similarly in mitochondria UGA codes for Trp not stop codon and many more. In a randome nucleotide sequence, 1 in every 20 codons in each reading frame is, on an average is a termination codon. Generaally, a reading frame without a termination codon among 50 or many more codon is known as an open reading frame (ORF).

Translation in Prokaryotes

Pretein synthesis (translation) takes place in three stages: initiation, elongation and termination.

Initiation

the initiation step requires 30S ribosomal subunit, the mRNA, the initiating formyl methionine tRNA (fMet - tRNA $^{f\,Met}$), initiation factors (Ifs), GTP, 50S subunit and Mg $^{2+}$ (Fig. 20). Synthesis of amino acyl - tRNA, amino acid activation, is an important for biniding of amino acid to the tRNA molecule in order to take part in protein synthesis and making enable the amino acid to react with the end of growing polypeptide chain forming a new polypeptide chain. The amino acids that fails to link with tRNA cannot bind to the growing polypeptide chain. Aminoacyl tRNA synthesis playes a key role in the attachment of amino acid to tRNA molecule, separate aminoacyl tRNA synthetase molecule responsible for every amino acid (20 synthatase in total). the first step of synthesis is formation of aminoacyl - AMP (aminoacyl adenylate) formation by reaction of amino acid with ATP. In The second step, aminoacyl group of aminoacyl - AMP is transferred to the 3' end of the tRNA molecule to form aminoacyl - tRNA. The A (aminoacyl - tRNA), site where aminoacyl r - RNA binds. The peptidyl - tRNA binding site (P - site) is the site where tRNA link to growing polypeptide chain. The exit - site (E - site) is the site from where tRNA binds following its role in translation and prior to release from the ribosome.

AUG, the first translated codon in all mRNA, codes for methionine and it is also called initiation codon (start codon). In an mRNA there is also AUG codon exist naturally exist, encodes methionine residue internal to protein. tRNA $_{f}^{Met}$ is used for the initiation codon, whereas, tRNA $_{m}^{Met}$ is used for internal AUG codon. A short purine rich sequence (5' - AGGAGGU - 3'), called Shine - Dalgano sequence, lies 5' to the AUG initiation coden and is complementary to part of the 16S rRNA in the small ribosomal subunit. The Shine - Dalgarno sequence is the binding site of the 30S ribosomal subunit which then migrates in the 3' direction along the mRNA until it encounters the AUG initiation codon. Ifs (IF1, IF2 and IF3) catalyze the protein synthesis in prokaryotes.

The process of initiation begins with the binding of IF1 and IF3 to the 30S subunit and then it binds to the Shine - Dalgarno sequence and moves along the 3' along the mRNA until encounters the AUG codon. The $_{f}^{Met}$ - tRNA fMET binds with the IF2 and GTP to form a complex and then IF3 is released. The large subunit then binds with the release of IF1 and IF2 and hydrolysis of GTP takes place to form 70S initiation complex. In prokaryotes, the binding of $_{f}^{Met}$ - tRNA fMet occurs directly at P - site.

Elongation

Elongation requires initial complex as described above, aminoacyl tRNAs, a set of three cytosolic proteins called elongation factors (FF - Tu, EF - Ts and EF - G) and GTP. cells use three steps called elongation cycle, aminoacyl - tRNA binding peptide, peptide bond formation and translocation (Fig. 21).

aminoacyl-tRNA binding: Corresponding appropriate incoming aminoacyl-tRNA binds to a complex of GTP-bound EF-Tu. The resulting aminoacyl tRNA-EF-Tu-GTP complex then binds to the A-site of the initiation complex. EF-Tu-GTP complex is released after the hydrolysis of the GTP and again EF-Tu-GTP complex is regenerated involving EF-Ts and GTP.

Peptide bond formation: A peptide bond is formed by the catalysis of peptidyl transferase, part of larger subunit, Carboxyl end of amino acid bound to the

tRNA in the P site is uncoupled from the tRNA and joined by a peptide bond to the amino group of the amino acid linked to the tRNA in the A site. This takes place by the transfer of the initiating N-formylmethionyl group from its tRNA to the amino group of its tRNA to the amino group of the second amino acid present now in the A site. The amino acid with α-amino group in the A site acts as a nucleophile, displaying the tRNA in the P-site to form the peptide bond.

Translocation: In the third stage of elongation, translocase (a complex of elongation EF-G) and GPT binds to ribosome. The deacylated tRNA displaces from the P-site to the E-site, the dipeptidyl-tRNA in the A site displaces to the A site to start a new cycle of elongation. The process of elongation continues, adding one amino acid to the C-terminus end of the growing protein chain for each codon that is read.

Termination: Elongation continues until one of the termination codon (or stop codon) becomes positioned in the A-site. Out of the three, two codons (UAA and UAG) are recognized by release factor (RF) 1 whereas, UAG is recognized by RF2, RF3 is also required to assist RF1 and RF2. Thus either RF1 and RF3 or RF2 and RF3 bind depending upon the exact termination codon, present om the A-site. The release factor directed the peptidyl transferase to transfer the polypeptide to a water molecule instead of the aminoacyl tRNA, cleaving the bond between the polypeptide and tRNA in the P-site. The mature polypeptide now leaves the ribosome followed by the mRNA and free tRNA and then ribosomal subunit dissociated into 30S and 50S subunit becomes ready to start new cycle of translation.

Termination

one of three termination (stop) codons (UAG, UAA and UGA) becomes positioned in the A-site (Fig. 22). Unlike other codons, prokaryotic cells do not contain aminoacyl-tRNAs complementary to stop codons. Instead, one of two release factors (RF1 and RF2) binds instead. RF1 recognizes UAA amd UAG whereas RF2 recognizes UGA. A third release factor, RF3, is also needed to

assist RF1 or RF2. Thus, either RF1+RF3 or RF2+RF3 bind depending on the exact termination codon in the A site.

RF1 (or RF2) binds at or near the A site whereas RF3/GTP binds elsewhere on the ribosome. The release factor cause the peptidyl transferase to transfer the polypeptide to a water molecule instead of aminoacyl-tRNA, effectively cleaving the bond between the polypeptide and tRNA in the P-site. The polypeptide, now ready to leave the ribosome, followed by the mRNA and tRNA, and the ribosomal subunit dissociated into 30S and 50S subunits and is now ready to start next cycle of translation.

Translation in Eukaryotes

The same basic mechanism of translation is followed by the eukaryotes as in prokaryotes with three stages described here as initiation, elongation and termination. However there are few differences especially in the initiation process.

Initiation

Initiation in the eukaryotic translation requires at least nine eukaryotic initiation factors (eIFs) as compared to three initiation factors in prokaryotes. The initiation begin with the charging of tRNA with methionine and known as Met-$tRNA_i^{met}$. In case of prokaryotes there were Shine-Dalgarno sequence, instead, in eukaryotes a 40S ribosomal subunit attaches at the 5 end of the mRNA and moves downstream (5' to 3' direction) until it finds the initials codon AUG. The first step is the formation of a pre-initiation complex consisting of the 40S small ribosomal subunit, Met-$tRNA_i^{met}$, eIF2 and GTP. the pre-initiation complex further binds to the 5' end of the eukaryotic mRNA which requires eIF4F (also called cap binding complex) and eIF3. The complex now moves along the mRNA in a downstream direction until it encounters the starting codon AUG. The 5' untranslated region of the eukaryotic mRNA varies in the nucleotide length but may also contain hair loop structure. It is believed that the

secondary structure as hair loops is removed by the initiation factor of initiating complex. Initiation complex is usually recognized by the short sequence called Kozak consensus sequence (5'-ACCAUGG-3'). The 60S ribosomal subunit binds to from an 80S initiation complex which is necessary for the hydrolysis of GTP and leads to release of many initiation factors.

Elongation

In eukaryotes, three elongation factors (eEF1α, eEF1βγand eEF2), have similar function as in prokaryotic counterparts EF-Tu, EF-Ts and EF-G. In eukaryotes, the deacylated tRNA ejects directly from the ribosome in contrast to the prokaryotes, where it moves from the P-site to E-site prior to leaving the ribosome.

Termination

The single eukaryotic release factor (eRF) uses the ATP and recognizes the termination codons for tertermination.

Inhibition of Translation

Protein synthesis is an essential function in cellular physiology and is the primary target of many naturally occurring antibiotics and toxins. Because nearly every step in protein synthesis can be specially inhibited by one antibiotic or another by interacting specifically with the protein and RNAs of prokaryotic and eukaryotic ribosome.

Puromycin: Its structure is similar to 3' end of aminoacyl-tRNA, enabling it to bind to A site of ribosome and causing premature termination of polypeptide chain.

Tetracycline: It blocks the A site of the ribosome in bacteria and prevent the binding of aminoacyl-tRNAs.

Chloramphenicol: It blocks the peptidyl transferase and inhibits the protein synthesis in bacteria (as well as chloroplast and mitochondria).

Streptomycin: It misreads the genetic code in bacteria (in low concentration) and inhibits initiation (at high concentration).

Cycloheximide: It blocks the peptidyl transferase of 80S eukaryotic ribosome but not that of 70S bacterial as well as mitochondrial and chloroplast ribosome.

CELL SIGNALING

Signaling at the Cell Surface

Many different kinds of molecules transmit information between the cells of multicellular organism as they have the ability to sense and respond to specific chemicals. Although these molecules acts as ligands that bind to receptor expressed by their target cells. Multicellular organisms are well organized into different tissues made up of many specialized cells, hence the problem of coordinating and regulating the various activities are very important. Furthermore, the specific function of these cells may be critical only for certain occasion and these processes are mediated through the chemical messenger. The most important signaling in the plant and animal system is extracellular signaling in which molecules are synthesized and released by signaling cell and produces a specific response target cell that have receptor for the signaling molecule. The extra cellular or membrane bound domains of the receptor attracts the signaling molecule which acts as ligand. The overall step by step process of converting signals to cellular response is termed as cell signaling. Signaling molecules are classified on the basis of distance between their sites of production and their target tissue. Some hormonal messengers acts as endocrine signals. The secretion (hormone) of the endocrine signals is produced at greater distance from their target tissues and they are carried by circulatory system to various sites in their body. Growth factors, secretes locally, signals which diffuses to the nearby tissues and such signals are called paracrine signals. Still other mediators act on the same cells that produce them; such signals are called autocrine signals. Some signaling molecule such as epinephrine can act both

short range and long range and produce endocrine as well as paracrine function. Messenger molecules can be chemically characterized as amino acids, or their derivatives, peptides, proteins, fatty acids, lipids, nucleotides or nucleosides.

Specific Interaction Between Ligand and their Receptor

Inside the ligand binding site on the receptor specific appropriate amino acid side chain must be positioned so that they can form chemical bond with the messenger molecule. Each receptor generally binds only a single or group of closely related signaling molecules. The binding reaction between a ligand and the receptor specific for it, known as cognate receptor which is similar to binding of enzyme with its substrate. The ligand can be either bound to a receptor or free in the solution, the receptor is said to be occupied when receptor binds its ligand. As we increase the concentration of the ligand, greater proportion of its cognate receptors will become occupied, the condition is known as saturation. The concentration of the ligand in solution and the number of receptors occupied can be described quantitatively in terms of receptor affinity. Each receptor protein is characterized by binding specificity toward a particular ligand and this receptor-ligand complex mediates specific cellular response called effector specificity. As we know that receptors have a characteristic affinity towards their ligand, cells are geared to sense changes in ligand concentration rather than fixed ligand concentration. When receptors are occupied with the ligands for a long time, the cells become non respondent or desensitized and for further increase in stimulus we have to increase the concentration of ligand. For cells to adapt to a permanent difference in the level of messenger concentration, sensitization provides a better way to do this.

Signaling Molecules with Intracellular Receptors

Small lipophilic hormones cross the plasma membrane by diffusion and then interact with intracellular receptor either in the cytosol or in the nucleus. The resulting receptor is structurally related, being part of the nuclear receptor

super-family. The resulting hormone receptor complex often binds to region of DNA and affects the transcription of many genes. Intracellular receptor of lipophilic hormones includes the steroid hormones (*e.g.*, estrogen and progesterone) which are synthesized from cholesterol, thyroxine which is produced by thyroid cells and is the chief iodinated compound in animals, retinoic acid which is derived from vitamin A, and vitamin D which is synthesized in the skin. Nitric oxide (NO), which is synthesized by deamination of arginine catalyzed by the enzyme NO synthase. NO binds to the active site of the guanylyl cyclase, stimulating the enzyme to produce the small intracellular mediator cGMP. Carbon monoxide (CO) is also used as a signaling molecule by stimulating guanylyl cyclase.

Signaling Molecule with Cell Surface Receptor

Hydrophilic molecule which is unable to diffuse across the hydrophobic interior of the lipid bilayer binds to receptor molecule present in plasma membrane. These include the peptide hormone (*e.g.*, insulin and glucagon) small biogenic amines (*e.g.*, adrenalin and histamine) that are derived from amino acids and function as hormones and neurotransmitter. There are some lipophilic hormones (*e.g.*, prostaglandins) which also bind to the receptors located in the plasma membrane.

Cell Surface Receptor

Hydrophilic as well as some lipophilic hormones binds to the cell surface receptor. The receptors are integral membrane protein situated in the plasma membrane that binds to the signaling molecule (ligand) with very high affinity. Binding of ligand to the receptor may cause a conformational change in the receptor or promote dimerization of two receptors that initiate a sequence of reactions in the target cell which leads to change in cellular function.

Cell surface receptor can be classified into three major classes depending on mode of transfer the information from ligand to the interior of the cell: enzyme linked receptors, ion channel linked receptor and G- protein coupled receptor.

Enzyme Linked Receptor

Binding of these ligands to their extracellular face leads to conformational change and activateactivity of enzyme. In case of insulin receptor, a complex of two α and two β subunits held together by disulfide bonds, binds to extracellular face. The receptor then undergoes a conformational change resulting in to the auto phosphorylation of the cytosolic domain of the β subunit. The phosphotylated receptor is then recognized by other protein in the cytosol that in turn modulates various intracellular events allowing the cell to respond. β subunit may also directly phosphorylate other target protein within the cell. The insulin receptor is an example of a receptor tyrosine kinase whereas the transforming growth factor-β (TGF-β) family receptor has serine/threonine kinase activity in their cytosolic domain.

Ion Channel Linked Receptor

Ion channel linked receptors are involved in the rapid synaptic signaling between the two cells which are electrically excitable. In this case binding of the ligand cause conformational change in the protein such that a specific ion channel is opened. This allows the certain ions to flow through that which subsequently alters the electric potential across the membrane. Neurotransmitter acetyl choline is the best example which bind to specific receptor that allow Na^+ ion to flow into, and K^+ ion out of, the target cell.

G-Protein Linked Receptor

Guanine nucleotide binding protein (G-protein), the largest family of the cell surface receptor is so named because ligand binding cause a change in a receptor conformation that activates a particular G-protein. It transmits signals to the intracellular targets via intermediary action of G-protein. The G-protein

coupled receptor are remarkable in that they all have a structurally and functionally related protein characterized by seven membrane spanning a-helices. The N-terminus of the protein is exposed to the extracellular fluids, while the C-terminus resides in the cytosol. The extracellular proportion of G-protein linked receptor has specific messenger binding site and the cytosolic loop connecting the fifth and sixth transmembrane a helices. Large heteromeric G-protein consists of three subunits designated as α, β and γ to distinguish them from momomeric G-protein such as Ras protein which regulates the cytoskeleton.

Protein kinase-Associated Receptor

Many cell surfaces are directly linked to intracellular enzymes in contrast to G-protein. These enzyme linked receptors are the receptor protein-tyrosine kinase which phosphorylated their substrate on tyrosine residue. The kinase associated receptor includes the receptor for most polypeptide growth factor and this is the main reason behind the study of signaling mechanism involved in the control and differentiation of animal cell. The human genome encodes 59 receptor protein tyrosine kinase and these entire receptor share a common structural organization: an N-terminal extracellular ligand binding domain, a single transmembrane α helix and a cytosolic C-terminal domain with protein kinase activity. Most of the protein-tyrosine kinase receptor consists of single polypeptide with an exception to insulin and some related receptor which consists of two polypeptide chain.

cAMP Pathway: Second Messenger

Cyclic AMP is formed from the enzyme adenylyl cyclase situated in the plasma membrane with protruded catalytic proportion into the cytosol. G-protein responds quickly to change in ligand concentration because they remain active for a while before the Gα subunit hydrolyses its bound GTP leading to an inactive state. Adenylyl cyclase cease to make cAMP due to inactive G-protein;

however the concentration of cAMP remain elevated until it is being degraded to AMP by cAMP phosphodiesterase. cAMP dependent protein kinase (protein kinase A), is a tetrameric protein consist of two regulatory and two catalytic subunit. cAMP bind to their regulatory subunit leading to their dissociation from catalytic subunit whereas the free catalytic subunit are enzymatically active and are able to phosphorylate serine residue on their target site. In the glycogen metabolism, protein kinase A phosphorylates two main enzymes. The first is phosphorylase kinase, phosphorylates and activates glycogen phosphorylase which catalyzes the breakdown of glycogen to glucose-1-phosphate.

The second is glycogen synthase which catalyze glycogen synthesis. Thus it is clear tha televation of cAMP leads to activation of protein kinase A thus blocks further glycogen synthesis at the same time as it stimulate glycogen breakdown.

Inositol Triphosphate and Diacyl Glycerol Receptor

Inositol-1,4,5-triphosphate (IP_3) is the breakdown product of inositol phospholipid which acts as a second messenger. IP_3 and diacyl glycerol (DAG) is generated from phophatidyl inositol-4,5- bisphosphate (PIP_2). PIP_2 plays a significant role in cell growth and survival, is phosphorylated on third position of inositol by the enzyme phosphatidylinositide (PI) 3 kinase. A specific G-protein for activation of phospholipase C called G_p generating IP3 and DAG. IP3 being water soluble quickly diffuses through the cytosol binds to IP3 receptor channel in the endoplasmic reticulum. After binding of the 1P3, calcium is released in the cytosol and then it binds to a protein calmodulin to from calcium-calmodulin complex which activates the desired physiological process. The DAG generated by phospholipase C remains in the membrane and activates protein kinase C (PKC). PKC are involved in the phosphorylation of specific serine and threonine groups on a variety of target proteins depending upon the cell type.

Calcium Ions

An increase in the level of Ca^{+2} in the cytosol may be achieved by many extracellular signals. For example, in the muscle cell Ca^{+2} triggers contraction. The concentration of Ca^{+2} in the cytosol is usually kept very low (0.1 µM), whereas it's concentration in the extracellular fluid and in the lumen of the ER is high (1 µM). Thus there is gradient of Ca^{+2} across the plasma membrane and ER membrane, such that when Ca^{+2} channel in these membranes are triggered to open, Ca^{+2} ions rapidly flow into the cytosol, raising the Ca^{+2} concentration by 10-20 folds.

CONCLUSIONS AND PROSPECTS

The basic concepts of the chapter “Cell and molecular biology” will stimulate curiosity among readers mind and thereby make the learning of cell biology easier and more rewarding. The extreme sophistication of cellular mechanisms will challenge the biologists throughout the new century to explore the molecular machinery. Science is progressing at an extraordinary and unrivalled pace. This is especially true of cell biology as well as molecular biology. With our ever-increasing knowledge of cell biology, we can better understand what controls and contributes to our development and individuality of various life processes. Combined with our understanding of molecular biology we can explore exciting scientific applications that will benefit society. The future of molecular biology, a very popular topic among biologists, is closely related to the cell biology and genetics. Recent technological advancements have engendered rapid development in this area. And this, in turn, has highlighted the need for scientists as well as ethicists to think carefully so that they will not be blamed by future generations for their actions or lack thereof. Our improved understanding of the molecular basis for life has opened up new approaches for the investigation, diagnosis and treatment of various diseases. As a consequence, we are in a new era in the development and

production of medical diagnostics, protein engineering, therapies and therapeutics and nanobiology.