Lecture 21: Structure of Prokaryotic Cells

Introduction- Higher eukaryote has multiple organs to perform specific functions such as liver, kidney and heart. Organs have specific tissues and each tissue is composed of cells. **"Cell is the structural and functional unit of life"** and it contains all necessary infrastructures to perform all functions. Based on cellular structure, cells are classified as prokaryotic and eukaryotic cells. In most of the cases, prokaryotes are single cells where as eukaryotes are either single cells or part of multi-cellular tissues system. Besides this, both types of cells have several structural and metabolic differences as given in Table 21.1 and are discussed later in the lecture.

TABLE 21.1 DIFFERENCE BETWEEN PROKARYOTIC AND EUKARYOTIC CELLS

Feature	Prokaryote	Eukaryote
Size	Small, in µm range	Variable size, upto 40µm in diameter.
Genetic material	Circular DNA present in cytosol as free material	DNA in the form of linear chromosome present in well defined double membrane nucleus, no direct connection with cytosol
Replication	Single origin of replication	Multiple origin of replication.
Genes	No Intron	Presence of Intron
Organelles	No membrane bound organelles	Membrane bound orgelles with well defined function.
Cell walls	Very complex cell wall	Except Fungi and plant, eukaryotic cells are devoid of a thick cell wall.
Ribosome	70S	80S
Trancription and translation	Occurs together	Transcription in nucleus and translation in cytosol

Structure of Prokaryotic cells- A prokaryotic cell is much simpler and smaller than eukarotic cells. It lacks membrane bound organelles including nucleus. A typical prokaryotic cells is shown in Figure 21.1, A. The description of different structural feature of prokaryotic cells is as follows-

1. Outer Flagella: A flagellum attached to the bacterial capsule is a central feature of most of the prokaryotic cell especially motile bacteria. It provides motion or locomotion to the bacteria and be responsible for chemotaxsis of bacteria. Movement of bacteria towards a chemical gradient (such as glucose) is known as chemotaxsis. Flagellum is a part of cell wall and its motion is regulated by motor protein present inside the cell. Flagellar motion is an energy consuming process and governed by ATPase present at the bottom of the shaft. It is made up of protein flagellin and reduction or suppression of flagellar protein reduces bacterial infectivity (pathogenicity) and ability to grow.

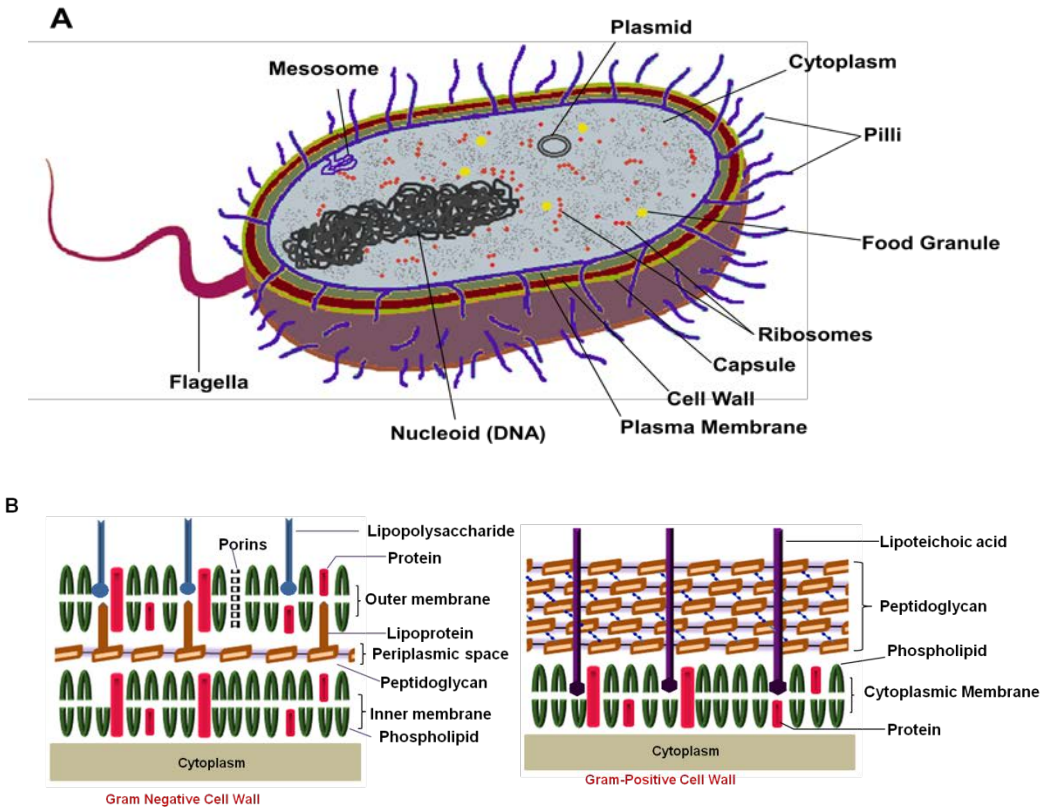


Figure 21.1: Structural details of a typical prokaryotic cell. (A) Whole cell and (B) composition of cell wall of gram negative and positive bacteria.

2. Bacterial surface layers: Bacteria posses 3 anatomical barriers to protect the cells from external damage. Bacterial capsule is the outer most layer and made up of high molecular weight polysaccharides. It is impermeable to the water or other aqueous solvent and it is responsible for antigenicity of bacterial cells. Cell wall in bacteria and its response to gram staining is the basis of classification of bacterial species.

WHAT IS GRAM STAINING? Gram staining is developed by Danish scientist Hans Christian Gram. This technique differentiates bacterial strains based on their cell wall composition, especially thickness of peptidoglycan layer. A detail staining procedure is given in following paper (**Use of the gram stain in microbiology. Beveridge, TJ (2001) *Biotech Histochem* 76 (3): 111–8. Pubmed ID: 11475313**). During the staining procedure bacterial sample is stained with two dyes, **crystal violet** and **safarin**. During a washing step with non-polar solvents such as alchol or acetone (decolorization), gram –ve bacteria leave the **blue** stain due to a thin peptidoglycan layer in cell wall whereas gram +ve bacteria retains both stains and appears as **Pink**.

Cell wall composition in gram-ve and gram +ve bacteria is different. Bacterial cell wall has different constituents and be responsible for their reactivity towards gram stain.

A. Peptidoglycan layer: peptidoglycan layer is thick in gram +ve bacteria and thin in gram –ve bacteria. Peptidoglycan is a polymer of NAG (N-acetyl-glucosamine) and NAM (N-acetyl-muramic glucosamine) linked by a β-(1,4) linkage. Sugar polymer are attached to peptide chain composed of amino acids, L-alanine, D-glutamic acid, L-lysine and D-alanine. Peptide chain present in one layer cross linked to the next layer to form a mesh work and be responsible for physical strength of cell wall. Peptidoglycan synthesis is targeted by antibiotics such as pencillin where as lysozyme (present in human saliva or tears) degrades the peptidoglycan layer by cleaving glycosidic bond connecting NAG-NAM to form polymer.

B. Lipoteichoic acids: Lipoteichoic acid (LTA) are only found in gram +ve bacteria cell wall and it is an important antigenic determinant.

C. Lipopolysaccharides (LPS)- Lipopolysaccharides (LPS) are found only in gram –ve bacterial cell wall and it is an important antigenic determinant.

3. Cytosol and other organelles-Prokaryotic cells do not contains any membrane bound organelle. The organelles are present in cytosol such as ribosome (70S), genetic material where as electron transport chain complexes are embedded within the plasma membrane.

4. Chromosome and extra chromosomal DNA-Prokaryote cell contains genetic material in the form of circular DNA, known as "**bacterial chromosome**". It contains genetic elements for replication, transcription and translation. Bacterial chromosome follows a rolling circle mode of DNA replication. The genes present on chromosome does not contains non coding region (introns) and it is co-translated to protein. Besides main circle DNA, bacteria also contains extra circular DNA known as "plasmid". Presence of plasmid containing resistance gene confers resistance towards known antibiotics. Exchange of extra-chromosomal DNA between different bacterial strains is one of the mechanisms responsible for spread of antibiotic resistance across the bacterial population.

Bacterial Plasmid: Plasmid are widely been used for cloning of foreign DNA into the bacteria as host strain. Before getting into the details of discussing bacterial plasmid we will discuss the basic properties of plasmids.

Different forms of plasmids: Bacterial plasmid is a double stranded circular DNA exists in 3 different forms (Figure 21.1). If the both strands of circular double strands are intact then it is called as **covalently closed circles** (CCC) where as if one of the strand has nick, then it acquire the conformation of **open circle** DNA (OC, DNA). During the isolation of plasmid DNA from bacteria, covalently closed circular DNA losses few number of turns and as a result it acquire **supercoiled** configuration. The interchange between these different forms are possible under the in-vitro or in-vivo conditions, such as DNA gyrase produces additional turn into the circular DNA to adopt supercoiled conformation.

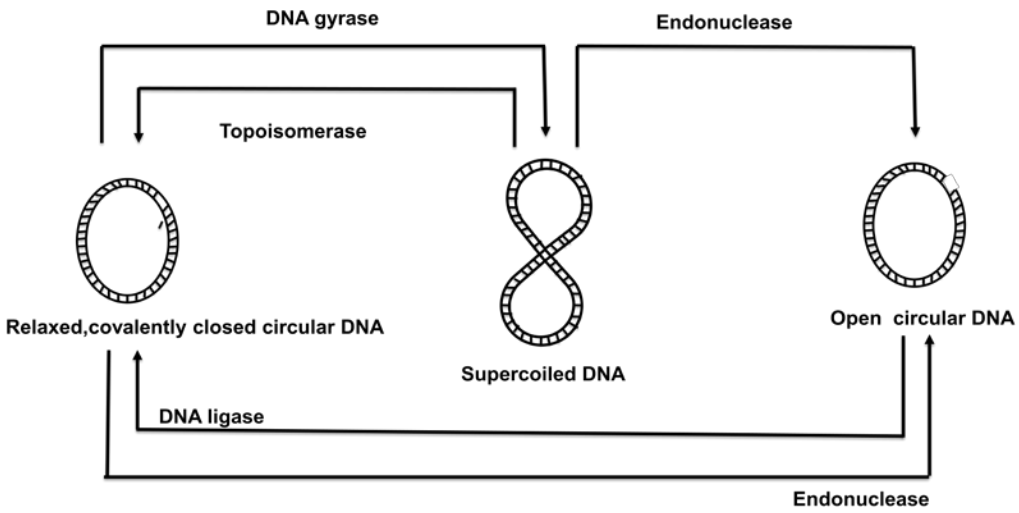


Figure 21.1: Different forms of plasmids

Features of different plasmids: There are minimum molecular components to assemble bacterial plasmid to perform the function of vector are as follows-

1. Origin of replication-Like any other replicating DNA, plasmid DNA needs its own independent origin of replication to provide replication start site to make more copies. It decides the range of bacterial host strain can be use with the particular plasmid vector. The plasmids containing ori region from Col E1 can be able to grow in limited bacterial species such as E.Coli etc. In contrast, plasmid containing ori from RP4 or RSF1010 can be able to grow in gram (-) bacteria and gram (+) bacteria.

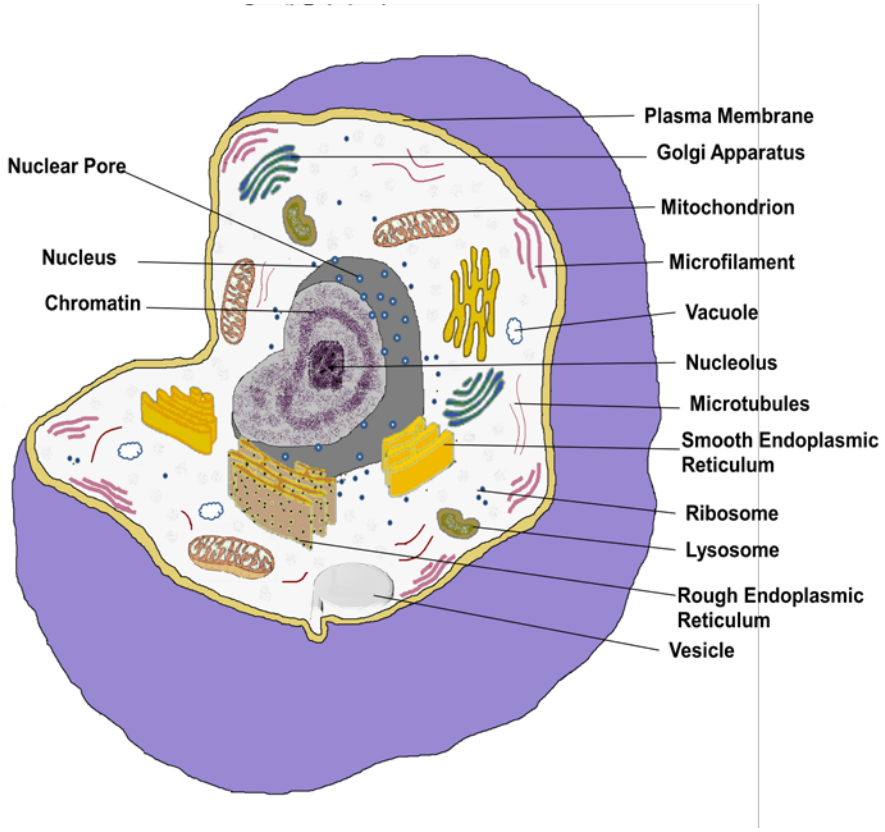
2. Selection marker- Selection marker in the form of either antibiotic resistance gene or enzymatic gene is essential to give phenotypic changes in host after entry of the plasmid.

3. Promoter- Plasmid replication in host is performed by the host provided proteins such as DNA gyrase, helicase, polymerase and DNA ligase. But proteins required for conferring antibiotic resistance or enzyme use for selecting transformed host cells is present on plasmid and a promoter adjacent is required to express genes present on plasmid DNA. In addition, promoter is also needed to express gene present on foreign DNA.

Lecture 22: Eukaryotic Cells (Part-I)

Structure of Eukaryotic cell- The eukaryotic cells are much more complex and it contains many membrane bound organelles to perform specific functions. It contains a nucleus isolated from cytosol and enclosed in a well defined plasma membrane. A typical eukaryotic animal and plant cell is shown in Figure 22.1 and the difference between these types of cell is given in Table 22.1.

A



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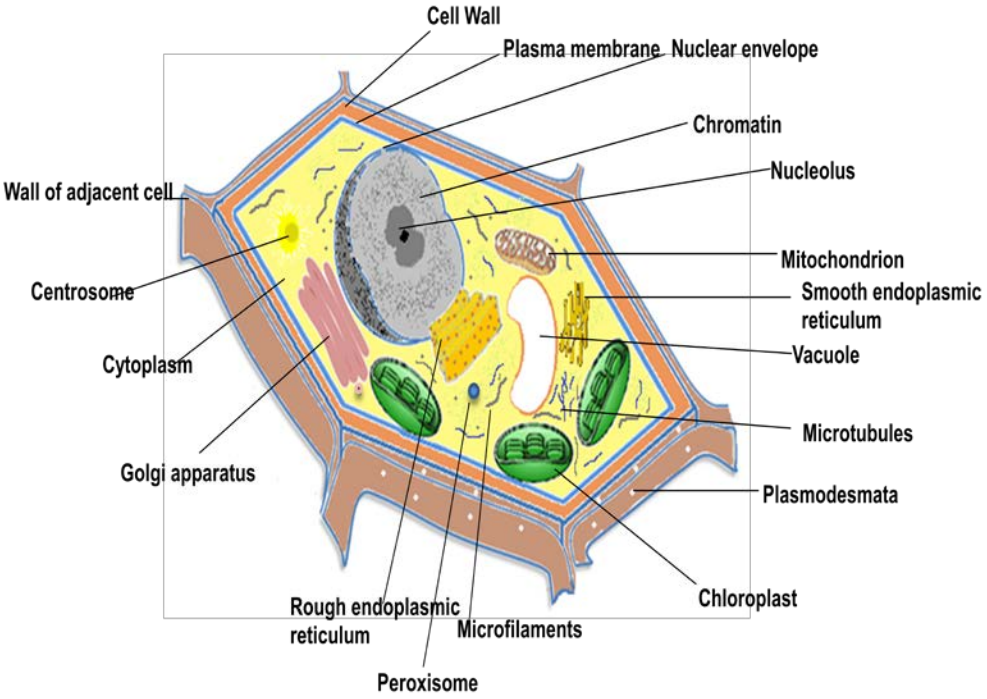


Figure 22.1 : Structure of Eukaryotic cell. (A) Animal Cell (B) Plant Cell

TABLE 22.1 DIFFERENCE BETWEEN ANIMAL AND PLANT CELLS

FEATURE	PLANT CELL	ANIMAL CELL
Cell wall	Present	Mostly absent
Size	Large	Comparatively small
Chlorophyll	Present	Absent
Vacuole	Large Central	Small and many in number
Mitochondria	Few	More
Lysosome	Almost absent	Present
Glyoxysomes	Present	Absent
Cytokinesis	By Plate method	By constriction

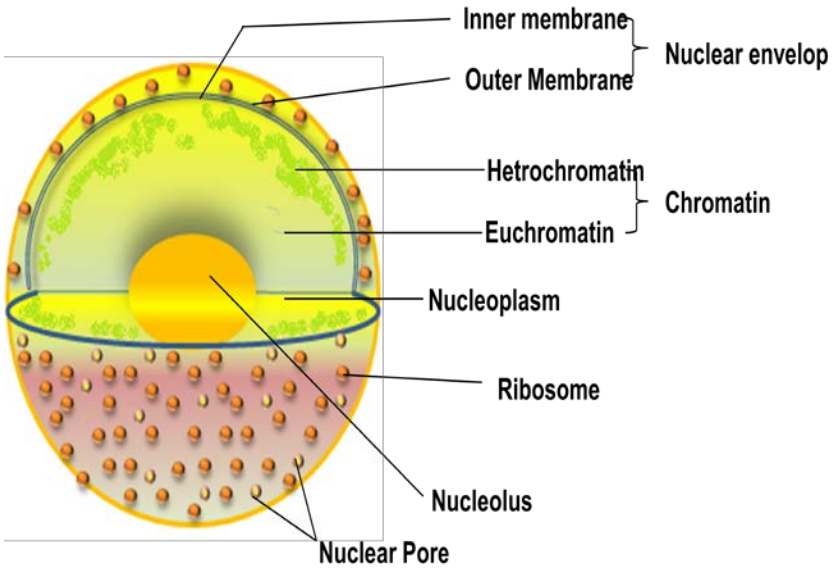
The description of different structural feature of eukaryotic cell is as follows-

Different organelles of Eukaryotic cells (Animal)

1. Cytosol-Cytosol is the liquid part filled inside the cell and it contains water, salt, macromolecules (protein, lipid, RNA). It has an array of microtubule fiber running through out the cytosol to give vesicular structure to its destination. Besides this, cytosol exhibits “Sol” to “Gel” transition and such transition regulates multiple biochemical and cellular processes.

2. Nucleus-Nucleus is the central processing unit of cell and homologus to the processor in a typical computer (Figure 22.2, A). The liquid filled inside nucleus is called as **nucleoplasm**. It is a viscous liquid containing nucleotides and enzymes to perform replication, transcription, DNA damage repair etc. It contains genetic material (DNA) in a complex fashion involving several proteins (histones) to pack into nuclear bodies or chromosomes. The chromatin in eukaryotic nucleus is divided into **euchromatin** or **heterochromatin**. Euchromatin is a part of chromatin where DNA is loosely packed and it is transcriptionally active to form mRNA where as Heterochromatin is more densily packed and it is transcriptionally inactive. Nucleus in eukarytotic cells are present in a double layer of membrane known as **nuclear envelope** (Figure 22.2, B). Outer membrane of nuclear envelope is continuous with the rough endoplasmic reticulum and has ribosome attached to it. The space between two membrane is called as **perinuclear space**. Nuclear envelope is often has **nuclear pore** and as per calculation an average nucleus has 3000-4000 pores per nuclear envelope.

A



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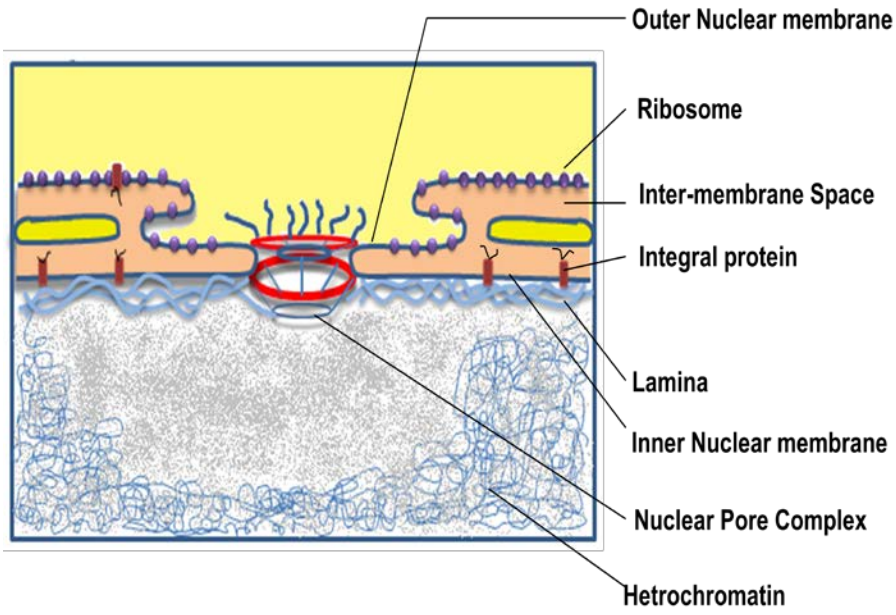


Figure 22.2: Structural details of nucleus. (A) whole and (B) enlarged view of nuclear pore.

Nuclear pore is 100nm is diameter and consists of several proteins. It is a gateway for transfer of material between nucleus and cytosol. RNA formed after transcription from DNA within the nucleus and move out of the nucleus into the cytosol through nuclear pore. Similarly protein from cytosol crosses nuclear pore to initiate replication, transcription and other processes.

1. Mitochondria- It is popularly known as "**power house of the cell**" as the organelle is actively involved in the generation of ATP to run the cellular activities. Mitochondria is a double layered membrane bound organelle with different structural properties (Figure 22.3, A). Outer membrane is smooth and cover the complete organelle with large number of integral protein, known as **porin**.

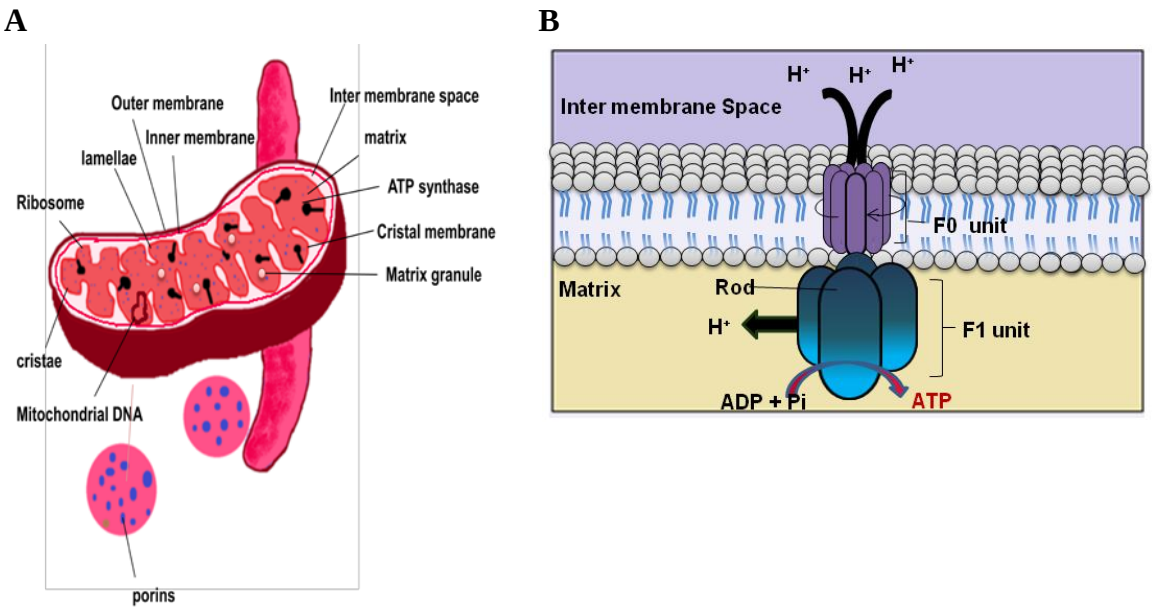


Figure 22.3: Mitochondria. (A) Struture of mitochondria and (B) enlarged view of ATP Synthase.

Porin allows free movement of molecules less than 5000da within and outside mitochondria. Where as larger molecules or proteins moves into the mitochondria through trasnporters involving signal peptides known as "**mitochondrial targeting sequence**". Inner membrane is folded into membrane projections to form **cristae**. Cristae occupies major area of membrane surface and house machinary for anaerobic oxidation and electron transport chain to produce ATP. Due to presence of inner and outer membrane, mitochondria can be divided into 2 compartments: first in between the inner and outer membrane, known as **intermembrane space** and second inside the inner

membrane known as **matrix**. The proteins present in intermembrane space have a role in executing "**programmed cell death**" or "**apoptosis**". Matrix is the liquid part present in the inner most of mitochondria and it contains ribosome, DNA, RNA, enzymes to run kreb cycle and other proteins. Mitochondrial DNA is circular and it has full machinery to synthesize its own RNA (mRNA, rRNA and t-RNA) and proteins. A number of difference exist between mitochondrial DNA and DNA present in nucleus and these difference are not discussed here due to space constrain. Electron transport chain components (complex I to complex V) are integral proteins, present in the inner membrane of mitochondria. During metabolic reactions such as glycolysis, kreb cycle [metabolic reaction are discussed later] produces large amount of reducing equivalent in the form of $NADH_2$ and $FADH_2$. Electron transport chain process reducing equivalent and flow of the electron through different complexes (Complex I to Complex IV) causes generation of proton gradient across the membrane. Proton expelled in the intermembrane space returned back to the matrix through complex V (ATP synthase) to generates ATP. ATP synthase (Figure 22.3, B) is a mushroom shaped multimeric protein complex, mainly composed of two proteins Fo and F1. Fo is a membrane bound portion where as F1 is the complex present into the lumen towards matrix. F_oF_1 complex of mitochondria harvest the proton motive force to catalyze phosphorylation reaction involving ADP and phosphate to generate ATP.

Functions of mitochondria-

1. Production of ATP

2. Generation of **Reactive Oxygen Species** (ROS) in immune cells to kill infectious agents.

3. Used to track tree of a family.

3. Role in programmed cell death or **"apoptosis"**

Apoptosis: Apoptosis is the programmed cell death involving a series of events involving cellular metalloprotease known as caspases. In an adverse event of exposure of cell to the cyto-toxic agent or environmental condition, it activates cell surface signaling to activate cytosolic caspases. In addition, it disturbs mitochondrial membrane potential to cause the release of CytC. Ultimately, these cellular events activates DNase activity within nucleus and degrade genominc DNA to cause cell death.

Lecture 23: Eukaryotic Cells (Part-II)

2. Chloroplast-Chloroplasts are found in plant, algae and other lower invertebrates such as euglena. Contrasting to mitochondria, chloroplast has outer membrane, an inner membrane and then light pigment containing inner most thylakoid membrane (Figure 23.1, A). Outer membrane is porous to the small molecules but protein or large molecule are transported by **TOC** (translocon on the outer chloroplast membrane) complex. Movement of material passed through outer membrane enters into the inner membrane through **TIC** (translocon on the inner chloroplast membrane) complex. In between outer and inner membrane is intermembrane space filled with aqueous liquid.

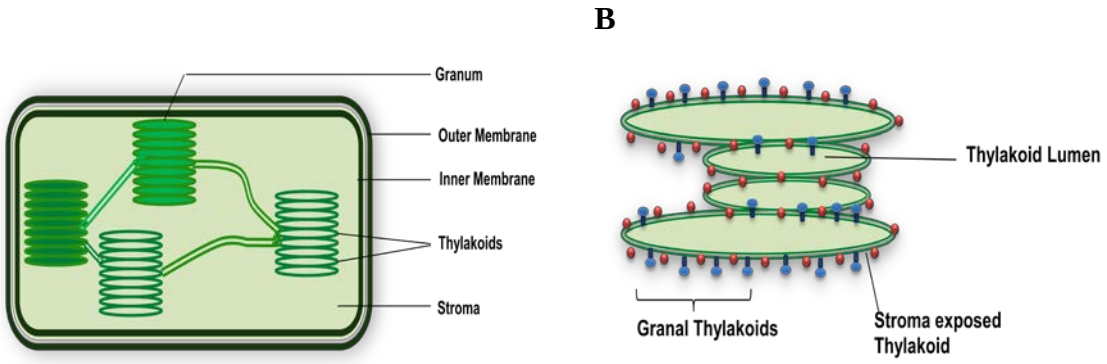


Figure 23.1: (A) Structure of Chloroplast, (B) Arrangement of thylakoid membrane in chloroplast.

The inner membrane of the chloroplast further folds into flattend membrane system known as **thylakoids**. The photosynthsis machinery such as light absorbing pigments, electron carriers and ATP synthesizing machinery is present on inner membrane as intergral protein complex. Thylakoid membranes are arranged like stack of coin to form **granum** (Figure 23.1, B). The granum through out the chloroplast are connected by tubule to share the material. Over-all structure of chloroplast is similar to mitochondria but it has few significant structural and biochemical differences. Thylakoid membrane contains photosynthetic green colored pigment chlorophyll.

$$6CO_2+6H_2O+Solar\ Energy \rightarrow C_6H_{12}O_6+6O_2 \quad \text{.....(23.1)}$$

Photosynthesis is a assimilation reaction involving CO_2 and water to produce sugar in the presence of solar energy (photons) to catalyze fusion reaction as given Eq. 23.1 and Figure 23.2. The photo system present on thylakoid membrane consists of two photo system, **photo system-I (PS-I)** and **photo system complex II (PS-II)**. PS-II absorbs the photon from solar energy to excite the electron to the higher energy state, and catalyze water break down into the proton and oxygen. The electron pass through multiple electron carrier and during this proton are exported out of the thylakoid membrane into the lumen. The proton passed through **ATP synthase** and returned back into the stroma to generate ATP. The electron from PS-II is eventually been received by PS-I and been excited after absorbing photon from sun light to high energy state. The energy associated with these electron are used to generate NADPH in the stroma. Hence as a result of photosynthesis, solar energy is been trapped by photo synthesis apparatus to generate **ATP** and **NADPH** into the lumen. Both of them is now been used to run **calvin cycle** to assimilate environmental CO_2 to form sugar.

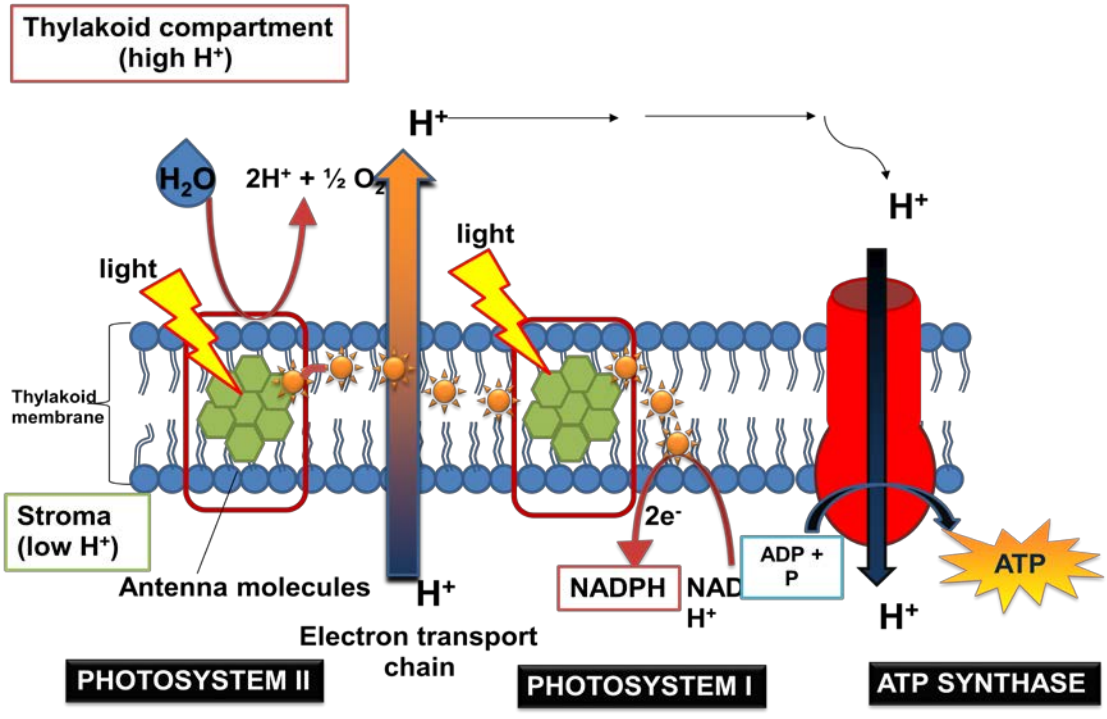


Figure 23.2: Different Steps of Photosynthesis.

5. Organelles of Vesicular Trafficking System: The main function of these organelles is to manage the distribution of material (food particles or proteins) through out the cells. 3 different organelles such as endoplasmic reticulum, golgi apparatus and lysosome co-ordinately work together to maintains vesicular transport of material across the cell (Figure 23.3). Eukaryotic cells takes up the solid material from outside the cells through a process called "**endocytosis**" where as uptake of liquid is through a process called as "**pinocytosis**". Similarly material is secreted out of the cells through "**exocytosis**". In addition, intravesicular system delivers protein synthesized in endoplasmic reticulum to different organelles.

During endocytosis, material present outside the cells binds to the cells surface through cell surface receptor and trapped it in a membraneous structure called as **endosome**. Endosomal vesicles are fused with the lysosomes to form late endosome. In late endosome, with the help of lysosomal enzymes material is digested and then endosome is fused with the golgi bodies and deliver the content for further distribution. In the similar manner, during secretion, vesicles originate from golgi bodies and fused with the plasma membrane to release the content outside of the cell.

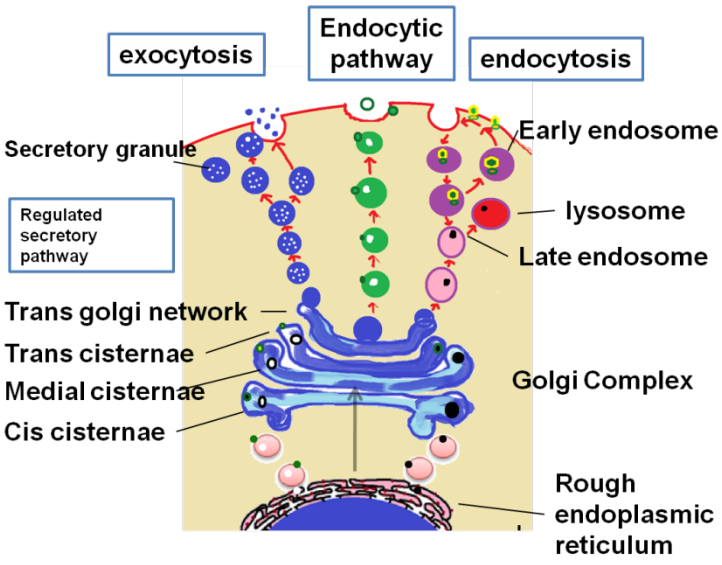


Figure 23.3: Intra cellular vesicular trafficking system of cell.

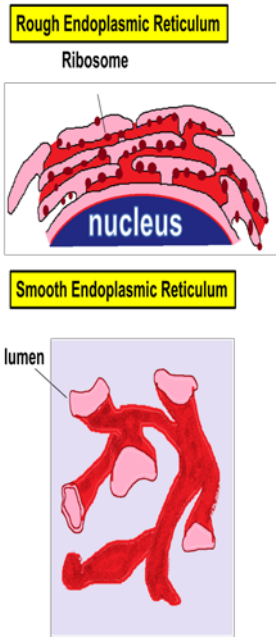


Figure 23.4: Endoplasmic reticulum.

Endoplasmic Reticulum- The vesicular network starts from nuclear membrane and spread throughout the cytosol constitutes endoplasmic reticulum (Figure 23.4). There are two different types of endoplasmic reticulum present in cell, Rough endoplasmic reticulum (RER) and smooth endoplasmic reticulum (SER). RER has ribosome attached to it to gives a rough appearance whereas smooth endoplasmic reticulum is devoid of ribosomes. Protein synthesis on ribosome attached to RER is sorted into 3 different catagories, such as integral membrane proteins, proteins for secretion and protein destinated for different organelles. Proteins are synthesized with a n-signal peptide and these signal peptides are recognized by signal recognition particle on their the target organelles. For example, if a protein is synthesized with a signal peptide for mitochondria, it will attach to signal recongnition particle and receptor onto the outer mitochondrial membrane to deliver the protein. The proteins without any signal peptide tags remained in cytsol.

Functions of endoplasmic reticulum:

1. Synthesis of steroid hormone in gonad cells.
2. Detoxification
3. Ca^{2+} sequesteration
4. Synthesis of protein, phospholipid and carbohydrate.
5. Protein sorting to different organelles.
6. Protein modifications such as glycosylation etc.

Golgi Bodies- Golgi bodies are first visualized by a metallic stain invented by **Camillo golgi** and it is made of flattend, disk like cisternae arranged in a stacked manner to give 3 distinct zones (Figure 23.5). **Cis-face** recieves material or vesicles from endoplasmic reticulum, **medial golgi** is the actual place where protein are covalently modified with the sugar. **Trans golgi** is the face of golgi towards plasma membrane and this site sorts vesicle for their destinated organelles or plasma membrane.

Functions of golgi bodies

1. Protein sorting

2. Protein modifications (Glycosylation)

3. Proteolysis

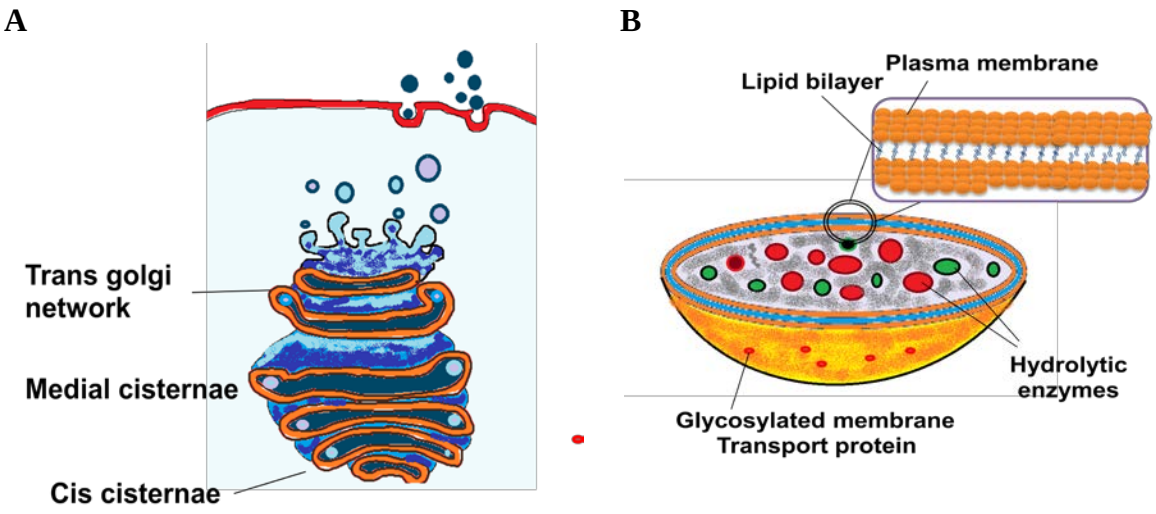


Figure 23.5: Schematic structure of (A) Golgi bodies and (B) Lysosome.

Lysosomes-Lysosome are discovered by De Duve. They are membrane bound organelles and an important component of intracellular vesicular system (Figure 23.5). They are popularly known as suicidal bags due to their role in autophagy, a cellular process probably operates in cells during starvation to meet their energy requirements. [for more details of molecular mechanism of autophagy and underlying signaling mechanism could be find here: **Annu Rev Genet. 2009;43:67-93. Regulation mechanisms and signaling pathways of autophagy**]. Lysosome lumen is extremely acidic and contains protease, cytolytic enzymes to degrade the ingested material.

Functions of lysosomes

1. Degradation of ingested food material for delivery through vesicular system.

2. Degradation of pathogenic bacteria

3. Degradation of old protein.

Lecture 24: Eukaryotic Cells (Part-III)

Introduction: The eukaryotic cell undergoes précised cell cycle and division to produce two daughter cells. Cell-cycle is the series of tightly regulated events leading to the division and duplication. It is a vital process used to single celled fertilized egg is developed into full organism. Cell division is the crucial event underlying the regeneration and repair in tissue, liver and heart. In prokaryotic cells, parent cell is divided simply by division into two halves through the process of binary fission. In eukaryotic cells, cell cycle has three phases; Interphase (synthesis of genomic DNA and cytoplasm), mitotic phase (division of DNA into two halves) and cytokinesis (division of cell). The over-view of these phases are given in Figure 24.1.

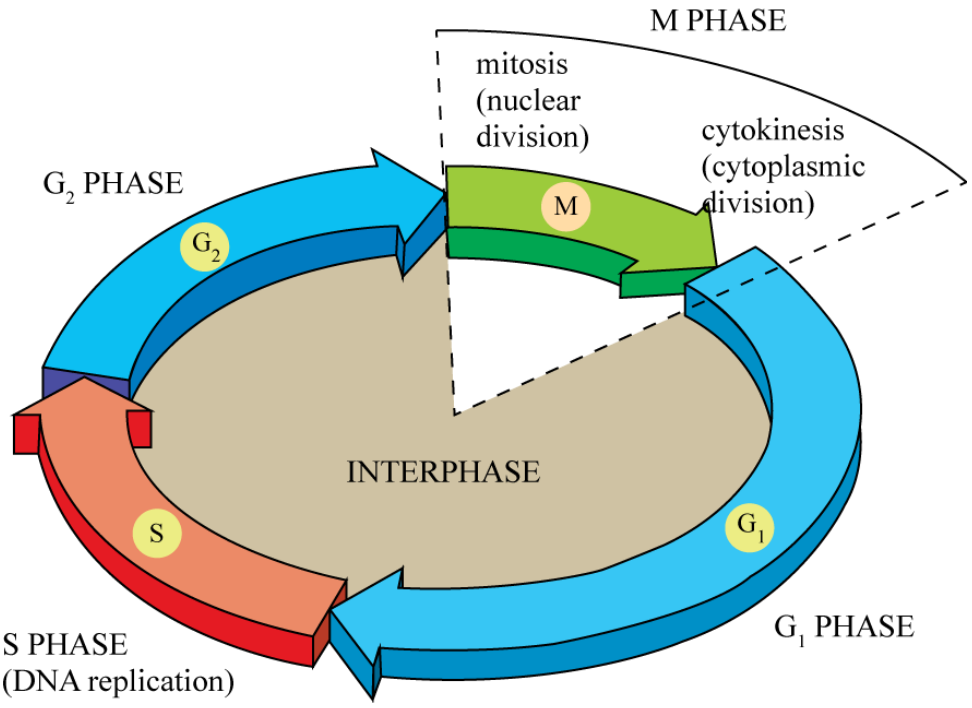


Figure 24.1: An Over-view of stages in eukaryotic cell cycle.

The details of these stages are as follows:

Interphase: Interphase is a preparatory phase required to perform requisite steps. These are series of events in the nucleus as well as in cytosol of daughter cells to enable it to enter into the division phase. This phase has several phases, these are as follows:

G1 Phase: it is also known as growth phase. It start from the end of the mitosis and until the beginning of S Phase. During this phase, cellular proteins, enzymes are synthesized. Most of these enzymes are required for DNA synthesis in S Phase. Duration of G1 Phase depends on cell type within the organism. G1 Phase is under the control of p53 gene products.

S Phase: Once cell grow and all factors, nucleotide is available, it starts DNA synthesis during S phase. At the end of this all chromosomes present in nuclei is replicated and DNA content doubled. No Change in ploidy. The synthesis of DNA occurs very fast to avoid exposure of newly synthesized DNA to mutagens.

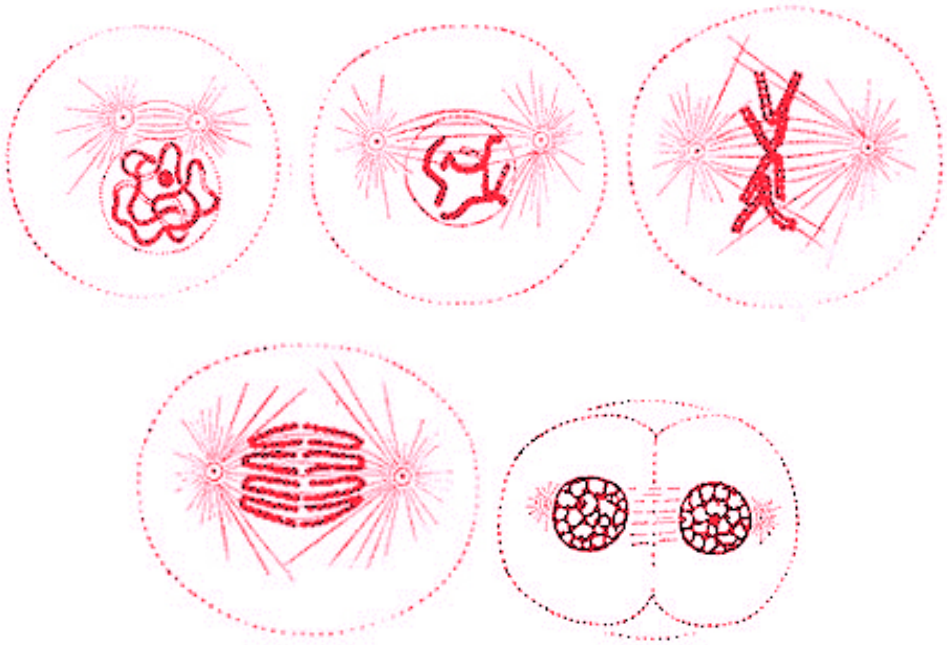
G2 Phase: The growth phase between DNA synthesis and mitosis. During this phase, cell grow and synthesize the protein and cellular factors required for mitosis and cytokinesis.

G0 Phase: After G1 phase, quiescent, senescent and non-proliferative multicellular eukaryotic cell enter into the G0 phase. Cells remain in this phase for long period or indefinite period, as in the case of neuron. It is also common in fully differentiated cells. The fast growing cell never enters into G0phase and hence it is not an regular cell cycle phase and under specific condition cell enter into G0 phase.

Mitosis or M-Phase: After G2 phase, cells enter into the mitosis or M-phase to divide the DNA equally between two daughter cells. Each mitosis has 4 distinct phases to precisely divide DNA content of the cell (Figure 24.2).

These are as follows:

Prophase: During this phase, nuclear membrane is dissolved and chromatin condenses into chromosomes. The nucleolus in the nucleus disappear. In the beginning each cell has one centrosome, which replicate along with DNA to give rise a pair of centrosome to co-ordinate down-stream events. Each centrisome has microtubule to form spindle and assist in distribution of nuclear content during mitosis. Centrioles are considered to organize the microtubule assembly but they are not essential.



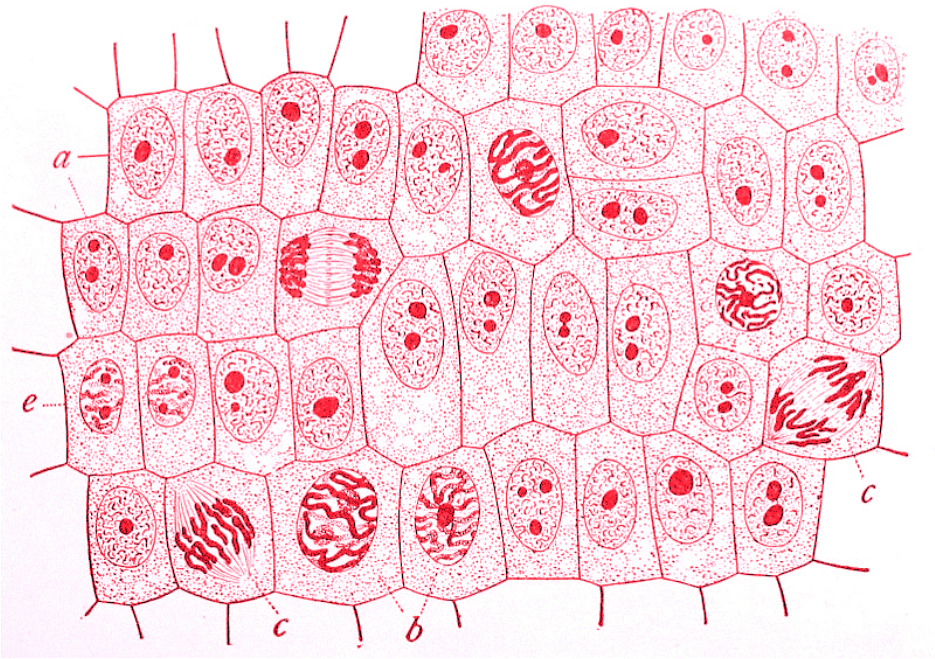


Figure 24.2: Different stages during mitosis in eukaryotic cell .

Metaphase: In this phase, the two centrosomes start pulling the chromosomes using the attached centromeres towards the ends of the cell. As a result, chromosome are aligned along the metaphase plate or equatorial plane. Since the pulling power of both centrosome is almost equal, it eventually arrange the chromosomes on metaphase plate. The alignment of chromosome along with metaphase plate is a crucial event to decide the entry of cells into the anaphase. The signal required for this control is created by mitotic spindle checkpoint.

Anaphase: The proteins attached to each chromatid are cleaved and sister chromatids are separated as daughter chromosomes. The chromosomes lined on metaphase plate are pulled by the microtubule and move towards their respective centrisome. Although the exact mechanism of generating force required for centrisome movement is unknown but it is suggested that rapid assembly and breakdown of microtubule may provide the force for this movement. At the end of this phase, chromosome are been prepared for distribution between sister cells.

Telophase: In this phase, daughter chromosomes moved and attached to the opposite end of the cell. A nuclear membrane forms around each set of separated daughter

chromosomes and nucleolus reappears. In this event, several processes during prophase are reversed to give two daughter nuclei.

Cytokinesis: At the end of telephase, mitosis is over but cell division requires distribution of cellular content equally between daughter cells. In animal cell, a cleavage furrow is formed along the metaphase plate and divides individual nuclei as separate cells. During this process, it is ensured that besides nuclei, all other cellular organelles should be distributed equally between daughter cells. In plant, cell plate is formed and divides the cellular content between daughter cells.

Role of Cell-cycle: Cell cycle plays important role in following events:

1. Development and growth: The development of single cell into the multi-cellular system is possible due to cell cycle and division.

2. Cell replacement: Eukaryotic cells have pre-defined life span and after that period it needs to be replaced with new one. It is possible due to cell division and making more cellular copies. For example, human RBC has life span of 3 months, new RBCs are formed from bone marrow by cell division.

3. Regeneration: Cellular damage and injury is the integral part of living system. Cell division is the primary event required for the synthesis of lost or damaged organ.

4. Asexual reproduction: Asexual reproduction is common in lower invertebrate (as discussed in earlier lecture). In these organisms cell divide to form new cells and these newly formed ceklls give rise to new organism. For example hydra.

Control of cell-cycle: Cell cycle at different step is tightly controlled by cell-cycle check points. These cell cycle check points are used to ensure the completion of different steps and repair of DNA damage. The main check points are present at G1/S, G2/M and M. Each check point is controlled by the mutual interaction between cyclin and cyclin dependent protein kinase. p53 gene products are known to control many events through G1/S and G2/M checkpoints.

The details of these events can be explored by following article, Stephen J. Elledge (6 December 1996). "Cell Cycle Checkpoints: Preventing an Identity Crisis". *Science* 274 (5293): 1664–1672. PMID 8939848

So what if any of these events goes wrong?

Disregulation of cell cycle and control mechanism give rise to tumor. After certain number of cell division, every cell enters into the G0 phase and ceases the cell division. In the case of tumor, cells lost the control mechanism and multiply infinitely to give rise to cell mass. These cells are taking nutrition but not performing functions. Retinoblastoma (RB), p53 are the crucial cellular factor responsible for cell cycle control and play crucial role in tumor development.

Lecture 25: Central Dogma of Life

Introduction: Life of an organism required co-ordinated function of different organs or tissues. Even in the unicellular system, cellular processes are controlled by a series of molecular events, mostly controlled by signal transduction. Signal transduction is a series of phosphorylation events performed by protein kinases. In addition, co-ordination between different organs through messenger molecules, which are also be proteinous in nature. In addition, the phenotypic changes (skin color, feather and pattern) in an organism are required for adaptation and biological demands such as selecting suitable life partner. Most of these examples are associated with the synthesis of suitable protein factor in a time and context dependent manner. The protein is made up of amino acids and every protein has unique amino acid arranged in a specific sequence. The information to synthesize proteins with unique amino acid sequence is provided by the nucleic acid present within the nucleus. In a preset sequence, DNA present in the nucleus give rise to the specific RNA sequence and that in turn guide the cellular machinery to synthesize protein (Figure 25.1). Scientist considered this as the fundamental event to run the life and considered as "central dogma of life". In another word Francis in his communication to jounrla Nature states "*The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states that such information cannot be transferred back from protein to either protein or nucleic acid*".

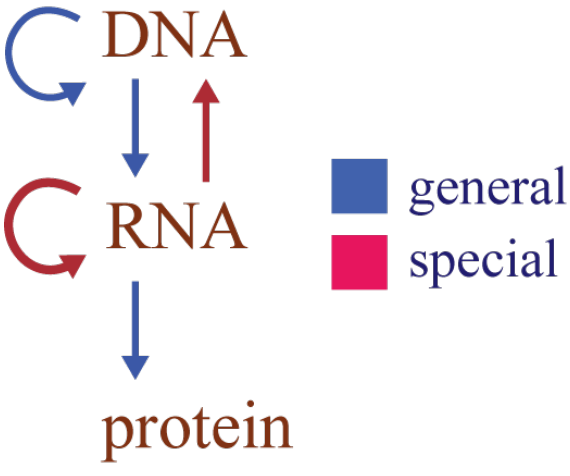


Figure 25.1: Sequence of Information Flow in Biological System.

The central dogma of life is the basis of life on earth and required to control the biological processes. Following this hierarchical flow of information from DNA to protein allows nucleus to control all biological activitires in a cell. Under normal conditions, the flow of information sequence-to-sequence requires 3 process (Figure 25.2). The three process required for flow of information is as follows:

(1) Sequence dependent synthesis of DNA from pre-exisitng DNA **[Replication]**.

(2) Sequence dependent synthesis of RNA from DNA **[Transcription]**.

(3) Sequence dependent synthesis of DNA from pre-exisitng DNA **[Translation]**.

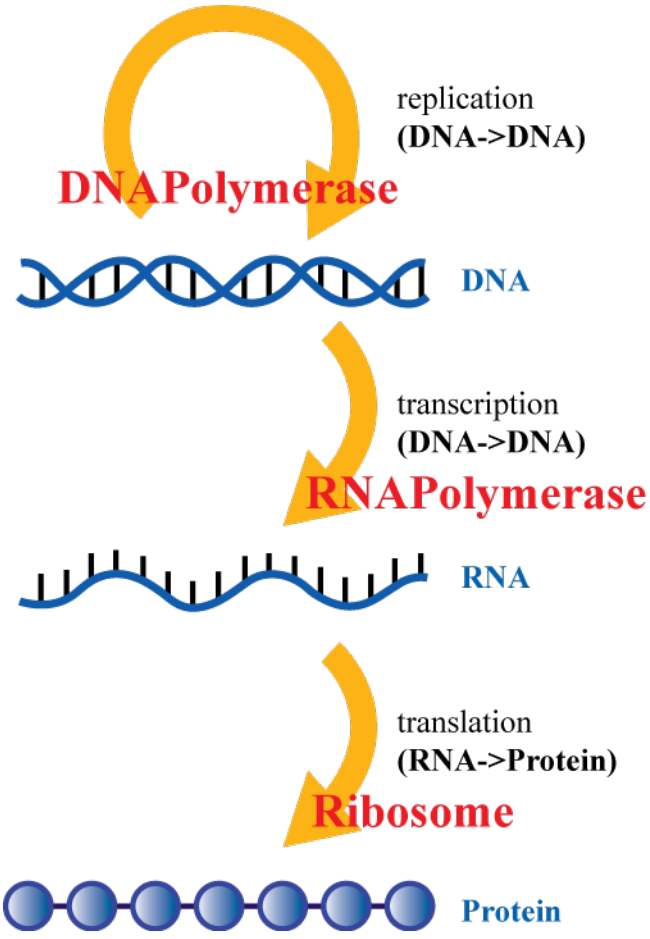


Figure 25.2: Flow of Information sequence-to sequence in Biological System.

The underlying process to flow the information in proposed central dogma of life is as follows:

1. Replication: Genomic content in an organism needs to be duplicated during S phase of cell-cycle. Duplication of DNA is done by replication utilizing the sequence information of parent DNA. The enzyme used for this purpose is DNA dependent DNA polymerase. **[The minor details of replication are discussed in a later lecture].**

2. Transcription: The DNA is present in nucleus whereas the protein synthesis machinery is present in cytosol. Hence the information present in DNA is used to synthesize RNA which has ability to transport outside the nucleus to participate into protin synthesis. Synthesis of RNA from DNA is done by transcription utilizing the sequence information of DNA. The enzyme used for this purpose is DNA dependent RNA polymerase. **[The minor details of transcription are discussed in a later lecture].**

3. Translation: The RNA present in cytosol is utilized by translation machinery to synthesize protein in a sequence dependent manner through a process known as translation. [The minor details of translation are discussed in a later lecture].

Under specific conditions, biological system does not follow the usual pathways to replay the information. Few of the special circumstances and underlying events are given in the Table 25.1. The processes occurring under the special circumstances are given in Figure 25.3. These are as follows:

Table 25.1: Flow of information under normal and special circumstances.

Normal Circumstances	DNA → DNA	DNA → RNA	RNA → protein
Special Circumstances	RNA → DNA	RNA → RNA	DNA → protein
Additional possibilities	protein → DNA	protein → RNA	protein → protein

1. Reverse Transcription: In most of the organism, genomic content is present in the form of DNA but in few organisms such as virus, RNA is present as genomic content and in these cases, RNA needs to be converted into DNA and replicate using the host replication machinery. It is done by reverse transcription utilizing the sequence information of parent RNA. The enzyme used for this purpose is RNA dependent DNA polymerase.

2. RNA replication: In most of the organism, genomic content is present in the form of DNA but in few organisms such as virus, RNA is present as genomic content and duplication of RNA is done by replication utilizing the sequence information of parent RNA. The enzyme used for this purpose is RNA dependent RNA polymerase.

3. DNA directly to Protein: Under in-vitro cell free system, it is possible to translate DNA directly into the protein in the presence of ribosome. It is not precisely controlled and it is not known whether it synthesizes the protein in a sequence dependent manner.

4. Protein to protein: These are considered as infectious protein which replicates to form indeitical copieson themselves. These proteins are known as prions. Although it represent the transfer of information but it is not the usual pathway and considered to be the exception of central dogma.

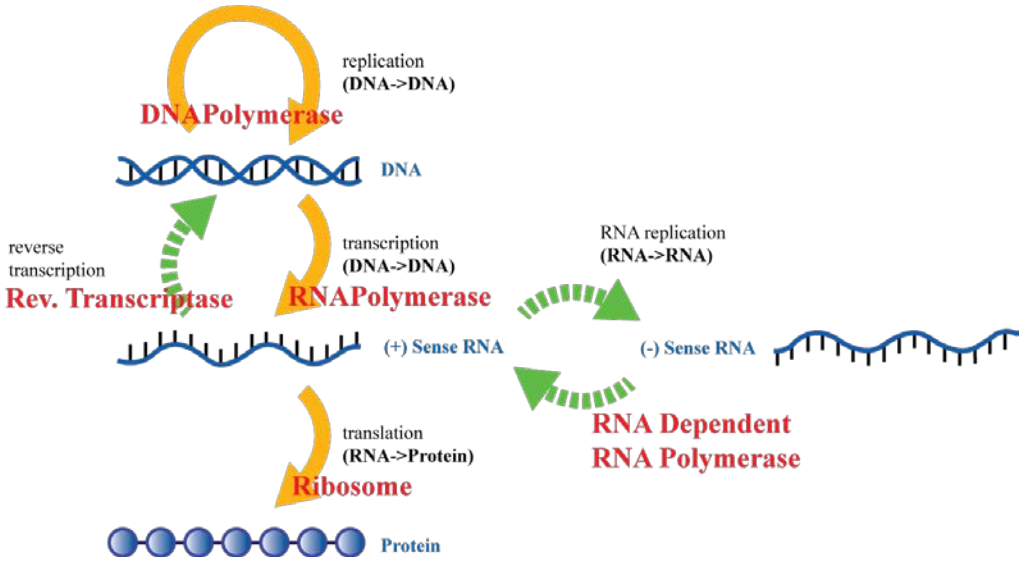


Figure 25.2: Flow of Information under special circumstances in Biological System. Special processes are colored in green.

Lecture 26: Structure of Nucleic Acid

Introduction: As discussed in previous lecture, genetic material of the organism controls the biological processes. Most of the organism (prokaryotic/eukaryotic) has DNA as the genetic whereas a minor fraction (virus etc) has RNA as genetic material. DNA or RNA is the biopolymer and is acidic in nature. In eukaryotic cells (animal or plant), nucleic acid is present within the nucleus whereas in prokaryotic cells, it is present as free form in the cytosol. The first nucleic acid was isolated by friedrich miescher in 1868. In the present lecture, we will discuss the structure of nucleic acid, its composition and other salient features. Understanding these properties will led us to understand the process of replication, transcription and translation (Discussed in later lectures).

Composition of nucleic acid: The nucleic acid (DNA or RNA) is composed of 3 components; (1) phosphoric acid, (2) base and (3) sugar. The phosphoric acid provides the backbone to the polymer where as sugar work as anchoring point for nitrogenous bases. The 9 membered nitrogenous bases give the diversity in the sequence of nucleic acid (Figure 26.1). The detail description of the individual component is as follows:

Phosphate backbone: Phosphoric acid serves as backbone of the molecule.

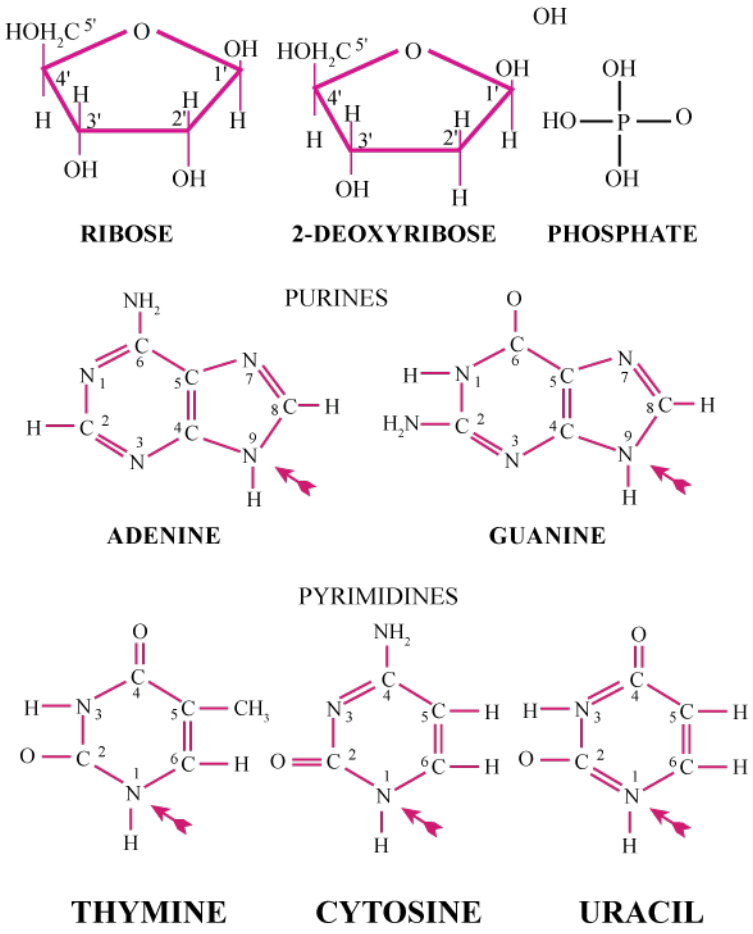


Figure 26.1: Components of Nucleic acid.

Sugar: The 5 membered cyclic reducing sugar is present in nucleic acid. These are two different varients, the sugar molecule which contains hydroxyl group at 3´ and known as ribose otherwise it is known as deoxyribose sugar. Based on sugar, nucleic acid is classified as RNA or DNA; Ribose sugar is present in RNA whereas deoxyribose is present in DNA. The purpose of sugar in the nucleic acid is to provide the attachment site for nitrogenous bases.

Nitrogenous Bases: There are two varients; These are 9 membered, conjugated double bond system, Purine such as Adenine (A) and Guanine (G). The 6 membered single ring system, pyrimidine such as Thymine (T), Uracil (U) and Cytosine (C). The presence of nitrogenous bases in DNA/RNA is pre-determined. DNA has Adenine, Guanine, Thymine and Cytosine (and strictly no Uracil) whereas RNA has Adenine, Guanine,

Uracil and Cytosine (and strictly no Thymine). The exact reasoning behind this distribution we will discuss in the later part of the lecture.

Over all Structure of Nucleic acid: The DNA is doubled stranded whereas RNA is single stranded (in most of the cases). The individual monomer responsible for making DNA or RNA is nucleotide and as a result, DNA or RNA can be considered as polynucleotide. Individual nucleotide is nucleoside attached to one or more phosphate group and can be termed as (1 phosphate group) monophosphate nucleoside, (2 phosphate group) di-phosphate nucleoside and (3 phosphate group) Tri-phosphate nucleoside. Each nucleoside is composed of nitroengous base attached to the sugar through glycosidic bonds (Figure 26.2).

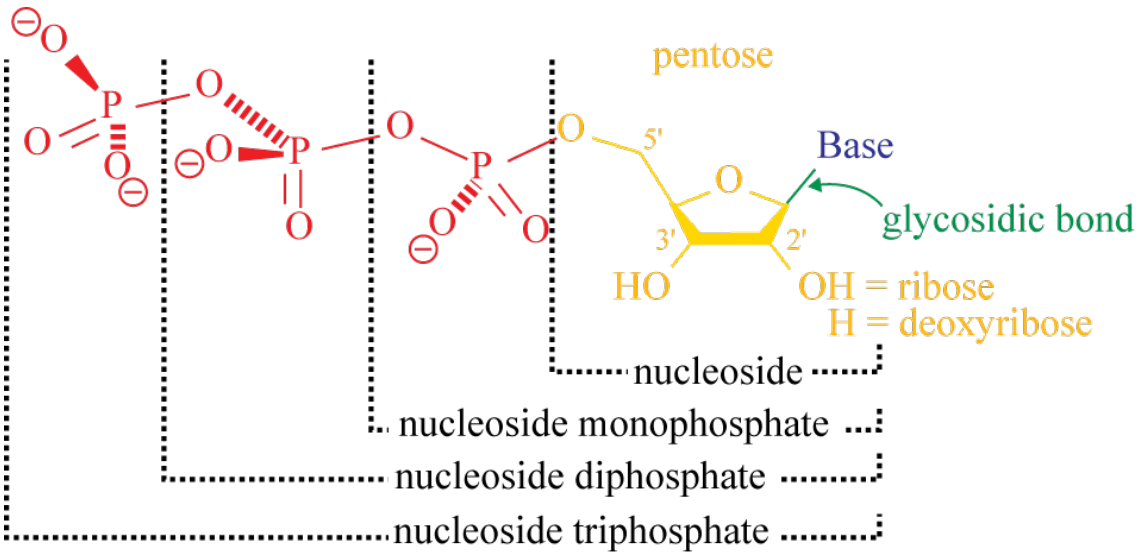


Figure 26.2: Nucleotide and its variants.

Nucleotide has free –hydroxyl group (-OH) at 3´ carbon and a phosphate group at 5´ of sugar moiety. The first nucleotide has a free phosphate and the 3´ hydroxyl group makes bond with the phosphate group at 5´ of next nucleotide (Figure 26.3). The propagation of nucleotide along the length of chain give rise polynucleotide. As a result of each polynucleotide chain has a free 5´ free phosphate group (on first nucleotide) and free 3´-hydroxyl group (on last nucleotide). It gives polarity to the polynucleotide chain and it

runs in the direction from 5´-3´. DNA is a double stranded whereas RNA is single stranded. Both strand of DNA are held together by hydrogen bonding between bases attached to the sugar. Adenine of one chain is always making 2 hydrogen bondings with the thymine of next chain. Similarly, guanine of one chain is making 3 hydrogen bondings with the cytosine of next chain (Figure 26.4).

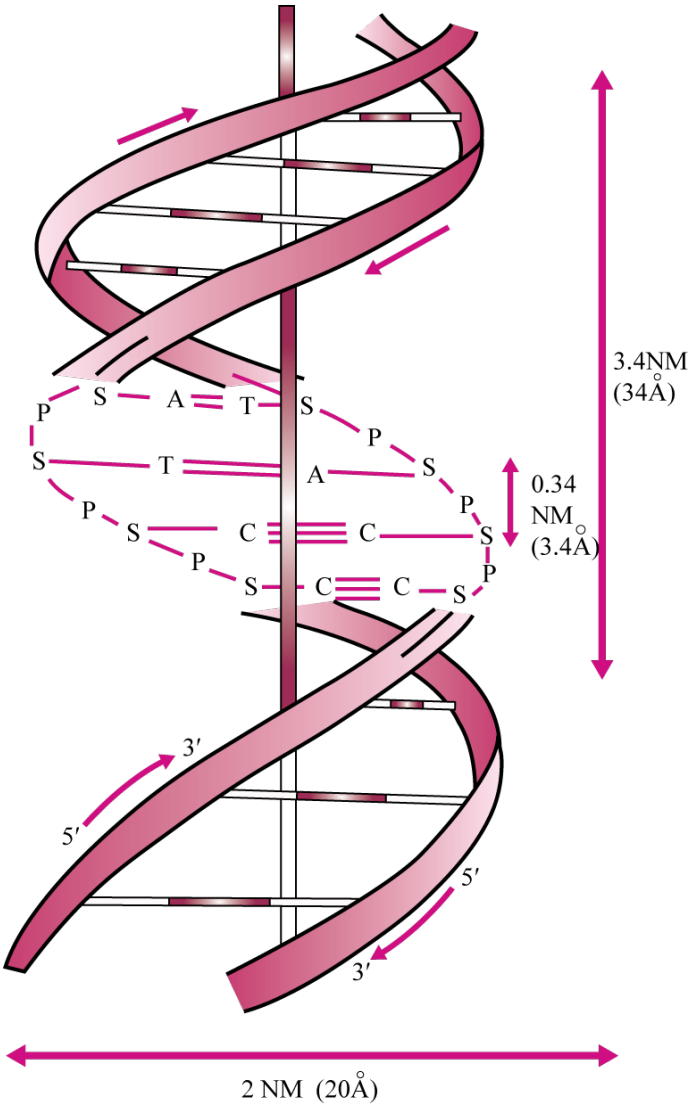


Figure 26.3: A small segment of DNA.

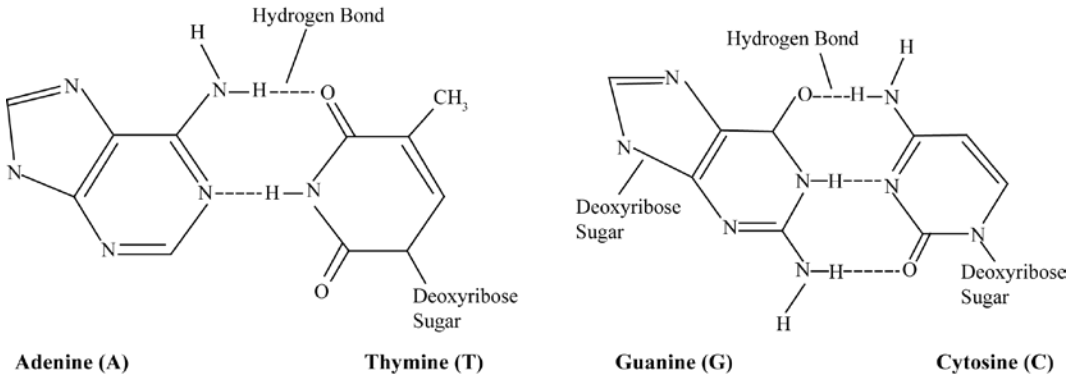


Figure 26.4: Hydrogen Bonding Between Base pairs in DNA.

WHY THERE IS RESTRICTION OF BASE PAIRING, ADENINE WITH THYMINE OR GUANINE TO CYTOSINE ?

As discussed earlier, adenine or guanine is purine and has 9 membered ring wheras thymine or cytosine is pyrimidine of 6 membered. As explained in Figure 26.5, presence of both purines (bulky side chain) or pyrimidines (small side chain) make it difficult to accommodate or too short to form hydrogen bonding within the DNA strands. In addition, purine and pyrimidine has perfect match of hydrogen accepter and donor sites. As a strict requirement of base pairing, two chains are complementary to each other.

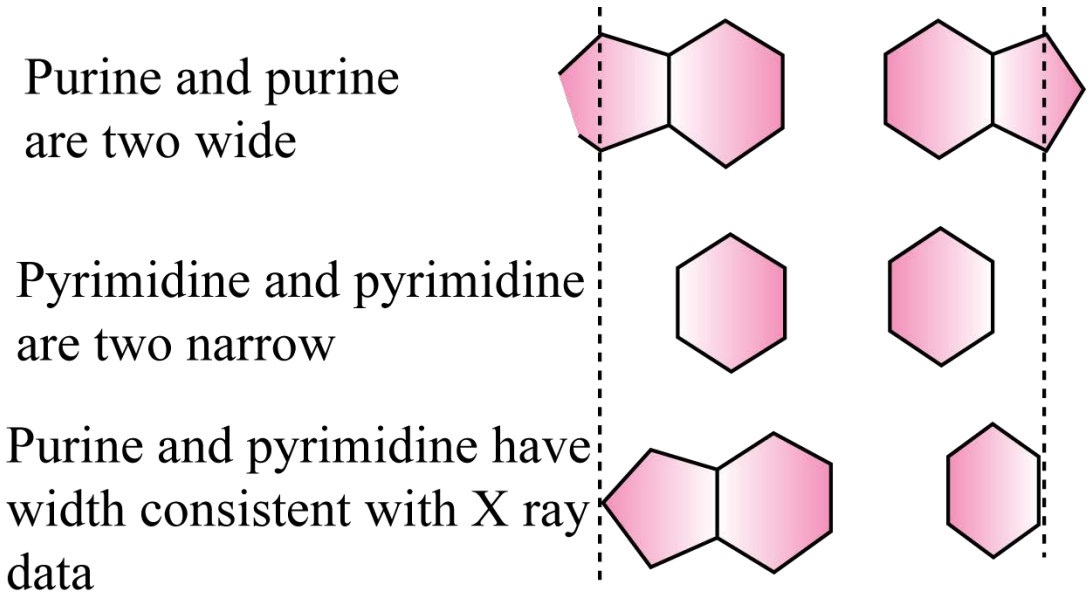


Figure 26.5: Packing of purine and pyrimidine in DNA double helix.

What is complementarity means to you????

It means, if I will provide you the sequence of nucleotide on one strand, it will let you to predict very precisely the sequence of nucleotide on other strand. For example as given in Figure 26.6, every appearance of “A” will give “T” and “G” will give “C” on the second strand.

5´-ATG-GCC-CTG-CAT-GAT-CCG-3´

3´-TAC-CGG-GAC-GTA-CTA-GGC-5´

Figure 26.6: Both strands in DNA are complementary in DNA double helix.

As discussed earlier, individual strand of DNA runs in the direction of 5´-3´ and the other strand run in the direction of 3´-5´. Hence, both strands are running in the antiparellel direction to maintain base complementarity. The presence of complementarity in base pairing and running of strand in the antiparellel direction allow precise duplication of DNA through replication (Figure 26.7). Understanding the base pairing required, chargaff’s has proposed rule about composition of DNA. The summary of this rule is as follows:

(1) The purines and pyrimidines are always in equal amount, A+G=C+T.

(2) The amount of adenine is equal to thymine, and the amount of cytosine is equal to guanine., A=T, G=C.

(3) The base ratio A+T/G+C may vary from one species to another but it will remain constant for a given species. He proposed that the these ratios can be used to identify the species and classify them.

(4) The deoxyribose sugar and phosphate components occur in equal proportions.

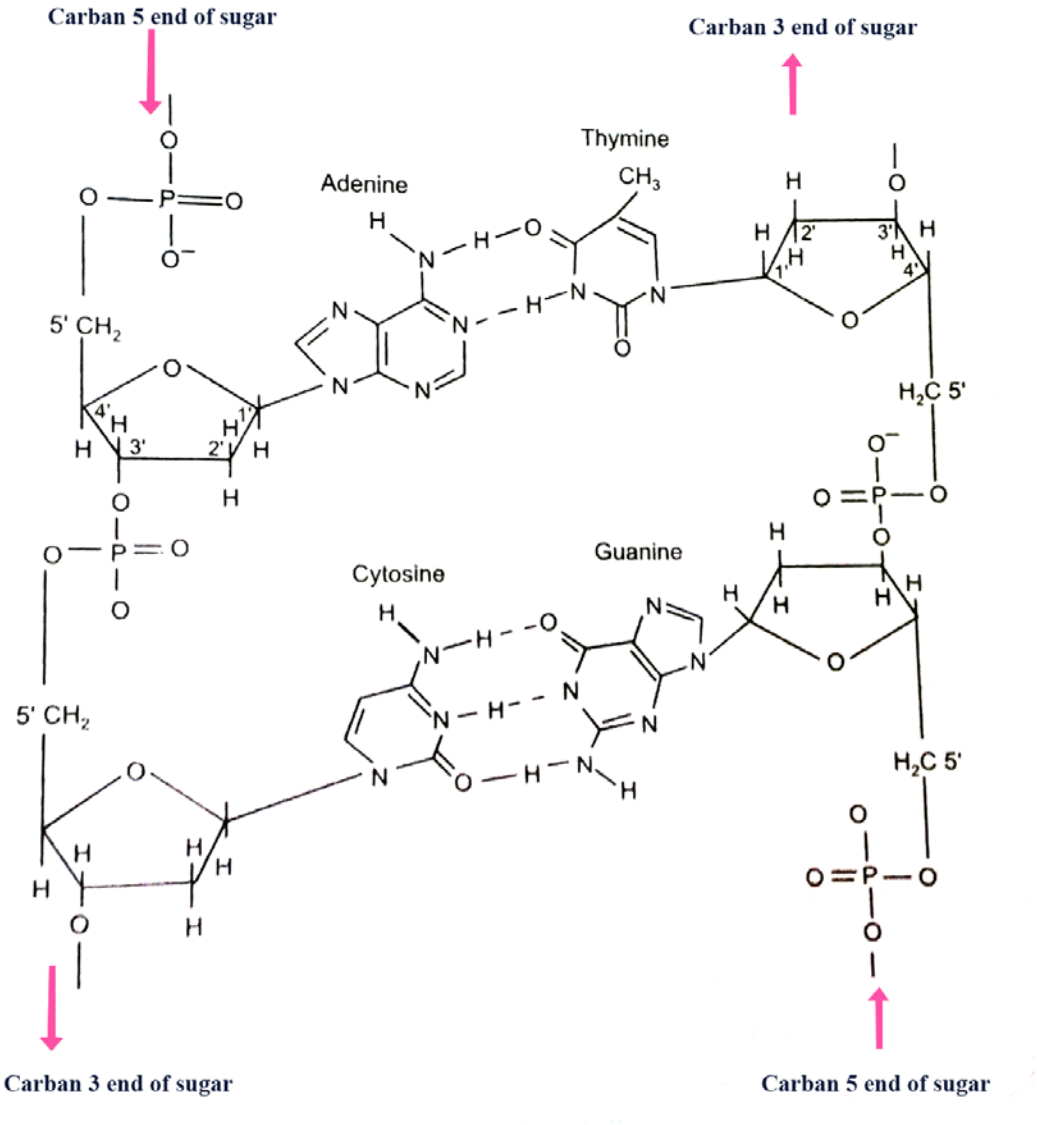


Figure 26.7: Packing of purine and pyrimidine in DNA double helix.

If DNA is double stranded, how it can denature to access the information nucleotide sequence. DNA double helix can be break open, if it is exposed to the high temperature or titrate with acid or an alkali. During this process, the hydrogen bonding between two strand breaks. This process is known as melting or denaturation. When the denatured DNA is incubated at low temperature, the separated strands reassociate to form duplex DNA. This process is known as renaturation. The denatutation/renaturation kinetics is used to understand the complexicity of the DNA and it has wide application in amplifying the strand using polymerase chain reaction (PCR).

Lecture 27: DNA Replication

Introduction: As discussed previously, duplication or re-synthesis of genomic content is essential to maintain the life of an organism. DNA has to be precisely replicate to maintain the sequence identical to the parent DNA. It will protect the appearance of potential mutation and resulting changed phenotype. In the current lecture, we will discuss salient features of replication and underlying factors and mechanism.

Replication of a DNA fragment has to be performed keeping following point into the consideration.

1. The replication machinery must duplicate whole fragment.

2. The replication must be free of errors.

3. The machinery must amplify the fragment in a given time frame. For ex. replication has to be complete within the doubling time of an organism.

Replication Machinery: The replication of DNA required following enzymes and factors.

1. Primase: This enzyme synthesizes the primer at the site of replication to start the replication.

2. DNA Polymerase: These enzymes are responsible for synthesis of DNA of pre-existing DNA fragment. These enzymes are incapable to perform denovo DNA synthesis but can be able to synthesize DNA on a pre-exisitng RNA or DNA primer. DNA polymerase has 3 different enzymatic activities; (1) 5´-3´ Polymerase, (2) 5´-3´ exonuclease and (3) 3´-5´ proofreading. E.Coli has 5 different DNA polymerase but only 3 are involved in replication and rest 2 are more involved in repair or recombination. Different properties of DNA polymerase is summarized in Table 27.1.

TABLE 27.1 Properties of different E.Coli DNA polymerase.

Feature	**I**	**II**	**III**
Structural Gene	*polA*	*polB*	*polC*
Subunits	1	7	>10
Molecular Weight	103,000	88,000	791,500
3′→5′ Exonuclease	Yes	Yes	Yes
5′→3′ Exonuclease	Yes	No	Yes
Polymerization Rate (nucleotides/sec)	16-20	40	250-1000
Processivity	3-200	1500	>500,000

3. DNA gyrase: This enzyme is required to unwind the double stranded DNA.

4. DNA ligase: The enzyme is required to join the DNA fragments.

5. Single Stranded DNA Binding (SSB) Protein: After unwind DNA, SSB binds to the single stranded DNA to avoid rewinding. It helps to reduce the energy required for unwinding.

In addition, replication machinery needs to complete this task in 4 steps:

1. Identify the site of occurrence: it is difficult to start the replication of circular or very large DNA fragment randomly. Hence, a particular pattern of nucleotide sequence exists on DNA to recognize and facilitates the replication initiation. The difficult task with regard to replication is to denature the double stranded DNA and presence of sequence with low melting temperature will facilitate the event. As discussed in earlier lecture, interaction between adenine and thymine is mediated by 2 hydrogen bonding whereas guanine and cytosine is mediated by three hydrogen bonding. As a result presence of AT rich region will facilitate earlier denaturation and assembly of replication machinery. These sequences are mostly present in a region and known as origin of replication. A typical example of E.coli origin of replication is given in the Figure 27.1.

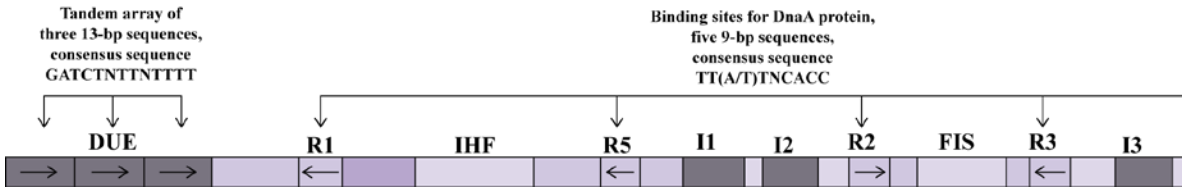


Figure 27.1: E.Coli Origin of Replication. [IMAGE REDRAW]

2. Initiation: The unzipping of DNA at the origin of replication forms a "Y" shaped structure known as replication fork (Figure 27.2). A short chain of RNA is formed at the 5´end. This is a RNA primer and synthesized by primase. Synthesis of RNA primer is essential as DNA polymerase cannot perform denovo DNA synthesis.

3. Elongation: In the elongation step, DNA polymerase start the synthesis of DNA on the pre-existing short oligomeric nucleotide strand. In this step, an incoming deoxynucleotide triphosphate get joined by hydrogen bonding to the appropriate nitrogen bases of the single DNA chain as per the base pairing rule; A-T, T-A, C-G and G-C. The nucleotide triphosphate joined to each DNA strand, break off their high energy bonds and set free pyrophosphate (P-P) molecules. Pyrophosphate undergoes hydrolysis with the help of an enzyme pyrophosphatase, and release energy and set free inorganic phosphate group 2Pi. The energy released in this process is used to derive the polymerization of nucleoside to form DNA (Eq 27.1). The released deoxynucleotide monophosphate joined to each single DNA chain become linked together to form new DNA chain. DNA polymerase.

$$P\text{-}P + H_20 \rightarrow 2Pi + Energy \text{----------} (27.1)$$

The DNA polymerase can polymerize the deoxyribonucleotides in the 5´-3´ direction. As the two DNA strands are antiparellel to each other, the new strand must be formed on the older strand in the opposite direction. The new strand formed continuously in the 5´-3 direction. This strand is known as leading strand. On the other parent DNA, the short DNA strands are synthesized in 5´-3 direction. These short fragments are known as okazaki fragment and these fragments are joined together to give lagging strand. The RNA primer is replaced by deoxyribonucleotide and the gap is joined by the enzyme DNA ligase (Figure 27.2).

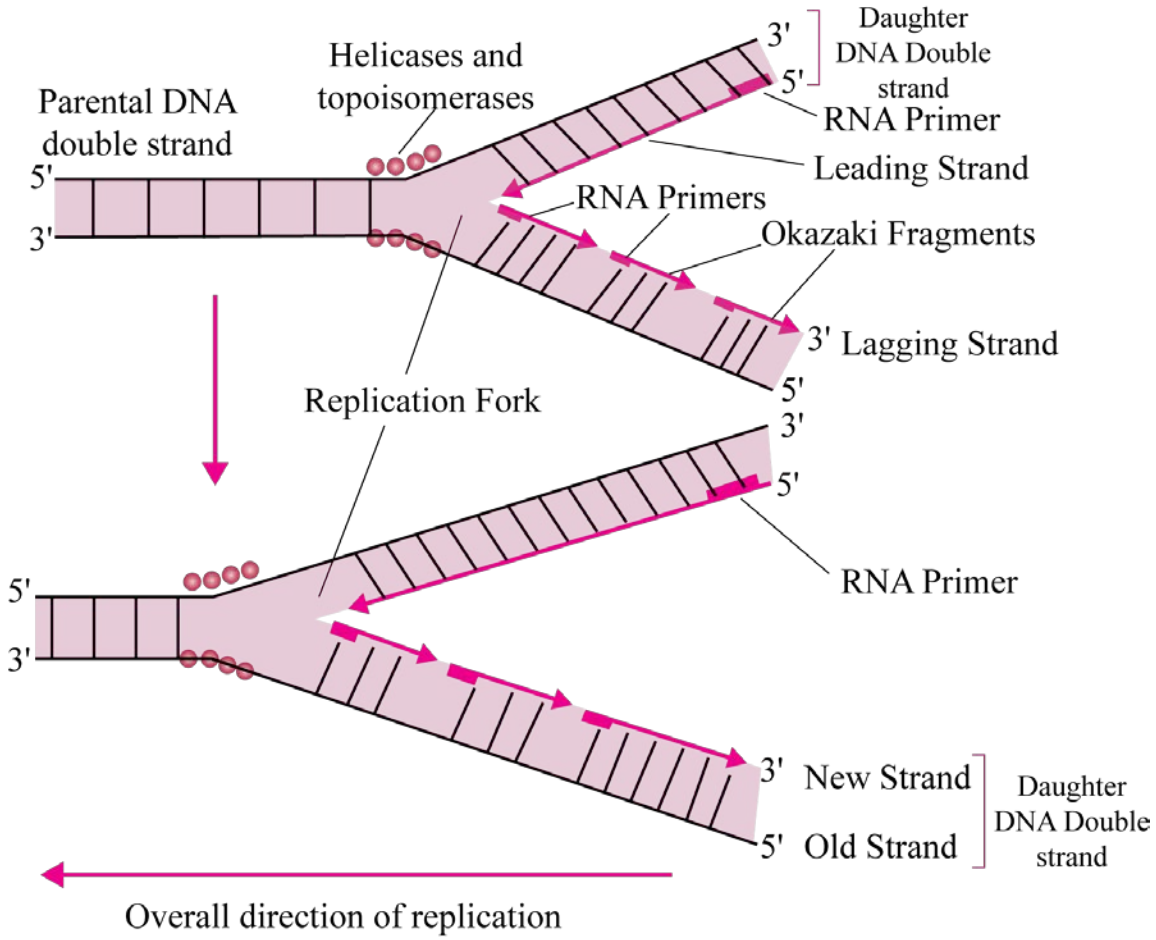


Figure 27.2: Replication of DNA.

4: Termination: The replication fork proceed to the end of the DNA or meet terminal region which contains multiple copies of ter sequences. The Ter sequences are a kind of trap to halt the replication.

Proof reading and DNA repairs: Template nucleotide sequence directs the accurate incorporation of incoming nucleotide and ensured accurate DNA replication. After every round of nucleotide incorporation, DNA polymerase runs in the backward direction and check for accuracy of incorporation. If any error detected, it is corrected at this stage.

Technological Development based on Replication: Polymerase chain reaction (PCR) is developed and used to amplify a DNA sequence to produce millions of copies. Kary Mullis discovered the PCR and got Nobel Prize in Chemistry in 1993 for his discovery. Since then, PCR has been used in various applications in medicine, animal science, plant science, food science etc.

Principle of the technique: The whole process of PCR involves three main events, Denaturation, Annealing and Elongation (Figure 27.3). A DNA fragment of interest is used as a template and a pair of primers which are short oligonucleotides complimentary to the both strands of the template DNA. The purpose of primer is to initiate the DNA synthesis in the direction of 5' to 3'. The number of amplified DNA or the amplicons increases exponentially per cycle thus one molecule of DNA give rise to 2,4,8,16 and so forth. This continuous doubling is carried out by a specific enzyme called DNA polymerase which sits at the unfinished double stranded DNA created by template DNA and primer. For further extension of the DNA, the polymerase enzyme require supply of other DNA-building blocks such as the nucleotides consisting of four bases Adenine (A), Thymine (T), Cytosine (C) and Guanine (G). The template, primer, polymerase and four bases are the main components for polymerase chain reaction.

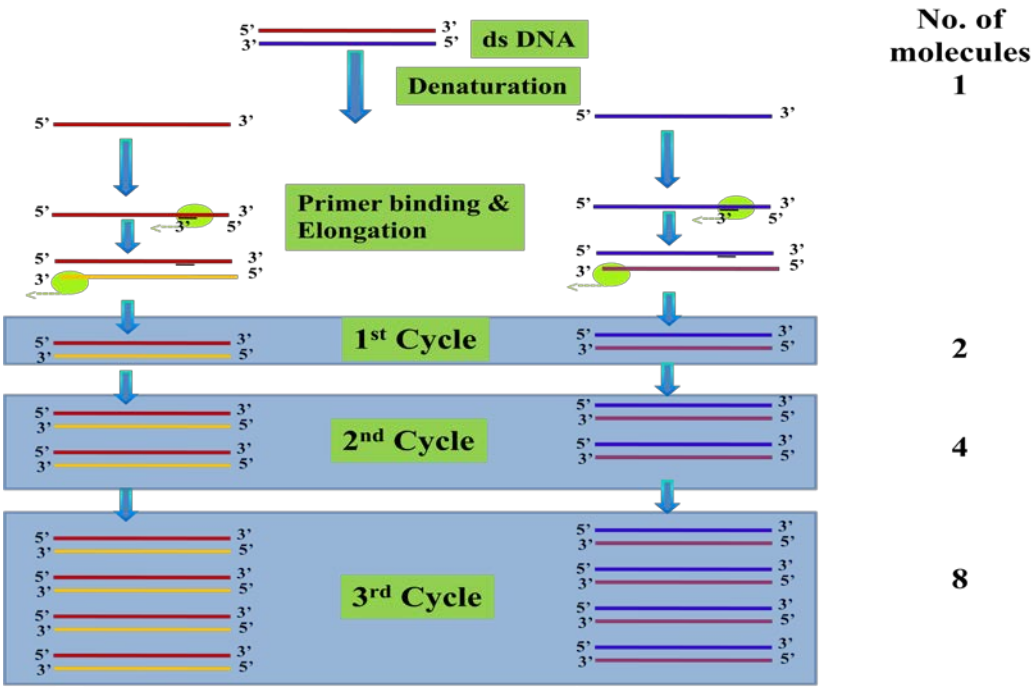


Figure 27.3: Basic Principle of polymerase chain reaction (PCR).

Methodology: PCR has three major events (Denaturation, Annealing and Elongation) to complete the amplification process (Figure 27.3). The complete process of PCR is as follows-

1. Initial denaturation: Heating the PCR mixture at 94°C to 96°C for 10min to ensure complete denaturation of template DNA. It is followed by the cyclic events which has different steps as described below:

A. Denaturation: This is the first step in which the double stranded DNA template is denatured to form two single strand by heating at 95°C for 15-30 secs.

B. Annealing: This is the annealing step where at lower temperature (usually 50-65^{0}C) primers are allowed to bind to template DNA, annealing time is 15-30 secs and it depends on the length and bases of the primers. Generally annealing temperature is about 3-5°C below the melting temperature (T_m) of the pair of the primers is used.

C. Elongation: This is the synthesis step where the polymerase perform synthesis of new strand in the 5' to 3' direction using primer and deoxyribonucleoside triphosphates (dNTPs). An average DNA polymerase adds about 1,000 bp/minute. Step 1,2,3 makes one cycle and in general 35-40 such cycles are performed in a typical PCR amplification.

2. After the cycles are completed, the reaction is held at 70-74°C for several minutes to allow final extension of the remaining DNA to be fully extended.

3. The reaction is complete and the resulting amplified nucleic acids are held at a low temperature (~4°C) until analysis.

Reagents: The reagents required for a complete PCR reaction volume is given in the table

Reagents	**Amount required**
Template DNA	1pg-1ng for viral or short templates 1ng-1µg for genomic DNA
Primers (forward and reverse primers)	0.1-0.5µM of each primer
Magnesium chloride	1.5-2.0 mM is optimal for *Taq* DNA polymerase
Deoxynucleotides (dNTPs)	Typical concentration is 200 µM of each dNTP
Taq DNA Polymerase	0.5–2.0 units per 50 µl reaction

Instrumentation: Thermal cycler is the instrument that carries out the amplification via polymerase chain reaction (Figure 27.4). Usually the three main events are repeated for 30-40 cycles to obtain detectable amount of product at the end of the cycles**.** The automated system performs the cyclic temperature changes required for enzymatic amplification of specific DNA segments in vitro using this PCR. The device has a thermal block with holes where tubes containing reaction mixtures can be inserted. The cycler varies the temperature of the block in discrete, pre-programmed steps using peltier effect.

Primers: A primer is a short oligonucleotide that serves as a starting point for DNA synthesis. In PCR, two primers are required to bind to each of the single stranded DNA (obtained after denaturation) flanking the target sequence. These are called Forward and Reverse primers. They primers are designed in such a way that they have a sequence complimentary to the sequence in the template DNA. Two restriction enzymes sites are added at the 5' end of each of the primer to facilitate cloning. The chosen restriction enzymes will not cut DNA fragment (non-cutters). Typically 3 to 4 nucleotides are added at the end of the restriction sites to allow efficient cutting by restriction enzymes.

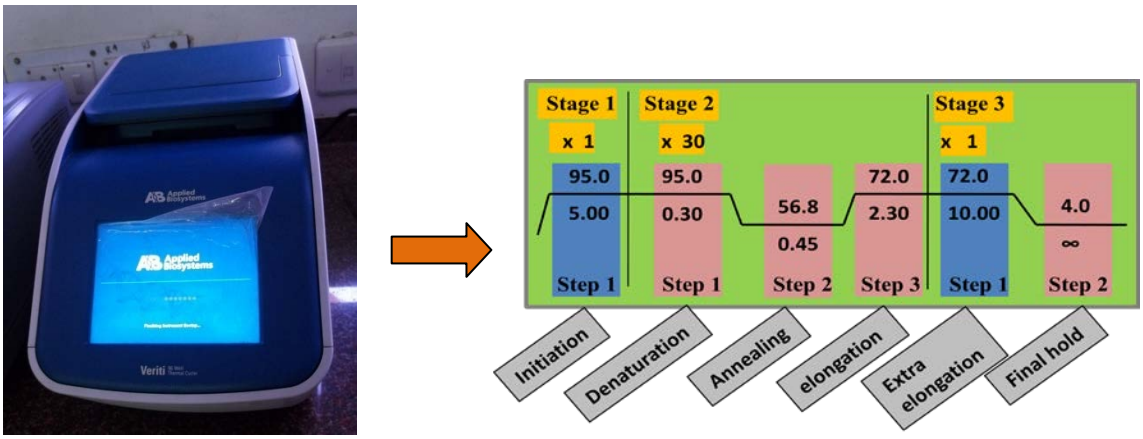


Figure 27.4. Representation of thermal cycler instrument showing the position of sample and schematic diagram of 30 cycle PCR.

Analysis of PCR results: Once PCR cycle is completed, the amplified product is loaded in the agarose gel and observed after ethidium bromide staining under UV light source (Figure 27.5). A water blank reaction is included to monitor the cross contaminating DNA source as template. The percentage of agarose gel depends on the size of DNA to

be visualized. Generally 0.8-1% agarose gel is used for analyzing 0.5-5 kb amplified DNA while a DNA of larger size or genomic DNA is visualized in gel as low as 0.5%.

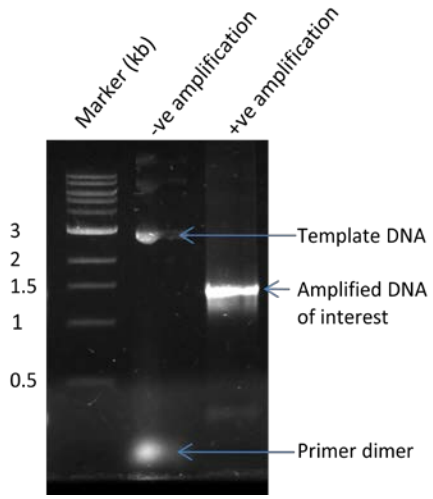


Figure 27.5: Analysis of PCR product on an agarose gel.

Lecture 28: Transcription (Part-I)

INTRODUCTION: Every cell mainly contains three types of RNA-transfer RNA (t-RNA), ribosomal RNA (rRNA), messenger RNA (mRNA). Synthesis of RNA from DNA templates with the help of DNA dependent RNA polymerases is known as transcription. It occurs unidirectionally in which chain is synthesized in 5' to 3' direction. The segment which is transcribed from DNA is known as **Transcription unit** (Figure 28.1). In eukaryotes **monocistronic** transcription unit occur in which coding sequence presents for only one polypeptide. But in prokaryotes **polycistronic** transcription unit occurs in which coding sequence presents for more than one polypeptide.

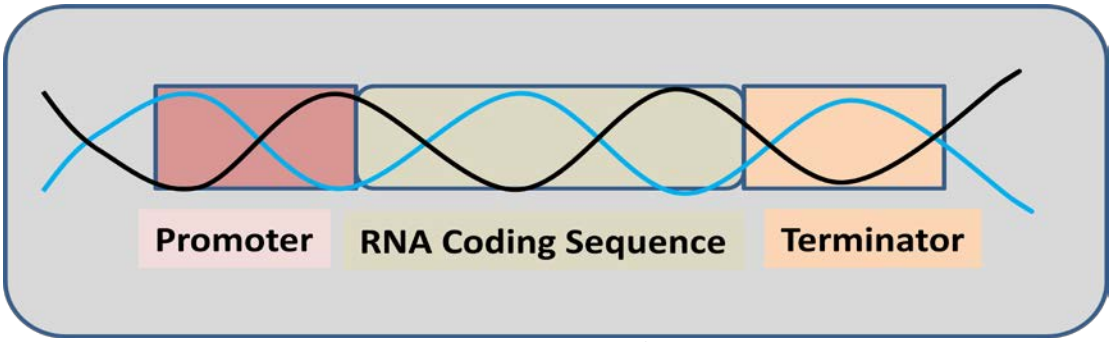


Figure 28.1 : Structure of gene

Start Point – It is the first base pair from where transcription starts and is called **start site**. RNA polymerase moves from start point along with template, synthesize RNA up to terminator sequence.

Upstream –It is nontemplate nucleotide in 5' end or minus direction; sequence before start point.

Downstream-It is nucleotide in 3' end or plus direction; sequence after start point. DNA is a double stranded structure. During transcription only one strand is transcribed so that transcribed sequence is identical with one strand of DNA, known as **Coding or sense strand** and other complementary strand is known as **template** or **antisense strand** (Figure 28.2).

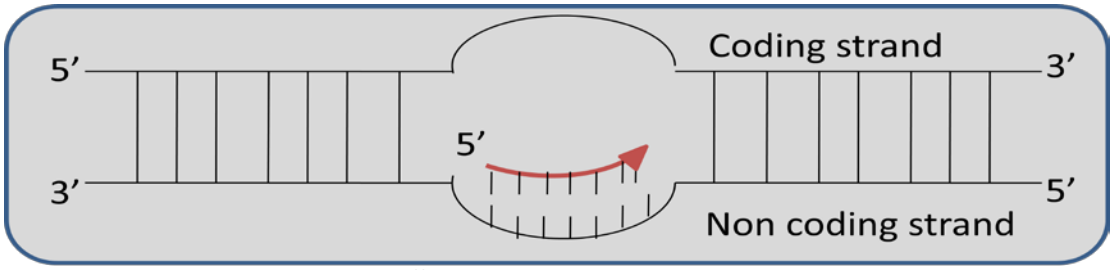


Figure 28.2 : Difference between coding and non coding strand

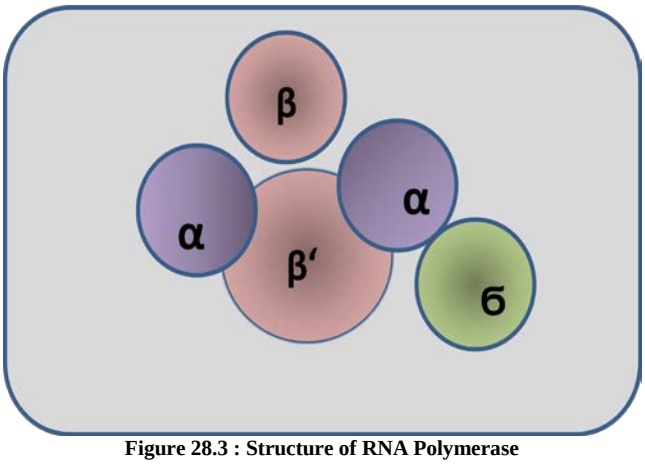
TRANSCRIPTION: The molecular process through which cell form RNA from DNA is called as transcription. The basic steps of transcription remain same between prokaryotic and eukaryotic organism and few of the selected differences are outlined in the Table 28.1.

Table 28.1: Difference between Prokaryotes and Eukaryotes Transcription

Prokaryotic Transcription	**Eukaryotic Transcription**
Polycistronic type of transcription	Monocistronic type of transcription
Occurs in cytoplasm	Occurs in nucleus
Coupled transcription –translation process occurs	Coupled transcription –translation process not occurs.
Single type of RNA Polymerase required for synthesis of all type of RNA	Three different type of RNA Polymerase required for synthesis of all type of RNA
No need of any transcription factor for initiation.	Its require transcription factor for initiation
RNA Polymerase are made up by 5 subunits	RNA Polymerase are made by 10-15 subunits

TRANSCRIPTION IN PROKARYOTES

A**) RNA Polymerase:** In prokaryotes single type of RNA polymerase is present which is responsible for synthesis of all type of RNA. Eubacterial RNA pol is named as **Holoenzyme** is a **multisubunit protein** which contain five subunits **ααββσ ,** structure of RNA polymerase is given in Figure 28.3.



α- assembly of core enzyme
β β'- performs all enzymatic and catalytic function
σ –recognizes promoter sequence.
ααββ' forms core enzyme.
Holoenzyme= core enzyme + sigma factor

Figure 28.3 : Structure of RNA Polymerase

B**) Prokaryotic promoter:** Promoter typically consists of 40 bp region located near to 5' end side of transcription start site. Promoter region consists of two 6 bp consensus sequences elements- **Pribnow box or TATA box and -35 region.** Pribnow box 10 bp upstream of start point is a consensus sequence **TATAAT**, -35 region has consensus sequence **TTGACA.** Structure of Prokaryotic promoter is given in Figure 28.4.

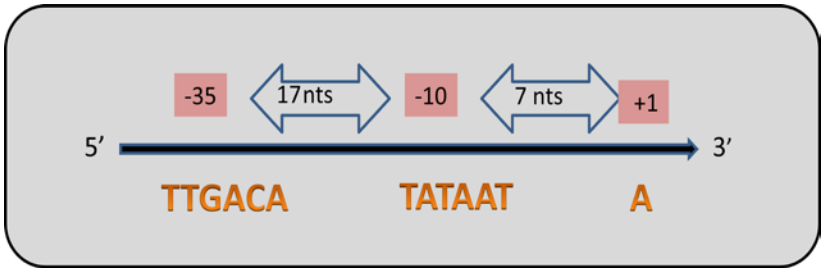


Figure 28.4 : Structure of Prokaryotic Promoter

TRANSCRIPITION OCCURS IN FOUR STAGES: 1) Template binding, 2) Chain initiation, 3) Chain elongation, 4) Termination.

1) Binding of RNA polymerases to template DNA and Chain initiation: DNA duplex should be opened so that RNA pol can approach to single stranded DNA template. Efficiency of initiation is inversely proportional to melting temperature Tm, and AT rich

region has lower Tm because of double hydrogen bond stabilizing them than triple bond in GC rich region and thus is more stable. Therefore, AT rich region is good for melting of duplex and easy to create open promoter complex than GC rich region. RNA polymerase has σ factor which recognize promoter sequence at which RNA polymerase Holoenzyme binds and form a complex which known as closed complex. In fact, sigma factor is released when chain reaches nearly up to 10 bases, leaving core enzyme for further elongation, which are given below in Figure 28.5.

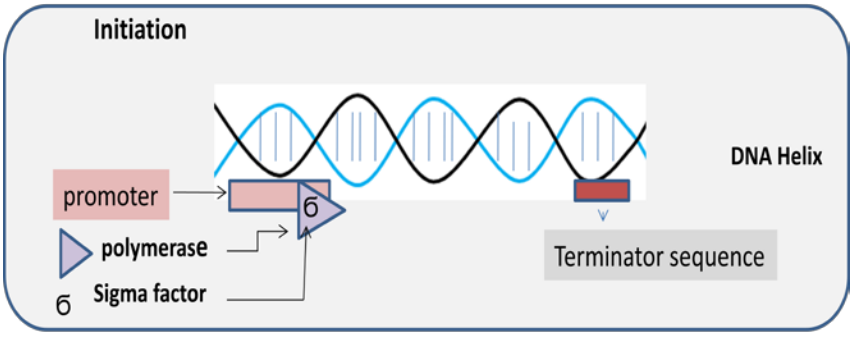


Figure 28.5 : Initiation of Transcription

Open complex- closed complex is converted into open complex by melting short region of DNA (-10bp). RNA polymerase bind at promoter region and unwind it and it covers (-55 to +1) 55 bp, and start initiation here one template strand available for incoming nucleotide for base pairing and synthesis of RNA occurs. -10 region of template is essential for recognition, the promoter region are double stranded in closed complex and single stranded in open complex. RNA polymerase has two binding sites for nucleotides**: 1) initiation sites 2) elongation sites.** Initiation site binds to first nucleotide within open promoter complex at +1 position which is usually a purine-A or G. It means first nucleotide will be ATP or GTP. Elongation site bind with second incoming nucleotide base pairing at +2 positions. Two nucleotides are joined together then the first base is released from the initiation site, and the initiation is complete.

2) Chain elongation: Chain elongation occurs in 5'-3' direction.RNA synthesis is carried out by transcription bubble which form due to transient separation of double stranded DNA into a single stranded DNA; and transcription takes place at template DNA strand, whichis given below in Figure 28.6.

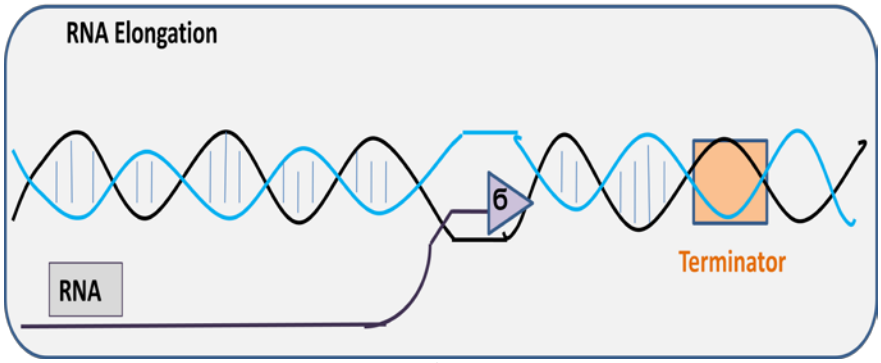


Figure 28.6 : Elongation of Transcription

RNA chain synthesis occur basically at 5' end to 3' end direction by adding nucleotide at 3' end .The 3' OH group of last nucleotide is combined to the incoming 5' ϒ phosphate nucleotide; α and β phosphate groups are removed and only ϒ phosphate is used in the formation of phosphodiester bond. Likewise other nucleotide added which are complementary to template DNA and thus RNA chain strand translocation occurs. In bacteria transcription rate is nearly 40 to 50 nucleotide per second at 37^0C which is nearly same as the translation in prokaryotes (50 amino acid per second). RNA polymerase bind to promoter and create a transcription bubble .RNA polymerase moves along with DNA and RNA chain grows continuously. The length of transcription bubble is approximately. 12 to 14 bp. Length of DNA RNA hybrid is about 8 to 9 bp. As the RNA polymerase moves, the duplex reforms again. RNA hangs as free polynucleotide chain. Transcription bubble moves continuously by disrupting the DNA structure. Nucleotides are added covalently to 3' end of the chain of RNA.β and ϒ phosphates are removed from incoming nucleotides and hydroxyl group is removed from 3' carbon nucleotide presents at end of chain.

3) Chain termination: When RNA polymerase stops adding nucleotide at RNA chain, it releases a completed product and RNA chain get free from the terminator sequence. During termination all the hydrogen bonds break down which hold RNA-DNA hybrid

together and when RNA chain is separated DNA again reform duplex. Site at which site enzyme stops adding nucleotides is known as chain termination site **(**Figure 28.7).

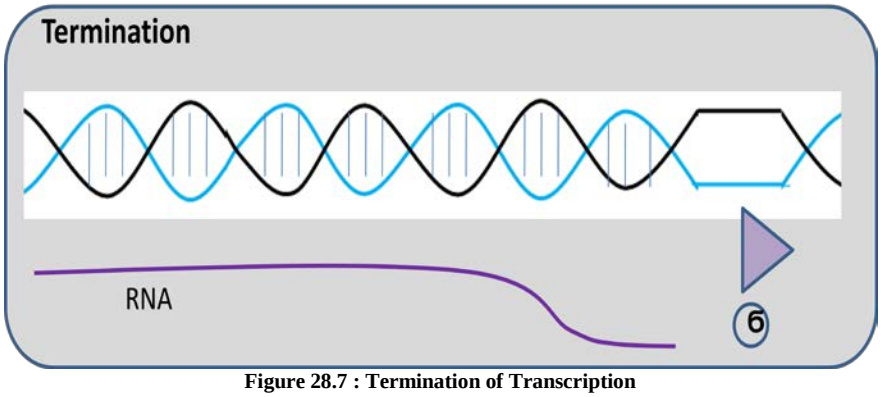


Figure 28.7 : Termination of Transcription

Two types of termination mechanism are present in bacteria: 1) Intrinsic termination, 2) rho factor dependent termination.

Intrinsic Termination: In this mechanism of termination rho factor is not required and termination depends on RNA product. It requires G-C rich hairpin; hairpin structure is followed by 7 U residues. RNA DNA hybrid requires forces for holding the elongation complex together thus when the hybrid gets detached; it collapses the elongation complex which causes the termination. In this type of termination dissociation of polymerase occurs by destabilizing the attachment of growing chain to the template. During this process hairpin structure is formed by the transcript via complementary base pairing. It includes palindromic sequences. This stem loop structure includes GC rich region followed by U rich region (Figure 28.8).

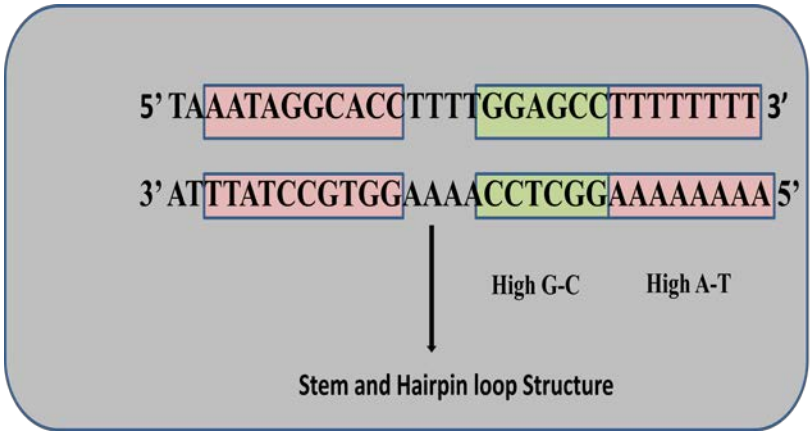


Figure 28.8 : Stem hairpin loop structure

Steps in intrinsic termination: Different steps which are involved in intrinsic termination are described here in figure 1.9. These steps are as follows:

1) Here, two inverted repeats GCCCGC are present in the DNA template which is transcribed

2) Nearly 6 adenines residues follow the second inverted repeat GCCCGC, given below in Figure 28.9(a).

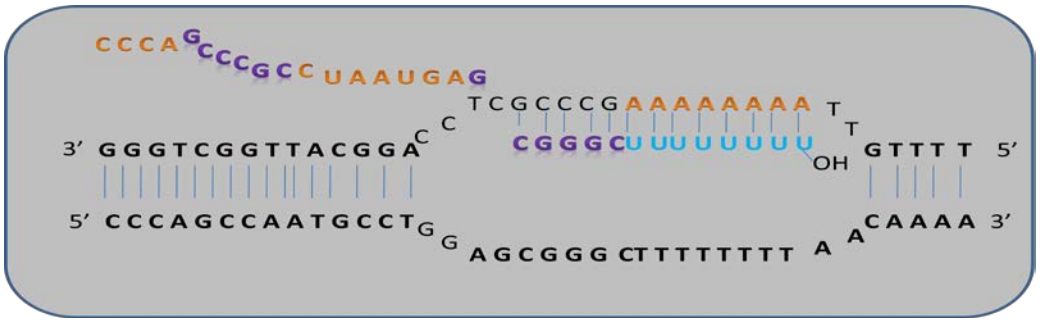


Figure 28.9(a) : Inverted repeat are following by adenine residues

Now inverted repeats are forming a hairpin structure which pause the polymerase

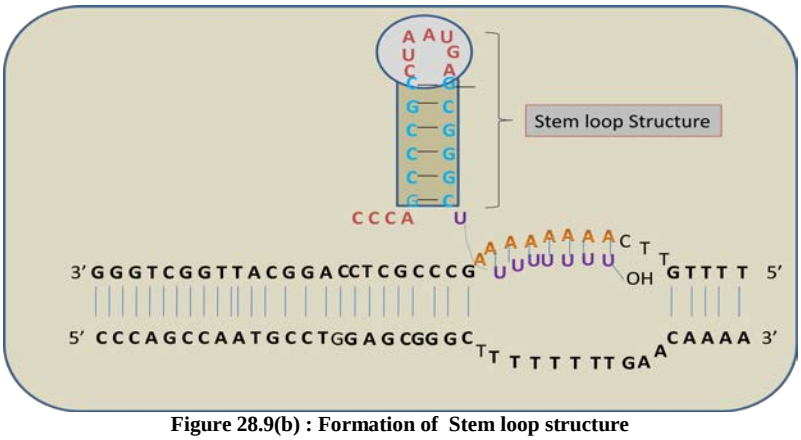


Figure 28.9(b) : Formation of Stem loop structure

Due to formation of stem loop structure, the A-U bonds get break down, leads termination and RNA molecules get separated

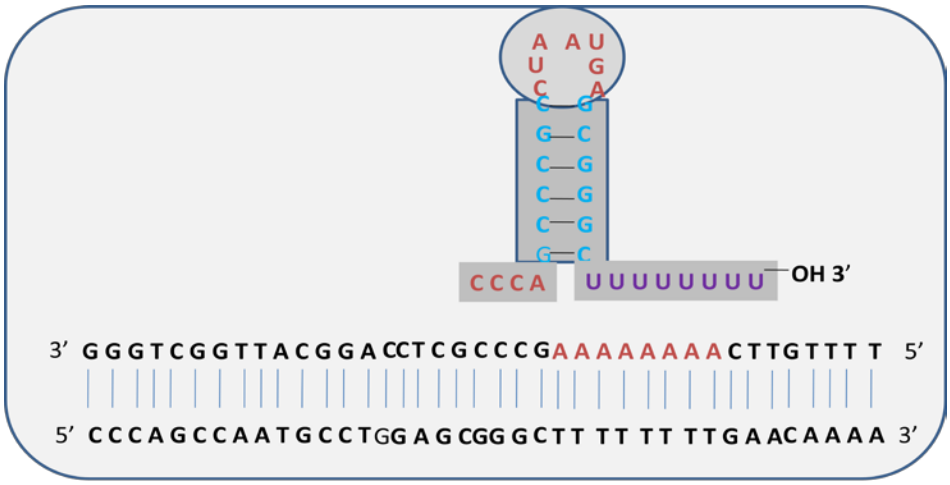


Figure 28.9(c) : Chain termination

2. Rho dependent termination: This type of termination requires Rho protein. Rho is an ATP dependent helicase that disrupts RNA –DNA hybrids. It is an essential protein which causes transcription termination, rho protein is hexamer ATP dependent helicases and its subunit contain RNA binding and ATP hydrolysis domain. These rho protein firstly bind to sequence which is present at upstream of termination site; these sites are called rut site. These site are rich in C residues rho factor followed to RNA polymerase until it do not catch RNA polymerase, which described in Figure 28.10(a). Rho follow the RNA polymerase by its helicase activity which is driven by ATP hydrolysis ,when RNA polymerase reached at terminator site the rho protein freeze the structure of polymerase which are given in fig 1.10(b) and when Rho factor collapse with the enzyme which cause termination and new RNA chain get released , given in figure 1.10(c). Steps in rho-dependant termination which described in figure 1.10(a),(b),(c).

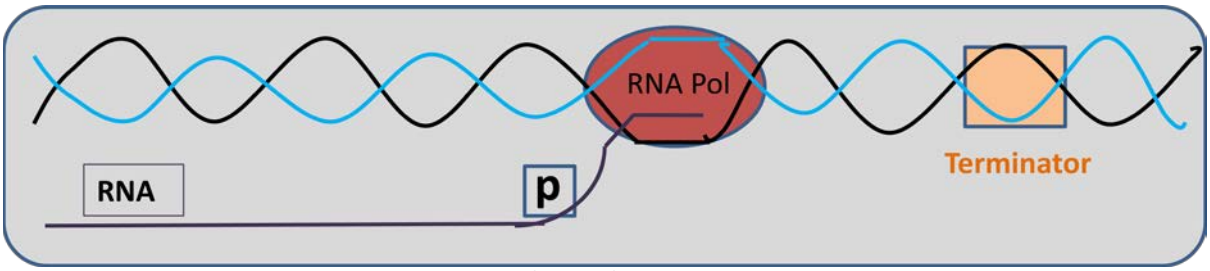


Figure 28.10(a) : Rho factor is following to RNA Polymerase

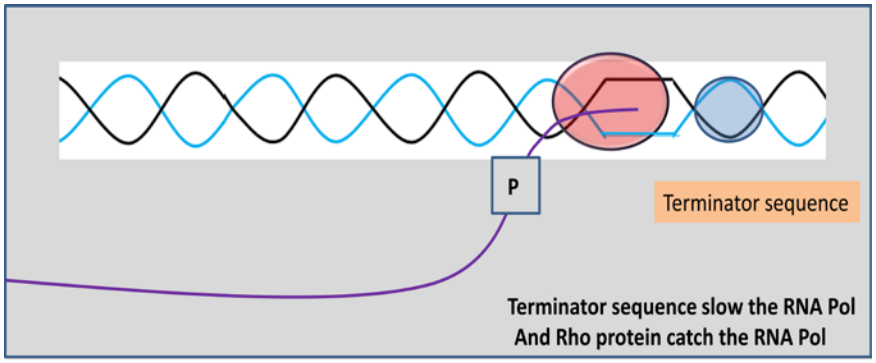


Figure 28.10(b) : Terminator sequence slow the RNA Pol and Rho protein catch the RNA Pol.

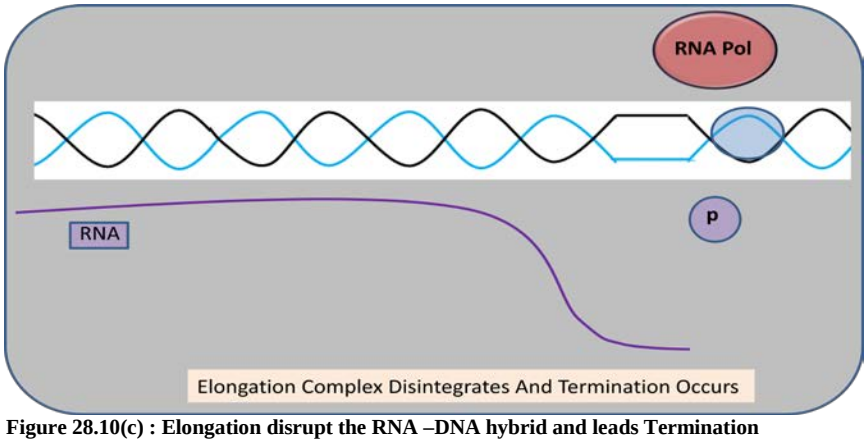


Figure 28.10(c) : Elongation disrupt the RNA –DNA hybrid and leads Termination

EUKARYOTIC TRANSCRIPTION: Eukaryotic transcription is different from prokaryotes transcription, eukaryotes transcription requires transcription binding factors ,enhancer along with RNA polymerase ,transcription factors are a protein which are essential for transcription but these are not the part of RNA polymerase, DNA template. These factors are bind at DNA template sequentially and then DNA polymerase bind and form a initiation complex. Basal transcription factor create a structure at the promoter, so RNA polymerase can easily recognise to promoter, in bacteria single RNA polymerase can transcribed the all type of RNA but in eukaryotes it requires different RNA polymerase for different RNA structure.

RNA Polymerase: The RNA polymerase of mitochondria and chloroplast are similar like bacteria. All eukaryotic RNA polymerases are multi subunit proteins which contain. Three different types of RNA polymerases are responsible for transcription (Table 28.2).

Table 28.2: Different types of RNA polymerasein eukaryotic system.

Type of polymerase	Synthesis of RNA	Amantin sensitivity	Occurrence
RNA Pol I	synthesis of r RNA	resistant	Nucleoli
RNA pol II	synthesis of m-RNA	Very sensitive	Nucleoplasm
RNA pol III	Synthesis of t-RNA	Less sensitive	Nucleoplasm

2) Eukaryotic promoter: Promoter of RNA POL l and POL ll are mostly located at upstream site, but promoter of rRNA lll are located on downstream site of the start point. Each promoter contain some specific sequence which get recognise by the transcription factor. Eukaryotic promoter has longer region than prokaryotic promoter because it contain all those sequences which are important regarding to initiation, it includes **core promoter** element at which RNA polymerase get attached and form initiation complex and also for efficient transcription it requires an **upstream promoter element**, which are basically G+C rich region and at which transcription factors are bind.

TRANSCRIPTION INITIATION BY RNA POLYMERASE II : Eukaryotic m RNA transcription requires initiation complex which consist general transcription factors (GTFs) and mediator. Various transcription factor and their functions are as follows:

General Transcription Factors (GTFs)	Function
TFIID **I) TBP (Tata binding protein)** **ii) TAFs (TBP Associated Factors)**	 recognize to core promoter (TATA box) recognize to core promoter (Non TATA box)
TFIIA	Stabilized TBP and TAFs binding
TFIIB	It help in RNA polymerase II and TFIIF recruitment and also help in start site selection
TFIIF	Help to RNA Pol in promoter binding
TFIIE	Help in TFIIH recruitment, modulation of TFIIH, Helicase, ATPase, Kinase activities
TFIIH	Help in promoter melting with helicase .activities prompter clearance by phosphorylation

These transcription factors are sequentially bind to TATA box DNA to form a pre initiation complex. At last when TFIIH get bind, It phosphorylates Pol II to initiate transcription in the presence of ATP which are described in Figure 28.11

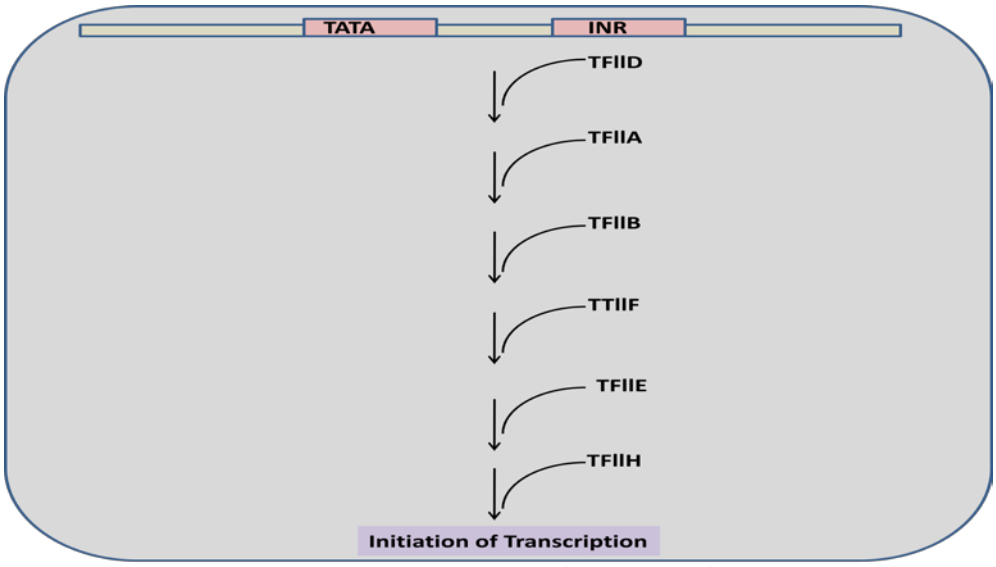


Figure 28.11 : Sequential addition of Transcription factor

Mediator: For transcription activation mediator is required. Transcription requires various activator proteins .Mediator is a protein complex which serves as bridge between co activator and enhancer, and between promoter and RNA polymerase.

TRANSCRIPTION TERMINATION

For Polymerase l gene	rho dependent termination
For polymerase lll gene	rho independent termination
For polymerase ll gene	It more complex, polII termination generally coupled with the RNA processing event, in which 3'end of transcript undergoes cleavage and polyadenylation.

RNA pol II transcription genes may continue up to hundreds or even thousands of nucleotides beyond the end of a coding sequence. Then the cleavage of RNA strand occurs by a complex which appears to associate with the polymerase. Cleavage of RNA is couple with termination process and it occurs at same consensus sequences. The polyadenylation of mature pol ll m RNA occurs at the 3′,which result in a poly(A) tail; this process is followed by cleavage and termination. Both processes polyadenylation and termination occurs at same consensus sequence, and both these processes are interdependent.

Poly A dependent termination: This type of termination are basically coupled with the RNA maturation process in which 3' end of nascent RNA undergo polyadenylation and cleavage, and these 3 ' end processing reaction are carried out in two steps:

Transcription of poly A followed by cleavage of nascent transcript, and then the upstream product is polyadenylated and downstream product is degraded. Basically 3' end processing starts when the cis acting element in the Poly A site of nascent RNA transcript is recognised by the binding factors .when these factors bind at 3'end, it form a very complex structure which result in high shear forces consequently processing slow down which causes disruption of polymerase ll and DNA –RNA hybrid complex; and ultimately termination occurs (Figure 28.12)

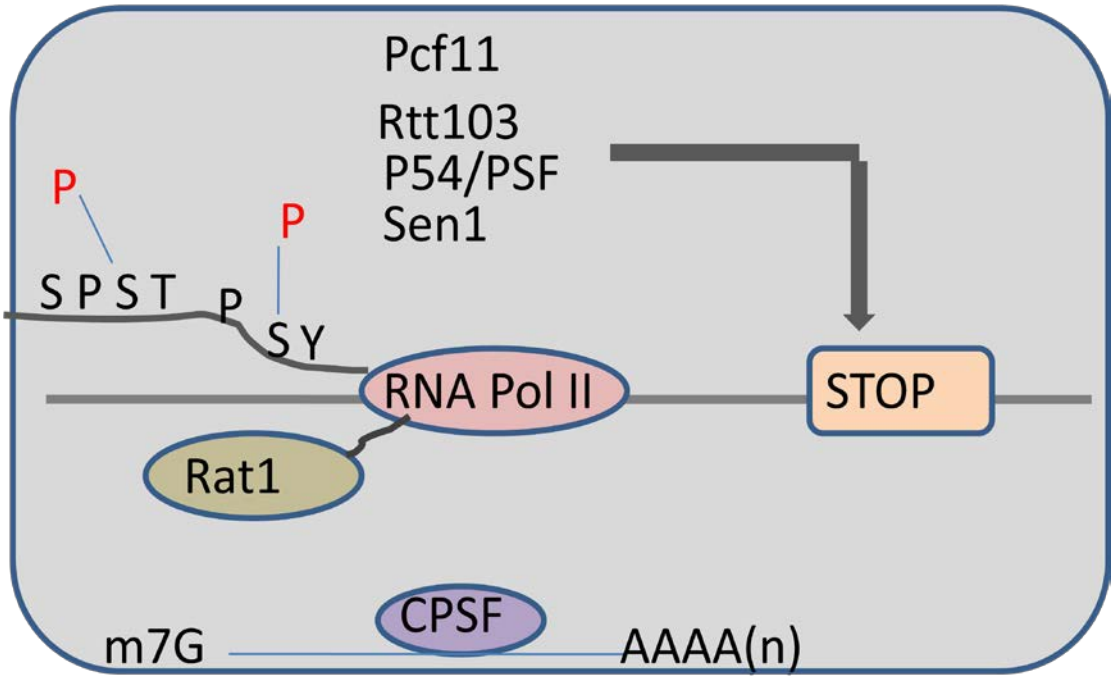


Figure 28.12 : Termination of RNA Pol II Transcription

Lecture 29: Transcription (Part-II)

Summary of Previous Lecture: In previous lecture we discussed about structure of gene which includes promoter, coding and terminator region. We also illustrated the differences between Transcription in Prokaryotes and Eukaryotes system. There was elaborate description of transcription in prokaryotes and eukaryotes. However, transcription in general is divided in 3 steps: 1) Initiation 2) Elongation and (3) Termination. In this lecture we will discuss about post-transcriptional modification.

POST-TRANSCRIPTIONAL MODIFICATION: In prokaryotes, transcription and translation both processes are carried out simultaneously. The RNA copy of a gene is m-RNA, and it is ready for translation into protein. In fact, translation starts even before transcription is finished. In eukaryotes, the primary RNA transcript of a gene are further processed prior to its translation into protein. This step is called "RNA processing". Also, Post RNA processing, it needs to be transported out of the nucleus into the cytoplasm through the nuclear pores. Many of the RNA molecules in bacteria and virtually all molecules in eukaryotes are processed to some degree after synthesis as a newly synthesised RNA molecule is immature and is called **primary transcript.** Most elaborated form of processing occurs for all eukaryotic mRNAs and tRNAs of both bacteria and eukaryotes.

A eukaryotic gene has several non-coding region (**introns**) interspersed with coding region (**exons**). When an m-RNA is transcribed, primary transcript has both introns and exons. Introns are removed from primary transcript via a process called **splicing.** In splicing, introns are removed and exons are joined together in a continuous sequence forming **mature m-RNA** transcript ready to be transported out of the nucleus and translated. Apart from splicing, eukaryotic m-RNA, also undergoes 5' capping and polyadenylation at 3' end.All three processes of splicing, capping and adenylation occurs inside nucleus. Elaborate protein complexes are involved in these three RNA processing which act in association with each other and with phosphorylated CTD of Pol II. The primary transcript of bacterial or eukaryotic tRNAs are processed by either **cleavage** which removal of bases from each end or in some cases **splicing.** Furthermore, many unusual bases, not found in any nucleic acid are also added to tRNA.

Steps in RNA processing: (1) Add a cap to the 5’ end, (2) Add a poly-A tail to the 3’ end, (3) splice out introns

Capping: In eukaryotic cells, m RNA is inherently unstable at the ends. So needs to modified the end to protect it againsed ribonucleases, mRNA is capped so that it is protected from ribonucleases as well as it is important in binding of m-RNA to ribosome for translation; it uses certain cap binding protein complexes. Capping reaction starts soon after transcription has started. As soon as 20-30 nucleotides are formed, capping occurs. At the 5’ end capping process occurs, a slightly modified guanine (7-methyl G) is attached “backwards”, by a 5’ to 5’ linkage, to the triphosphates of the first transcribed base. Capping reaction includes **condensation** of GTP with triphosphate at 5’ end followed by **methylation** of guanine at N-7. Further methylation occurs at 2’hydroxyls of 2nd and 3rd nucleotide adjacent to the cap which described below in Figure 29.1.

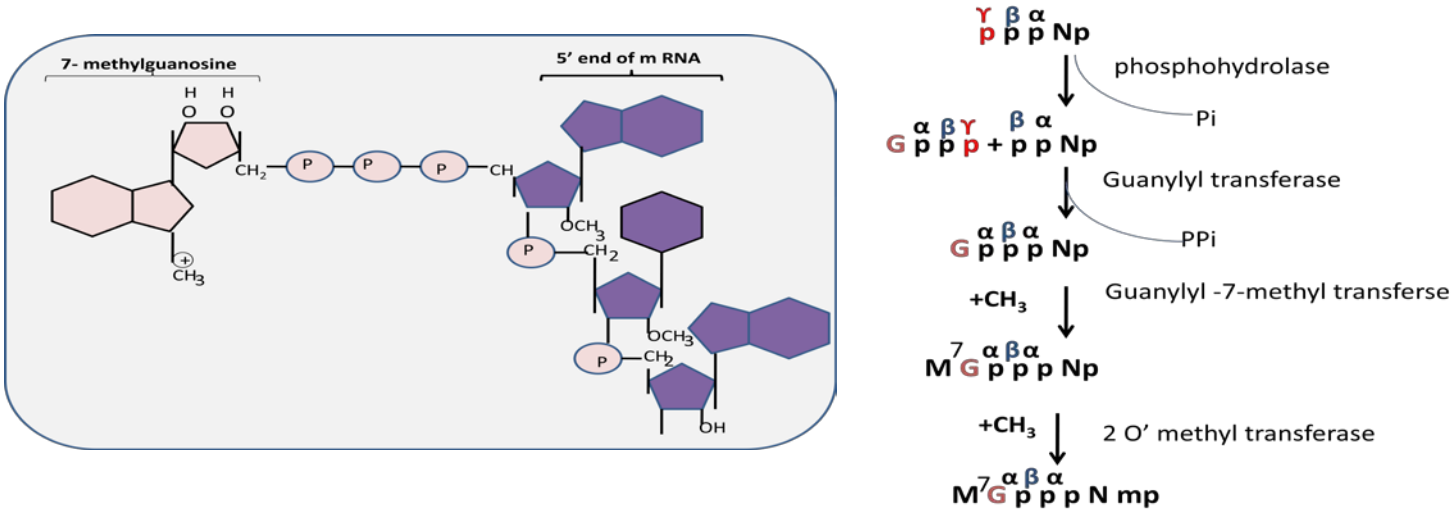


Figure 29.1: Capping process in m RNA

Tailing: Eukaryotic mRNA has series of adenosine residues ranging from 80 to 250 in number forming a poly (A) tail at 3' end of the primary transcript. This poly (A) tail has several uses- 1) it can export mature mRNA out of the nucleus. 2) It increases stability of mRNA. 3) It serves as recognition signal for binding of translational factors during ignition of translation. The process requires template-independent RNA polymerase called as **poly (A) polymerase**. Tailing is described below in different steps in Figure 29.2

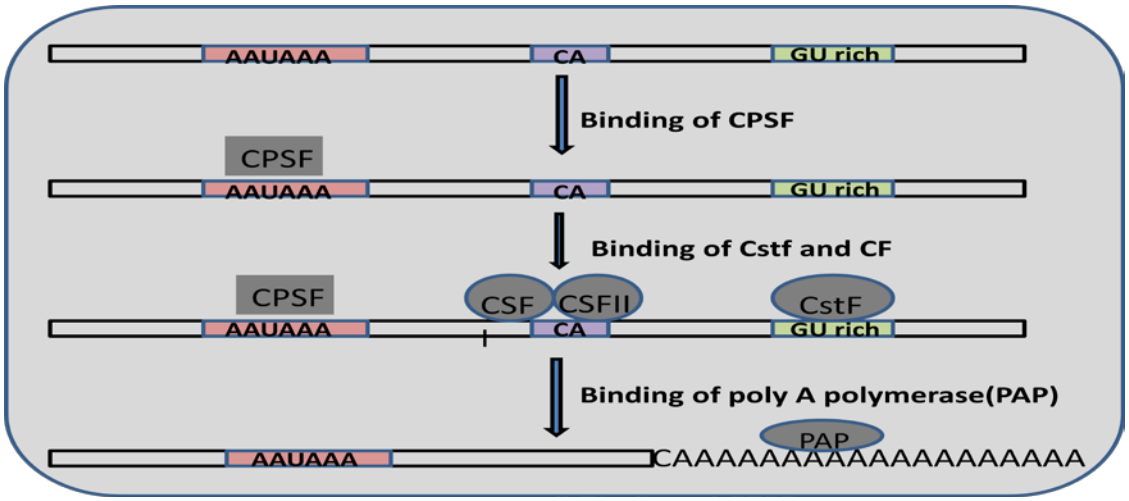


Figure 29.2:Tailing process in m RNA

INTRONS SPLICING: Introns are noncoding nucleotide sequence within a gene that don't code for protein and don't appear in the final mRNA molecule and are remove by the splicing. Protein-coding sequence of a gene (known as exons), which are interrupted by introns. The vast majority of eukaryotic genes are interrupted with non-coding a region (introns), which needs to be spliced out; however, histone protein coding genes in vertebrate is one among few exceptions. The occurrence of introns varies in eukaryotic species, some yeast species lack introns and many genes in eukaryotes carry a dozen of them. Few bacterial and Archeal genes also have introns. Introns can vary in length from 50 to 20000 nucleotides. In higher animals as humans, introns are more than exons. There are four classes of introns: (1) Group I, (2) Group II; both are self-splicing introns and does not involve any protein enzymes.(3) Spliceosomal introns- they are not self-splicing. (4) Introns that require ATP for splicing.

Splicing mechanism of group I and Group II introns: Splicing mechanism of both Group I and Group II involve similar steps of two **trans-esterificastion** reactions in which a ribose 2' or 3' hydroxyl group makes a nucleophilic attack on phosphorus and a new phosphodiester bond is formed at the expense of the old. Mechanisms of these groups differ in nucleophile which is used. Group I uses 3' hydroxyl group of guanine nucleotide as nucleophile. Group I introns are found in some nuclear, mitochondrial, and chloroplast that code for rRNAs, mRNAs, and tRNAs. Group I and Group II splicing mechanism are described below in figure 29.3(a) and in 2.3(b) respectively.

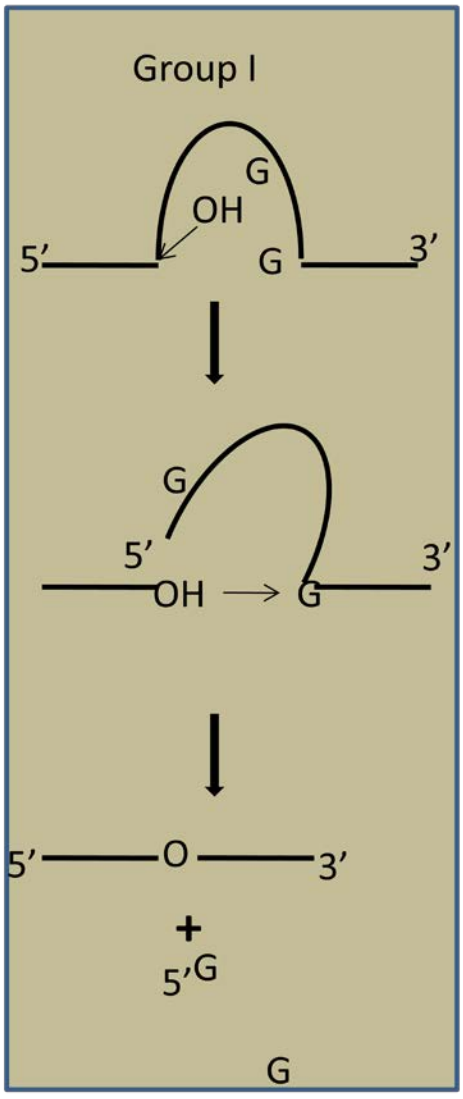


Figure 29.3(a):group I splicing

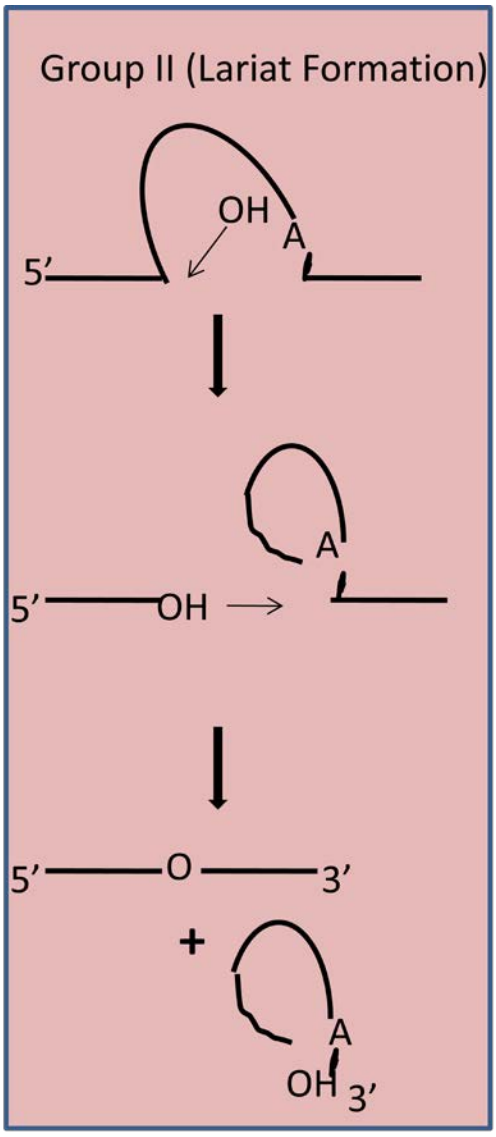


Figure 29.3(b):group II splicing

Alternative splicing: Alternative splicing is a method substantially used for many mammalian genes can result in multiple products that vary structurally and functionally from the same primary transcript.Sometimes, alternate splicing is unregulated phenomenon while in some it is strictly regulated. One of the best examples of regulated alternative splicing occurs in **sex determination in drosophila**. In drosophila three genes are involved in sex determination sex lethal gene (sxl), transformer gene (tra), doublesex gene (dsx). Due to alternative splicing, functional genes are produced in females and non-functional genes are produced in males. Alternative splicing occurs using two mechanisms - one when two poly (A) or cleavage sites are available in primary transcript. Cleavage occurs at either site resulting in two different products. Such mechanism is followed by **variable domains of immunoglobulins heavy chains** and their diversity is due to this mechanism of alternative splicing. Similarly alternative splicing with such mechanism results in **production of two different hormones**- calcium regulating hormone in rat thyroid and calcitonin-gene related peptide in rat brain. Other mechanism involves more than one 3' splice site for one 5' splice site. Hence splicing occurs by taking either of those 3' splice site resulting in different products. Here in **Figure 29.4** we are describing the steps of alternate splicing in which protein A formed by exon 1, 2 ,3 and protein B formed by exon1,3,4

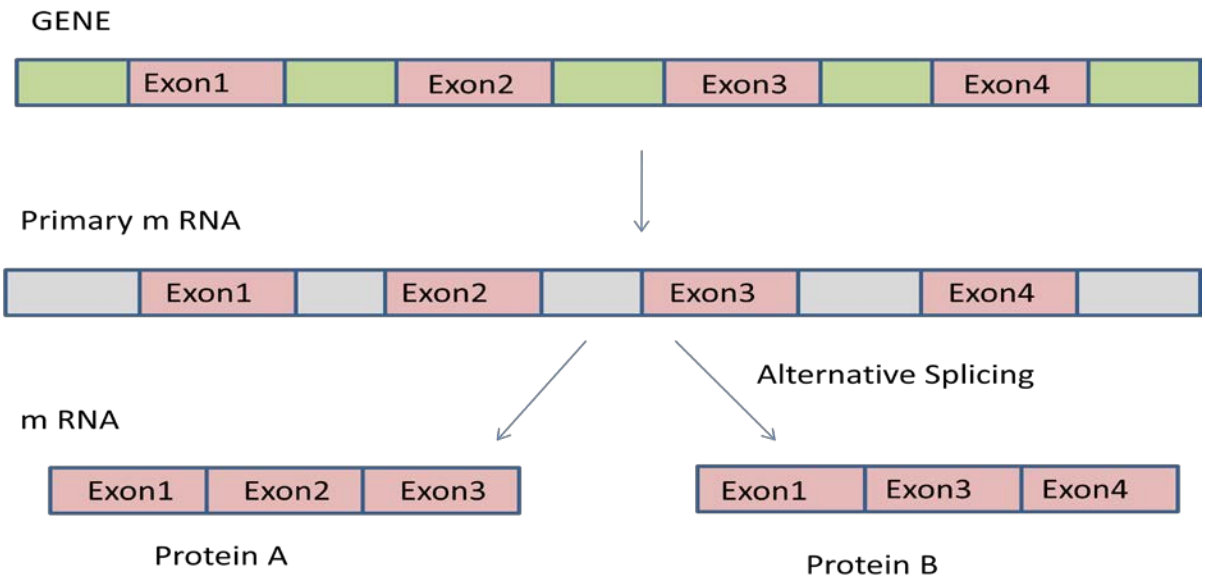


Figure 29.4:Alternate splicing occurs in 2 steps and form 2 different protein

Processing of rRNA: Eukaryotes have 80s ribosomes and prokaryotes have 70s ribosomes. Ribosomal RNAs are transcribed as long precursor sequence which is then modified at specific bases and cleaved to give mature products. In both bacteria and eukaryotes rRNA processing involves two basic steps of **cleavage and base modification**.

rRNA processing in Bacteria: rRNA precursor in bacteria is a 30s rRNA which modified and cleaved to give 23s rRNA, 16s rRNA, 5s rRNA and some t-RNA segments in between are also there sometimes. **30s pre- rRNA transcript** consist of 16s rRNA sequence followed by spacer which may have t-RNA sequence in some cases and then there is 23s rRNA sequence followed by 5s rRNA sequence near to 3' end. At times there is one more t-RNA sequence after 5s rRNA sequence at 3' end. There are seven different genes for rRNA in E.coli, they are essentially similar in sequence of rRNA segments but differ with number and sequence of t-RNA segments. Maturation process involves methylation of 30s rRNA precursor at specific sites occurring at 2'hydroxyl groups of bases. Some bases such as uridine is modified to pseudouridine or dihydrouridine. Further cleavage process is carried out using enzymes RNase III, RNase P, and RNase E at sites 1, 2 and 3 respectively as shown in the Figure 29.5. Intermediate products are formed namely 17s, tRNA, 25S and 5S. These are acted on by certain nucleases to give final products of 16s, tRNA, 23s, 5s rRNA respectively which is given in figure 29.5.

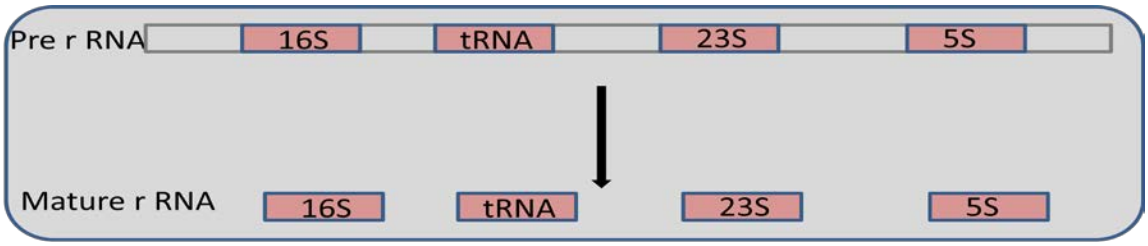


Figure 29.5:RNA splicing in Bacteria

rRNA processing in Vertebrates (Eukaryotes): In Eukaryotes nucleolus is the centre of processing ribosomal RNA. A **45s precursor** is formed by RNA polymerase I and processed in 90s **preribosomal nucleolar complex** to give 18s, 28s, and 5.8s rRNA which described in **Figure 29.6**. There is tight coupling of RNA processing with ribosomal assesmbly.5s rRNA is transcribed by RNA polymerase III from a separate gene. Precursor RNA undergoes methylation at more than 100 bases from 14000

nucleotides at 2’ hydroxyl group. Furthermore there is modification of bases such as uridine to pseudouridine etc. followed by series of cleavage reaction. Cleavage and modifications are guided by **snoRNAs (small nucleolar RNA**). In yeast, entire processing involves pre-RNA, 170 non-ribosomal protein, 70 snoRNA and 78 ribosomal proteins. snoRNA are supposed to be remnant of spliceosomes.

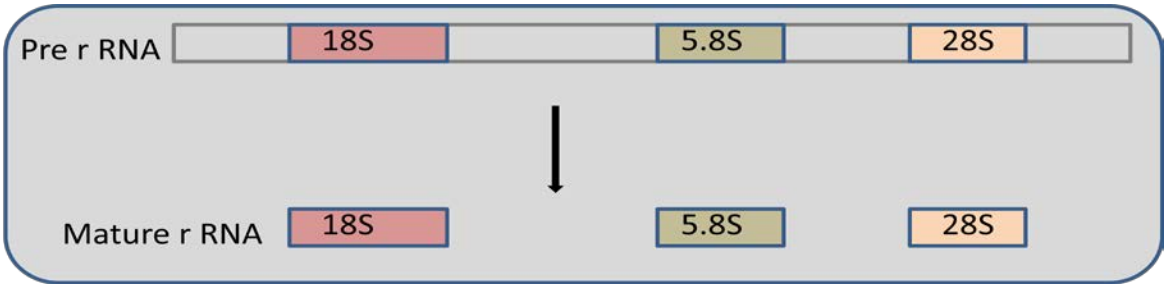


Figure 29.6:RNA splicing in Eukaryotes

Processing of t-RNA: In both Eukaryotes and prokaryotes t-RNA processing occurs. It is transcribed as long precursor; sometimes single primary transcripts carry more than one t-RNA segments which are separated by cleavage. Processing of pre tRNA involves cutting off of extra sequences by **endonucleases** such as **RNase P** at 5’ end and **RNase D** at 3’ end. RNase P is a ribozyme with RNA exhibiting catalytic activity. After removal of sequences from 3’ end, CCA sequence is added via enzyme tRNA nucleotidyltransferase This enzyme binds to CCA sequence at its active site and phosphodiester bond is formed with 3’ end. Furthermore, there is base modification occurring simultaneously such as methylation, deamination or reduction; in case of pseudouridine, uracil is removed and reattached to sugar through C5.Processing of tRNA is described in **figure 29.7.**

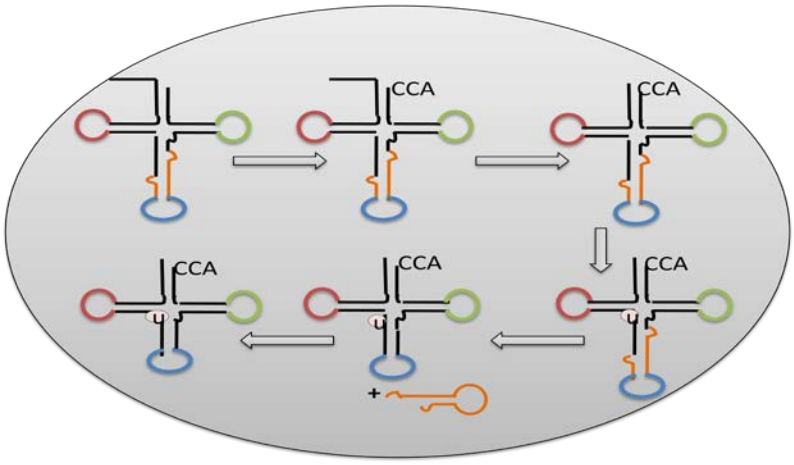


Figure 29.7: Processing of t-RNA

Lecture 30: Translation (Part-I)

Introduction: mRNA is the random sequence of nucleotides differentiated by bases attached to them which are **Uracil (U), Adenine (A), Cytosine (C), and Guanine (G).** Three nucleotides together code for specific amino acid and are called as **codons**. For example, GAU codes for Asparagine, GGU codes for glycine and so forth. These are called **genetic codes**. Following is the Figure 30.1, showing genetic code for 20 amino acids.

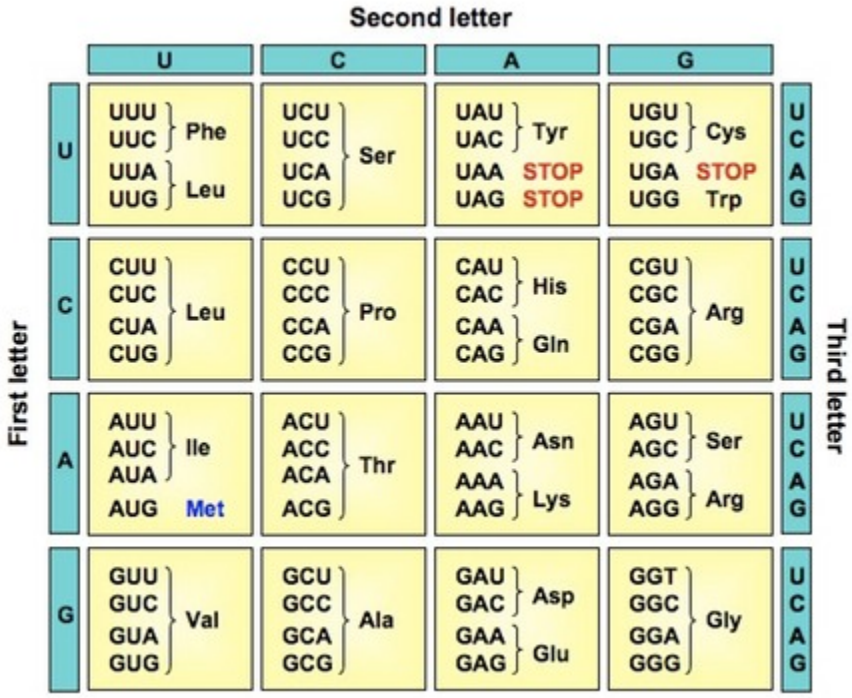


Figure 30.1 :Genetic code

General features of Genetic code

- The genetic code is **triplet** code called as codon: It is known that we have only 4 types of nucleotides that make the whole genome. It is also known that each codon consists of 3 nucleotides which means there are 4^3 = 64 possible amino acids. However, since there are only 20 amino acids it is obvious that more than one codon codes for single amino acid. This also illustrates wobble hypothesis.

- Each coding sequence has start and stop codon to initiate and terminate translation respectively. Usually start codon is AUG which code for methionine and stop codons are UAA, UAG, and UGA. In some cases, starting codons are GUG or UUG.

- The code is **unambiguous** which suggests that code is for only one amino acid.
- There is **no comma, gap** in the code.
- The code is **degenerate**. This means that one amino acid has more than one codon. For example phenylalanine is specific to two codons UUU and UUC. Only tryptophan and methionine are coded by single codon.
- The codon is **non-overlapping**. For example a code as AUGCUGGGUGAUUUUGUA then codons will be AUG, CUG, GGU and so on and not AUG, UGC, GCU and so on.
- Genetic code is **universal**, which suggests that genetic code and its meaning is common for all life forms. However there are some exceptions to this rule. For example, UGA is a stop codon but it codes for tryptophan in *Mycoplasma*, *Spiroplasma*, and mitochondria of several species. Similarly, CUG codes for Leucine in general but in yeast mitochondria it codes for threonine.

The basic process that occurs in protein synthesis is the formation of a peptide bond between the carboxyl group of one amino acid at the end of growing polypeptide and a free amino group on an amino acid. Peptide synthesis requires several components- mRNA, tRNAs, Ribosomes and several factors and enymes for conducting the process.

Messenger RNA (mRNA) : mRNA has a 5’ end, 5’ UTR, ribosomal binding site, coding sequence, 3’ UTR. In eukaryotes there are additional structures as 5’ Guanine cap and poly (A) tail (detail already discussed in earlier lecture) Figure 30.2. Messenger RNA (mRNA) has 3 reading frames out of which only one codes for desired protein. If in the sequence of bases there is no stop codon to interrupt the translation then that synthesis entire polypeptide chain and is that is called as **open reading frames (ORF)**.

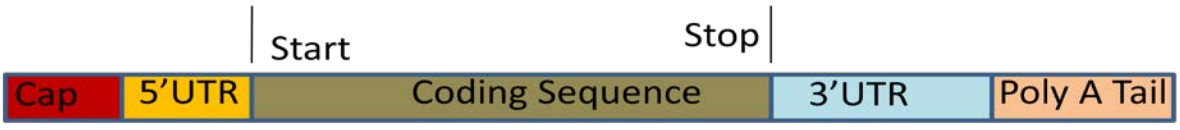


Figure 30.2 :Structure of m RNA

Transfer RNA (t-RNA): Transfer RNA (tRNA) has clover leaf structure in two dimension and L- shaped structure in 3 dimension. tRNA is 73 to 94 ribo-nucleotides in length. A tRNA molecule consists of 5' phosphate terminal, an **acceptor arm** that ends in **CCA terminal** at 3', **D loop** which often contains dihydrouridine, **anticodon loop**, and **T arm** which has TΨC where Ψ is pseudouridine. CCA sequence is important as it is important for recognition of tRNA and is also site of attachment of amino acid.Structure of t RNA are given below in Figure 30.3

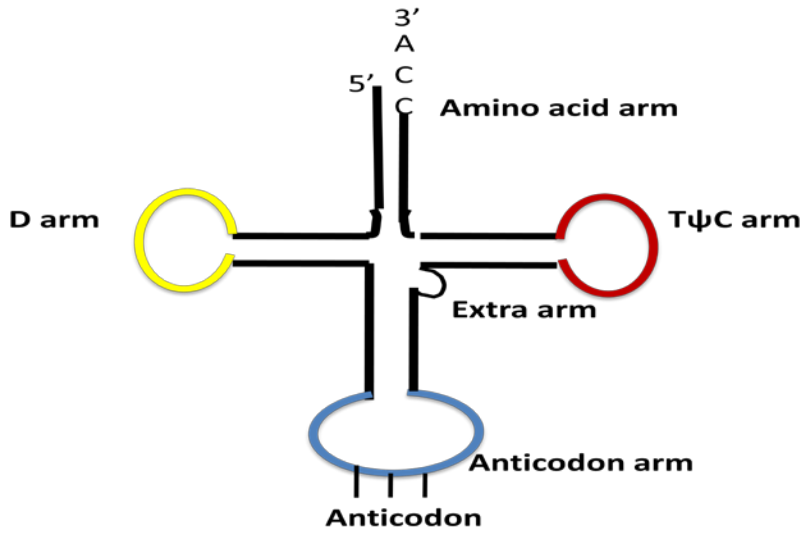


Figure 30.3 :Structure of T RNA

Each t-RNA is specific to amino acid that it carries it in CCA arm. There are 30-45 different tRNA in prokaryotes and 50 types in eukaryotes which suggest that there is more than one tRNA for single amino acid. For example for glycine there are two tRNA which are represented as $tRNA^{Gly1}$ and $tRNA^{Gly2}$.

Ribosomes: Ribosomes are ribonucleoprotein particles that contain r-RNA and proteins. Each ribosome is made of two subunits. In prokaryotes, mitochondria and chloroplast of prokaryotes there is **70S ribosome** which is composed of 50s and 30s subunits. In E.coli, 30s subunit consist of 16s rRNA (1541 nucleotides) and 21 r-proteins and 50s subunit contains 23s rRNA (2904 ntds), 5s rRNA (120 ntds) and 31 proteins. In eukaryotes there is **80S ribosome** which consists of 60s and 40s ribosomal subunit. 60s subunit consists of 28s rRNA (4718 nucleotides), the small 5s rRNA (120 nucleotides), 5.8s rRNA (160 nucleotides) and approximately 50 proteins. The 40s subunit consists of the 18s rRNA (1874 nucleotides) and 33 r-proteins. (Note:'s' means Svedberg's unit of sedimentation

coefficient). The 70s ribosome has three tRNA binding sites- **P-site** (or peptidyl-tRNA binding site), **A-site** (aminoacyl-tRNA-binding site), and **E-site** (deacylated tRNA, also called the **exit site**) Figure 30.4.

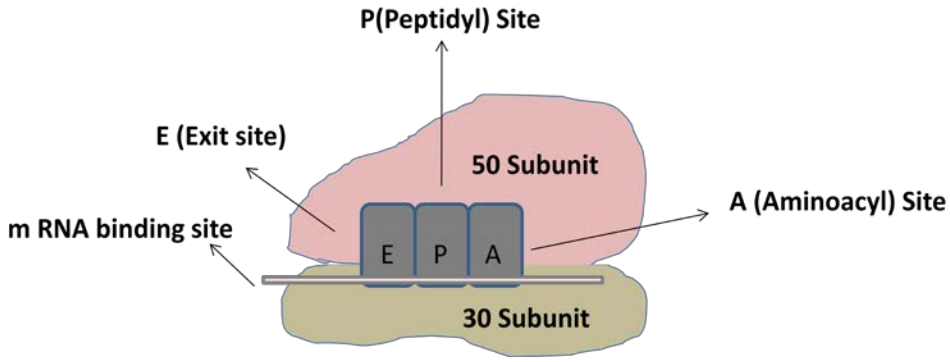


Figure 30.4 :Structure of 70s Ribosome

Activation of amino acid: During this process amino acids are attached to the t-RNA in the presence of enzyme Amino acyl-t-RNA synthetase, this enzyme activate the amino acids by attaching covalently to the t RNA, when t RNA get charged, its named as aminoacyl-t RNA. During this process amino acids are attached to t-RNA with high energy bond, so called as activated amino acids.

Amino Acids+ tRNA +ATP $\xrightarrow{\text{amino acyl t-RNA synthase}}$ **Amino acyl-tRNA+AMP+PPi**

Initiation: In eubacteria, first amino acid in the polypeptide chain is N formyl-methionine which is specific to three codes as AUG, GUG, and UUG. Whenever these codes are present at the initiation point they code for N-formyl-methionine but if they are present in between the coding sequence then they code for methionine and valine respectively. How does this happen? This is because of difference in initiator tRNA and the one used in between the process of translation (Figure 30.5). Initiator tRNA has unique features that distinguish from elongating tRNA in eubacteria. Firstly, there is no base pairing between 1^{st} and 72^{nd} bases in the acceptor arm. In anti-codon stem there are three consecutive GC base pairs. These base pairs are important to insert tRNA directly into P-site. These features are useful for interaction of this initiation tRNA with IF-2. Three GC base pairs are also found in Eukaryotes and archaebacteria just like in prokaryotes .Several initiation factors are required in the process, which include IF-1, IF-2, and IF-3.

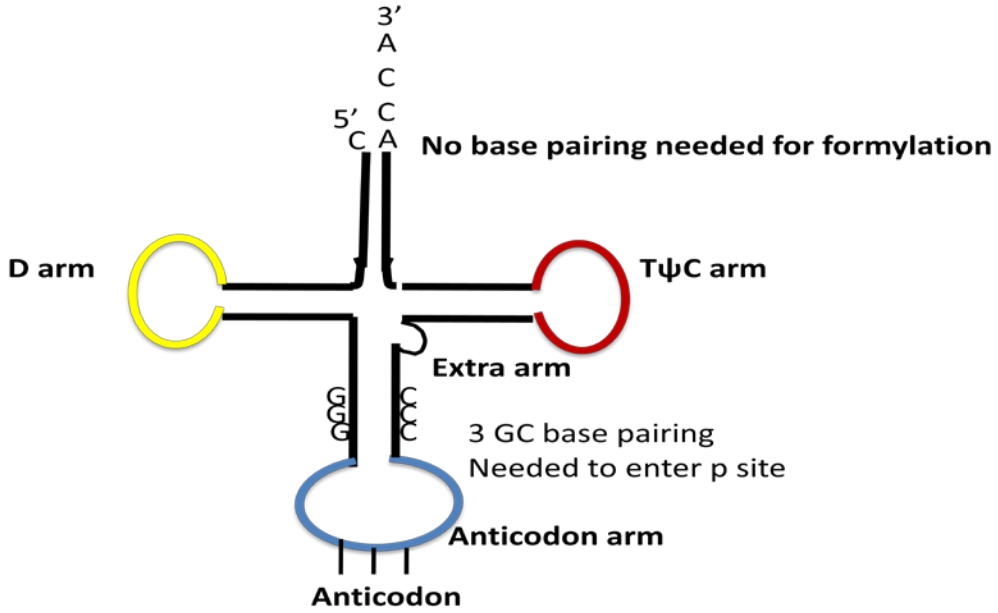


Figure 30.5 :Difference between initiator and elongation t RNA

Steps involved in initiation process: In the first step, small subunit of ribosome binds to mRNA such that initiation codon lies in partial P site. This gets possible due to activity of IF-3. It basically prevents untimely re-association of large and small subunit of ribosome. Moreover it promises accuracy of initiation site selection. In mRNA there is ribosomal binding site which consists of **Shine -dalgarno** sequence and initiation codon. This Shine-dalgarno sequence which is **5'-AGGAGGU-3'** and is located 10bp upstream of initiation codon is complementary to region near 3' end of 16s rRNA, a component of small subunit of RNA as discussed in given below Figure 30.6.

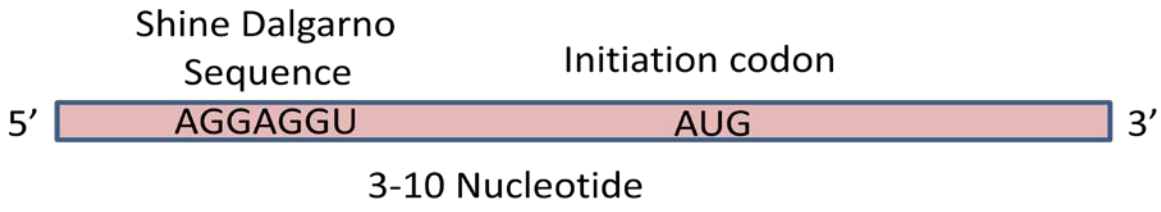


Figure 30.6 :Ribosome binding site

In the next step, initiator tRNA carrying N-formyl methionine enters partial P site and binds to mRNA via its anti-codon loop. IF-2 is responsible for this activity. It directs initiator tRNA to its correct position in the initiation complex. It also exhibits ribosome dependant GTPase activity. Once GTP is hydrolysed then 50S subunit joins to form complete ribosome, the different steps of initiation are given in Figure 30.7. Finally, when larger subunit also joins the complex it forms complete P site and A-site. Second charged tRNA (amino acid laden) enters **A** site. This tRNA as per the rule has anticodon corresponding to codon in mRNA.

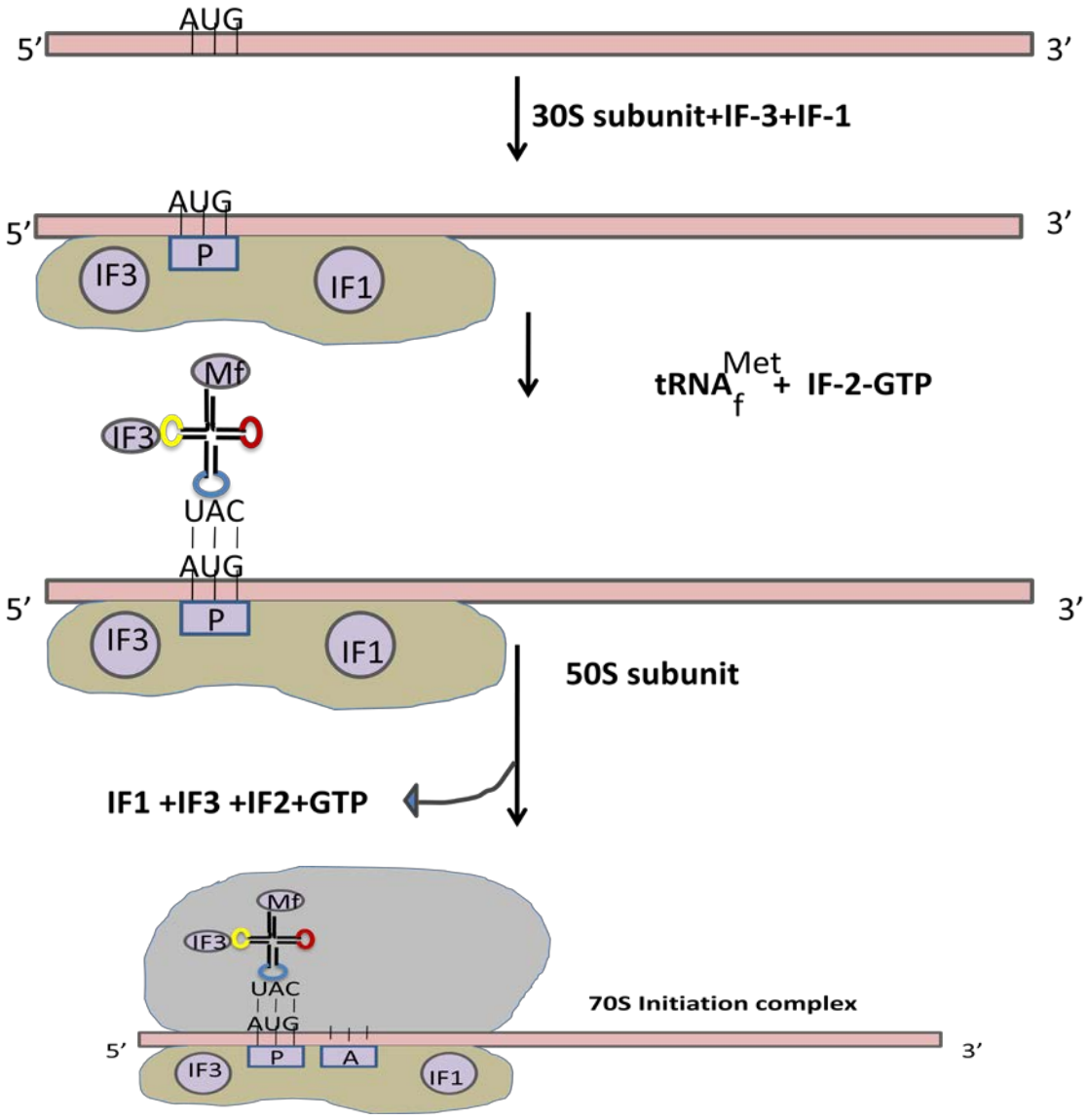


Figure 30.7 :Initiation of protein synthesis

Differences between initiation of prokaryotes and eukaryotes: In translation process, main difference between eukaryotes and prokaryotes is in the initiation process itself. Some major differences between Eukaryotic initiation and prokaryotic initiation are as follows. In Eukaryotes, there is only one start codon for Eukaryotes AUG and it codes for methionine and not N-formyl-methionine. Eukaryotic cells need more initiation factors than prokaryotes. Eukaryotic cells require 12 initiation factors. In Eukaryotes, process of association of mRNA with smaller subunit (40s) is more complex than prokaryotes. 40s first subunit identifies 5' methylated cap of m-RNA and then there is scanning process involved wherein initiation codon is recognized. This recognition is aided by ATP dependant helicases that hydrolyse ATP. This recognition of initiation codon is also aided by **Kozak sequences 5'-ACCAUGG-3'** similar to Shine-dalgarno sequence in prokaryotes Figure 30.8.

Figure 30.8 : Ribosome binding site in eukaryotes

Important thing to notice that in prokaryotes there is no scanning process, 16s subunit directly binds to region so that initiation codons is in P-site whereas in eukaryotes there is proper scanning process.

However, at first, initiation factor eIF3 and eIF2 binds 40s subunit where the former factor prevents premature association of 60s subunit with 40s and later factor which is GTP binding trimeric protein binds to $tRNA^{Met}$ (ternary complex= eIF2+GTP+$tRNA^{Met}$) . **Ternary complex** and eIF3 binds to 40s subunit forming **43s initiation complex**. All this happens without the presence of mRNA.

Then mRNA binding to 40s takes place with the help of initiation factor eIF4F. It is a heterotrimer which consists of eIF4G, eIF4E, and eIF4A. eIF4E binds to 5' cap and eIF4A acts as helicase to unwind any secondary structure at 5' end. eIF4G interacts with poly (A) tail via poly (A) binding protein associated (PBP). It implies that there is some sort of circular organization during protein synthesis.

eIF4F –mRNA complex is associated with another initiation factor eIF4B which also stimulate helicase activity of eIF4A. Now, the complex eIF4F-mRNA-eIF4A joins 43s initiation complex via protein-protein interaction.

Scanning process as mentioned earlier is carried out with the help of factors eIF1 and eIF1A. 43s pre-initiation complex translocation along with mRNA till it finds AUG. As soon as it reaches AUG, it forms 48s complex. Furthermore eIF5A also joins which results in GTP hydrolysis by eIF2. Hydrolysis releases eIF2 and eIF3 and ultimately eIF5B causes addition of 60s subunit also. (Note: eIF2 can participate in another round of initiation, its bound GDP must be replaced with GTP. This process is catalysed by the eIF3B, a guanine nucleotide exchange factor (GEF).

ELONGATION : It is the cyclic process, elongation process start from formation of first peptide bond to addition of last of amino acid.ahe amino acid added to the chain one at the time to the nascent peptide chain. Addition of amino acid is very rapid process. The peptide sequence is in order of codon and anticodons in m-RNA, Rate of elongation is nearly 15 amino acids per second.

There are some requirements regarding to elongation-

(1) m-RNA and 70S ribosome
(2) Amino acyl t RNA
(3) Elongation factors

Elongation is carried out by ribosome in three different stages:

(1) Decoding : It is codon directed binding During the process of ribosome select and bind of incoming amino acyl t RNA at a site whose anticodon is complementary to the codon of m RNA, Decoding region of 16s RNA confirm the proper base pairing between codon and anticodon

(2) Peptide bond formation: In this process peptidyl group of p site of t RNA is transferred to the amino acyl group in a site through the peptide bond.

(3) Translocation: In this case t-RNA of A site is transferred to p site to make a space for next amino acyl t RNA at A site, and A site of t-RNA is shifted at E site. This shift is also coupled with ribosome movement along with m-RNA.

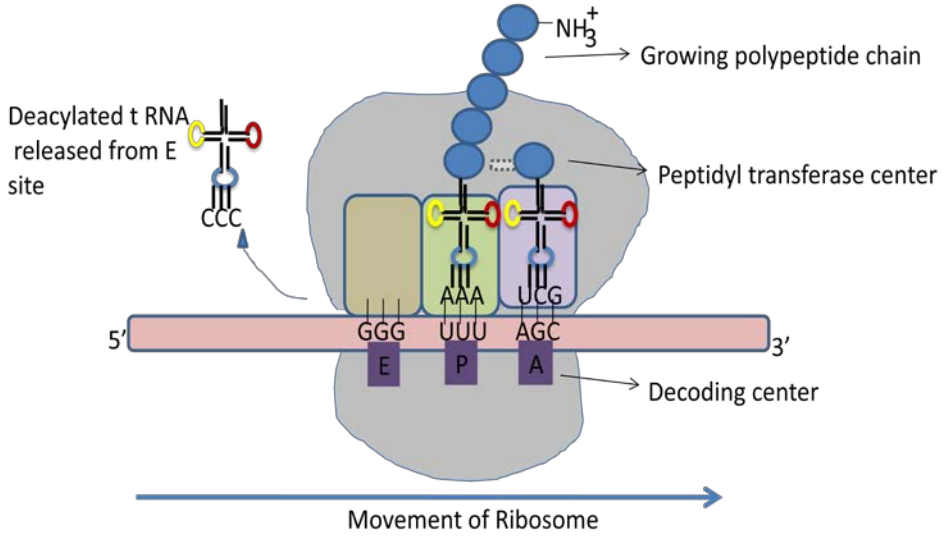


Figure 30.9 :Movement of Ribosome ot m RNA

Elongation process requires three elongation factors

EF-Tu	It is G protein which binds to amino acyl t RNA and directs it to correct position at ribosome a site.
EF-Ts	Its main function is to regenerate to EF-Tu and hydrolysis of GTP
EF-G	It is also a G protein which mediates translocation.

Process of chain elongation on ribosome: EF-TU promotes the entry of amino acyl t-RNA into the A site of 70 s ribosome. Firstly EF-TU bind to GTP and it activated the EF-TU GTP complex which bind t-RNA. When codon and anti-codon base pairing stabilised then hydrolysis of GTP occurs which converts into GDP and Pi, which helps in binding of aminoacyl t RNA to A site and after this EF-TU is released. EF-TS is catalysed the release of EF-TUGDP from ribosome and regenerate EF-TUGTP, Its main work is to recycle the EF-TU,given in Figure 30.10.

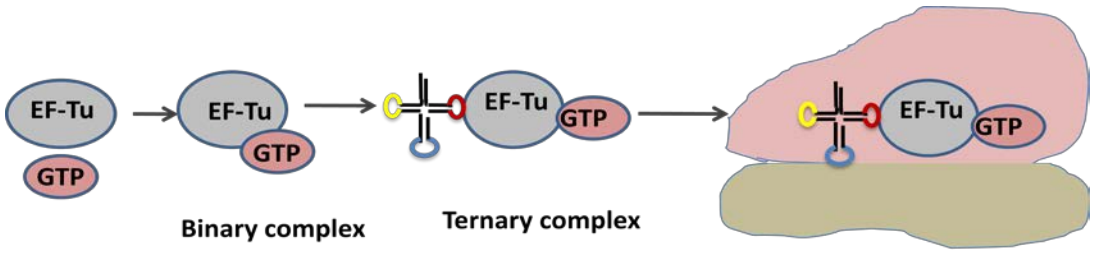


Figure 30.10 :Working of EF-TU

2) Peptidyl Transfer: it is peptide bond formation steps which the amino group of peptide bond are linked to t-RNA molecule in A site and carboxyl end of polypeptide chain uncoupled from t-RNA molecule in P site. This reaction is carried out by the enzyme peptidyl transferase. Peptidyl transferase is an enzyme which is associated with 23s r-RNA of 50S-ribosomal subunit, peptide bond formation involves O to N migration and conversion of ester into amide sa given in Figure 30.11..

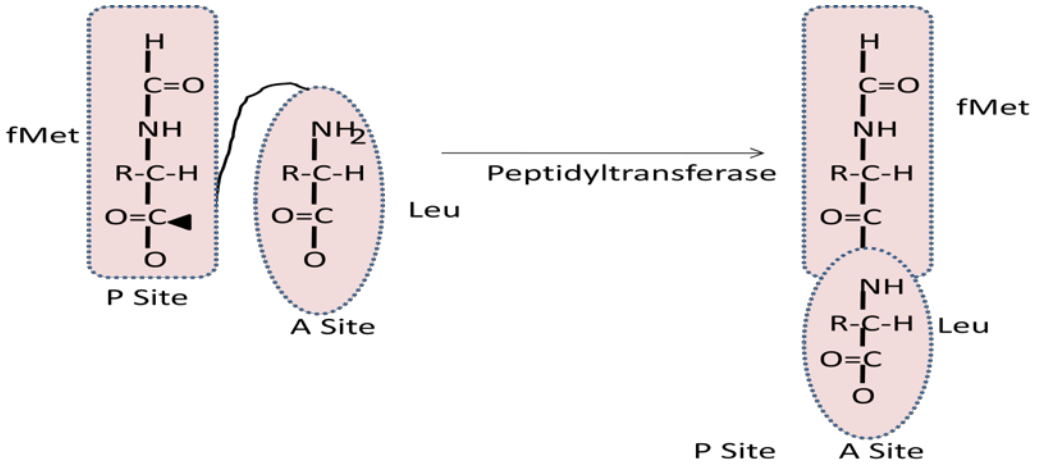


Figure 30.11:Structure of 70s Ribosome

TRANSLOCATION: Three things are necessary for translocation as given in Figure 30.12

(1) Deacylated RNA remove from P site

(2) Peptidyl t-RNA move from A to P site.

(3) Ribosome should move on m-RNA, one codon down, so next codon can come at A site.

Translocation step carried out by EF-G factor, During translocation accepter end of both t-RNA of A and P site are interact with peptidyl transferase centre of 23s r-RNA of 50 subunit. In translocation A and P t-RNA transfer to P and E site respectively, as ribosome move three nucleotide along m-RNA chain in 5' to 3' direction. During translocation step GTP is converted into GDP; and uncharged t-RNA released from P site to E site (exit site) and newly formed peptidyl t-RNA from A site to P site. Elongation process is nearly same in both prokaryotes and eukaryotes.

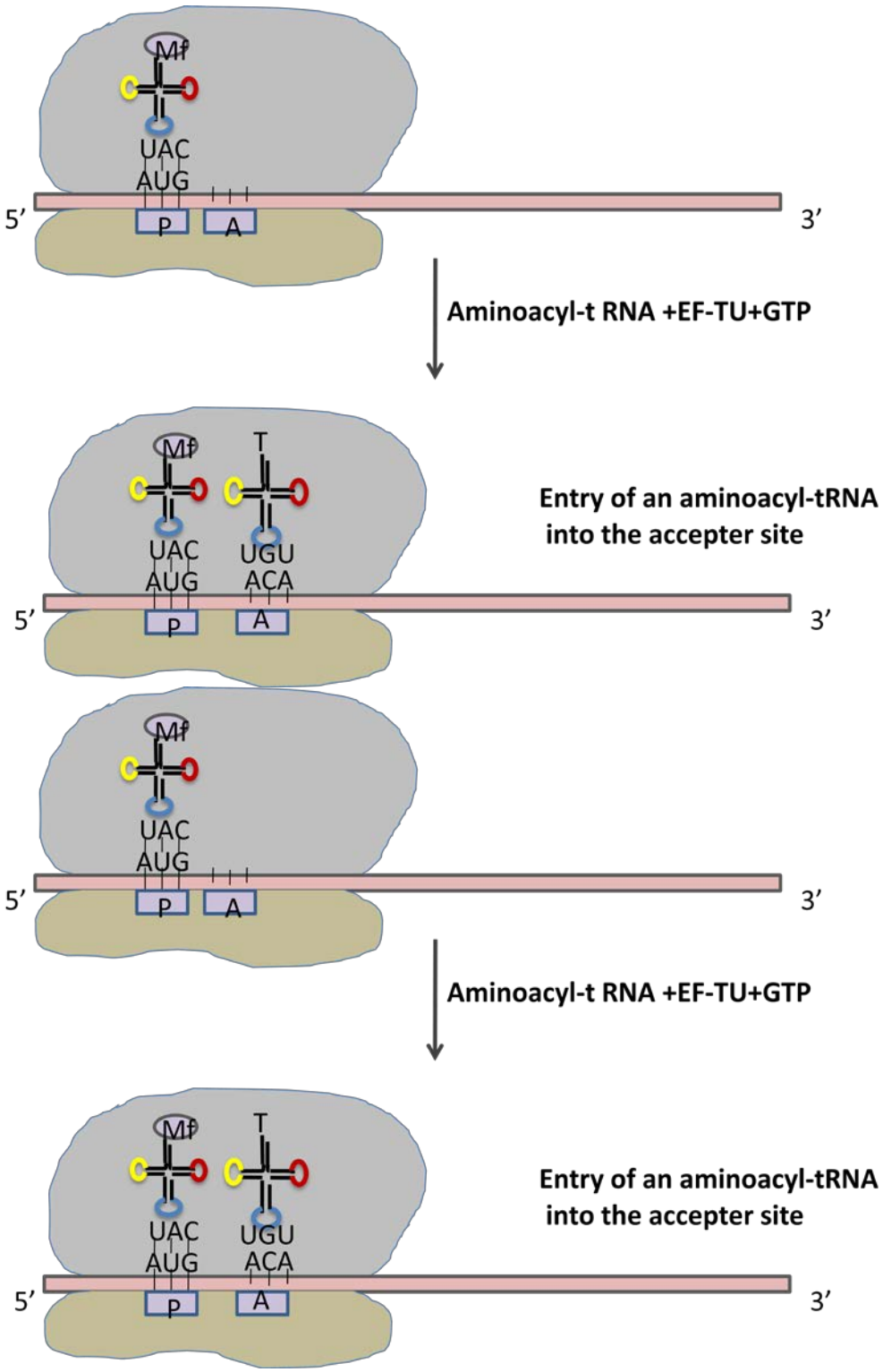


Figure 30.12: Elongation of protein synthesis

Termination: Termination of translation occurs due to the stop codon, There is three stop codon (UAA, UAG and UGA) present. Out of these three when one of the stop codons appears in the A site of the ribosome it cause termination because there is no tRNAs present corresponding to these codons, so tRNA is not bind codon and cause **termination**. During termination release factors (RF) are involved. when UAA or UAG is in the A site, RF1 binds to the ribosome. when UAA or UGA is in the A site, RF2 binds to the ribosome. RF3 is a type of GTPase which main function is to catalyse the release process, through **GTP** binding and hydrolysis.

1. Release factor (RF1) or Release factor (RF2) binds to the ribosome nearly to the A site.

2.Polypeptide chain are released from the ribosome by the peptidyl transferase complex, peptidyl transferase complex transfer the carboxy terminal residue of polypeptide chain from t-RNA of P site to water molecule.

3. Now the release factors (RF)and **GDP** released, and t RNA also freed.

4. Now 70S ribosome is unstable due the presence of initiation factors IF3 and IF1 and ribosome recycling factors. as a result, 70S ribosome disrupts into 30S and 50S subunits and prepared for initiation. The sequential events of termination are shown in Figure 30.13. It occurs as follows:

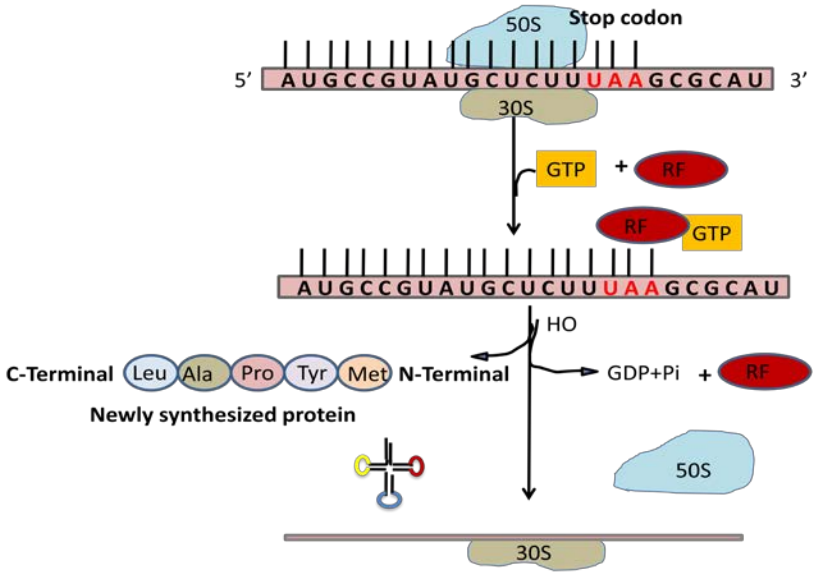


Figure 30.13 :Structure of 70s Ribosome

Lecture 31: Translation (Part-II)

Summary of Previous Lecture: In lecture 30 we discussed about the protein synthesis. As discussed, it is completed in three steps; initiation, elongation, and termination. Translation started by the initiation codon AUG or GUG, when charged t RNA come at the ribosome site which are moving at the m RNA chain.Anticodon which are present in the charged t RNA is complementary of m RNA codon. so ribosome is sliding on the m RNA chain, and when termination cordons come it cause chain termination because there is no any antic don present on t RNA which is complementary to codon of m-RNA. Now in this lecture we are going to discuss about the post translational modifications.

POST TRANSLATIONAL MODIFICATIONS: Post Translational modifications are chemical modifications which play a critical role in functional proteomics, because it regulate position, activity and interaction with other cellular molecules like proteins, lipids, nucleic acids, and cofactors etc. Post Translational modifications occur at different amino acids side chains or at peptide linkages and is carried out by different enzymes like phosphatases, kinases, transferases and ligases which main function are mainly addition or removal of different functional groups or sugars to or from amino acids side chain. And it also involves the proteases, which main function is to cleave peptide bonds or to remove specific sequences or regulatory subunits from a large polypeptide.

Post Translational modification is not a rapid process, but it can occur at any step in protein life cycle, Some proteins are modified soon after translation because it require proper protein folding of nascent protein for stability. Some other modifications occur after folding and localization to activate or inactivate catalytic activity or to stimulate the biological activity of the protein. Proteins are also linked covalently with the tags that target a protein.

Protein Post Translational modifications can also be reversible or irreversible, it basically depend upon the nature of the protein modification. Example, kinase phosphorylate side chains of specific amino acid of any protein, it is a very common method of protein activation or inactivation. Phosphatases hydrolyze the phosphate group to remove it from

the side chain of specific amino acids of a protein and reverse the biological activity against the kinases.

PHOSPHORYLATION: Phosphorylation is an important post-translational modification. It is prevalent from bacteria to higher eukaryote sustaining as mainly two types. First it acts to functionally regulate the catalytic activity of the protein by defining a rigid and permanent 3-D protein structure. Secondly, temporarily phosphorylate proteins serve as anchors for other protein substrates in signal transduction pathways. As, such it acts as a key-player in the regulation of many cellular processes like cell-cycle, cell growth, apoptosis and regulation of signal transduction pathways.

Mechanism of phosphorylation: In eukaryotic cells, phosphorylation is known to occur only at the side chains of three amino acids, serine, threonine and tyrosine. This is because these amino acids harbour a nucleophilic (–OH) group. The terminal phosphate group (γ-PO32-) on the universal phosphoryl donor adenosine triphosphate (ATP) serves as the point of nucleophilic attack from that -OH group, which results in the transfer of the phosphate group to the amino acid side chain.Magnesium (Mg2+) ions acts as catalyst by chelating the γ- and β-phosphate groups resulting in lowering of the threshold for phosphoryl transfer to the nucleophilic (–OH) group as we discussed in Figure 31.1 .

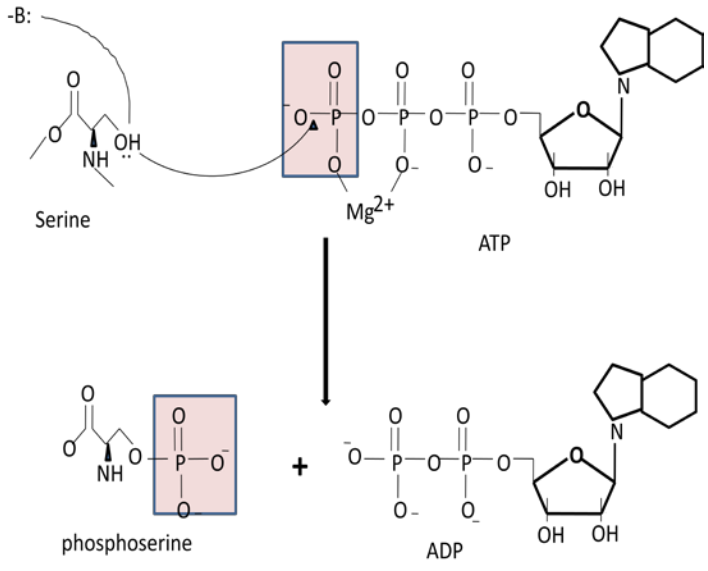


Figure 31.1 : Mechanism of phosphorylation

These conformational changes can affect the protein in two different ways; (1) Phosphorylation causing conformational changes in the phosphorylated proteins, These conformational changes stimulate the catalytic activity of protein, so any protein can be activated or inactivated by the phosphorylation. (2) Phosphorylated proteins employ the neighbouring proteins which have structurally conserved domains that distinguish and bind to phosphomotifs. These domains are specific for diverse amino acids. Protein phosphorylation is a reversible Post translation modification which is carried by kinases which phosphorylate and phosphatases which dephosphorylate to substrates (Figure 31.2). These two type of enzymes make possible the dynamic nature of phosphorylated proteins. So the balance concentration of kinase and phosphatase is very important for the cell and it is also important for the catalytic efficiency of a particular phosphorylation site.

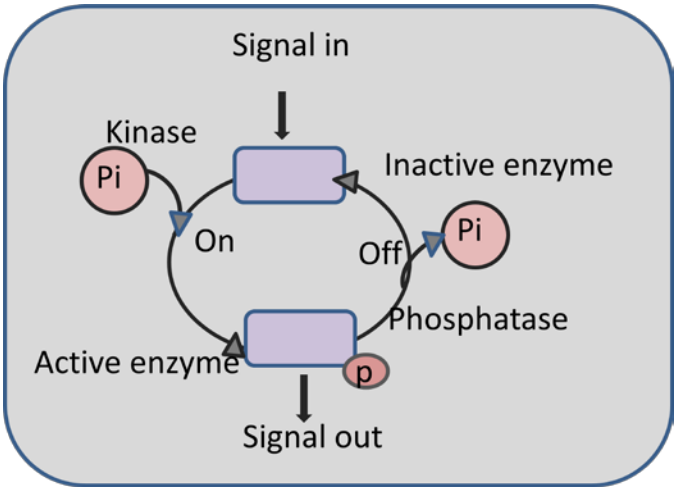


Figure 31.2 : Working of phosphatases and kinases

Glycosylation: Glycosylation is a dire function of the biosynthetic-secretory pathway in the endoplasmic reticulum (ER) and Golgi apparatus. Approximately 50% proteins characteristically expressed in a cell go through this alteration, which involves the covalent addition of sugar moieties to specific amino acids. Mostly, soluble and membrane-bound proteins expressed in the endoplasmic reticulum undergo glycosylation , including all secreted proteins, surface receptors and ligands . Moreover, some proteins that are transferred from the Golgi to the cytoplasm are also glycosylated.

Protein glycosylation has several roles to play. In the ER, glycosylation is important to govern the standing of protein folding. it is a quality control mechanism to assurance that only correctly folded proteins will be transferred to the Golgi. Glycosylated proteins (glycoprotein) are discovered in nearly every living organism including eukaryotes, eubacteria and archae. Eukaryotes hold the highest variety of organisms that express.

Types of Glycosylation: There are several types of glycosylation N- glycosylation O-glycosylation and C-linked glycosylation, glypiation and phosphoglycosylation as per the type of the sugar-peptide bond and the oligosaccharide attached.

N-glycosylation: In this type of glycosylation, glycans are covalently bound to the carboxamido nitrogen on asparagine (Asn or N) residues. This is the most common type of glycosylation- 90 percent of glycoproteins are N-glycosylated. It occurs as soon as protein is synthesised, rather almost simultaneously with the translation process.

N-glycosylation process and enzymes involved in the process are conserved across very wide range of species of eukaryotes and archae. N-glycosylation can be broken down into several events which include-

1. Precursor glycan assembly
2. Attachment
3. Trimming
4. Maturation

Initial steps of glycosylation are identical for all proteins but there is difference in trimming and maturation steps which generate diversity in glycosylated proteins.

Precursor glycan assembly: The purpose is to assemble 14 sugar molecules which consist of 3 Glucose (Glc), 2 N-acetylglucosamine (GlcNAc), and 9 Mannose sugar molecules on the ER membrane via dolichol. Dolichol is a polyisoprenoid lipid carrier rooted in the ER membrane via a pyrophosphate linkage (-PP-). Firstly, first 7 sugar molecules obtained from sugar nucleotides (UDP- and GDP-sugars) in the cytoplasm, are added. After this assembly, complex flips to ER lumen side and seven more sugars are added to form $Gcl_3Man_9GlcNAc_2$-PP-dolichol precursor glycan.

Glycan attachment: The pre-assembled glycan is attached to newly formed polypeptide chain via enzyme **oligosaccharide transferase (OSTase).** OSTase looks for consensus sequence Asn-X-Thr/Ser, where X may be any amino acid except proline. Once it is recognized then 14- mer precursor glycan is attached to carboxamido nitrogen on Asn of the developing polypeptide chain. β-OH group of serine and threonine residue acts as hydrogen bond donor for the reaction. Here in given figure we are discuss about the diagrammatical arrangement of glycogen arrangement. Glycan synthesis starts at the cytosolic face of the ER and when the structure is flipped into the ER lumen then it completed. Now OSTase enzyme transfer the precursor glycan to the Asn residue on the nascent protein as we discuss in given Figure 31.3.

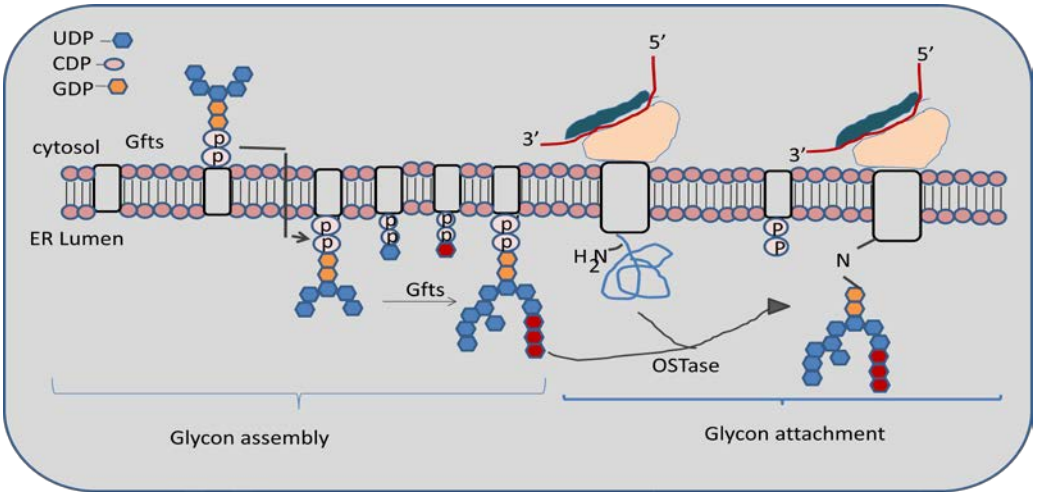


Figure 31.3: Glycon assembly and arrangement

Trimming in ER: Sugar molecules are trimmed off by glycosidases via hydrolysis. This process occurs both in golgi and ER and both serves different purposes. In ER, trimming is done to both screen protein folding and specify when proteins should be degraded. There is continuous cycle of removal and addition of terminal glucose molecules that enables proper protein folding. At first, two terminal Glc are removed followed by binding of calnexin and calreticulin to the chain via remaining Glucose molecules. These then act as chaperons for folding of proteins. Final Glc is hydrolysed by glycosidase II to discharge glycoprotein from chaperons. Folded output from this process has not yet acquired native conformation. Now, UDP-glucose glycoprotein glucosyltransferase acts

on non-native folded protein and transfers a Glc to the glycoprotein, and the protein again is constrained to the lectin chaperones to aid proper protein folding**.** The cycle continues till the protein is properly folded. Properly folded proteins are trafficked to golgi bodies for further processing. Those proteins which are not properly folded are identified by ER-resident mannosidase (ERManI). These proteins are deglycosylated by glycanase N and then forwarded to ER-associated degradation (ERAD).

Glycan maturation in the Golgi: In golgi bodies both trimming and addition of sugar molecules takes place which generates diversity in glycan structure of glycoproteins. Up to previous step of trimming in ER, glycan structure of all proteins is same. In golgi bodies, each cisternae carries specific enzyme and step-wise processing occurs. The ultimate glycan structure can be majorly classified in two catagories. **Complex oligosaccharides – contain multiple sugar types.**

High-mannose oligosaccharides – multiple mannose residues
Hybrid – branches of both high mannose and complex oligosaccharides

When the glycan is accessible to Golgi mannosidase I and II it forms complex oligosaccharide as given in Figure 31.4. These enzymes cut off several mannose residues and then get glycosylated by GlcNAc transferase. This process results in formation of common core region. After this, via several Gtfs, multiple sugar moieties are added to the core which could be of variable length or could be branched also. These variable length chains or branched chains commonly include sugars such as GlcNAc, galactose (Gal), N-acetylneuraminic acid (NANA or sialic acid) and fucose. Complex oligosaccharide is resistant to endoglycosidase H (endo H) unlike high mannose containing glycan, and thus forms the basis of differentiating the two categories of glycan. High mannose oligosaccharide has high mannose content and do not carry other sugar moieties. Apparently, conformation of glycoproteins is such that enzymes required to process glycan to form complex oligosaccharide are inaccessible. There are certain glycoproteins that have hybrid oligosaccharides, including a combination of complex and high-mannose glycans,

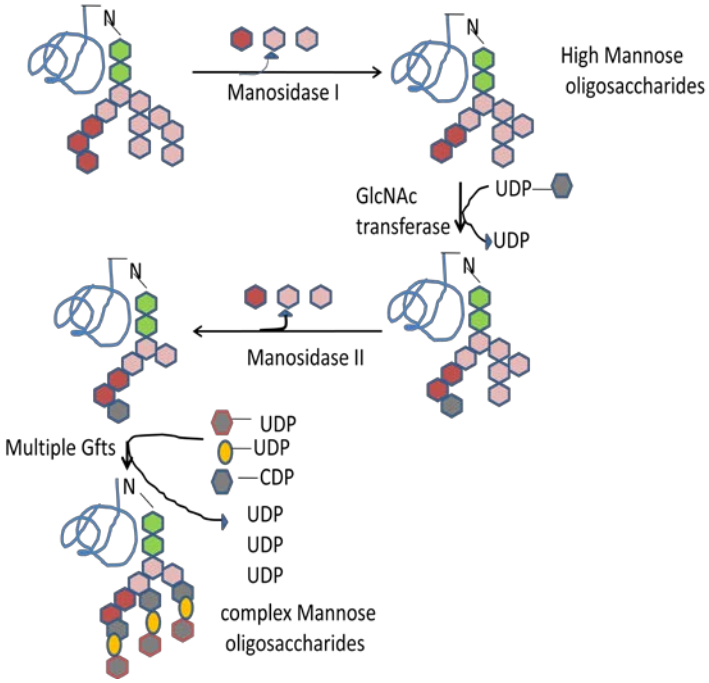


Figure 31.4: Glycon maturation

O- Glycosylation: O-glycosylation is common for high molecular weight proteins found in mucus secretions and proteoglycan core protein that form extra-cellular matrix. O-glycosylation is also common in antibodies. Even though N-glycosylation has already occurred in these proteins it does not exclude O-glycosylation.

O-glycosylation occurs in Golgi apparatus after translation on serine and threonine side chains.O-glycosylation can also occur in the cytosol and nucleus to regulate gene expression or signal transduction through other Gtfs ,basic O gycosylation of serine or threonine are given below in Figure 31.5.

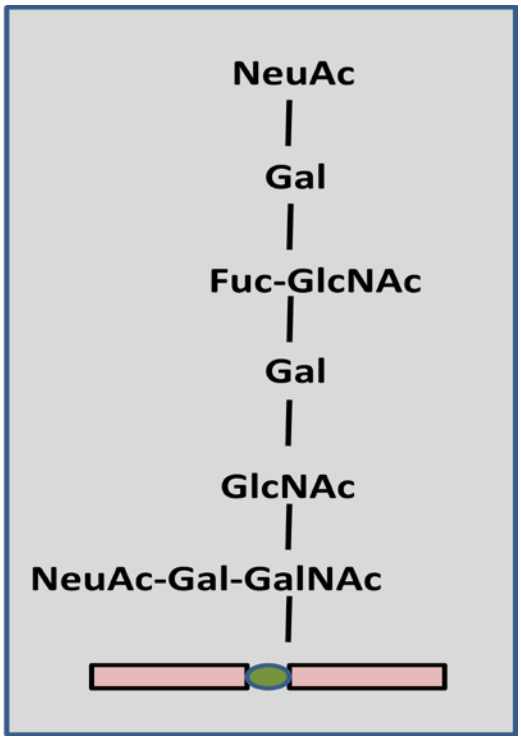


Figure 31.5: O-Glycosylation of threonine or serine

O-glycosylation is carried out by enzyme N-acetylgalactosamine (GalNAc) transferase, which transfers a single GalNAc residue to the β-OH group of serine or threonine. Some proteins are glycosylated with GalNAc, some with glucose, xylose, and mannose and so on. It is based on which cell type and species glycosylation is occurring. Sugar moieties are obtained from sugar nucleotides just like in N-glycosylation. O-glycosylation forms less complex structure compared to that in N-glycosylation.

Phosphoglycosylation: This type of modification is limited to parasites such as Leishmania and Trypanosomaand slime moulds like Dictyostelium. It is abundant in leishmania and is used to make proteophosphoglycans which is important for the organism to protect it from host's defence mechanism. In this modification glycans are linked to serine and threonine residues by phosphodiester bond.

C-glycosylation or C-mannosylation: This is different from other types of glycosylation. In others there is bonding between carbon-nitrogen and carbon-oxygen but C-mannosylation is characterized by Carbon-carbon bonding. This process is carried out by enzyme *C*-mannosyltransferase (c-Mtf) which links C1 of mannose to C2 of indole ring of tryptophan. The enzyme identifies the precise sequence Trp-X-X-Trp and relocates a mannose residue from dolichol-P-Man to the first Trp in the sequence. C-mannosylation has been spotted in multiple cell lines (42) and rat liver microsomes.

Ubiquitinization: This is one another post-translational modification where ubiquitin is added to protein. Ubiquitin is the eukaryotic protein coded by 4 different genes in mammals UBA52,RPS27AUBB, and UBC. Protein is made of 76 amino acids and has a molecular mass of about 8.5 kDa. It is characterized by presence of C-terminal tail and 7 lysine residues. In ubiquitinization, basically, carboxylic acid of the terminal glycine from the di-glycine motif in the activated ubiquitin forms an amide bond to the epsilon amine of the lysine in the modified protein. It marks the cellular protein for the process of degradation via proteosome, changes protein's location, prevent or promote protein-protein interaction.

Steps followed in ubiquitinization process.

Activation of Ubiquitin: It occurs in a two-step reaction process. At first, ubiquitin interacts with ATP and forms ubiquitin-adenylate intermediate. In the next step, ubiquitin is transferred to E1 active site containing cysteine residue. This causes formation of thioester linkage between the C-terminal carboxyl group of ubiquitin and the E1 cysteine sulfhydryl group.

Transfer of Ubiquitin from E1 active site to E2 active site via trans-esterification reaction occurs.

E1 + ATP + Ub → E1.Ub-AMP + PPi

E1.Ub-AMP + Ub → E1-s-co-Ub-AMP-Ub

E1-s-co-Ub-AMP-Ub + E2-SH → E2-s-co-Ub + E1.AMP-Ub

E2-s-co-Ub + Protein-NH2 → E2-SH + Protein-NH-co-Ub

In the last step of the ubiquitylation cascade there is formation of an isopeptide bond between a lysine of the target protein and the C-terminal glycine of ubiquitin via activity of one of the hundreds of E3 ubiquitin-protein ligases. All steps are are given in Figure 31.6. In E1-E2-E3 cascade, one E1 molecule causes binding to several E2 which in turn bind to hundreds of E3 in hierarchical fashion.

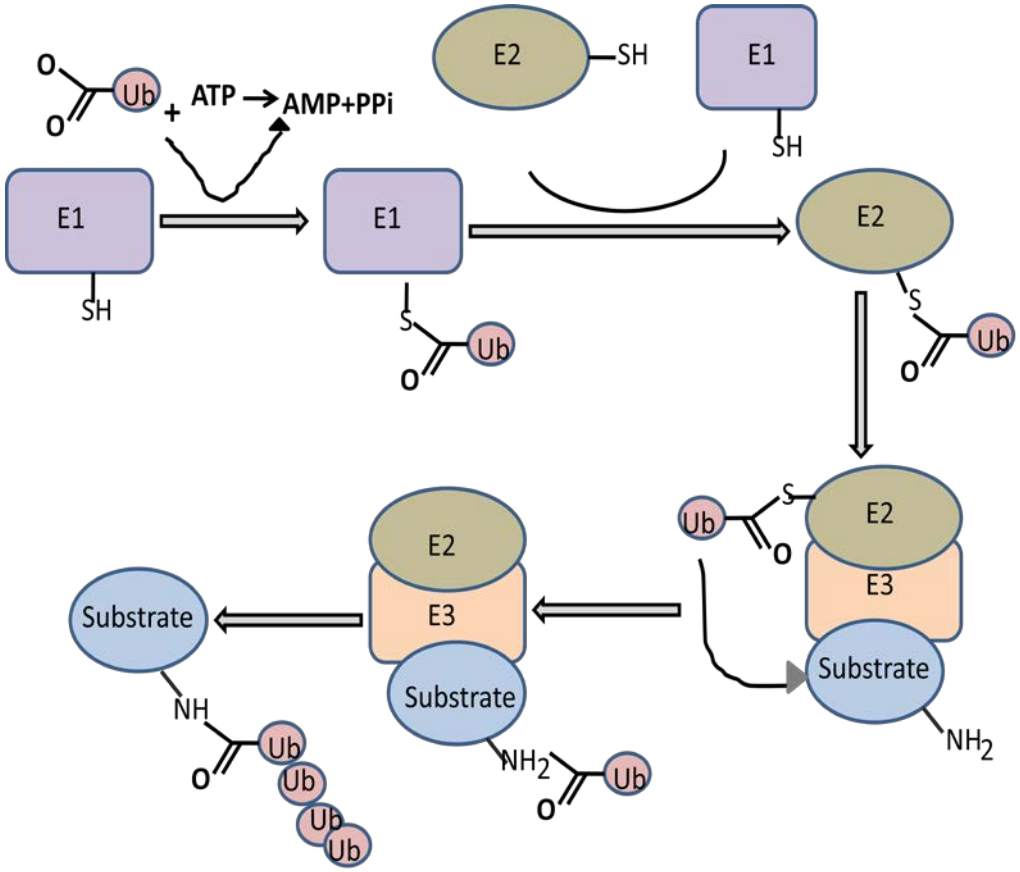


Figure 31.6 : Sequential steps of ubiquitylation

Methylation: This process refers to addition of methyl group to Nitrogen (N-methylation) or Oxygen (O-methylation) to amino acid side chain. N-methylation is irreversible whereas O-methylation is potentially reversible. Methylation enhances hydrophobicity of amino acid and neutralizes negative charge when attached to carboxylic acid. Main methyl group contributor for such reaction is SAM (S-adenosyl methionine). This reaction is mediated by enzyme methyltransferases. Methylation process is involved in epi-genetic regulation as histone methylation and demethylation.