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CELL AND MOLECULAR BIOLOGY

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Units (1-14)

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SYLLABI-BOOK MAPPING TABLE

Cell And Molecular Biology

Syllabi	Mapping in Book
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Unit 1: Cell theory - Structural organization of Prokaryotic and Eukaryotic cells. **Unit 2:** Ultrastructure of Cell membrane, Nucleus, Chromosomes, Mitochondria. **Unit 3:** Endoplasmic reticulum, Golgi apparatus, Lysosomes, Ribosomes, Peroxisomes and their functions. **Unit 4:** The cytoskeleton - Microtubules and Microfilaments - Cell cycle - Mitosis and Meiosis.	**Unit 1:** Cell Theory – Structural Organization **(Pages 1-19);** **Unit 2:** Cell Components **(Pages 20-33);** **Unit 3:** Endoplasmic Reticulum **(Pages 34-57);** **Unit 4:** Cytoskeleton **(Pages 58-73)**
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BLOCK - III: TRANSCRIPTION AND TRANSLATION	
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BLOCK - IV: REGULATIONS OF GENE EXPRESSION	
Unit 12 Concept of operon - Lac and trp operons, Positive and negative control, Repressor and Inducer. **Unit 13:** Hormonal regulation of gene expression, Transcription factors, Steroid receptors; DNA binding motifs in pro- and eukaryotes. **Unit 14:** Analysis of Gene expression using Molecular Methodolgy.	**Unit 12:** Concept of Operon **(Pages 197-210);** **Unit 13:** Harmonal Regulation of Gene Expression **(Pages 211-223);** **Unit 14:** Analysis of Gene Expression Using Molecular Biology **(Pages 224-262)**

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INTRODUCTION

NOTES

Cell biology is a branch of biology that studies cells physiological properties, their structure, the organelles they contain, interactions with their environment, their life cycle, division, cell function and death. This is done both on a microscopic and molecular level. Cellular biology is also referred to as Cytology and mainly revolves around the basic and fundamental concept that cell is the fundamental unit of life. The most important concept of cellular biology is the cell theory which states three important principles that all organisms are composed of one or more cells, the cell is the basic unit of life in all living things and all cells are produced by the division of pre-existing cells.

Molecular cell biology is the study of how molecules interact to give rise to the properties of cells, their ability to grow, maintain themselves and divide. Molecular cell examines how the interaction of macromolecules gives rise to life, i.e., to the functioning of living organisms. The integration of molecular structure, function, and behaviour can lead to a functional living cell. Molecular genetics is a branch of genetics and molecular biology that deals with the structure and function of genes at a cellular and molecular level. Molecular genetics is concerned with the arrangement of genes on DNA molecule, the replication of DNA, the transcription of DNA into RNA, and the translation of RNA into proteins.

Cell and Molecular Biology is an interdisciplinary field of science that deals with the fields of chemistry, structure and biology as it helps to understand life and cellular processes at the molecular level. Molecular biology explores cells, their characteristics, parts and chemical processes, and pays special attention to how molecules control a cell's activities and growth.

This book, *Cell and Molecular Biology*, is divided into four block, which is further divided into fourteen units which will help you understand about the basics of cell theory, structural organization of prokaryotic and eukaryotic cells, ultrastructure of cell membrane (nucleus, chromosomes, mitochondria), endoplasmic reticulum, Golgi apparatus, lysosomes, ribosomes, peroxisomes and their functions, cytoskeleton (microtubules and microfilaments), cell cycle (mitosis and meiosis), structure and functions of DNA, types of RNA and its function, enzymes involved in molecular biology (DNA polymerases, RNA polymerase, helicase, primase, ligase, etc.), mechanism of prokaryotic and eukaryotic replication, synthesis of leading and lagging strands, Okazaki fragments, prokaryotic transcription, eukaryotic transcription (promoters, enhancers, factors, properties of RNA polymerase I, II and III), post transcriptional modification, reverse transcription, protein synthesis (machinery, formation of initiation complex, translocation, chain elongation and termination), cell free protein synthesis, comparison of protein biosynthesis in prokaryotes and eukaryotes, concept of operon, positive and negative control, hormonal regulation of gene expression, transcription factors, steroid receptors, analysis of gene expression using molecular methodology.

The book follows the self-instruction mode or the SIM format wherein each unit begins with an 'Introduction' to the topic followed by an outline of the 'Objectives'. The content is presented in a simple and structured form interspersed with 'Check Your Progress' questions and answers for better understanding. A list of 'Key Words' along with a 'Summary' and a set of 'Self Assessment Questions and Exercises' is provided at the end of the each unit for effective recapitulation.

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BLOCK - I

CELL STRUCTURE

UNIT 1 CELL THEORY – STRUCTURAL ORGANIZATION

Structure

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1.0 INTRODUCTION

In biology, cell theory is the historic scientific theory and is universally accepted that all living organisms are made up of 'cells'. The scientists have defined that the cells are the basic structural and organizational unit of all living organisms, and that all cells originate from pre-existing cells. Principally, the cells are the basic unit of structure in all organisms and also the basic unit of reproduction. With the discovery and advancement of microscopes and magnification technology over time, Robert Hooke was the first who studied the cork cells under the microscope and gave the very significant theory on scientific study of cells, also known as 'cell biology' or 'cell theory'. Cell theory was eventually formulated in 1839. The three principles to the cell theory include that, all living organisms are composed of one or more cells, the cell is the basic unit of structure and organization in organisms and the cells arise from pre-existing cells.

In this unit, you will study about the significance and characteristic features of a cell, cell as basic unit of life, various components of a cell, and the structural organization of Prokaryotic and Eukaryotic cells.

NOTES

1.1 OBJECTIVES

After going through this unit, you will be able to:

- Explain the basics of cell theory
- Evaluate that why cells are termed as the fundamental unit of all organisms
- Understand the structure of cell
- Define the various components of a cell
- Discuss the structural organization of Prokaryotic and Eukaryotic cells

1.2 FUNDAMENTALS OF CELL THEORY

By definition, a '**cell**' is the fundamental and structural unit of all living organisms. It is the smallest biological, structural and functional unit of all plants and animals. Therefore, cells are called the '**Building Blocks of Life**' or the '**Basic units of Life**'. Organisms made up of a single cell are termed as '**unicellular**' whereas organisms made up of many cells are termed as '**multicellular**'. Cells perform many different functions within a living organism, such as digestion, respiration, reproduction, etc., and keep it alive. For example, within the human body there are various types of cells which perform specific functions. Basically, the cells form tissues and multiple tissues make up an organ, different organs create an organ system, such as digestive system, respiratory system, circulatory system, nervous system, etc., to perform specific functions in the human body and any other living organism. Therefore,

$$\text{Cells} \rightarrow \text{Tissue} \rightarrow \text{Organ} \rightarrow \text{System} \rightarrow \text{Organism/Human Body}$$

Fundamentally, all organisms are composed of structural and functional units of life called '**cells**'. The body of some organisms like bacteria, protozoans and some algae is made up of a single cell while the body of fungi, plants and animals are composed of many cells. Human body is built of about one trillion cells. Cells vary in size and structure as they are specialized to perform different functions. But the basic components and functions of the cell are common to all cells.

Landmarks in Cell Study

Soon after Anton van Leeuwenhoek invented the microscope, Robert Hooke in 1665 observed a piece of cork under the microscope and found it to be made of small compartments which he called 'cells', in Latin 'cell' means 'small room'. In 1672, Leeuwenhoek observed bacteria, sperm and red blood corpuscles, all of which were cells. In 1831, Robert Brown, an Englishman observed that all cells had a centrally positioned body which he termed the **nucleus**.

Anton van Leeuwenhoek is another scientist who saw these cells soon after Hooke. He made use of a microscope containing improved lenses that could magnify objects almost 300-fold or 270x. Leeuwenhoek studied the tiny organisms under the microscope and named them 'animalcules', which included protozoa and other unicellular organisms, like bacteria. Antony van Leeuwenhoek is regarded as the 'Father of Microbiology'. He is known for the discovery of bacteria.

In 1838, M.J. Schleiden and Theodore Schwann formulated the 'cell theory'. The cell theory formulates the following three principles:

- All organisms are composed of cells.
- Cell is the structural and functional unit of life.
- Cells arise from pre-existing cells.

1.2.1 Fundamentals of Cell

The cell is the fundamental unit of life. All living organisms on planet Earth are composed unicellular (single cell) or multicellular (many cells). Cells range in its size from a millimeter to microns and generally varies in their shapes. Few cells are flat, oval, rod, curved, spherical, concave, rectangular, and various other shapes are also found. Most of the cells are microscopic in size and can only be seen under the microscope. Some cells are fairly long and large. For example, a neuron in the human body is approximately 100 microns or 1 meter long and the ostrich egg is the largest cell which ranges from 14-15 cm long and 12-13 cm wide (Refer Figures1.1 and 1.2).

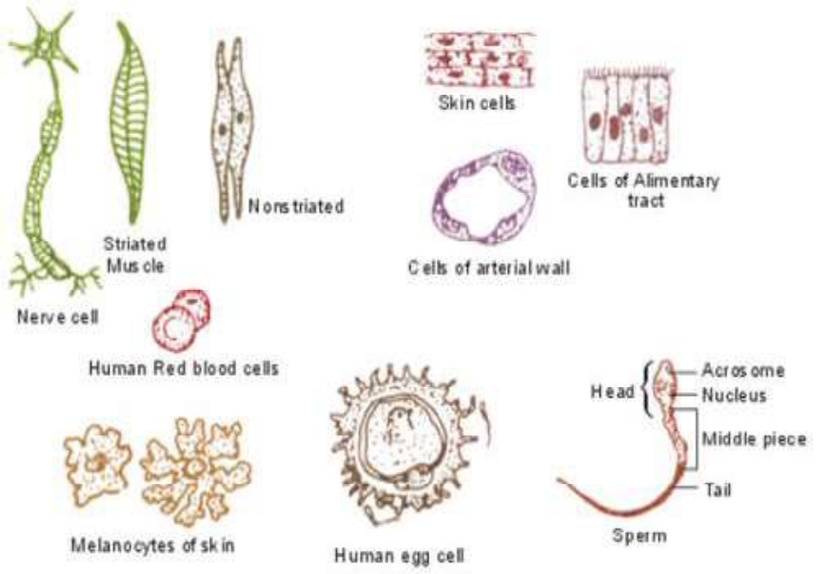


Fig. 1.1 *Different Shapes of Cells*

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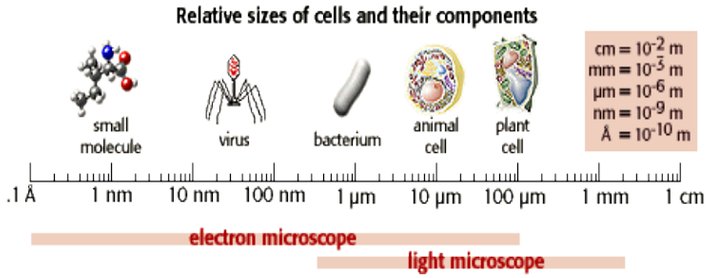


Fig. 1.2 *Different Sizes of Cells and Their Components*

Animal cells are a typical eukaryotic cell with a membrane-bound nucleus with the presence of DNA inside the nucleus. They also comprise of other organelles and cellular structures which carry out specific functions necessary for the cell to function properly.

A cell may be defined as a unit of protoplasm bounded by a plasma or cell membrane and possessing a nucleus. Protoplasm is the life giving substance and includes the cytoplasm and the nucleus. The cytoplasm has in it organelles, such as Ribosomes, Mitochondria, Golgi Bodies, Plastids, Lysosomes and Endoplasmic Reticulum. Plant cells have in their cytoplasm large vacuoles containing non-living inclusions like crystals, pigments, etc. The bacteria have neither organelles nor a well formed nucleus. But every cell has following three major components:

- Plasma Membrane
- Cytoplasm
- DNA (naked in bacteria and covered by a membrane in all other organisms)

1.3 PROKARYOTIC AND EUKARYOTIC CELLS

There are many different types of cells in the organism, such as blood cells, skin cells, bone cells and even bacteria. All cells, whether from bacteria, human, or any other organism will be one of two general types. There is another basic cell structure that is present in many but not all living cells, termed as '**nucleus**'. The nucleus of a cell is a structure in the **cytoplasm** that is surrounded by a membrane (the **nuclear membrane**) and contains, and protects, most of the cell's DNA. Based on whether they have a nucleus, the cytologists recognize two basic types of cells as **Eukaryotic** cell and **Prokaryotic** cell (Refer Figure 1.3). Their differences have been tabulated below in Table 1.1. Organisms which do not possess a well formed nucleus are prokaryotes, such as the bacteria. All others possess a well-defined nucleus, covered by a nuclear membrane and are termed as eukaryotes.

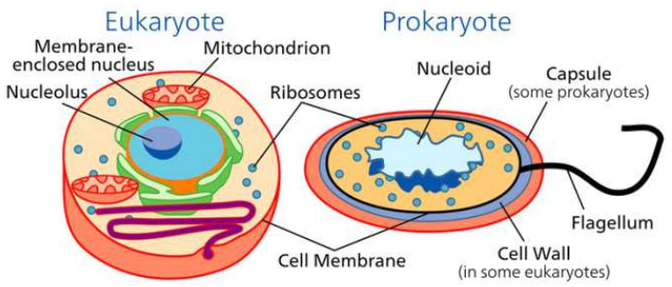


Fig. 1.3 *Two Basic Types of Cells*

Higher eukaryotes have multiple organs to perform specific functions, such as liver, kidney and heart. Each organ has specific tissue and each tissue is composed of cells. Hence, cell is the structural and functional unit of life as it contains all necessary infrastructure 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 whereas eukaryotes are either single cells or part of multicellular tissues system. Besides this, both types of cells have several structural and metabolic differences as given in Table 1.

Table 1.1 *Difference between Eukaryotic Cell and Prokaryotic Cell*

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

1.3.1 Structure of Prokaryotic Cells

A prokaryotic cell is much simpler and smaller than eukaryotic cells. It lacks membrane bound organelles including nucleus. The structure and components of a typical prokaryotic cell is shown in Figure 1.4(A). The description of different structural feature of prokaryotic cells is as follows.

NOTES

1. Outer Flagella

A flagellum attached to the bacterial capsule is a central feature of most of the prokaryotic cell especially of the 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 proteins present inside the cell. Flagellar motion is an energy consuming process and it is governed by an 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 or pathogenicity and ability to grow.

2. Bacterial Surface Layers

Bacteria possess 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.

Gram Staining

Gram staining is developed by a Danish scientist Hans Christian Gram. This technique differentiates bacterial strains based on their cell wall composition, especially thickness of the Peptidoglycan Layer. During the staining procedure bacterial sample is stained with two dyes, Crystal Violet and Safarnin. During a washing step with non-polar solvents, such as alcohol 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 appear as Pink.

Cell Wall

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 (Refer Figure 1.4(B)).

A. Peptidoglycan Layer: The 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 Acid) linked by a ²-(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 the cell wall. Peptidoglycan synthesis is targeted by antibiotics, such as penicillin whereas lysozyme (present in human saliva or tears) degrades the peptidoglycan layer by cleaving glycosidic bond connecting NAGNAM 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: The LipoPolySaccharides (LPS) are found only in Gram –ve bacterial cell wall and it is an important antigenic determinant.

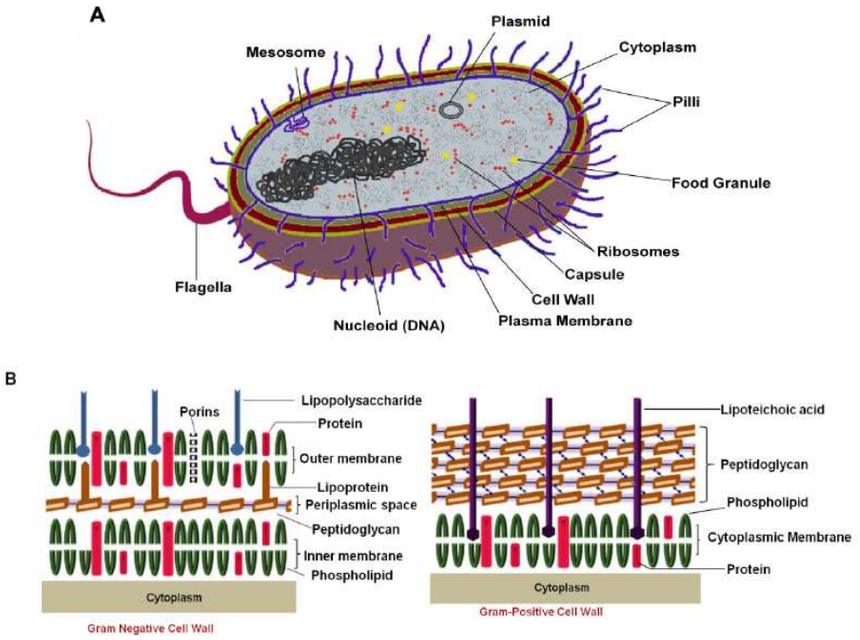


Fig. 1.4 *Structural Details of a Typical Prokaryotic Cell (A); Composition of Cell Wall of Gram Negative and Gram Positive Bacteria (B)*

3. Cytosol and Other Organelles

Prokaryotic cells do not contain any membrane bound organelle. The organelles are present in cytosol, such as ribosome (70S), genetic material whereas 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 chromosomal 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.

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1.3.2 Structure of Eukaryotic Cell

The eukaryotic cell is 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 double membrane. Eukaryotic cells are usually larger than prokaryotic cells, and they are found mainly in multicellular organisms. Organisms with eukaryotic cells are called eukaryotes, and they range from fungi to humans.

Eukaryotic cells also contain other organelles besides the nucleus. An organelle is a structure within the cytoplasm that performs a specific job in the cell. Organelles called mitochondria, for example, provide energy to the cell, and organelles called vacuoles store substances in the cell. Organelles allow eukaryotic cells to carry out more functions than prokaryotic cells can. This allows eukaryotic cells to have greater cell specificity than prokaryotic cells. Ribosomes, the organelle where Proteins are made, are the only organelles in prokaryotic cells. A typical eukaryotic animal and plant cell is shown in Figure 1.5.

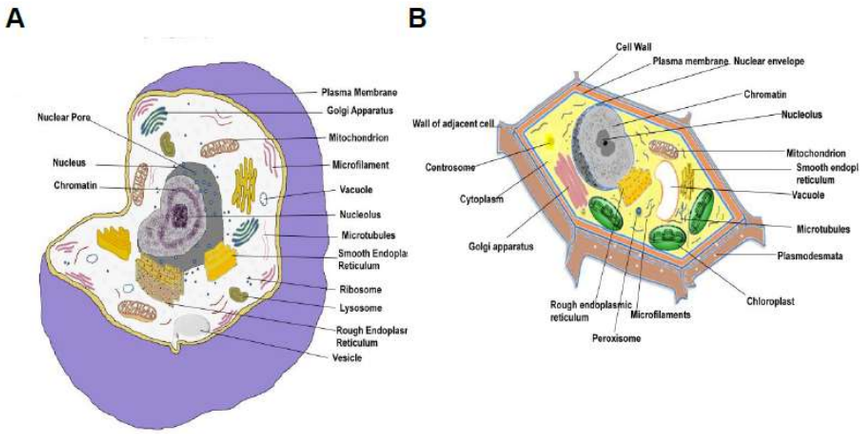


Fig. 1.5 *Structure of Eukaryotic Cell - Animal Cell (A), Plant Cell (B)*

Different Organelles of Eukaryotic Cells (Animal)

Following is the description of different structural features of eukaryotic cell.

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 throughout 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 Eukaryotic cell and homologous to the processor in a typical computer (Refer Figure 1.6(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 and Heterochromatin. Euchromatin is a part of chromatin where DNA is loosely packed and it is transcriptionally active to form mRNA whereas Heterochromatin is more densely packed and it is transcriptionally inactive. Nuclei in eukaryotic cells are present in a double layer of membrane known as nuclear envelope (Refer Figure 1.6(B)). Outer membrane of nuclear envelope is continuous with the rough endoplasmic reticulum and has ribosome attached to it. The space between these two membranes is called as perinuclear space. Nuclear envelope often has nuclear pore and as per calculation an average nucleus has 3000-4000 pores per nuclear envelope.

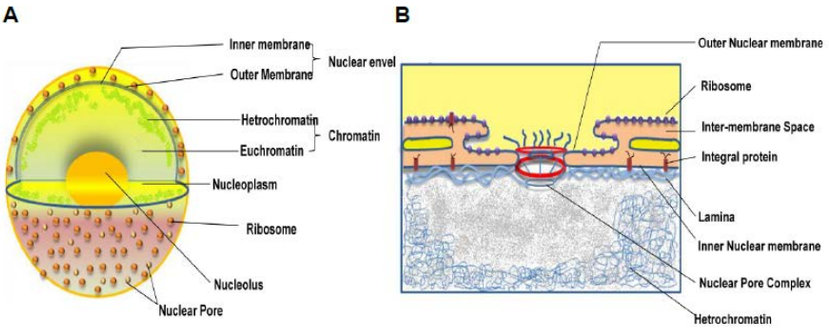


Fig. 1.6 *Structural Details of Eukaryotic Nucleus - Entire Nucleus (A) and Enlarged View of Nuclear Pore (B)*

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.

3. Mitochondria

Mitochondria 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 (Refer Figure 1.7(A)). Outer membrane is smooth and cover the complete organelle with large number of integral proteins, known as porins.

Porin allows free movement of molecules less than 5000da within and outside mitochondria. Whereas larger molecules or proteins moves into the mitochondria through transporters 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 machinery for anaerobic oxidation and electron transport chain to produce ATP. Due to presence

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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 compartment of the mitochondria and it contains ribosome, DNA, RNA, enzymes to run Krebs's cycle and other proteins.

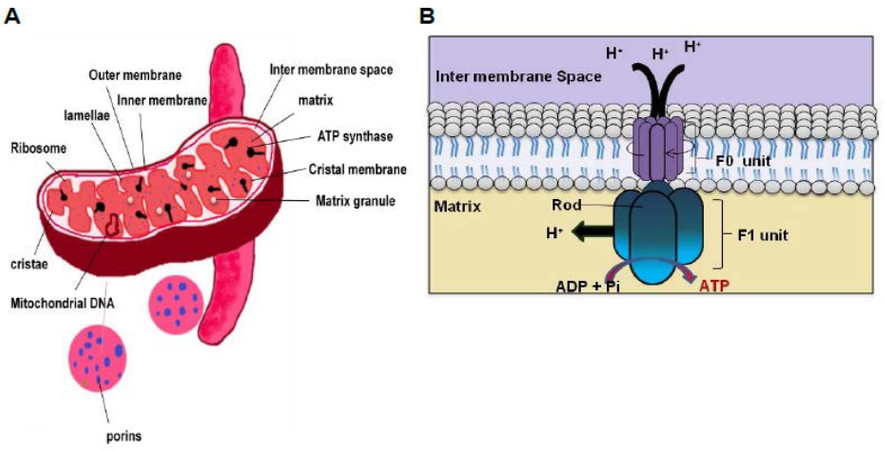


Fig. 1.7 *Mitochondria - Structure of Mitochondria (A) and Enlarged View of ATP Synthase (B)*

Mitochondrial DNA is circular and it has full machinery to synthesize its own RNA (mRNA, rRNA and t-RNA) and proteins. Marked differences exist between mitochondrial DNA and DNA present in nucleus and these differences 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, Krebs's cycle produces large amount of reducing equivalent in the form of NADH2 and FADH2. 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 generate ATP. ATP Synthase (Refer Figure 1.7(B)) is a mushroom shaped multimeric protein complex, mainly composed of two proteins F0 and F1. F0 is a membrane bound portion whereas F1 is the complex present into the lumen towards matrix. F0F1 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.
4. Role in programmed cell death or 'apoptosis'.

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4. 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 (Refer Figure 1.8(A)). Outer membrane is porous to the small molecules but protein or large molecules are transported by TOC (translocon on the outer chloroplast membrane) complex. Movement of material passed through outer membrane gets 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.

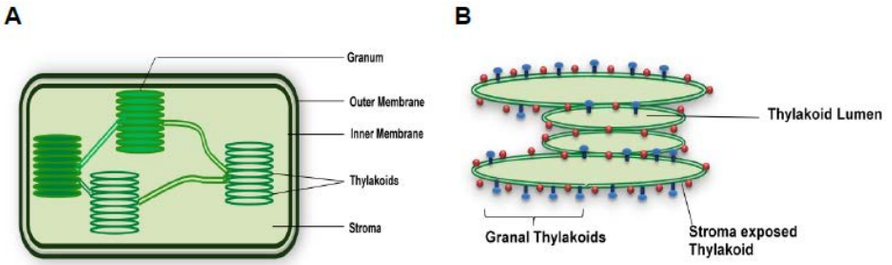


Fig. 1.8 *Structure of Chloroplast (A) and Arrangement of Thylakoid Membrane in Chloroplast (B)*

The inner membrane of the chloroplast further folds to a flattened membrane system known as **thylakoids**. The photosynthesis machinery, such as light absorbing pigments, electron carriers and ATP synthesizing machinery is present on inner membrane as integral protein complex. Thylakoid membranes are arranged like stack of coin to form granum (Refer Figure 1.8(B)). The granum throughout the chloroplast are connected by tubule to share the material. Overall structure of chloroplast is similar to mitochondria but it has few significant structural and biochemical differences. Thylakoid membrane contains photosynthetic green colored pigment chlorophyll and undergoes the following reaction to produce Glucose ($C_6H_{12}O_6$) and release Oxygen (O_2).

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

Photosynthesis is an assimilation reaction involving CO_2 and water to produce sugar in the presence of solar energy (photons) that catalyzes fusion reaction as shown in the above Equation. 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 passes through

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ATP synthase and returns back into the stroma to generate ATP (Refer Figure 1.9). 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 electrons are used to generate NADPH in the stroma. Hence as a result of photosynthesis, solar energy is trapped by photo synthesis apparatus to generate ATP and NADPH into the lumen. Both of them are used to run Calvin cycle to assimilate environmental CO_2 to form sugar.

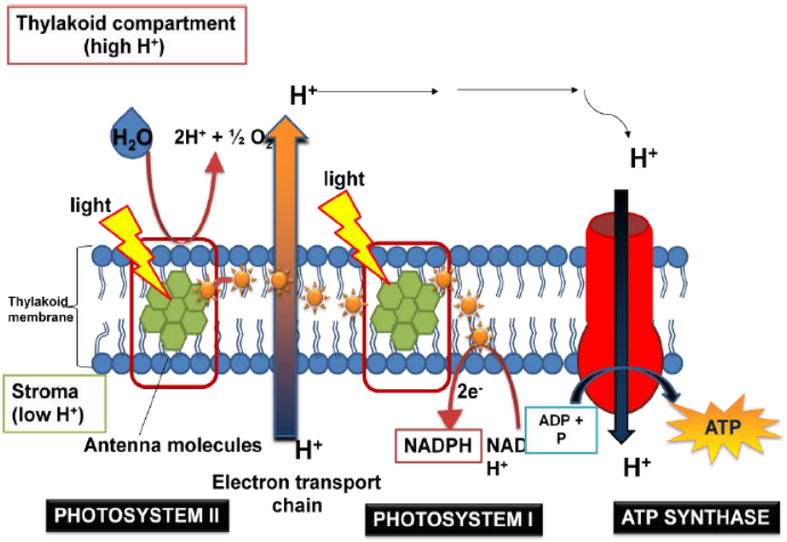


Fig. 1.9 Different Steps of Photosynthesis

5. Organelles of Vesicular Trafficking System

The main function of these organelles is to manage the distribution of material (food particles or proteins) throughout the cell. Three different organelles, such as Endoplasmic Reticulum (ER), Golgi apparatus and Lysosome, coordinately work together to maintain vesicular transport of material across the cell (Refer Figure 1.10). Eukaryotic cell takes up the solid material from outside through a process called '**endocytosis**' whereas 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 receptors and trap it in a membranous 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 fuse with the plasma membrane to release the content outside of the cell.

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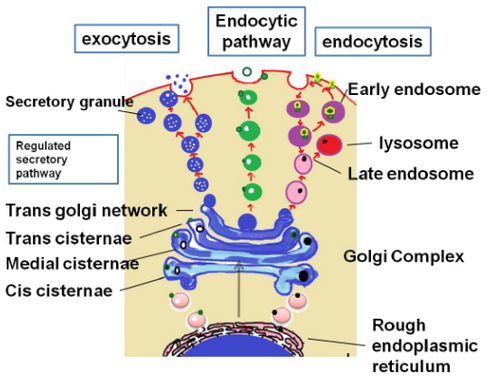


Fig. 1.10 *Intra-Cellular Vesicular Trafficking System of Cell*

Endoplasmic Reticulum (ER)

The vesicular network starts from nuclear membrane and spread throughout the cytosol constitutes endoplasmic reticulum (Refer Figure 1.11). There are two different types of Endoplasmic Reticulum (ER) present in the cell, Rough Endoplasmic Reticulum (RER), and Smooth Endoplasmic Reticulum (SER). RER has ribosome attached to it to give a rough appearance whereas smooth endoplasmic reticulum is devoid of ribosomes. Protein synthesis on ribosome attached to RER are sorted into three different categories, such as integral membrane proteins, proteins for secretion and protein destined for different organelles. Proteins are synthesized with **n-signal peptide** and these signal peptides are recognized by signal recognition particle on their target organelles. For example, if a protein is synthesized with a signal peptide for mitochondria, it will attach to signal recognition particle and receptor onto the outer mitochondrial membrane to deliver the protein. The proteins without any signal peptide tags are supposed to remain in the cytosol.

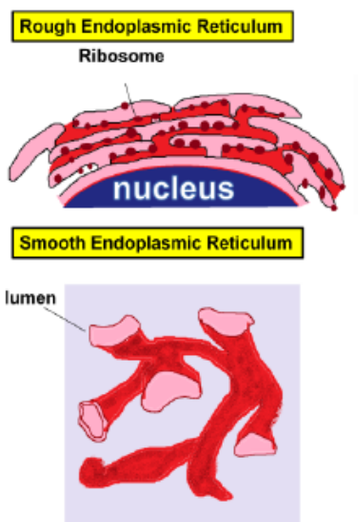


Fig. 1.11 *Endoplasmic Reticulum*

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Functions of Endoplasmic Reticulum

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

Golgi Bodies

Golgi bodies were first visualized by a metallic stain invented by Camillo Golgi and it is made of flattened, disk like cisternae arranged in a stacked manner to give three distinct zones (Refer Figure 1.12(A)). Cis-face receives 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 sort's vesicle for their destined organelles or plasma membrane.

Functions of Golgi Bodies

1. Protein Sorting
2. Protein Modifications (Glycosylation)
3. Proteolysis

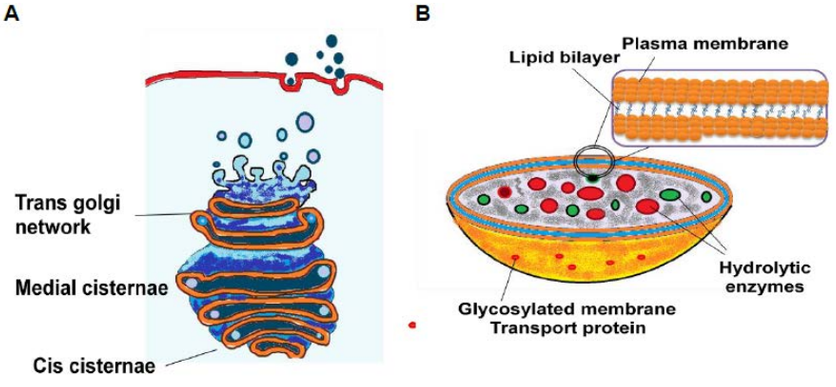


Fig. 1.12 *Schematic Structure - Golgi Bodies (A) and Lysosomes (B)*

Lysosomes

Lysosomes are discovered by De Duve. They are membrane bound organelles and an important component of intracellular vesicular system (Refer Figure 1.12(B)). 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. Lysosome lumen is extremely acidic and contains protease, cytolytic enzymes to degrade the ingested material.

Functions of Lysosomes

Following are the functions of lysosomes.

1. Degradation of Ingested Food Material for delivery through Vesicular System.
2. Degradation of Pathogenic Bacteria.
3. Degradation of Old Protein.

Check Your Progress

1. What is cell? Give definition.
2. How the cell performs specific functions in the human body?
3. Give the cell theory formulated by M.J. Schleiden and Theodore Schwann.
4. What are the different types of cells in an organism?
5. Define eukaryotic cell.
6. What is cytosol?
7. Define nucleus of a eukaryotic cell.

1.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. By definition, a 'cell' is the fundamental and structural unit of all living organisms. It is the smallest biological, structural and functional unit of all plants and animals. Therefore, cells are called the 'Building Blocks of Life' or the 'Basic Units of Life'. Organisms made up of a single cell are termed as 'unicellular' whereas organisms made up of many cells are termed as 'multicellular'. Cells perform many different functions within a living organism, such as digestion, respiration, reproduction, etc., and keep it alive.
2. The cells form tissues and multiple tissues make up an organ, different organs create an organ system, such as digestive system, respiratory system, circulatory system, nervous system, etc., to perform specific functions in the human body and any other living organism. Therefore,

   Cells → Tissue → Organ → System → Organism/Human Body

   Human body is built of about one trillion cells. Cells vary in size and structure as they are specialized to perform different functions. But the basic components and functions of the cell are common to all cells.
3. In 1838, M.J. Schleiden and Theodore Schwann formulated the 'cell theory'. The cell theory formulates the following three principles:
   - All organisms are composed of cells.
   - Cell is the structural and functional unit of life.
   - Cells arise from pre-existing cells.

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4. There are many different types of cells in the organism, such as blood cells, skin cells, bone cells and even bacteria. All cells, whether from bacteria, human, or any other organism will be one of two general types. There is another basic cell structure that is present in many but not all living cells, termed as 'nucleus'. The nucleus of a cell is a structure in the cytoplasm that is surrounded by a membrane (the nuclear membrane) and contains, and protects, most of the cell's DNA. Based on whether they have a nucleus, the cytologists recognize two basic types of cells as Eukaryotic cell and Prokaryotic cell.
5. The eukaryotic cell is 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 double membrane. Eukaryotic cells are usually larger than prokaryotic cells, and they are found mainly in multicellular organisms. Organisms with eukaryotic cells are called eukaryotes, and they range from fungi to humans.
6. 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 throughout 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.
7. Nucleus is the central processing unit of Eukaryotic cell and homologous to the processor in a typical computer. 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.

1.5 SUMMARY

- By definition, a 'cell' is the fundamental and structural unit of all living organisms. It is the smallest biological, structural and functional unit of all plants and animals. Therefore, cells are called the 'Building Blocks of Life' or the 'Basic Units of Life'.
- Organisms made up of a single cell are termed as 'unicellular' whereas organisms made up of many cells are termed as 'multicellular'.
- Cells perform many different functions within a living organism, such as digestion, respiration, reproduction, etc., and keep it alive.
- The cells form tissues and multiple tissues make up an organ, different organs create an organ system, such as digestive system, respiratory system, circulatory system, nervous system, etc., to perform specific functions in the human body and any other living organism. Therefore,

  Cells → Tissue → Organ → System → Organism/Human Body

NOTES

- Human body is built of about one trillion cells. Cells vary in size and structure as they are specialized to perform different functions. But the basic components and functions of the cell are common to all cells.
- Cells range in its size from a millimeter to microns and generally varies in their shapes. Few cells are flat, oval, rod, curved, spherical, concave, rectangular, and various other shapes are also found. Most of the cells are microscopic in size and can only be seen under the microscope while some cells are fairly long and large.
- Animal cells are a typical eukaryotic cell with a membrane-bound nucleus with the presence of DNA inside the nucleus. They also comprise of other organelles and cellular structures which carry out specific functions necessary for the cell to function properly.
- There are many different types of cells in the organism, such as blood cells, skin cells, bone cells and even bacteria.
- All cells, whether from bacteria, human, or any other organism will be one of two general types. There is another basic cell structure that is present in many but not all living cells, termed as 'nucleus'.
- The nucleus of a cell is a structure in the cytoplasm that is surrounded by a membrane (the nuclear membrane) and contains, and protects, most of the cell's DNA. Based on whether they have a nucleus, the cytologists recognize two basic types of cells as Eukaryotic cell and Prokaryotic cell.
- Higher eukaryotes have multiple organs to perform specific functions, such as liver, kidney and heart. Each organ has specific tissue and each tissue is composed of cells.
- A prokaryotic cell is much simpler and smaller than eukaryotic cells. It lacks membrane bound organelles including nucleus.
- The eukaryotic cell is 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 double membrane.
- Eukaryotic cells are usually larger than prokaryotic cells, and they are found mainly in multicellular organisms.
- Organisms with eukaryotic cells are called eukaryotes, and they range from fungi to humans.
- 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 throughout 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.
- Nucleus is the central processing unit of Eukaryotic cell and homologous to the processor in a typical computer. The liquid filled inside nucleus is called

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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.

- Mitochondria 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.
- There are two different types of Endoplasmic Reticulum (ER) present in the cell, Rough Endoplasmic Reticulum (RER), and Smooth Endoplasmic Reticulum (SER).

1.6 KEY WORDS

- **Cell:** A cell is the fundamental and structural unit of all living organisms and are called the 'Building Blocks of Life' or the 'Basic Units of Life'.
- **Unicellular:** Organisms made up of a single cell are termed as 'unicellular'.
- **Multicellular:** Organisms made up of many cells are termed as 'multicellular'.
- **Nucleus:** The nucleus of a cell is a structure in the cytoplasm that is surrounded by a membrane (the nuclear membrane) and contains, and protects, most of the cell's DNA.
- **Cytosol:** It is the liquid part filled inside the cell and it contains water, salt, macromolecules (Protein, Lipid, RNA).
- **Nucleus:** It is the central processing unit of Eukaryotic cell and the viscous liquid filled inside nucleus is called as 'nucleoplasm' which contains nucleotides and enzymes to perform replication, transcription, DNA damage repair, etc.
- **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.

1.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answers Questions

1. What is the significance of cell in living organisms?
2. Who formulated the cell theory?
3. Differentiate between eukaryotic and prokaryotic cells.

4. What is the significance of nucleus?
5. What is DNA?
6. Define the terms nucleus, mitochondria, endoplasmic reticulum, cytosol and Golgi apparatus.

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Long Answers Questions

1. Discuss the significance of cell with reference to cell theories and cell structure giving appropriate examples.
2. Briefly explain the structural organization of prokaryotic and eukaryotic cells.
3. How will you differentiate between eukaryotic and prokaryotic cells? Draw labelled diagrams of both.
4. Explain the various components of prokaryotic cells giving diagrams.
5. Discuss about the various components of eukaryotic cells giving diagrams.

1.8 FURTHER READINGS

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UNIT 2 CELL COMPONENTS

NOTES

Structure

2.0 Introduction
2.1 Objectives
2.2 Components of the Cell
  2.2.1 Ultra Structure of Cell Membrane (Plasma Membrane)
  2.2.2 Mitochondria - The Energy Transformers (Singular = Mitochondrion)
  2.2.3 Nucleus: The Hereditary Organelle
2.3 Answers to Check Your Progress Questions
2.4 Summary
2.5 Keywords
2.6 Self Assessment Questions and Exercises
2.7 Further Readings

2.0 INTRODUCTION

All living organisms are made up of cells and new cells are produced when live cells divide. The cell is the smallest unit of life in an organism. The cell lives and, as a result, the organism lives. Whatever an organism does for survival it does for the survival of its cells. All the living matter of a cell is called protoplasm. The cell is surrounded by a cell or plasma membrane. The nucleus is the control centre of the cell. The cytoplasm surrounds the nucleus. The cytoplasm is everything within the cell except for the nucleus. There are many small organelles within the cytoplasm. The fine detail of a cell when seen by an electron microscope is called ultrastructure. The major components of the cell are cell membrane (plasma membrane), cytoplasm and nucleus.

In this unit, you will study about the various components of cell, the ultrastructure of cell membrane, nucleus, chromosomes and mitochondria.

2.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the basic components of cell
- Explain the ultrastructure of cell membrane
- Define the features of nucleus, chromosomes and mitochondria
- Discuss the structure and functioning of nucleus, chromosomes and mitochondria

2.2 COMPONENTS OF THE CELL

NOTES

All living organisms are made up of cells and new cells are produced when live cells divide. The cell is the smallest unit of life in an organism. The cell lives and, as a result, the organism lives. Whatever an organism does for survival it does for the survival of its cells. All the living matter of a cell is called protoplasm. The cell is surrounded by a cell or plasma membrane. The nucleus is the control centre of the cell. The cytoplasm surrounds the nucleus. The cytoplasm is everything within the cell except for the nucleus. There are many small organelles within the cytoplasm. The fine detail of a cell when seen by an electron microscope is called ultrastructure.

The major components of the cell are (1) Cell Membrane, (2) Cytoplasm and (3) Nucleus.

2.2.1 Ultra Structure of Cell Membrane (Plasma Membrane)

Each cell has a limiting boundary, the cell membrane, plasma membrane or plasmalemma. It is a living membrane, outermost in animal cells but next to cell wall in plant cells. It is flexible and can fold in (as in food vacuoles of Amoeba) or fold out (as in the formation of pseudopodia of Amoeba). The plasma membrane is made of proteins and lipids and several models were proposed regarding the arrangement of proteins and lipids. The fluid mosaic model proposed by Singer and Nicholson (1972) is widely accepted as shown in Figure 2.1.

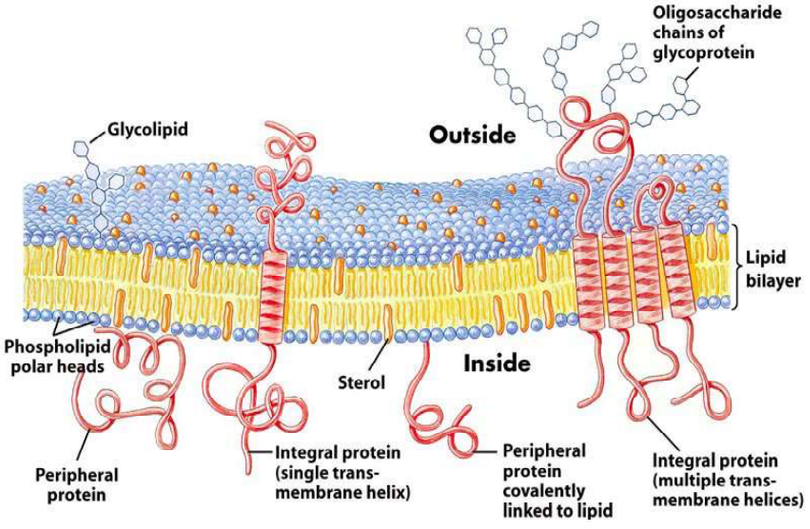


Fig. 2.1 *Fluid Mosaic Model of Cell Membrane*

According to the fluid mosaic model,

(i) The plasma membrane is composed of a lipid bilayer of phospholipid molecules into which a variety of globular proteins are embedded.

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(ii) Each phospholipid molecule has two ends, an outer head hydrophilic, i.e., water attracting, and the inner tail pointing centrally hydrophobic, i.e., water repelling.

(iii) The protein molecules are arranged in following two different ways:

- Peripheral Proteins or Extrinsic Proteins: These proteins are present on the outer and inner surfaces of lipid bilayer.
- Integral Proteins or Intrinsic Proteins: These proteins penetrate lipid bilayer partially or wholly.

Functions

(i) The plasma membrane encloses the cell contents.

(ii) It provides cell shape (in animal cells), for example the characteristic shape of red blood cells, nerve cells, bone cells, etc.

(iii) It allows transport of certain substances into and out of the cell but not all substance, so it is termed selectively permeable.

Transport of small molecules (such as, glucose, amino acids, water, mineral ions, etc.). Small molecules can be transported across the plasma membrane by any one of the following three methods:

Diffusion: Molecules of substances move from their region of higher concentration to their region of lower concentration. This does not require energy. For example, absorption of glucose in a cell.

Osmosis: The movement of water molecules from the region of their higher concentration to the region of their lower concentration through a semipermeable membrane. There is no expenditure of energy in osmosis. This kind of movement is along concentration gradient.

Active Transport: When the direction of movement of a certain molecules is opposite that of diffusion, i.e., from region of their lower concentration towards the region of their higher concentration, it would require an 'active effort' by the cell for which energy is needed. This energy is provided by ATP (Adenosine TriPhosphate). The active transport may also be through a carrier molecule.

Transport of Large Molecules (Bulk Transport): During bulk transport the membrane changes its form and shape. It occurs in two ways: (i) Endocytosis (taking the substance in) and (ii) Exocytosis (passing the substance out).

Cell membrane regulates movement of substance into and out of the cell. If the cell membrane fails to function normally the cell dies. Endocytosis, as shown in Figure 2.2, is of following two types:

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Endocytosis

Phagocytosis	Pinocytosis
1. intake of solid particles	1. intake of fluid droplets
2. membrane folds out going round the particle, forming a cavity and thus engulfing the particle	2. membrane folds in and forms a cup like structure sucks in the droplets

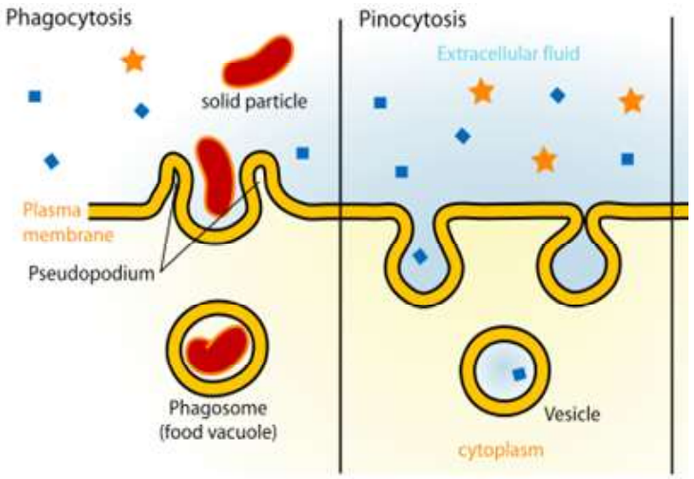


Fig. 2.2 *Diagrammatic Representation of Phagocytosis and Pinocytosis*

Cell Wall

In bacteria and plant cells the outermost cell cover, present outside the plasma membrane is the cell wall. Bacterial cell wall is made of peptidoglycan. Figure 2.3 illustrates the bacterial cell wall.

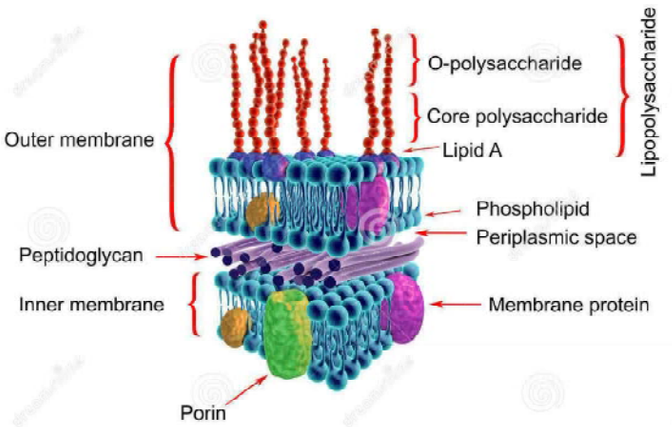


Fig. 2.3 *Bacterial Cell Wall*

NOTES

Cytosol and Other Organelles

The Ccytoplasm contains many cell organelles, such as

1. Those that trap and release energy - mitochondria and chloroplasts.
2. Those that are secretory or involved in synthesis and transport - Golgi, ribosomes and endoplasmic reticulum.
3. The organelles for motility - cilia and flagella.
4. The suicidal bags – lysosomes.
5. The nucleus which controls all activities of the cell and carries the hereditary material.

2.2.2 Mitochondria - The Energy Transformers (Singular = Mitochondrion)

Mitochondria (found in plant and animal cells) are the energy releasers.

- Appear as tiny thread like structure under light microscope. Approximately 0.5 - 1.00 ¼m (micrometer).
- Number usually a few hundred to a few thousand per cell. Smallest number is just one as in an alga (**Micromonas**).

Structure: The general plan of the internal structure of a mitochondria observed by means of electron microscope is shown in Figure 2.4.

The wall is made of double membrane. The inner membrane is folded inside to form projections called cristae which project into the inner compartment called matrix.

Function: Oxidises **Pyruvic Acid** (breakdown product of glucose) to release energy which gets stored in the form of ATP for ready use. This process is also called **cellular respiration**.

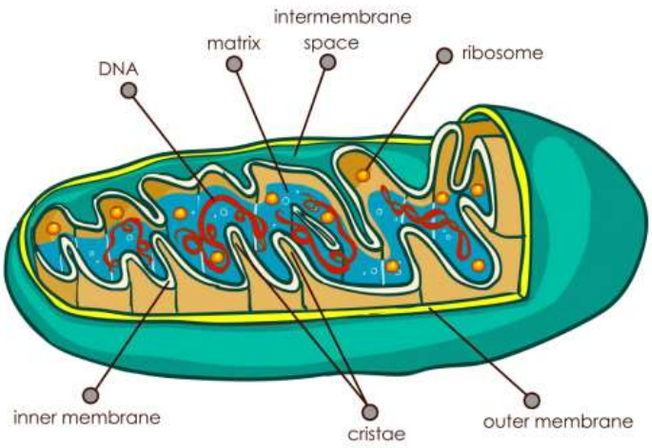


Fig. 2.4 *Structure of a Mitochondrion*

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Centriole

It is present in all animal cells (but not in *Amoeba*), located just outside the nucleus. It is cylindrical, 0.5 ¼m in length and without a membrane. It has 9 sets of peripheral tubules but none in the centre. Each set has three tubules arranged at definite angles (Refer Figure 2.5). It has its own DNA and RNA and therefore it is self duplicating.

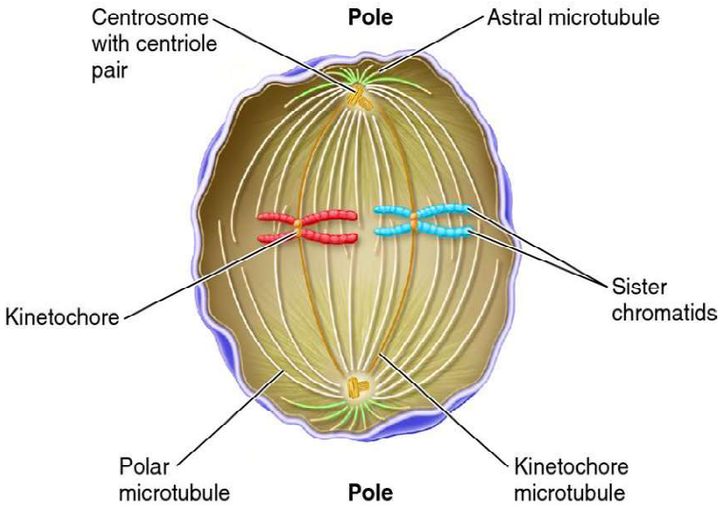


Fig. 2.5 *Structure of Centriole*

Function: Centrioles are involved in cell division. They give orientation to the 'mitotic spindle' which forms during cell division.

Basal Bodies

These are structures similar to centrioles. They have the same nine sets of triplet organization, as in the centrioles. The cilia and flagella appear to arise from the basal bodies.

2.2.3 Nucleus: The Hereditary Organelle

General structure of nucleus includes:

(i) It is the largest organelle seen clearly when the cell is not dividing.

(ii) It stains deeply, is mostly spherical, WBC have lobed nuclei.

(iii) It is mostly one in each cell (uninucleate having single nuclei, multinucleate in which some cells have many nuclei).

(v) Double layered nuclear membrane enclosing nucleoplasm which contains chromatin network and a nucleolus (Refer Figure 2.6).

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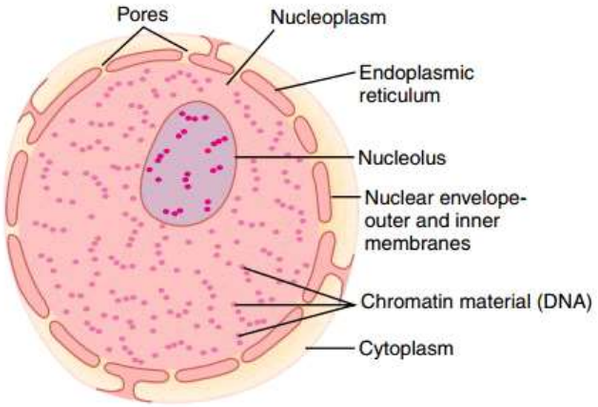


Fig. 2.6 *Structure of Nucleus*

Functions

- Maintains the cell in a working order.
- Co-ordinates the activities of organelles.
- Takes care of repair work.
- Participates directly in cell division to produce genetically identical daughter cells, this division is called mitosis.
- Participates in production of gametes through another type of cell division called meiosis.

Parts of Nucleus

Following are the parts of a nucleus.

Nuclear Membrane

- Double layered membrane is interrupted by large number of pores.
- Membrane is made up of lipids and proteins (like plasma membrane) and has ribosomes attached on the outer membrane which make the outer membrane rough.
- The pores allow the transport of large molecules in and out of nucleus, and the membranes keep the hereditary material in contact with the rest of the cell.

Chromatin

Within the nuclear membrane there is jelly like substance (**karyolymph** or **nucleoplasm**) rich in proteins as shown in Figure 2.7. In the karyolymph, fibrillar structures form a network called **chromatin fibrils**, which gets condensed to form distinct bodies called **chromosomes** during cell division. On staining the chromosomes, two regions can be identified in the chromatin material

heterochromatin dark and autromaticn (light). Heterochromatin has less DNA and genetically less active than euchromatin which has more DNA and genetically more active.

NOTES

Number of chromosomes is fixed in an organism. During cell division chromosomes divide in a manner that the daughter cells receive identical amounts of hereditary matter.

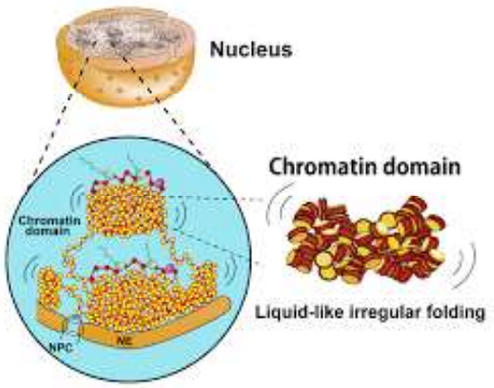


Fig. 2.7 *Structure of Chromatin*

Nucleolus

- Membraneless, spheroidal bodies present in all eukaryotic cells except in sperms and in some algae.
- Their number varies from one to few, they stain uniformly and deeply.
- It has DNA, RNA and proteins.
- Store house for RNA and proteins; it disappears during cell division and reappears in daughter cells.
- Regulates the synthetic activity of the nucleus.
- Thus nucleus and cytoplasm are interdependent, and this process is equal to nucleo–cytopalsmic interaction.

2.2.4 Chromosome

The darkly stained, rod shaped bodies visible under light microscope in a cell during metaphase stage of mitosis are referred to as chromosomes. Strasburger was the pioneer man who discovered chromosomes in 1875, and the term chromosome was coined by Waldeyer in 1888. The main features of eukaryotic chromosomes are given below:

- Chromosomes are not visible during interphase under light microscope. During other stages of cell division, they are visible, but are more clearly visible during mitotic metaphase. Hence, they are studied during metaphase.

NOTES

- Chromosomes bear genes in a linear fashion and thus are concerned with transmission of characters from generation to generation.
- Chromosomes of eukaryotes are enclosed by a nuclear membrane, while in prokaryotes, they remain without such envelope free in the cytoplasm.
- Chromosomes vary in shape, size and number in different species of plants and animals.
- Chromosomes have property of self-duplication, segregation and mutation.
- Chromosomes are composed of DNA, RNA an histones. DNA is the major genetic constituent of chromosomes.

Shape of Chromosomes

Chromosome shape is usually observed during anaphase. The shape of chromosomes is determined by the position of centromere, a part of chromosome on which spindle fibres are attached during metaphase.

Chromosomes have generally three different shapes, viz., rod shape, J shape and V shape. These shapes are observed when the centromere occupies terminal, sub-terminal and median (middle) position on the chromosomes, respectively.

Size of Chromosomes

Chromosome size is measured with the help of micrometer at mitotic metaphase. It is measured in two ways, viz., in length and in diameter. Plants usually have longer chromosomes than animals. Moreover, species or individuals which have fewer chromosome numbers have larger chromosomes.

The maximum length of chromosome is observed during interphase and minimum during anaphase. Thus chromosome size varies from species to species. Giant chromosomes have length up to 300 μ.

Number of Chromosomes

There are three types of chromosome numbers, viz., haploid, diploid and basic number.

Haploid: It represents half of the somatic chromosome number of a species and is denoted by **n**. Since haploid chromosome number is usually found in the gametes, it is also known as gametic number.

Diploid: It refers to somatic chromosome number of a species and is represented by **2n**. Since diploid chromosome number is found in zygotic or somatic cells it is also referred to as zygotic or somatic number.

Basic Number: The gametic chromosome number of a true diploid species is called basic number. It is the minimum haploid chromosome number of any species which is denoted by **x**. For example, in wheat, the basic number is 7, whereas the

haploid number is 7, 14 and 21 for diploid, tetraploid and hexaploid species, respectively.

Thus haploid chromosome number differs from basic number. Both are same in case of true diploid species but differ in case of polyploid species. Thus, basic number can be a haploid number but all haploid numbers cannot be basic number. Chromosome number differs from species to species.

NOTES

Role of Chromosomes in Heredity

Chromosomes are considered as physical basis of inheritance. The first conclusive evidence that chromosomes carry the units of inheritance was put forward by Sutton in 1903. Working with grasshopper, he gave a hypothesis that chromosomes contain genes and their behaviour during meiosis is the physical basis of Mendelian laws of heredity. Thus his work formed the basis of chromosomal theory of heredity. Now this theory is universally accepted. Various evidences which support that genes are located on the chromosomes which form the physical basis of heredity include the following:

- Two copies of each in somatic cells one copy in gametic cell.
- Self-duplication or replication capacity.
- Segregation during meiosis.
- Mutability.

All these parallel features between gene and chromosomes suggest that chromosomes carry genes and represent the physical basis of heredity.

Biochemical Studies: Biochemical studies reveal that hereditary units (genes) are composed of DNA in eukaryotes and RNA in some prokaryotes. The major part of DNA is found in chromosomes which prove beyond doubt that chromosomes are the carriers of hereditary units what we call genes.

Check Your Progress

1. What are the different cell parts?
2. What is cell membrane?
3. How are the protein molecules arranged?
4. Define active transport.
5. What is the function of mitochondria?
6. Define the shapes of chromosomes.

2.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

NOTES

1. The cell is surrounded by a cell or plasma membrane. The nucleus is the control centre of the cell. The cytoplasm surrounds the nucleus. The cytoplasm is everything within the cell except for the nucleus. There are many small organelles within the cytoplasm. The fine detail of a cell when seen by an electron microscope is called ultrastructure. The major components of the cell are (1) Cell Membrane, (2) Cytoplasm and (3) Nucleus.
2. Each cell has a limiting boundary, the cell membrane, plasma membrane or plasmalemma. It is a living membrane, outermost in animal cells but next to cell wall in plant cells. It is flexible and can fold in (as in food vacuoles of Amoeba) or fold out (as in the formation of pseudopodia of Amoeba). The plasma membrane is made of proteins and lipids and several models were proposed regarding the arrangement of proteins and lipids.
3. The protein molecules are arranged in following two different ways:
   - Peripheral Proteins or Extrinsic Proteins: These proteins are present on the outer and inner surfaces of lipid bilayer.
   - Integral Proteins or Intrinsic Proteins: These proteins penetrate lipid bilayer partially or wholly.
4. Active transport is when the direction of movement of a certain molecules is opposite that of diffusion, i.e., from region of their lower concentration towards the region of their higher concentration, it would require an 'active effort' by the cell for which energy is needed. This energy is provided by ATP (Adenosine TriPhosphate). The active transport may also be through a carrier molecule.
5. Function of mitochondria is that it oxidises Pyruvic Acid (breakdown product of glucose) to release energy which gets stored in the form of ATP for ready use. This process is also called cellular respiration.
6. Chromosomes have generally three different shapes, viz., rod shape, J shape and V shape. These shapes are observed when the centromere occupies terminal, sub-terminal and median (middle) position on the chromosomes, respectively.

2.4 SUMMARY

- The cell is surrounded by a cell or plasma membrane. The nucleus is the control centre of the cell. The cytoplasm surrounds the nucleus. The cytoplasm is everything within the cell except for the nucleus. There are

many small organelles within the cytoplasm. The fine detail of a cell when seen by an electron microscope is called ultrastructure.

- The major components of the cell are (1) Cell Membrane, (2) Cytoplasm and (3) Nucleus.
- Each cell has a limiting boundary, the cell membrane, plasma membrane or plasmalemma. It is a living membrane, outermost in animal cells but next to cell wall in plant cells. It is flexible and can fold in (as in food vacuoles of Amoeba) or fold out (as in the formation of pseudopodia of Amoeba). The plasma membrane is made of proteins and lipids and several models were proposed regarding the arrangement of proteins and lipids.
- The plasma membrane is composed of a lipid bilayer of phospholipid molecules into which a variety of globular proteins are embedded.
- Each phospholipid molecule has two ends, an outer head hydrophilic, i.e., water attracting, and the inner tail pointing centrally hydrophobic, i.e., water repelling.
- Diffusion: Molecules of substances move from their region of higher concentration to their region of lower concentration. This does not require energy. For example, absorption of glucose in a cell.
- Osmosis: The movement of water molecules from the region of their higher concentration to the region of their lower concentration through a semipermeable membrane. There is no expenditure of energy in osmosis. This kind of movement is along concentration gradient.
- Active Transport: When the direction of movement of a certain molecules is opposite that of diffusion, i.e., from region of their lower concentration towards the region of their higher concentration, it would require an 'active effort' by the cell for which energy is needed. This energy is provided by ATP (Adenosine TriPhosphate). The active transport may also be through a carrier molecule.
- In mitochondria, the wall is made of double membrane. The inner membrane is folded inside to form projections called cristae which project into the inner compartment called matrix.
- Centrioles are involved in cell division. They give orientation to the 'mitotic spindle' which forms during cell division.
- Number of chromosomes is fixed in an organism. During cell division chromosomes divide in a manner that the daughter cells receive identical amounts of hereditary matter.
- Chromosomes bear genes in a linear fashion and thus are concerned with transmission of characters from generation to generation.
- Chromosomes of eukaryotes are enclosed by a nuclear membrane, while in prokaryotes, they remain without such envelope free in the cytoplasm.

NOTES

- Chromosomes vary in shape, size and number in different species of plants and animals.
- Chromosomes have property of self-duplication, segregation and mutation.
- Chromosomes are composed of DNA, RNA an histones. DNA is the major genetic constituent of chromosomes.
- Chromosomes have generally three different shapes, viz., rod shape, J shape and V shape. These shapes are observed when the centromere occupies terminal, sub-terminal and median (middle) position on the chromosomes, respectively.

2.5 KEY WORDS

- **Ultrastructure:** The fine detail of a cell when seen by an electron microscope is called ultrastructure.
- **Diffusion:** Molecules of substances move from their region of higher concentration to their region of lower concentration. This does not require energy.
- **Osmosis:** The movement of water molecules from the region of their higher concentration to the region of their lower concentration through a semipermeable membrane.

2.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answers Questions

1. What are the various cell components?
2. Define the term ultrastructure of cell membrane.
3. Explain the structure and functions of nucleus, chromosomes and mitochondria.

Long Answers Questions

1. Discuss the different cell components with the help of figures.
2. Explain the cell with reference to ultrastructure of cell membrane. Draw diagrams where necessary.
3. Write short notes on - Nucleus, Chromosomes, Mitochondria.

2.7 FURTHER READINGS

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NOTES

UNIT 3 ENDOPLASMIC RETICULUM

NOTES

Structure

3.0 Introduction
3.1 Objectives
3.2 Endoplasmic Reticulum (ER)
3.3 Golgi Apparatus
3.4 Lysosomes
3.5 Ribosomes
3.6 Peroxisomes
3.7 Answers to Check Your Progress Questions
3.8 Summary
3.9 Key Words
3.10 Self Assessment Questions and Exercises
3.11 Further Readings

3.0 INTRODUCTION

The Endoplasmic Reticulum (ER) is a type of organelle found in eukaryotic cells that forms an interconnected network of flattened, membrane-enclosed sacs or tube-like structures known as cisternae. The membranes of the ER are continuous with the outer nuclear membrane. The endoplasmic reticulum occurs in most types of eukaryotic cells, but is absent from red blood cells and spermatozoa. There are two types of endoplasmic reticulum: rough (granular) and smooth (agranular). The outer (cytosolic) face of the rough endoplasmic reticulum is studded with ribosomes that are the sites of protein synthesis. The rough endoplasmic reticulum is especially prominent in cells such as hepatocytes. The smooth endoplasmic reticulum lacks ribosomes and functions in lipid manufacture and metabolism, the production of steroid hormones, and detoxification. The smooth ER is especially abundant in mammalian liver and gonad cells.

In this unit, you will study about Endoplasmic Reticulum, Golgi bodies, Lysosomes, Ribosomes, Peroxisomes and their functions in detail.

3.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand about endoplasmic reticulum
- Explain about Golgi bodies and its functions
- Discuss about lysosomes and its functions
- Expalin what are ribosomes and peroxisomes and their function

3.2 ENDOPLASMIC RETICULUM (ER)

NOTES

The Endoplasmic Reticulum (ER) is an important organelle in eukaryotic cells. It plays a major role in the production, processing, and transport of proteins and lipids. The ER produces transmembrane proteins and lipids for its membrane and for many other cell components including lysosomes, secretory vesicles, the Golgi apparatus, the cell membrane, and plant cell vacuoles. It was first reported by Porter in 1945. This continuous membrane system connects the nuclear membrane on one end and the cell membrane on the other.

Structure of Endoplasmic Reticulum (ER)

Endoplasmic Reticulum contains three different types of structure. These are cisternae, vesicles and tubeless (Refer Figure 3.1):

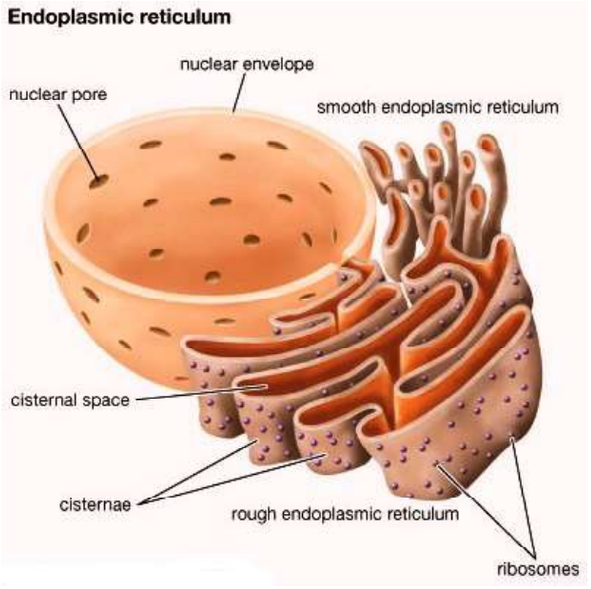


Fig. 3.1 *Structure of Endoplasmic Reticulum*

(a) Cisternae

These are long, flat and unbranched plates or lamellae arranged in parallel rows.

(b) Vesicles

They are usually round or ovoid sacs. They often occur isolated in the cytoplasm.

(c) Tubules

They are irregularly branched tube-like structures having a diameter of 50-100n. These are surrounded by this unit membrane of 50-60 thickness and their lumen is filled with the secretary products of the cell.

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There are two types of ER, such as smooth walled and rough walled. They may be present in the same or different types of cells namely:

(i) Smooth Endoplasmic Reticulum (SEE)

The surface of this type of reticulum is smooth as ribosome's not attached to it. Smooth ER is actively engaged in steroid synthesis, carbohydrate metabolism, pigment production etc. in cells.

(ii) Rough Endoplasmic Reticulum (RER)

The Rough Endoplasmic Reticulum is named so because of its appearance. It is a series of connected flattened sacs that have many ribosomes on their outer surface, hence the name. It synthesizes and secretes proteins in the liver, hormones and other substances in the glands. This type of endoplasmic reticulum is especially prominent in certain kinds of cells like hepatocytes where active protein synthesis occurs.

Functions of Endoplasmic Reticulum (ER)

- It is mainly responsible for the transportation of proteins and other carbohydrates to another organelle, which includes lysosomes, Golgi apparatus, plasma membrane, etc.
- They provide the increased surface area for cellular reactions.
- They help in the formation of nuclear membrane during cell division.
- They play a vital role in the formation of the skeletal framework.
- They play a vital role in the synthesis of proteins, lipids, glycogen and other steroids like cholesterol, progesterone, testosterone, etc.

Check Your Progress

1. Define endoplasmic reticulum.
2. What are cisternae?
3. What are vesicles?
4. Write few functions of ER.

3.3 GOLGI APPARATUS

The Golgi apparatus, like the endoplasmic reticulum, is a canalicular system with sacs that performs some important cellular functions like biosynthesis of polysaccha rides and packaging of cellular products.

In 1898, an Italian biologist, Camillo Golgi discovered a dark yellow network located near the nucleus of nerve cells. This network, which was later identified in

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other cell types, was named the Golgi complex or Golgi body. Since originally these were known to be networks, they were also called 'dictyosomes' (Gr., dictyes = net).

The Golgi complex occur in all cells except the prokaryotic cells and some eukaryotic cells like mature sieve tubes of plants, mature sperm and red blood cells of animals. In animal cells, there usually occurs a single Golgi apparatus however, its number may vary.

Thus, Paramoeba species has two Golgi apparatuses, and nerve cells, liver cells and chordate oocytes have multiple Golgi apparatuses. In animal cells, the Golgi apparatus is a localized organelle. Usually it remains polar and occurs in-between the nucleus and the periphery (for example, thyroid cells, goblet cells).

In nerve cells it occupies a circum-nuclear position. However, in the cells of higher plants, the Golgi bodies or dictyosomes are usually found scattered through-out the cytoplasm.

Morphology of Golgi Complex

The Golgi apparatus is highly pleomorphic. In some cells it is compact and limited, in others it is reticular. Typically, however, it consists of flattened disk like cisternae with dilated rims and associated vesicles and tubules (Refer Figure 3.2).

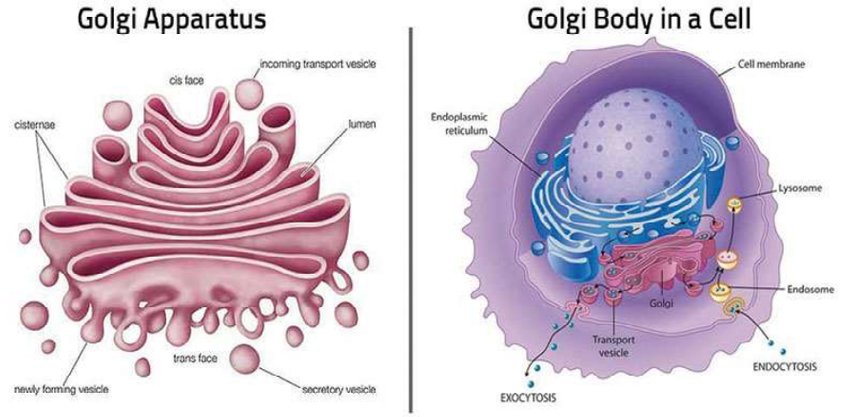


Fig. 3.2 *Structure of Golgi Apparatus*

The detailed structures of the basic components of the Golgi apparatus are as follows:

Cisternae

The cisternae are flattened, plate or saucer-like closed compartments. These are arranged in an orderly stack, much like a stack of pancakes. Typically, a Golgi stack contains fewer than eight cisternae. Depending on the cell type, an individual cell may contain from a few to several thousand stacks per cell.

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The diameter of cisternae varies from 0.5 to 1.0 nm and in each stack, cisternae are separated by a space of 20 to 30 nm, which may contain rod-like elements or fibres. Each cisterna is bounded by a smooth unit membrane and is curved in a manner resembling a shallow cup.

The cisterna closest to the Endoplasmic Reticulum (ER) is usually convex and is said to be at the cis face or proximal face or forming face, while the cisterna at the opposite end of the stack is of concave shape and said to be at the trans or distal or maturing face. This polarization is called cis-trans axis of the Golgi apparatus.

Functionally the Golgi complex is also divided into four distinct compartments, the cis, medial, trans cisternal, and the Trans Golgi Network (TGN). Newly synthesized membrane, secretory and lysosomal proteins leave the ER and enter the Golgi through its cis face and then pass across the stack to the trans face (Refer Figure 3.3).

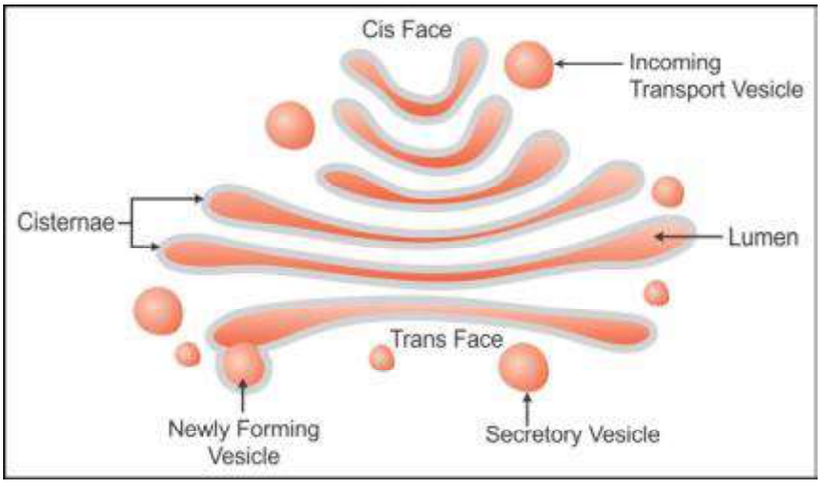


Fig. 3.3 *Cis and Trans face of Golgi Apparatus*

Tubules

The cis or forming face is characterized by the presence of small transi-tion vesicles or tubules that converge upon the Golgi cisternae, forming a kind of fenestrated plate.

Vesicles

Vesicles are those that bud from the ER or from the cis and medial cisternae of the Golgi complex and are seen in the electron microscope to be covered by a indistinct fuzzy coat that contains a GTP- binding protgin, called ARF (adenosylation ribose factor).

Functions of Golgi Complex

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Golgi complex represents a special mem-branous compartment interposed between the ER and the extracellular space, through which there is a continuous traffic of substances. During transportation through different compartments of Golgi, substances are modified, transformed and then are directed to their proper destinations.

The over-all functions of Golgi apparatus are as follows:

a. Synthesis of Glycosphingolipids and Glycoproteins:

The Golgi plays a major role in the glycosidation of lipids and proteins to produce glycosphingolipids and glycoproteins. Following synthesis on the membrane-bound ribosomes of the RER, the polypeptides reach the Golgi complex, where the terminal side chains of galactose, fucose, and sialic acid are added by the corresponding transferases present in the Golgi apparatus.

The Golgi also appears to be involved in the addition of sulfate to the carbohydrate moiety of the glycoproteins in cartilage cells.

b. Secretion

The main function of Golgi complex is cell secretion, not only of exportable proteins but also of the enzymes present in lysosomes and peroxisomes. Secretion may be continuous where the product is discharged as soon as it is elaborated, as found in liver cells and plasma cells. In other cells the secretory cycle is discontinuous, with storage in secretory or zymogen granules,for example pancreas, parotid gland.

In the pancreas, following six steps can be recognized.

(i) The Ribosomal Stage

The synthesis of proteins by polysomes attached to the RER.

(ii) The Cisternal Stage

The protein is processed and stored within the ER.

(iii) Intracellular Transport

The secreted proteins enter the transitional tubules and vesicles that lead to the Golgi complex, in which they fuse with large condensing vacuoles present at the maturing face of the Golgi. This transport requires the use of energy (ATP) and is same in all protein-producing cells.

(iv) Concentration of the Secretion

By the processes of progressive filling, and concentration, the condensing vacu-oles are converted into the zymogen granules that have characteristic electron-opaque content. The conver-sion does not require energy and is probably due to the formation of

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osmotically inactive aggregates of sulfated peptidoglycans. It involves movement of water from vacuoles to the cytosol.

(v) Intracellular Storage

The previous step culminates with the storage of the secretory product into secretory gra-nules, which are released following appropriate stimulus.

(vi) Exocytosis

The discharge of secre-tory granules involves its movement towards the apical region and fusion between its membrane and the lumi-nal plasma membrane. Exocytosis requires energy (ATP) and Ca++ and this requirement is related to the process of fusion-fission of membranes. Thus, Golgi complex acts as a centre of reception, finishing, packaging and dispatching for a variety of cellular products.

c. Recycling of Membranes

Membranes do not arise de novo. Actually, membrane components flow by means of vesicles from ER through Golgi to the plasma membrane. During exocytosis, the secretory granules become fused with the plasma membrane.

To remove the excess membranes from the api-cal region of the cell, it has been suggested that patches of membranes are invaginated from the surface as small vesicles that move back into the Golgi, to be reutilized in the packing of more secretion. Thus a dual func-tion at the cis and trans faces has been postulated.

d. Formation of Primary Lysosome

The Golgi complex is involved in the formation of primary lysosomes and glycosylation of many lysosomal enzymes that are mostly glycoprotein in nature.

e. Formation of Melanin

According to Novikoff, the GERL of the Golgi complex acts as the site for the formation of melanin granules; processing and packaging of secretory materials of many endo and exocrine cells.

f. Molecular Processing of Secretion

Many proteins are synthesized as biologically inactive precursors, which are activated later by the removal of a portion of the polypeptide chain by enzymes present in the Golgi apparatus. For example, the hormone insulin has two precursors – preproinsulin and pro-insulin.

The former is activated in the ER into pro-insulin which is then activated in the Golgi through the removal of the C pep-tide by the converting

enzyme and is pack-aged as active hormone insulin within the secretory granules.

g. Acrosome Formation

Electron micro-scopic studies have showed that acrosomes of spermatozoa are developed from Golgi cisternae and vacuoles.

h. Other Cellular Functions

In addition, Golgi apparatus is also involved in many other cellular functions, such as secretion of materials of primary and secondary cell walls in plants, secretion of cortical granules of a variety of oocytes, formation of yolk and vitelline membrane of growing primary oocytes.

Check Your Progress

5. What are Golgi apparatus ?
6. Where does Golgi apparatus occur?
7. What are vesicles in Golgi apparatus ?

3.4 LYSOSOMES

In the cytoplasmic matrix of the cells, there occur variously shaped bodies usually bounded by a single surface membrane and containing hydrolytic enzymes. These are called lysosomes. These enzyme containing bodies play important role in the digestion or lysis of intracellular substances, so they are called lysosomes.

Lysosomes were first reported by Christian de Duve and co-workers in Belgium in 1955 following their extensive work on the biochemical identification of certain hydrolytic enzymes in the liver cells of rats. During that period pioneering works were carried out by Novikoff in America when he was able to complement the biochemical work on Lysosome with observations at light microscopic and ultra-structural levels.

Occurrence of Lysosomes

Lysosomes appear to be absent in prokaryotes. Cytochemical and electron microscopic studies have revealed the presence of membrane bound enzyme containing bodies in animal tissues. It is not certain whether the structures equivalent to animal Lysosomes are present in plant cells.

The presence of lysosomes has been demonstrated in several slime moulds, fungal hyphae and algae. In 1964, P. Matile reported the occurrence of lysosomes in neumspora. Among the algae acid phosphatase has been located in lysosomes of Euglena and a few other species.

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Structure of Lysosomes

Lysosomes represent a class of morphologically heterogeneous cytoplasmic particles. The polymorphic nature of lysosomes has been attributed to specific functions, substances they contain and stage of digestion of those substances. Their size ranges from 0.25 to 0.8μ in diameter. In mammalian kidney cells they may be as large as 5μ and they may be even larger in phagocytes.

The density of lysosomes lies between that of mitochondria and microsomes (fragments of endoplasmic reticulum). Initially they were discovered by differential centrifugation and not by electron microscopes. They were originally obtained by sub-fractionation of the classical mitochondrial fraction. The classical mitochondrial fraction was sub-fractionated into the heavier and lighter components (Refer Figure 3.4).

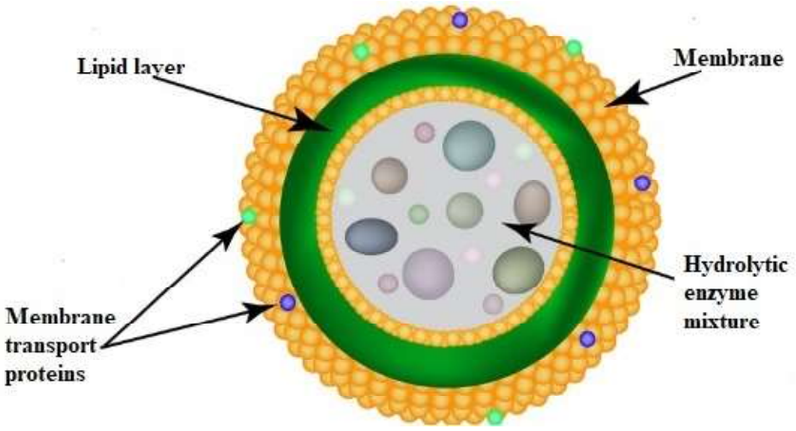


Fig. 3.4 *Structure of Lysosome*

The light mitochondrial sub-fraction or L-fraction was later named lysosome (lytic bodies).

The lighter fraction (lysosomes) lacked cytochrome oxidase but had high concentration of acid phosphatase which became active only after severe mechanical disruption. The absence of cytochrome oxidase in lysosomes distinguishes them from mitochondria.

Lysosomes are bounded by a single limiting membrane of lipoprotein that is homologous with the unit membrane. Chemically lysosome is a bag packed with a variety of hydrolysing enzymes. The surface membrane is impermeable or very little permeable to substrates of the enzymes contained in the lysosome.

The internal organization is quite variable; some lysosomes have solid or very dense content, others have a dense outer zone with a less dense core and still others have a cavity or vacuoles.

On the basis of morphological and functional criteria, a variety of lysosomes can be recognised in different cells as well as within a single cell. Two basic forms

of lysosomes have teen distinguished; (i) the primary lysosomes; and (ii) the secondary lysosomes.

NOTES

i. Primary Lysosomes (Storage Granules)

They originate from the endoplasmic reticulum or are cut off indirectly from the tips of golgi saccules and have not yet been involved in digestive process.

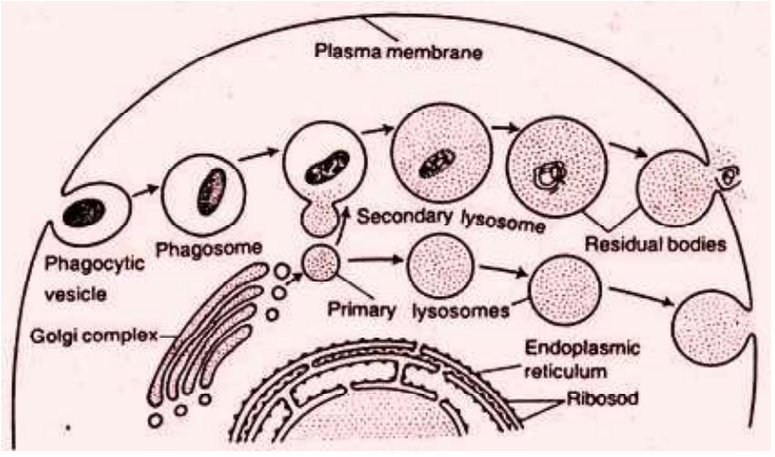


Fig. 3.5 *Formation of Primary and secondary Lysosomes*

ii. Secondary Lysosomes

These are digestive vacuoles which are sites of digestive activity.

Lysosomes belonging to this group may be classified into two separate types;

(a) Heterophagic

(b) Autophagic on the basis of exo or endogenous origin of the material undergoing digestion

Each of these types may be further sub-divided as follows:

(i) Pre-Lysosomes whose enzymes have never been engaged in hydrolysis.

(ii) Lysosomes which are sites of present digestive activity.

(iii) Post-Lysosomes which have lost their enzymes.

Lysosomes start functioning when the cell takes up substances either by phagocytosis or pinocytosis. Undigested substances or residues within the lysosomes usually remain packed with different inclusions. Under certain circumstances lysosomes start digesting the very cell in which they occur by opening their membrane and setting free their digestive acid hydrolases. The Lysosomes contain some 40 enzymes. Some of the important acid hydrolases identified from lysosomal fractions.

NOTES

It is not necessary that all the hydrolytic enzymes should occur in a single lysosome. These enzymes have been located in a variety of tissue types. Lysosomes obtained from one source do not present a homogeneous picture under the electron microscope. This suggests that a particular lysosome may contain only a few or even a single species of enzymes. Some evidences do indicate that various hydrolytic enzymes are associated with different lysosomes in a variety of cells. These hydrolases have an acid optimum of about pH5. The enzymes of lysosomes become active only when the surface membrane is ruptured.

Functions of Lysosomes

1. Extracellular Digestion

Lysosomes are small bags containing digestive enzymes. They behave like tiny time bombs waiting for their explosion in the cytoplasm. When the limiting membrane ruptures, the digestive enzymes are released which take part in the digestion.

Sometimes lysosomal enzymes may be released outside the cell where they digest extracellular substances. Saprophytic fungi and other micro-organisms utilize extracellular digestion of complex substrates in the habitat and degrade them into simpler soluble forms which are then absorbed.

2. Intracellular Digestion

The digestive enzymes released in the cytoplasm may be involved in autophagy or heterophagy.

(a) Autophagy refers to digestion of endogenous materials or breakdown of molecules and pieces of cytoplasmic materials within the cell. The simpler substances formed after the digestion are then utilized in the synthesis of some other substances.

This recycling of cell component is called turnover. Actually the digestive materials are non-functional part of other organelles like mitochondria, endoplasmic tubules, enclosed in a vesicle or digestive vacuole called autophagic vesicle or autophagosome.

Primary lysosomes fuse with the autophagic vesicles as a result of which lysosomal enzymes are discharged into them. In this way secondary Lysosomes are formed. The presence of hydrolytic enzymes in the autophagosomes and progressive disintegration of enclosed organelles give indication of digestive activity.

Autophagy may bring about cellular digestion after the death of a cell and so it brings about the self-clearance of dead cells. This is why Christian de-Duve called lysosomes as the suicide bags.

(b) Heterophagy refers to intake of extraneous matter into the cell and subsequent break-down of that material by acid hydrolases. The bulk intake of exogenous material is called endocytosis (Refer Figure 3.6). The intake of

liquid material is pinocytosis while the intake of solid matter is referred to as phagocytosis. The process of phagocytosis is illustrated by Figure. 3.6 below.

NOTES

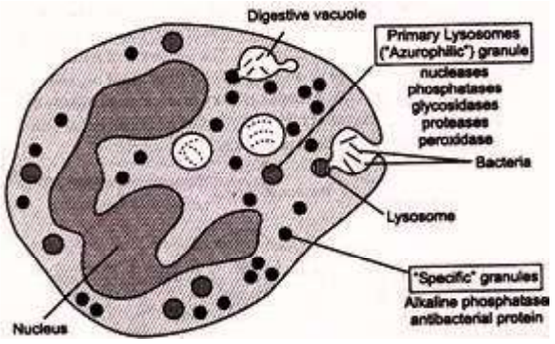


Fig. 3.6 *Phagocytosis in a cell*

3. Role in the Release of Hormones

There is evidence that lysosomal acid hydrolases are involved in release of certain hormones from secretory cells of certain glands, e.g., thyroid hormones are released by hydrolysis of thyroglobulin.

4. Role in the Penetration of Sperm Nucleus into the Egg

The enzymes released from acrosome vesicle, the giant lysosomes of sperms, dissolve the cortical granules, the structure surrounding the egg nucleus and help in the penetration of sperm nucleus into the egg.

5. Role in Metamorphosis

During the development of embryo, several tissues become functionless which are digested by the enzymes released from lysosomes and the digested materials are then absorbed by the surrounding cells. Rudolph Weber has reported the absorption tail of tadpole in this way.

6. Lysosomal Activity in Relation to Pathology

Several recent pathological studies have indicated that irregularities in lysosomal activity may cause fever, congestive heart failure, hepatitis, Pylonephritis, hypertension, joint injuries, leucocyte granules and tissue injuries.

Release of nucleases (DNAase and RNAase) which attack DNA and RNA respectively may cause chromosomal breaks and rearrangement and in this way it can result in structural abnormalities in chromosomes.

The chromosomal abnormality may induce certain harmful mutations in characters. Sometime it may lead to carcinogenesis (cancer formation). Malignant cells are also found to have abnormal chromosomes. Partial deletion of chromosome 21 of man has been associated with chronic myeloid leukemia.

NOTES

Accumulation of certain indigestible materials such as silica, asbestos particles, crystals of sodium ureate in the cells under certain conditions may result in cell inflammation. The inflammations of cells in such cases results due to release of enzymes after lysosomal break down in the cells containing ingested particles.

The actively dividing cells have been found to contain only a few lysosomes which are located generally near the periphery of the cell rather than near the nucleus. This suggests that the release of hydrolases from lysosomes has something to do with the division of cell.

7. Protection

Lysosomes of leucocytes help in defence against infection by bacteria and other microbes and guard against toxic molecules by digesting them. A mature leucocyte or white blood cell entering the circulation contains many lysosomes. During the life-time a leucocyte may ingest a foreign body such as a bacterium.

In this event the lysosomes disappear releasing their enzymes into the digestive vacuoles containing the bacterial cell. The lysosomes rupture, bacterium is digested, and the leucocyte ultimately dies having performed its major function in the body.

In animal body several kinds of cells have short lives such as, outer layer of skin and the mucous membrane lining the body. The short lived cells are being continuously replaced and the lysosomes of dead or degenerating cells release their enzymes into the body of the cells so that the whole cell may be digested. The process of tissue degeneration or necrosis can be attributed partly to lysosomal function.

Check Your Progress

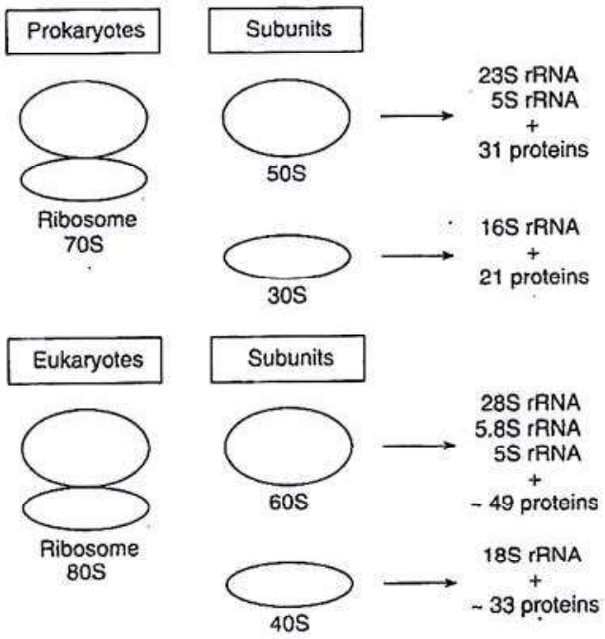
8. Define lysosomes.
9. Where does lysosomes appear?
10. When does lysosomes function?

3.5 RIBOSOMES

Ribosomes are cytoplasmic non- membranous ribonucleoprotein granules of 150 -200 A diameter. They have a typical binary and constricted structure with the two units being unequal in size. The prokaryotic and eukaryotic ribosomes are differentiated on the basis of the sedimentation coefficient.

These are of two basic types, 70S and BOS ribosomes found in prokaryotes and eukaryotes respectively. The 'S' (Svedberg units) refers to sedimentation coefficient which shows how fast a cell organelle sediments in an ultracentrifuge. The heavier a structure the more is its sedimentation coefficient.

NOTES



Composition of typical prokaryotic and eukaryotic ribosomes

The 70S ribosome of prokary-otes is relatively smaller and consists of a large 50S subunit and a small 30S subunit. The ribosomes of eukaryotes are heavier and made up of large 60S subunit and small 40S subunit (Refer Figure 3.7).

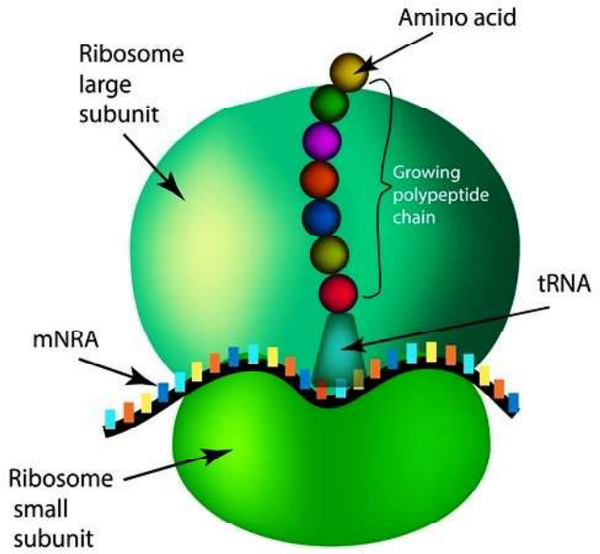


Fig 3.7 *Structure of Ribosome*

NOTES

Chemically ribosome is made up of rRNA, proteins and some divalent metallic ions. The subunits of ribosome can dissociate and asso-ciate on the basis of Mg++ concentration. Ribosomes may occur in the free form in prokaryotes, and are then called monosomes, or may be associated with mRNA to form poly-somes as in eukaryote. Ribosomes reported in plastids and mitochondria have a sedimentation coefficient of 70S and similar to prokaryotes in size and are different from cytoribosomes.

The structural model of ribosomes, as pro-posed by Stoffier and Wittmann, has the frontal face of the 30S subunit with its hollow facing the vaulted seat of the SOS subunit. The long axis of the BOS subunit is oriented transversely to the central protuberance of the SOS subunit. A tunnel is formed between the hollow of the small sub unit and the vaulted seat of the large subunit (Refer Figure 3.8).

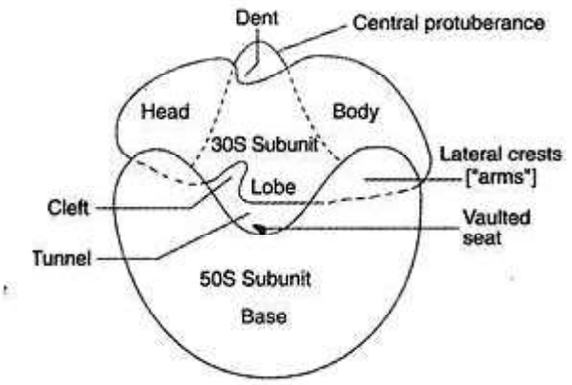


Fig. 3.8 *Stoffier and Wittmann model of Ribosome*

Functions of Ribosomes in Cell

Ribosomes are the site of protein synthesis and are string together by mRNA to form polysomes or polyribosomes. Interaction of the tRNA-amino acid complex with mRNA, which brings about translation of the genetic code, is coordinated by the ribosomes.

During protein synthesis, the messenger RNA moves through a channel between two subunits of ribo-some. Each ribosome has two functional sites – amino acyl or acceptor (A) site and the peptidyl or donor (P) site (Refer Figure 3.9). The acceptor sites receive the tRNA amino acid complex, and the donor site binds the growing polypeptide tRNA. As such, they perform most important function in protein synthesis.

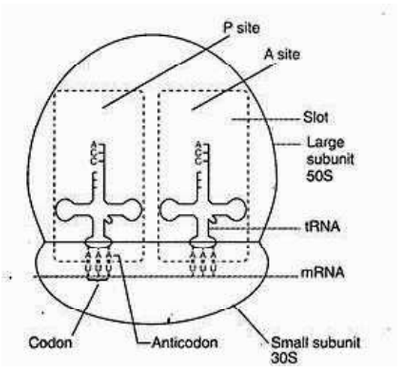


Fig 3.9 *Amino acyl or acceptor (A) site and the peptidyl or donor (P) site*

3.6 PEROXISOMES

In addition to lysosomes, a group of smaller particles than mitochondria and lysosomes are found in liver cells. These particles are rich in the enzymes peroxidase, catalase, D-amino acid oxidase and to a lesser extent, urate oxidase and are called peroxisomes (Refer Figure 3.10).

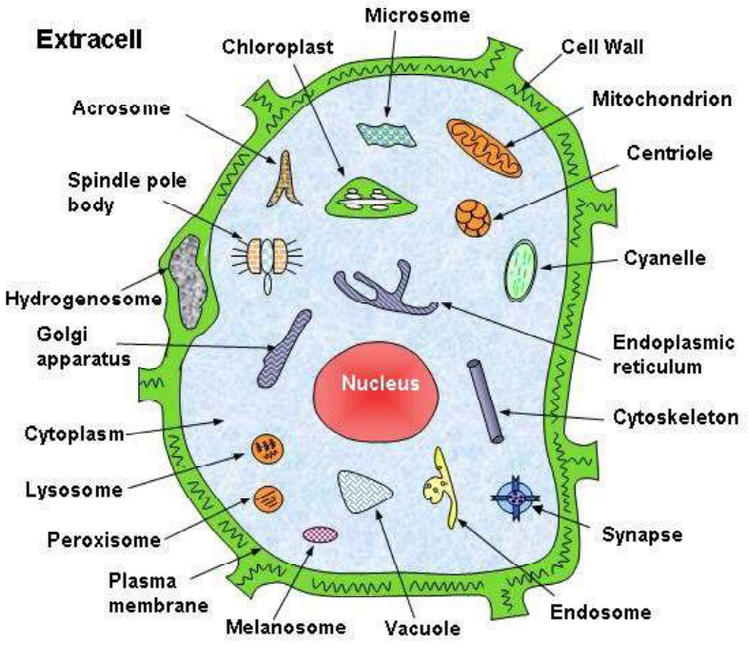


Fig 3.10 *Peroxisome*

Peroxisomes are found in liver, kidney, Protozoa, yeast and many cell types of higher plants. Peroxisomes present in plant cells show some morphological

similarities to the peroxisomes in animal cells. But plant peroxisomes have different enzymes including the enzymes of the glyoxylate cycle. Hence their name is glyoxysomes.

NOTES

Structure

Peroxisomes are ovoid granules limited by a single membrane. They contain a fine, granular substance which may condense in the centre, forming an opaque and homogeneous core or nucleoid.The average size of the peroxisomes in rat liver cells was shown to be 0.6 to 0.7 µm. The number of peroxisomes per cell varied between 70 and 100, whereas 15 to 20 lysosomes were found per liver cell. In many tissues peroxisomes show a crystal-like body made of tubular subunits.

In contrast to the nucleoid-containing peroxisomes found in liver and kidney, there are others which are smaller and lack a nucleoid. These are called microperoxisomes found in all cells and are related to endoplasmic reticulum. These may be considered as regions of ER in which catalase and other enzymes are found.

Origin

Both types of peroxisomes are formed in the endoplasmic reticulum, and the enzymes they contain are synthesized by ribosomes bound to the granular ER. It is assumed that the peroxisome grows and is destroyed, probably by autophage after four or five days.

Peroxisomal enzymes

The enzymes of peroxisomes are synthesized in the ribosomes attached to the rough endoplasmic reticulum. Liver peroxisomes contain four enzymes related to the metabolism of H_2O_2. Three of them are urate oxidase, D-amino oxidase, and oc-hydroxylic acid oxidase, which produce peroxide (H_2O_2), and fourth is catalase which destroys peroxide.

Catalase is found in the matrix of peroxisomes and represents upto 40% of the total protein. H_2O2 is toxic so catalase destroys it and it probably plays a protective role. The enzyme urate oxidase, D-amino oxidase and hydroxylic acid oxidase present in amphibian and avian peroxisomes are related to the catabolism of purines.

Functions of Peroxisomes

Peroxisomes derive their name from their use of molecular oxygen for metabolic processes. These organelles are largely associated with lipid metabolism and the processing of reactive oxygen species. Within lipid metabolism, peroxisomes mostly deal with â–oxidation of fatty acids, the mobilization of lipid stores in seeds, cholesterol biosynthesis and steroid hormone synthesis.

NOTES

β–oxidation

The main reason for the high energy density of fats is the low proportion of oxygen atoms in every fatty acid molecule. For instance, palmitic acid, a fatty acid containing 16 carbon atoms and having a molecular mass of over 250 gms/mole, has only two oxygen atoms. While this makes lipids good storage molecules, they cannot be directly burnet as fuel or quickly catabolized in the cytoplasm through glycolysis. They need to be processed before they can be shunted into the mitochondria for complete oxidation through the citric acid cycle and oxidative phosphorylation.

When these molecules need to be oxidized to release ATP, they need to be first broken down into smaller molecules before they can be processed in the mitochondria. Within peroxisomes, long chain fatty acids are progressively broken down to generate acetyl coenzyme A (acetyl CoA) in a process called β–oxidation. Acetyl CoA then combines with oxaloacetate to form citrate. While most carbohydrates enter the citric acid cycle as a three-carbon molecule called pyruvate which is then decarboxylated to form acetyl CoA, peroxisomal β–oxidation allows fatty acids to access the citric acid cycle directly.

One of the main byproducts of β–oxidation is hydrogen peroxide which can be harmful for the cell. This molecule is also carefully detoxified by the enzyme catalase within peroxisomes.

Lipid Biosynthesis and Detoxification

In animal cells, peroxisomes are the sites for some amount of lipid biogenesis, especially of special phospholipids called plasmalogens that form the myelin sheath in nerve fibers. Peroxisomes are also necessary for the synthesis of bile salts. About 25% of the alcohol we consume is oxidized to acetaldehyde in these organelles. Their role in detoxifying and oxidizing a number of molecules, metabolic byproducts and drugs makes them a prominent part of kidney and liver cells.

Disorders Relating to Peroxisome Function

Disorders arising from deficient peroxisome function could arise from defects in peroxisome biogenesis, mutated peroxisomal enzymes, or non-functional transporters that recognize PTS1 and PTS2 in cytoplasmic proteins. The most severe of these are rare genetic disorders that result in impaired brain development and neuronal migration, along with myelin deficiency. Other organs affected include the skeletal system, liver, kidney, eyes, heart and lungs.

These disorders are usually caused by mutations in PEX genes, which are necessary for organelle biogenesis – from the formation of the subcellular membrane, to the recognition of cytoplasmic proteins and their import into the matrix of the organelle. For instance, PEX16 is involved in the synthesis of peroxisomal membranes, while PEX2 forms the translocation channel for the import of matrix proteins. PEX5, on the other hand is the receptor for recognizing the PTS1 signal sequence.

Defects in these proteins can cause the accumulation of long chain fatty acids in blood plasma or urine as well as the inappropriate presence of phospholipids like plasmalogens in red blood cells.

NOTES

Check Your Progress

11. Define ribosome.
12. How many types of ribosomes are there?
13. What is the composition of ribosomes?

3.7 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. The endoplasmic reticulum (ER) is a type of organelle found in eukaryotic cells that forms an interconnected network of flattened, membrane-enclosed sacs or tube-like structures known as cisternae.
2. Cisternae are long, flat and unbranched plates or lamellae arranged in parallel rows.
3. Vesicles are usually round or ovoid sacs. They often occur isolated in the cytoplasm.
4. Functions of Endoplasmic Reticulum (ER)
   - It is mainly responsible for the transportation of proteins and other carbohydrates to another organelle, which includes lysosomes, Golgi apparatus, plasma membrane, etc.
   - They provide the increased surface area for cellular reactions.
   - They help in the formation of nuclear membrane during cell division.
5. The Golgi apparatus, also known as the Golgi complex, Golgi body, or simply the Golgi, is an organelle found in most eukaryotic cells.
6. The Golgi complex occur in all cells except the prokaryotic cells and some eukaryotic cells like mature sieve tubes of plants, mature sperm and red blood cells of animals. In animal cells, there usually occurs a single Golgi apparatus however, its number may vary.
7. Vesicles are those that bud from the ER or from the cis and medial cisternae of the Golgi complex and are seen in the electron microscope to be covered by a indistinct fuzzy coat that contains a GTP- binding protgin, called ARF (adenosylation ribose factor).
8. Lysosome is a membrane-bound organelle found in many animal cells and most plant cells. They are spherical vesicles that contain hydrolytic enzymes that can break down many kinds of biomolecules.

9. Lysosomes appear to be absent in prokaryotes. Cytochemical and electron microscopic studies have revealed the presence of membrane bound enzyme containing bodies in animal tissues. It is not certain whether the structures equivalent to animal Lysosomes are present in plant cells.

   The presence of lysosomes has been demonstrated in several slime moulds, fungal hyphae and algae. In 1964, P. Matile reported the occurrence of lysosomes in neumspora. Among the algae acid phosphatase has been located in lysosomes of Euglena and a few other species.

10. Lysosomes start functioning when the cell takes up substances either by phagocytosis or pinocytosis.
11. The ribosome is a complex molecular machine, found within all living cells, that serves as the site of biological protein synthesis.
12. These are of two basic types, 70S and BOS ribosomes found in prokaryotes and eukaryotes respectively.
13. Chemically ribosome is made up of rRNA, proteins and some divalent metallic ions.

3.8 SUMMARY

- The Endoplasmic Reticulum (ER) is an important organelle in eukaryotic cells. It plays a major role in the production, processing, and transport of proteins and lipids.
- The ER produces transmembrane proteins and lipids for its membrane and for many other cell components including lysosomes, secretory vesicles, the Golgi apparatus, the cell membrane, and plant cell vacuoles. It was first reported by Porter in 1945.
- The rough endoplasmic reticulum is named so because of its appearance. It is a series of connected flattened sacs that have many ribosomes on their outer surface, hence the name.
- It synthesizes and secretes proteins in the liver, hormones and other substances in the glands. This type of endoplasmic reticulum is especially prominent in certain kinds of cells like hepatocytes where active protein synthesis occurs.
- The Golgi apparatus, like the endoplas-mic reticulum, is a canalicular system with sacs that performs some important cellular functions like biosynthesis of polysaccha-rides and packaging of cellular products.
- In 1898, an Italian biologist, Camillo Golgi dis-covered a dark yellow network located near the nucleus of nerve cells. This network, which was later identified in other cell types, was named the Golgi complex or Golgi body. Since originally these were known to be net-works, they were also called "dictyosomes" (Gr., dictyes = net).

NOTES

- The Golgi complex occur in all cells except the prokaryotic cells and some eukaryotic cells like mature sieve tubes of plants, mature sperm and red blood cells of animals. In animal cells, there usually occurs a single Golgi apparatus however, its number may vary.
- Thus, Par-amoeba species has two Golgi apparatuses, and nerve cells, liver cells and chordate oocytes have multiple Golgi apparatuses. In animal cells, the Golgi apparatus is a localized organelle. Usually it remains polar and occurs in-between the nucleus and the periphery (for example, thyroid cells, goblet cells).
- In nerve cells it occupies a circum-nuclear position. However, in the cells of higher plants, the Golgi bodies or dictyosomes are usually found scattered through-out the cytoplasm.
- The cisternae are flattened, plate or saucer-like closed compartments. These are arranged in an orderly stack, much like a stack of pancakes. Typically, a Golgi stack contains fewer than eight cisternae. Depending on the cell type, an individual cell may contain from a few to several thousand stacks per cell.
- The diameter of cisternae varies from 0.5 to 1.0 nm and in each stack, cisternae are separated by a space of 20 to 30 nm, which may contain rod-like elements or fibres. Each cisterna is bounded by a smooth unit membrane and is curved in a manner resembling a shallow cup.
- The cisterna closest to the Endoplasmic Reticulum (ER) is usually convex and is said to be at the cis face or proximal face or forming face, while the cisterna at the opposite end of the stack is of concave shape and said to be at the trans or distal or maturing face. This polarization is called cis-trans axis of the Golgi apparatus.
- Functionally the Golgi complex is also divided into four distinct compartments, the cis, medial, trans cisternal, and the trans Golgi network (TGN). Newly synthesized membrane, secretory and lysosomal proteins leave the ER and enter the Golgi through its cis face and then pass across the stack to the trans face.
- Golgi complex represents a special mem-branous compartment interposed between the ER and the extracellular space, through which there is a continuous traffic of substances. During transportation through different compartments of Golgi, substances are modified, transformed and then are directed to their proper destinations.
- The Golgi also appears to be involved in the addition of sulfate to the carbohydrate moiety of the glycoproteins in cartilage cells.
- The main function of Golgi complex is cell secretion, not only of exportable proteins but also of the enzymes present in lysosomes and peroxisomes.

- Secretion may be continuous where the prod-uct is discharged as soon as it is elaborated, as found in liver cells and plasma cells. In other cells the secretory cycle is discontinu-ous, with storage in secretory or zymogen granules, for example, pancreas, parotid gland
- By the processes of progressive filling, and concentration, the condensing vacu-oles are converted into the zymogen granules that have characteristic electron-opaque content.
- The conversion does not require energy and is probably due to the formation of osmotically inactive aggregates of sulfated peptidoglycans. It involves movement of water from vacuoles to the cytosol.
- By the processes of progressive filling, and concentration, the condensing vacu-oles are converted into the zymogen granules that have characteristic electron-opaque content. The conversion does not require energy and is probably due to the formation of osmotically inactive aggregates of sulfated peptidoglycans.
- In the cytoplasmic matrix of the cells, there occur variously shaped bodies usually bounded by a single surface membrane and containing hydrolytic enzymes. These are called lysosomes. These enzyme containing bodies play important role in the digestion or lysis of intracellular substances, so they are called lysosomes.
- Lysosomes were first reported by Christian de Duve and co-workers in Belgium in 1955 following their extensive work on the biochemical identification of certain hydrolytic enzymes in the liver cells of rats. During that period pioneering works were carried out by Novikoff in America when he was able to complement the biochemical work on Lysosome with observations at light microscopic and ultra-structural levels.
- Lysosomes represent a class of morphologically heterogeneous cytoplasmic particles. The polymorphic nature of lysosomes has been attributed to specific functions, substances they contain and stage of digestion of those substances. Their size ranges from 0.25 to 0.8μ in diameter. In mammalian kidney cells they may be as large as 5μ and they may be even larger in phagocytes.

3.9 KEY WORDS

- **Endoplasmic reticulum:** The endoplasmic reticulum (ER) is a type of organelle found in eukaryotic cells that forms an interconnected network of flattened, membrane-enclosed sacs or tube-like structures known as cisternae.
- **Golgi apparatus:** The Golgi apparatus, also known as the Golgi complex, Golgi body, or simply the Golgi, is an organelle found in most eukaryotic cells.

NOTES

- **Lysosome:** Lysosome is a membrane-bound organelle found in many animal cells and most plant cells. They are spherical vesicles that contain hydrolytic enzymes that can break down many kinds of biomolecules.
- **Ribosome:** The ribosome is a complex molecular machine, found within all living cells, that serves as the site of biological protein synthesis.
- **Peroxisome:** Peroxisome, membrane-bound organelle occurring in the cytoplasm of eukaryotic cells. Peroxisomes play a key role in the oxidation of specific biomolecules. They also contribute to the biosynthesis of membrane lipids known as plasmalogens.

3.10 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Write a short note on endoplasmic reticulum.
2. What is RER?
3. Write a short note on SEE.
4. Write the functions of RE.
5. Brief a note on morphology of Golgi complex.
6. Write a brief note on structure of lysosomes.
7. Write short note on functions of ribosomes in the cell.

Long Answer Questions

1. Give a detailed note on ER explaining about its functions and structure.
2. Write a detailed note on Golgi apparatus mentioning about its morphology and functions.
3. Explain about lysosomes, its occurrence, functions and structure.
4. Discuss about ribosomes along with its functions in detail.
5. Describe in detail about peroxisomes , its structure, origin, functions and disorders related to peroxisomes functions.

3.11 FURTHER READINGS

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NOTES

UNIT 4 CYTOSKELETON

NOTES

Structure

4.0 Introduction
4.1 Objectives
4.2 The Cytoskeleton
    4.2.1 Microfilaments
    4.2.2 Microtubules
4.3 Cell Cycle
    4.3.1 Mitosis
    4.3.2 Meiosis
4.4 Answers to Check Your Progress Questions
4.5 Summary
4.6 Key Words
4.7 Self Assessment Questions and Exercises
4.8 Further Readings

4.0 INTRODUCTION

A cytoskeleton is present in all cells of all domains of life - archaea, bacteria, eukaryotes. It is a complex network of interlinking filaments and tubules that extend throughout the cytoplasm, from the nucleus to the plasma membrane. The cytoskeletal systems of different organisms are composed of similar proteins. In eukaryotes, the cytoskeletal matrix is a dynamic structure composed of three main proteins, which are capable of rapid growth or disassembly dependent on the cell's requirements at a certain period of time.

The structure, function and dynamic behavior of the cytoskeleton can be very different, depending on organism and cell type. Even within one cell the cytoskeleton can change through association with other proteins and the previous history of the network.

A multitude of functions can be performed by the cytoskeleton. Its primary function would arguably be to give the cell its shape and mechanical resistance to deformation, and through association with extracellular connective tissue and other cells it stabilizes entire tissues. The cytoskeleton can also contract, thereby deforming the cell and the cell's environment and allowing cells to migrate.

In this unit, you will study about cytoskeleton, microtubules and microfilaments, cell cycle , mitosis and meiosis.

4.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand about cytoskeleton

- Discuss about microtubules and microfilaments
- Expalin what cell cycle is
- Describe about mitosis and meiosis

NOTES

4.2 THE CYTOSKELETON

The cytoskeleton is a network of filaments and tubules that extends throughout a cell, through the cytoplasm, which is all of the material within a cell except for the nucleus. It is found in all cells, though the proteins that it is made of vary between organisms. The cytoskeleton supports the cell, gives it shape, organizes and tethers the organelles, and has roles in molecule transport, cell division and cell signaling (Refer Figure 4.1).

Structure of the Cytoskeleton

All cells have a cytoskeleton, but usually the cytoskeleton of eukaryotic cells is what is meant when discussing the cytoskeleton. Eukaryotic cells are complex cells that have a nucleus and organelles. Plants, animals, fungi, and protists have eukaryotic cells. Prokaryotic cells are less complex, with no true nucleus or organelles except ribosomes, and they are found in the single-celled organisms bacteria and archaea. The cytoskeleton of prokaryotic cells was originally thought not to exist; it was not discovered until the early 1990s.

The eukaryotic cytoskeleton consists of three types of filaments, which are elongated chains of proteins: microfilaments, intermediate filaments, and microtubules.

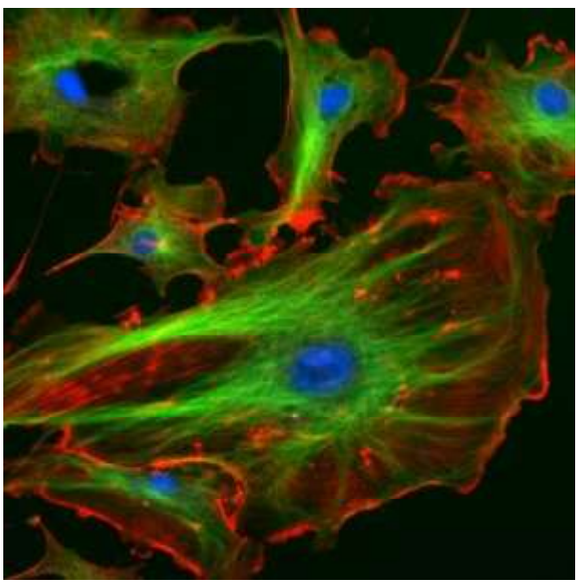


Fig 4.1 *Cytoskeleton*

NOTES

4.2.1 Microfilaments

Microfilaments are also called actin filaments because they are mostly composed of the protein actin; their structure is two strands of actin wound in a spiral. They are about 7 nanometers thick, making them the thinnest filaments in the cytoskeleton. Microfilaments have many functions. They aid in cytokinesis, which is the division of a cytoplasm of a cell when it is dividing into two daughter cells. They aid in cell motility and allow single-celled organisms like amoebas to move. They are also involved in cytoplasmic streaming, which is the flowing of cytosol (the liquid part of the cytoplasm) throughout the cell. Cytoplasmic streaming transports nutrients and cell organelles. Microfilaments are also part of muscle cells and allow these cells to contract, along with myosin. Actin and myosin are the two main components of muscle contractile elements (Refer Figure 4.2).

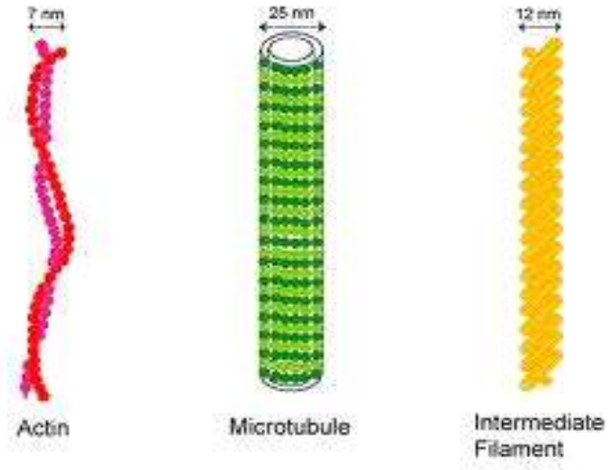


Fig 4.2 *Microfilaments- Actin, Microtubule and Intermediate Filament*

Intermediate Filaments

Intermediate filaments are about 8-12 nm wide; they are called intermediate because they are in-between the size of microfilaments and microtubules. Intermediate filaments are made of different proteins such as keratin (found in hair and nails, and also in animals with scales, horns, or hooves), vimentin, desmin, and lamin. All intermediate filaments are found in the cytoplasm except for lamins, which are found in the nucleus and help support the nuclear envelope that surrounds the nucleus. The intermediate filaments in the cytoplasm maintain the cell's shape, bear tension, and provide structural support to the cell.

4.2.2 Microtubules

Microtubules are the largest of the cytoskeleton's fibers at about 23 nm. They are hollow tubes made of alpha and beta tubulin. Microtubules form structures like flagella, which are 'tails' that propel a cell forward. They are also found in structures like cilia, which are appendages that increase a cell's surface area and in some

cases allow the cell to move. Most of the microtubules in an animal cell come from a cell organelle called the centrosome, which is a microtubule organizing center (MTOC). The centrosome is found near the middle of the cell, and microtubules radiate outward from it. Microtubules are important in forming the spindle apparatus (or mitotic spindle), which separates sister chromatids so that one copy can go to each daughter cell during cell division. They are also involved in transporting molecules within the cell and in the formation of the cell wall in plant cells.

Function of the Cytoskeleton

As described above, the cytoskeleton has several functions. First, it gives the cell shape. This is especially important in cells without cell walls, such as animal cells, that do not get their shape from a thick outer layer. It can also give the cell movement. The microfilaments and microtubules can disassemble, reassemble, and contract, allowing cells to crawl and migrate, and microtubules help form structures like cilia and flagella that allow for cell movement.

The cytoskeleton organizes the cell and keeps the cell's organelles in place, but it also aids in the movement of organelles throughout the cell. For example, during endocytosis when a cell engulfs a molecule, microfilaments pull the vesicle containing the engulfed particles into the cell. Similarly, the cytoskeleton helps move chromosomes during cell division.

One analogy for the cytoskeleton is the frame of a building. Like a building's frame, the cytoskeleton is the 'frame' of the cell, keeping structures in place, providing support, and giving the cell a definite shape.

Check Your Progress

1. Define cytoskeleton.
2. What is the function of cytoskeleton?
3. What is the composition of eukaryotic cytoskeleton?
4. What are microtubules?

4.3 CELL CYCLE

The cell cycle involves many repetitions of cellular growth and reproduction. With few exceptions (for example, red blood cells), all the cells of living things undergo a cell cycle. The cell cycle is generally divided into two phases: interphase and mitosis. During interphase, the cell spends most of its time performing the functions that make it unique. Mitosis is the phase of the cell cycle during which the cell divides into two daughter cells.Actively dividing eukaryote cells pass through a series of stages known collectively as the cell cycle: two gap phases (G1 and G2); an S (for synthesis) phase, in which the genetic material is duplicated; and an M phase, in which mitosis partitions the genetic material and the cell divides (Refer Figure 4.3).

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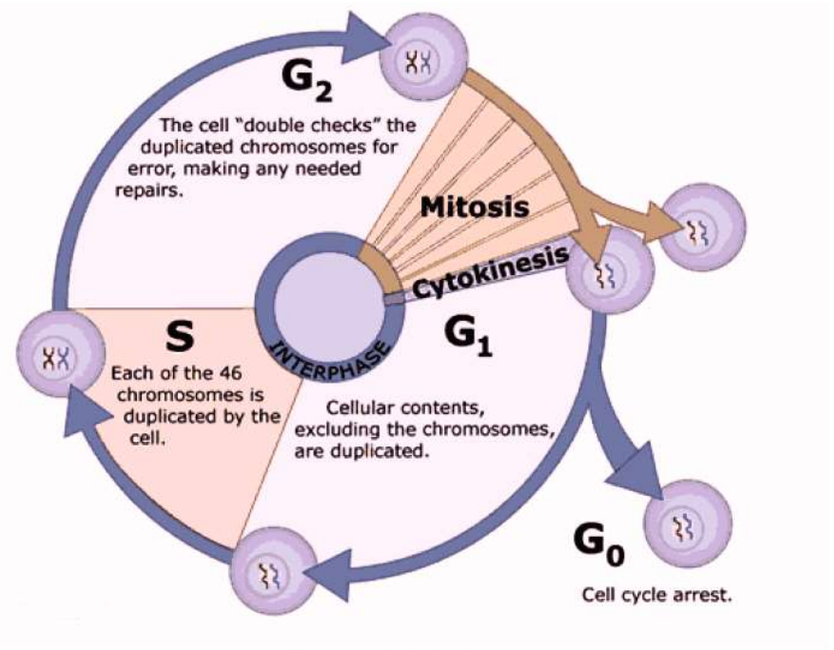


Fig. 4.3 *Cell Cycle*

G1 phase: Metabolic changes prepare the cell for division. At a certain point - the restriction point - the cell is committed to division and moves into the S phase.

S phase: DNA synthesis replicates the genetic material. Each chromosome now consists of two sister chromatids.

G2 phase: Metabolic changes assemble the cytoplasmic materials necessary for mitosis and cytokinesis.

M phase: A nuclear division (mitosis) followed by a cell division (cytokinesis).

The period between mitotic divisions - that is, G1, S and G2 - is known as interphase.

4.3.1 Mitosis

Mitosis is a form of eukaryotic cell division that produces two daughter cells with the same genetic component as the parent cell. Chromosomes replicated during the S phase are divided in such a way as to ensure that each daughter cell receives a copy of every chromosome. In actively dividing animal cells, the whole process takes about one hour. The replicated chromosomes are attached to a 'mitotic apparatus' that aligns them and then separates the sister chromatids to produce an even partitioning of the genetic material. This separation of the genetic material in a mitotic nuclear division (or karyokinesis) is followed by a separation of the cell cytoplasm in a cellular division (or cytokinesis) to produce two daughter cells. In some single-celled organisms mitosis forms the basis of asexual reproduction. In diploid multicellular organisms sexual reproduction involves the fusion of two haploid gametes to produce a diploid zygote. Mitotic divisions of the zygote and daughter

cells are then responsible for the subsequent growth and development of the organism. In the adult organism, mitosis plays a role in cell replacement, wound healing and tumour formation. Mitosis, although a continuous process, is conventionally divided into five stages: prophase, prometaphase, metaphase, anaphase and telophase (Refer Figure 4.4)

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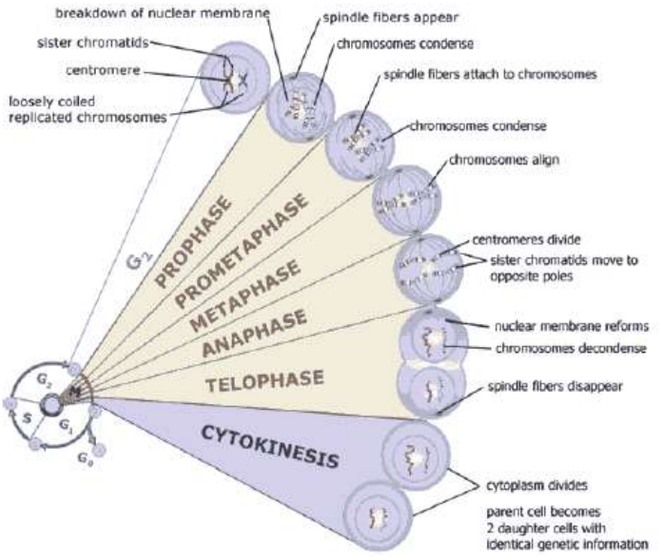


Fig. 4.4 *Mitosis - Prophase, Prometaphase, Metaphase, Anaphase and Telophase*

Prophase

Prophase occupies over half of mitosis. The nuclear membrane breaks down to form a number of small vesicles and the nucleolus disintegrates. A structure known as the centrosome duplicates itself to form two daughter centrosomes that migrate to opposite ends of the cell. The centrosomes organise the production of microtubules that form the spindle fibres that constitute the mitotic spindle. The chromosomes condense into compact structures. Each replicated chromosome can now be seen to consist of two identical chromatids (or sister chromatids) held together by a structure known as the centromere.

Prometaphase

The chromosomes, led by their centromeres, migrate to the equatorial plane in the midline of cell - at right-angles to the axis formed by the centrosomes. This region of the mitotic spindle is known as the metaphase plate. The spindle fibres bind to a structure associated with the centromere of each chromosome called a kinetochore. Individual spindle fibres bind to a kinetochore structure on each side of the centromere. The chromosomes continue to condense.

NOTES

Metaphase

The chromosomes align themselves along the metaphase plate of the spindle apparatus.

Anaphase

The shortest stage of mitosis. The centromeres divide, and the sister chromatids of each chromosome are pulled apart - or 'disjoin' - and move to the opposite ends of the cell, pulled by spindle fibres attached to the kinetochore regions. The separated sister chromatids are now referred to as daughter chromosomes. (It is the alignment and separation in metaphase and anaphase that is important in ensuring that each daughter cell receives a copy of every chromosome.)

Telophase

The final stage of mitosis, and a reversal of many of the processes observed during prophase. The nuclear membrane reforms around the chromosomes grouped at either pole of the cell, the chromosomes uncoil and become diffuse, and the spindle fibres disappear.

Cytokinesis

The final cellular division to form two new cells. In plants a cell plate forms along the line of the metaphase plate; in animals there is a constriction of the cytoplasm. The cell then enters interphase - the interval between mitotic divisions.

4.3.2 Meiosis

Meiosis is the form of eukaryotic cell division that produces haploid sex cells or gametes (which contain a single copy of each chromosome) from diploid cells (which contain two copies of each chromosome). The process takes the form of one DNA replication followed by two successive nuclear and cellular divisions (Meiosis I and Meiosis II). As in mitosis, meiosis is preceded by a process of DNA replication that converts each chromosome into two sister chromatids.

Meiosis I: Meiosis I separates the pairs of homologous chromosomes (Refer Figure 4.5). In Meiosis I a special cell division reduces the cell from diploid to haploid.

Prophase I: The homologous chromosomes pair and exchange DNA to form recombinant chromosomes. Prophase I is divided into five phases:

- **Leptotene:** chromosomes start to condense.
- **Zygotene:** homologous chromosomes become closely associated (synapsis) to form pairs of chromosomes (bivalents) consisting of four chromatids (tetrads).
- **Pachytene:** crossing over between pairs of homologous chromosomes to form chiasmata (sing. chiasma).

- **Diplotene:** homologous chromosomes start to separate but remain attached by chiasmata.
- **Diakinesis:** homologous chromosomes continue to separate, and chiasmata move to the ends of the chromosomes.

Prometaphase I

Spindle apparatus formed, and chromosomes attached to spindle fibres by kinetochores.

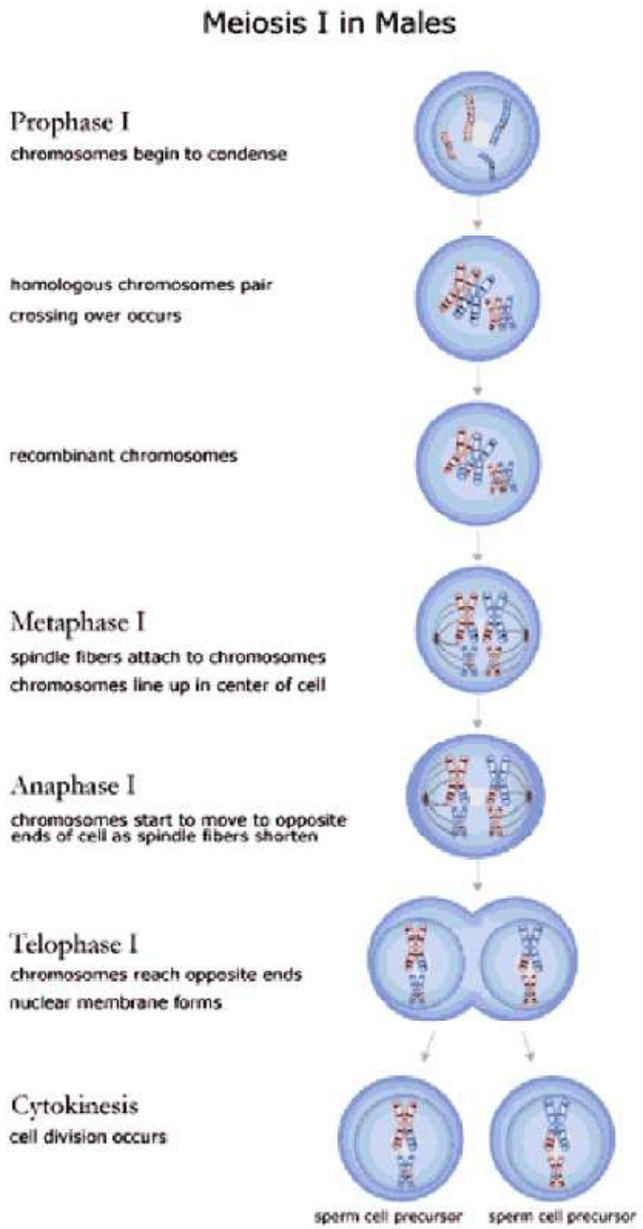


Fig. 4.5 *Meiosis 1 in Males*

NOTES

Metaphase I

Homologous pairs of chromosomes (bivalents) arranged as a double row along the metaphase plate. The arrangement of the paired chromosomes with respect to the poles of the spindle apparatus is random along the metaphase plate. (This is a source of genetic variation through random assortment, as the paternal and maternal chromosomes in a homologous pair are similar but not identical. The number of possible arrangements is 2n, where n is the number of chromosomes in a haploid set. Human beings have 23 different chromosomes, so the number of possible combinations is 223, which is over 8 million).

Anaphase I

The homologous chromosomes in each bivalent are separated and move to the opposite poles of the cell.

Telophase I

The chromosomes become diffuse and the nuclear membrane reforms.

Cytokinesis

The final cellular division to form two new cells, followed by Meiosis II. Meiosis I is a reduction division: the original diploid cell had two copies of each chromosome; the newly formed haploid cells have one copy of each chromosome.

Meiosis II

Meiosis II separates each chromosome into two chromatids (Refer Figure 4.6).

The events of Meiosis II are analogous to those of a mitotic division, although the number of chromosomes involved has been halved. Meiosis generates genetic diversity through: the exchange of genetic material between homologous chromosomes during Meiosis I. The random alignment of maternal and paternal chromosomes in Meiosis I. The random alignment of the sister chromatids at Meiosis II.

The events of Meiosis II are analogous to those of a mitotic division, although the number of chromosomes involved has been halved. Meiosis generates genetic diversity through: the exchange of genetic material between homologous chromosomes during Meiosis I. The random alignment of maternal and paternal chromosomes in Meiosis I. The random alignment of the sister chromatids at Meiosis II.

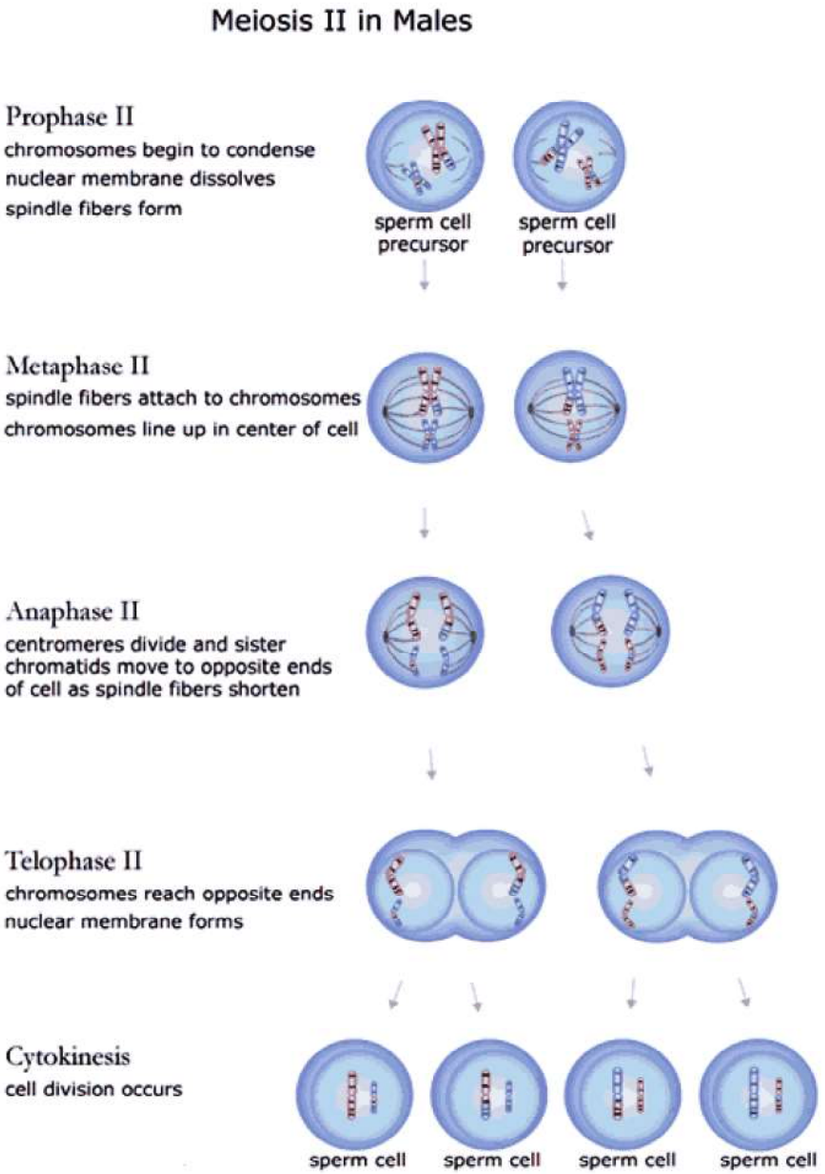


Fig. 4.6 *Meiosis II in Males*

NOTES

Meiosis in Females (Refer Figure 4.7 and 4.8)

NOTES

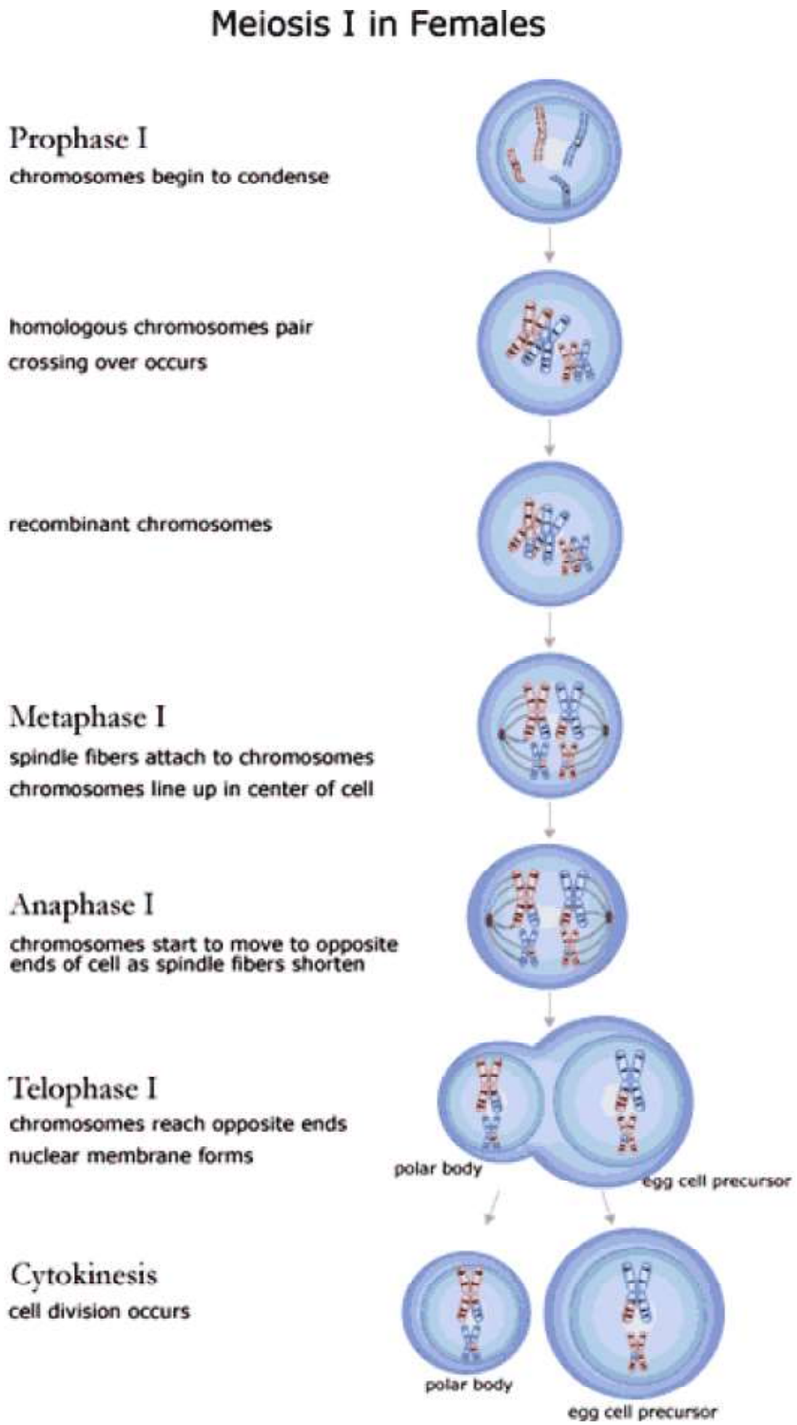


Fig. 4.7 *Meiosis 1 in Females*

NOTES

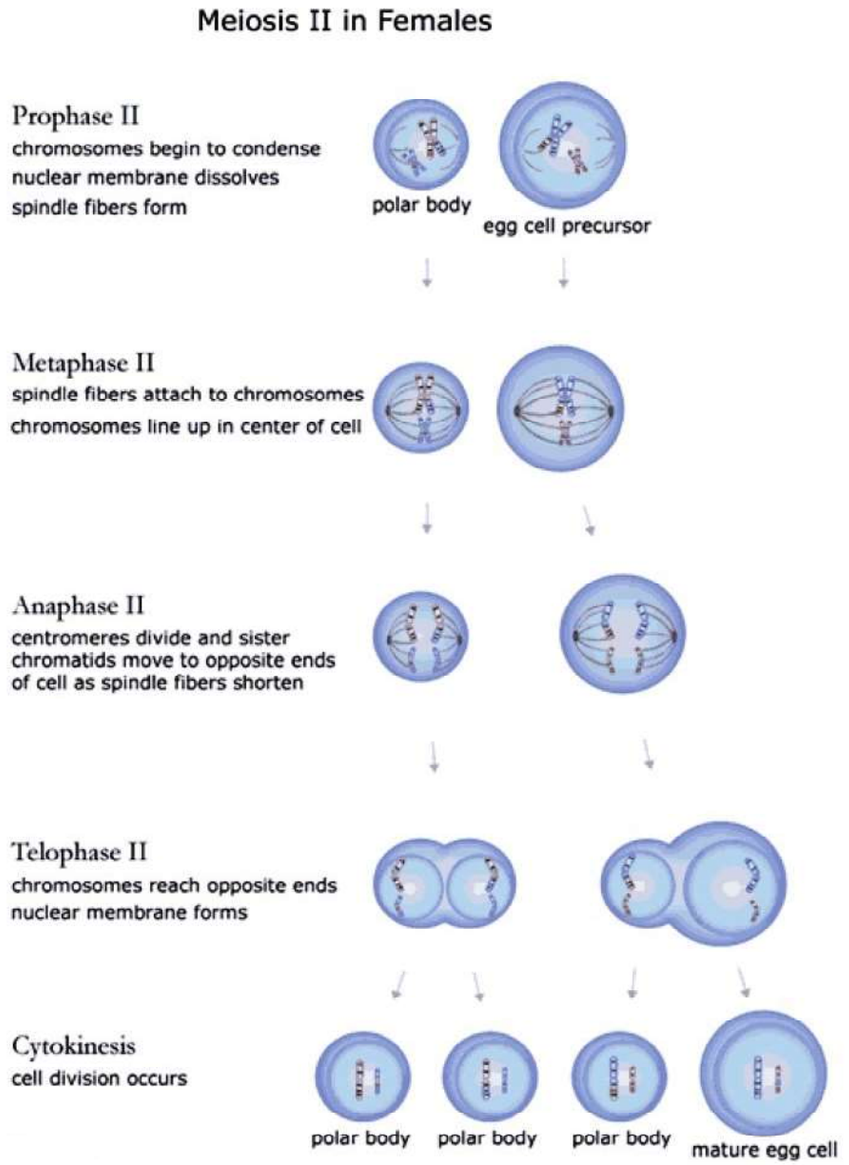


Fig. 4.8 *Meiosis II in Females*

Check Your Progress

5. What is cell cycle?
6. What is mitosis?
7. What is the function of mitosis in adult organisms.
8. In how many stages mitosis divided?
9. Define meiosis.

4.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

NOTES

1. The cytoskeleton is a network of filaments and tubules that extends throughout a cell, through the cytoplasm, which is all of the material within a cell except for the nucleus.
2. The cytoskeleton supports the cell, gives it shape, organizes and tethers the organelles, and has roles in molecule transport, cell division and cell signaling.
3. The eukaryotic cytoskeleton consists of three types of filaments, which are elongated chains of proteins: microfilaments, intermediate filaments, and microtubules.
4. Microtubules are the largest of the cytoskeleton's fibers at about 23 nm. They are hollow tubes made of alpha and beta tubulin. Microtubules form structures like flagella, which are 'tails' that propel a cell forward. They are also found in structures like cilia, which are appendages that increase a cell's surface area and in some cases allow the cell to move.
5. The cell cycle involves many repetitions of cellular growth and reproduction. With few exceptions, for example red blood cells, all the cells of living things undergo a cell cycle. The cell cycle is generally divided into two phases: interphase and mitosis.
6. Mitosis is a form of eukaryotic cell division that produces two daughter cells with the same genetic component as the parent cell.
7. In the adult organism, mitosis plays a role in cell replacement, wound healing and tumour formation.
8. Mitosis, although a continuous process, is conventionally divided into five stages: prophase, prometaphase, metaphase, anaphase and telophase
9. Meiosis is the form of eukaryotic cell division that produces haploid sex cells or gametes (which contain a single copy of each chromosome) from diploid cells (which contain two copies of each chromosome).

4.5 SUMMARY

- The cytoskeleton is a network of filaments and tubules that extends throughout a cell, through the cytoplasm, which is all of the material within a cell except for the nucleus.
- It is found in all cells, though the proteins that it is made of vary between organisms. The cytoskeleton supports the cell, gives it shape, organizes and tethers the organelles, and has roles in molecule transport, cell division and cell signaling

NOTES

- All cells have a cytoskeleton, but usually the cytoskeleton of eukaryotic cells is what is meant when discussing the cytoskeleton. Eukaryotic cells are complex cells that have a nucleus and organelles.
- Plants, animals, fungi, and protists have eukaryotic cells. Prokaryotic cells are less complex, with no true nucleus or organelles except ribosomes, and they are found in the single-celled organisms bacteria and archaea.
- The cytoskeleton of prokaryotic cells was originally thought not to exist; it was not discovered until the early 1990s.
- The eukaryotic cytoskeleton consists of three types of filaments, which are elongated chains of proteins: microfilaments, intermediate filaments, and microtubules.
- Microfilaments are also called actin filaments because they are mostly composed of the protein actin; their structure is two strands of actin wound in a spiral.
- Microfilaments have many functions, like they aid in cytokinesis, which is the division of a cytoplasm of a cell when it is dividing into two daughter cells. They aid in cell motility and allow single-celled organisms like amoebas to move.
- Microfilaments are also part of muscle cells and allow these cells to contract, along with myosin. Actin and myosin are the two main components of muscle contractile elements Intermediate filaments are about 8-12 nm wide; they are called intermediate because they are in-between the size of microfilaments and microtubules.
- Intermediate filaments are made of different proteins such as keratin (found in hair and nails, and also in animals with scales, horns, or hooves), vimentin, desmin, and lamin. All intermediate filaments are found in the cytoplasm except for lamins, which are found in the nucleus and help support the nuclear envelope that surrounds the nucleus.
- Microtubules are the largest of the cytoskeleton's fibers at about 23 nm. They are hollow tubes made of alpha and beta tubulin. Microtubules form structures like flagella, which are 'tails' that propel a cell forward.
- Microtubules are important in forming the spindle apparatus (or mitotic spindle), which separates sister chromatids so that one copy can go to each daughter cell during cell division.
- The microfilaments and microtubules can disassemble, reassemble, and contract, allowing cells to crawl and migrate, and microtubules help form structures like cilia and flagella that allow for cell movement.
- The cell cycle involves many repetitions of cellular growth and reproduction. With few exceptions (for example, red blood cells), all the cells of living things undergo a cell cycle. The cell cycle is generally divided into two phases: interphase and mitosis.

NOTES

- During interphase, the cell spends most of its time performing the functions that make it unique.
- Mitosis is a form of eukaryotic cell division that produces two daughter cells with the same genetic component as the parent cell. Chromosomes replicated during the S phase are divided in such a way as to ensure that each daughter cell receives a copy of every chromosome.
- Meiosis is the form of eukaryotic cell division that produces haploid sex cells or gametes (which contain a single copy of each chromosome) from diploid cells (which contain two copies of each chromosome). The process takes the form of one DNA replication followed by two successive nuclear and cellular divisions (Meiosis I and Meiosis II).

4.6 KEY WORDS

- **Cytoskeleton:** A cytoskeleton is present in all cells of all domains of life. It is a complex network of interlinking filaments and tubules that extend throughout the cytoplasm, from the nucleus to the plasma membrane.
- **Microfilaments:** Microfilaments, also called actin filaments, are filaments in the cytoplasm of eukaryotic cells that form part of the cytoskeleton and are primarily composed of polymers of actin, but in cells are modified by and interact with numerous other proteins.
- **Intermediate filaments:** Intermediate filaments (IFs) are cytoskeletal components found in the cells of vertebrate animal species, and perhaps also in other animals, fungi, plants, and unicellular organisms. They are composed of a family of related proteins sharing common structural and sequence features.
- **Microtubule:** Microtubules are filamentous intracellular structures that are responsible for various kinds of movements in all eukaryotic cells.
- **Actin:** Actin is a family of globular multi-functional proteins that form microfilaments.
- **Myosins :** Myosins are a superfamily of motor proteins best known for their roles in muscle contraction and in a wide range of other motility processes in eukaryotes.
- **Cell cycle:** The cell cycle, or cell-division cycle, is the series of events that take place in a cell leading to its division and duplication of its DNA (DNA replication) to produce two daughter cells.
- **Mitosis:** Mitosis is a part of the cell cycle when replicated chromosomes are separated into two new nuclei. Cell division gives rise to genetically identical cells in which the number of chromosomes is maintained.
- **Meiosis:** Meiosis is a specialized type of cell division that reduces the chromosome number by half, creating four haploid cells, each genetically distinct from the parent cell that gave rise to them.

4.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

NOTES

Short Answer Questions

1. Write a short note on cytoskeleton.
2. Brief about the structure of cytoskeleton.
3. What is the function of cytoskeleton?
4. Write a short note on intermediate filaments.
5. What is cell cycle?
6. Give a difference between mitosis and meiosis.

Long Answer Questions

1. Explain about cytoskeleton in detail writing about its structure and function.
2. Discuss about microtubules and microfilaments in detail.
3. Explain cell cycle in detail.
4. Give a detailed note on mitosis along with a diagram.
5. Discuss in detail about meiosis.

4.8 FURTHER READINGS

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NOTES

BLOCK - II
NUCLEIC ACIDS

UNIT 5 STRUCTURE AND FUNCTION: DNA AND RNA

Structure

5.0 Introduction
5.1 Objectives
5.2 Dna: Structure and Function
5.2.1 Structure of DNA; 5.2.2 Functions of DNA
5.3 RNA: Structure, Function and Types
5.4 Answers to Check Your Progress Questions
5.5 Summary
5.6 Key Words
5.7 Self Assessment Questions and Exercises
5.8 Further Readings

5.0 INTRODUCTION

DNA stands for deoxyribonucleic acid, while RNA is ribonucleic acid. Although DNA and RNA both carry genetic information, there are quite a few differences between them. While there is some evidence DNA may have occurred first, most scientists believe RNA evolved before DNA. RNA has a simpler structure and is needed in order for DNA to function. Also, RNA is found in prokaryotes, which are believed to precede eukaryotes. RNA on its own can act as a catalyst for certain chemical reactions. The real question is why DNA evolved, if RNA existed. The most likely answer for this is that having a double-stranded molecule helps protect the genetic code from damage. If one strand is broken, the other strand can serve as a template for repair. Proteins surrounding DNA also confer additional protection against enzymatic attack.

While the most common form of DNA is a double helix. there is evidence for rare cases of branched DNA, quadruplex DNA, and molecules made from triple strands. Scientists have found DNA in which arsenic substitutes for phosphorus. Double-stranded RNA (dsRNA) sometimes occurs. It is similar to DNA, except thymine is replaced by uracil. This type of RNA is found in some viruses. When these viruses infect eukaryotic cells, the dsRNA can interfere with normal RNA function and stimulate an interferon response. Circular single strand RNA (circRNA) has been found in both animals and plants. At present, the function of this type of RNA is unknown.

In this unit, you will study about DNA- its structure and functions, RNA- its types, function and structure in detail.

5.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand about DNA
- Discuss about structure and functions of DNA
- Explain about RNA
- Describe about the types and functions of RNA

NOTES

5.2 DNA: STRUCTURE AND FUNCTION

Deoxyribonucleic acid is a molecule composed of two chains that coil around each other to form a double helixcarrying the geneticinstructions used in the growth, development, functioning, are reproductionof all known living organismsand many viruses. DNA and ribonucleic acid(RNA) are nucleic acids; alongside proteins, lipidsand complex carbohydrates (polysaccharides), nucleic acids are one of the four major types of macromoleculesthat are essential for all known forms of life.

The two DNA strands are also known as polynucleotidesas they are composed of simpler monomericunits called nucleotides. Each nucleotide is composed of one of four nitrogen-containingnucleobases (Cytosine[C], Guanine[G], Adenine[A] or Thymine[T]), a sugarcalled deoxyribose, and a phosphate group. The nucleotides are joined to one another in a chain by covalent bondsbetween the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone. The nitrogenous bases of the two separate polynucleotide strands are bound together, according to base pairing rules (A with T and C with G), with hydrogen bonds to make double-stranded DNA.

History

Nucleic acids were first isolated by Friedrich Miescher (1869) from pus cells.They were named nuclein. Hertwig (1884) proposed nuclein to be the carrier of hereditary traits. Because of their acidic nature they were named nucleinic acids and then nucleic acids (Altmann, 1899).

Fisher (1880s) discovered the presence of purine and pyri-midine bases in nucleic acids. Levene (1910) found deoxyribose nucleic acid to contain phosphoric acid as well as deoxyribose sugar.

He characterised four types of nucleotides present in DNA. In 1950, Chargaff found that purine and pyrimidine content of DNA was equal. By this time W.T. Astbury had found through X-ray diffraction that DNA is a polynucleotide with nucleotides arranged perpendicular to the long axis of the molecule and separated from one another by a distance of 0.34 nm.

NOTES

In 1953, Wilkins and Franklin got very fine X-ray photographs of DNA. The photographs showed that DNA was a helix with a width of 2.0 nm. One turn of the helix was 3.4 nm with 10 layers of bases stacked in it. Watson and Crick (1953) worked out the first correct double helix model from the X-ray photo-graphs of Wilkins and Franklin. Wilkins, Watson and Crick were awarded Nobel Prize for the same in 1962.

Watson and Crick (1953) built a 3D, molecular model of DNA that satisfied all the details obtained from X-ray photographs. They proposed that DNA consisted of a double helix with two chains having sugar phosphate on the outside and nitrogen bases on the inner side.

The nitrogen bases of the two chains formed complementary pairs with purine of one and pyrimidine of the other held together by hydrogen bonds (A-T, C-G). Comple-mentary base pairing between the two polynucleotide chains is considered to be hall mark of their proposition. It is of course based on early finding of Chargaff that A = T and Ñ = G Their second big proposal was that the two chains are antiparallel with $5' \rightarrow 3'$ orien-tation of one and Y $\rightarrow 5'$orientation of the other.

The two chains are twisted helically just as a rope ladder with rigid steps twisted into a spiral. Each turn of the spiral contains 10 nucleotides. This double helix or duplex model of DNA with antiparallel polynucleotide chains having complementary bases has an implicit mechanism of its replication and copying.

Here both the polynucleotide chains function as templates forming two double helices, each with one parent chain and one new but complementary strand. The phenomenon is called semi conservative replication. In-vitro synthesis of DNA has been carried out by Kornberg in 1959.

Types of DNA

DNA duplex model proposed by Watson and Crick is right handed spiral and is called B-DNA (Balanced DNA). In the model the base pairs lie at nearly right angles to the axis of helix. Another right handed duplex model is A-DNA (Alternate DNA). Here, a single turn of helix has 11 base pairs.

The base pairs lie 20° away from perpendicular to the axis. C-DNA has 9 base pairs per turn of spiral while in D-DNA the number is only 8 base pairs. Both are right handed. Z-DNA (Zigzag DNA) is left-handed double helix with zigzag back-bone, alternate purine and pyrimidine bases, single turn of 45 Å length with 12 base pairs and a single groove (Refer Figure 5.1).

B-DNA is more hydrated and most frequently found DNA in living cells. It is physiologically and biologically active form. However, it can get changed into other forms. Right handed DNA is known to change temporarily into the left handed form at least for a short distance. Such changes may cause changes in gene expression.

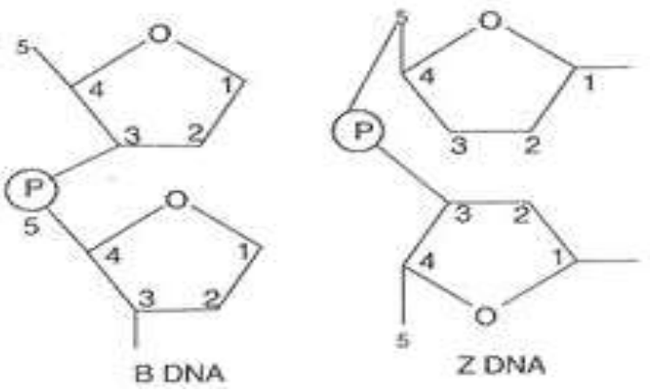


Fig. 5.1 *Orientation of Adjacent Sugar Molecules in B and Z DNA*

Circular and Linear DNA

In many prokaryotes the two ends of a DNA duplex are covalently linked to form circular DNA. Circular DNA is naked, that is, without association with histone proteins, though polyamines do occur. In linear DNA the two ends are free. It is found in eukaryotic nuclei where it is associated with histone proteins.

Linear DNA, without association with histone proteins, also occurs in some prokaryotes, for example Myco-plasma. In semi-autonomous cell organelles (mitochondria, plastids) DNA is circular, less commonly linear. It is always naked.

Chargaff's Rules

Chargaff (1950) made observations on the bases and other compo-nents of DNA. These observations or generalizations are called Chargaff's base equivalence rule.

(i) Purine and pyrimidine base pairs are in equal amount, that is, adenine + guanine = thymine + cytosine. [A + G] = [T + C], i.e., [A+G] / [T+C] = 1

(ii) Molar amount of adenine is always equal to the molar amount of thymine. Similarly, molar concentration of guanine is equalled by molar concentration of cytosine.

[A] = [T], i.e., [A] / [T] = 1; [G] = [C], i.e., [G] / [C] = 1

(iii) Sugar deoxyribose and phosphate occur in equimolar proportions.

(iv) A-T base pairs are rarely equal to Ñ—G base pairs.

(v) The ratio of [A+T] / [G+C] is variable but constant for a species (Refer Table 5.1). It can be used to identify the source of DNA. The ratio is low in primitive organisms and higher in advanced ones.

NOTES

Table 5.1 *Base Composition of DNA from Various Sources*

Species	A	G	C	T	A+T/ C+G
1. Man	30.4	19.0	19.9	30.1	1.55
2. Calf	29.0	21.2	21.2	28.5	1.35
3. Wheat germ	28.1	21.8	22.7	27.4	1.25
4. Pea	30.8	19.2	18.5	30.5	1.62
5. Euglena	22.6	27.7	25.8	24.4	0.88
6. Escherichia coli	24.7	26.0	25.7	23.6	0.93

5.2.1 Structure of DNA

DNA or deoxyribonucleic acid is a helically twisted double chain polydeoxyribonucleotide macromolecule which constitutes the genetic material of all organisms with the exception of rhinoviruses. In prokaryotes it occurs in nucleoid and plasmids. This DNA is usually circular. In eukaryotes, most of the DNA is found in chromatin of nucleus.

It is linear, in smaller quantities of circular, double stranded DNA are found in mitochondria and plastids (organelle DNA). Small sized DNAs occur in viruses, $\phi \times 174$ bacteriophage has 5386 nucleotides. Bacteriophage lambda (Phage X) possesses 48502 base pairs (bp) while number of base pairs in Escherichia coli is 4.6 x 106. A single genome (haploid set of 23 chromosomes) has about 3.3 x 109 bp in human beings. Single-stranded DNA occurs as a genetic material in some viruses, for example phage $\phi \times 174$, coliphage fd, M13. DNA is the largest macromolecule with a diameter of 2 nm (20Å or 2×10-9m) and often having 3 length in millimetres.

It is negatively charged due to phosphate groups. It is a long chain polymer of generally several hundred thousands of deoxyribonucleotides. A DNA molecule has two un-branched comple-mentary strands. They are spirally coiled. The two spiral strands of DNA are collectively called DNA duplex (Refer Figure 5.2).

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The two strands are not coiled upon each other but the whole double strand (DNA duplex) is coiled upon itself around a common axis like a rope stair case with solid steps twisted into a spiral. Due to spiral twisting, the DNA duplex comes to have two types of alternate grooves, major (22 Å) and minor (12 Å).

In B-DNA, one turn of the spiral has about 10 nucleotides on each strand of DNA. It occupies a distance of about 3.4 nm (34 Å or 3.4×10-9 m) so that adjacent nucleotides or their bases are separated by a space of about 0.34 nm (0.34×10-9 m or 3.4 Å).

A deoxyribonucleotide of DNA is formed by cross-linking of three chemicals ortho- phosphoric acid (H3PO4), deoxyribose sugar (C5H10O4) and a nitrogen base. Four types of nitrogen bases occur in DNA. They belong to two groups, purines (9-membered double rings with nitrogen at 1,3,7 and 9 positions) and pyrimidines (six membered rings with nitrogen at 1 and 3 positions). DNA has two types of purines (adenine or A and guanine or G) and two types of pyrimidines (cytosine or Ñ and thymine or T).

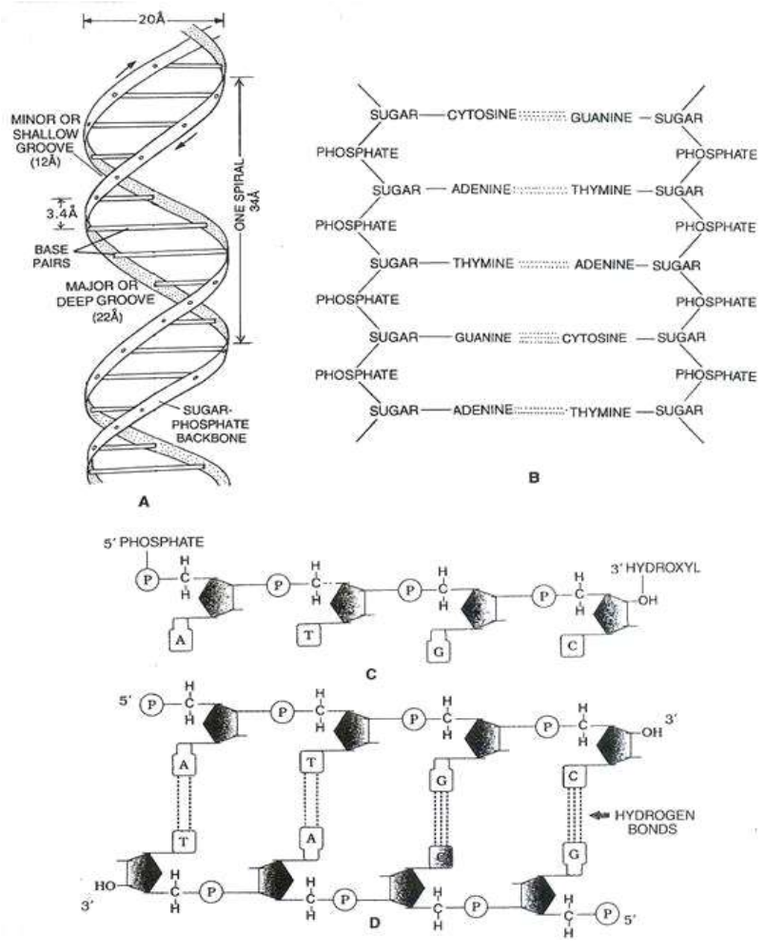


Fig. 5.2 *Structure of DNA*

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Depending upon the type of nitrogen base, DNA has four kinds of deoxyribonucleotides —deoxy adenosine 5- monophosphate (dAMP), deoxy guaninosine 5-monophosphate (d GMP), deoxy thymidine 5-monophosphate (d TMP) and deoxy cytidine 5- monophosphate (d CMP).

The back bone of a DNA chain or strand is built up of alternate deoxyribose sugar and phosphoric acid groups. The phosphate group is connected to carbon 52 of the sugar residue of its own nucleotide and carbon Ó of the sugar residue of the next nucleotide by (3 '—5') phosphodiester bonds. -H of phosphate and -OH of sugar are eliminated as H20 during each ester formation.

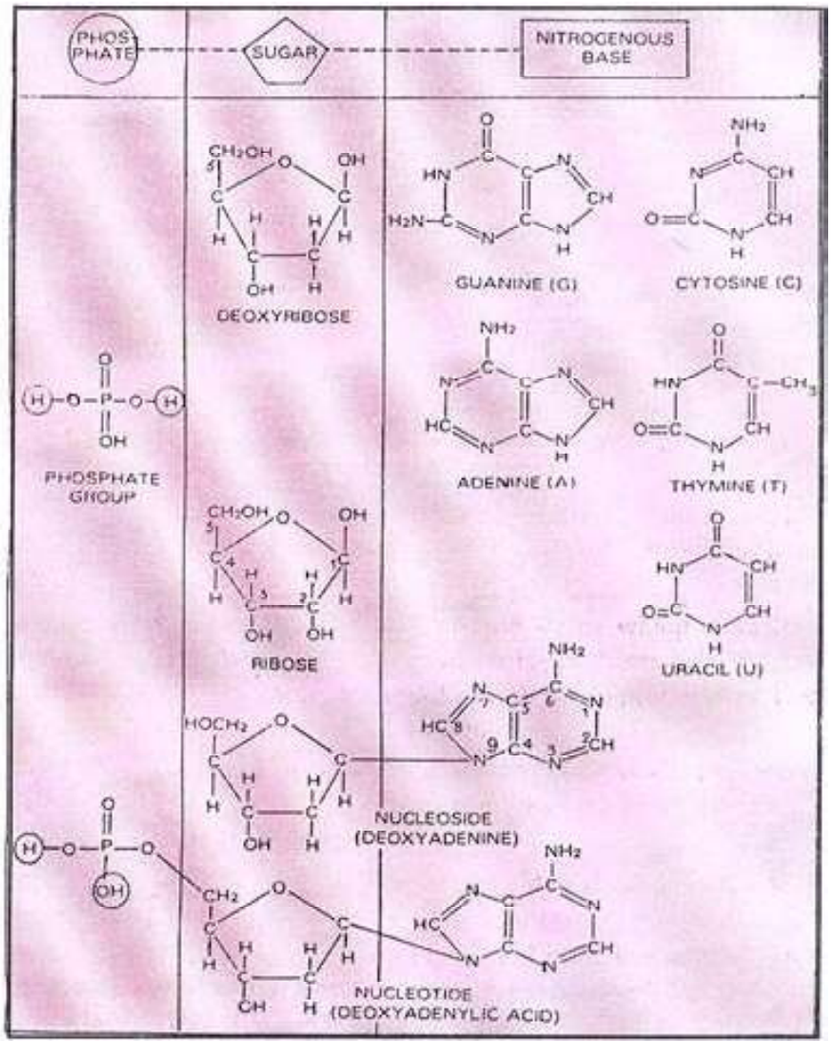


Fig. 5.3 *Building Blocks of Nucleotides*

Phosphate group provides acidity to the nucleic acids because at least one of its side group is free to dissociate. Nitrogen bases lie at right angles to the longitudinal axis of DNA chains. They are attached to carbon atom 1 of the sugars

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by N-glycosidic bonds. Pyrimidine (C or T) is attached to deoxyribose by its N-atom at 1 position while a purine (A or G) does so by N-atom at 9 position.

The two DNA chains are antiparallel that is, they run parallel but in opposite directions. In one chain the direction is 5′ → Y while in the opposite one it is 3′ → 5′ (Refer Figure 5.2). The two chains are held together by hydrogen bonds between their bases. Adenine (A), a purine of one chain lies exactly opposite thymine (T), a pyramidine of the other chain. Similarly, cytosine (C, a pyrimidine) lies opposite guanine (G a purine). This allows a sort of lock and key arrangement between large sized purine and small sized pyrimidine.

It is strengthened by the appearance of hydrogen bonds between the two. Three hydrogen bonds occur between cytosine and guanine (C = G) at positions 1′–1′, 2′ – 6′ and 6′ – 2′ . There are two such hydrogen bonds between adenine and thymine (A=T) which are formed at positions 1′– 3′ and 6′ – 4′ . Hydrogen bonds occur between hydrogen of one base and oxygen or nitrogen of the other base. Since specific and different nitrogen bases occur on the two DNA chains, the latter are complementary.

Thus the sequence of say AAGCTCAG of one chain would have a complementary sequence of TTCGAGTC on the other chain. In other words, the two DNA chains are not identical but complementary to each other. It is because of specific base pairing with a purine lying opposite a pyrimidine. This makes the two chains 2 nm thick.

A purine- purine base pair will make it thicker while a pyrimidine- pyrimidine base pair will make it narrower than 2 nm. Further, A and Ñ or G and T do not pair because they fail to form hydrogen bonds between them. 5′ end of each chain bears phosphate radical while the 3′ end possesses a sugar residue (3′ – OH).

Salient Features of B model of DNA of Watson and Crick

- DNA is the largest biomolecule in the cell.
- DNA is negatively charged and dextrorotatory.
- Molecular configuration of DNA is 3D.
- DNA has two polynucleotide chains.
- The two chains of DNA have antiparallel polarity, 5′ → 3′ in one and 3′ → 5′ in other.
- Backbone of each polynucleotide chain is made of alternate sugar-phosphate groups. The nitrogen bases project inwardly.
- Nitrogen bases of two polynucleotide chains form complementary pairs, A opposite T and C opposite G.
- A large sized purine always comes opposite a small sized pyrimidine. This generates uniform distance between two strands of helix.

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- Adenine (A) of one polynucleotide chain is held to thymine (T) of opposite chain by two hydrogen bonds. Cytosine (C) of one chain is similarly held to guanine of the other chain by three hydrogen bonds.
- The double chain is coiled in a helical fashion. The coiling is right handed. This coiling produces minor and major grooves alternately.
- The pitch of helix is 3.4 nm (34 A) with roughly 10 base pairs in each turn. The average distance between two adjacent base pairs comes to about 0.34 nm (0.34 × 10-9 m or 3.4 A).
- Planes of adjacent base pairs are stacked over one another. Alongwith hydrogen bonding, the stacking confers stability to the helical structure.
- DNA is acidic. For its compaction, it requires basic (histone) proteins. The histone proteins are +vely charged and occupy the major grooves of DNA at an angle of 30° to helix axis.

Sense and Antisense Strands

Both the strands of DNA do not take part in controlling heredity and metabolism. Only one of them does so. The DNA strand which functions as template for RNA synthesis is known as template strand, minus (-) strand or antisense strand.

Its complementary strand is named nontemplate strand, plus (+) strand, sense and coding strand. The latter name is given because by convention DNA genetic code is written according to its sequence.

(5′) GCATTCGGCTAGTAAC (3′)

DNA Nontemplate, Sense (+) or Coding Strand

(3′) CGTAAGCCGATCATTG (5′)

DNA Template, Antisense, or Noncoding or (–) Strand

(5′) GCAUUCGGCUAGUAAC (3′)

RNA Transcript

RNA is transcribed on $3' \rightarrow 5'$ (-) strand (template/antistrand) of DNA in $5 \rightarrow 3$ direction. The term antisense is also used in wider prospective for any sequence or strand of DNA (or RNA) which is complementary to mRNA.

Denaturation and Renaturation

The H-bonds between nitrogen bases of two strands of DNA can break due to high temperature (82-90°C) or low or high pH, so that the two strands separate from each other. It is called denaturation or melting. Since A-T base pair has only 2H bonds, the area rich in A-T base pairs can undergo easy denaturation (melting). These areas are called low melting areas because they denature at comparatively low temperature. The area rich in G- C base pairs (called high melting area) is comparatively more stable and dense because three hydrogen bonds connect the G-C bases.

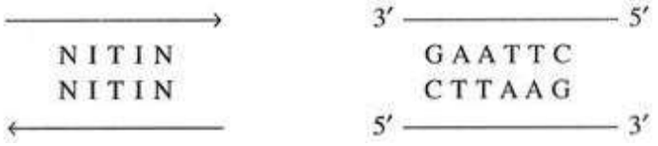
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These areas have high temperature of melting (Tm). On melting the viscosity of DNA decreases. The denatured DNA has the tendency to reassociate, i.e., the DNA strands separated by melting at 82-90°C can reassociate and form duplex on cooling to temperature at 65°C. It is called renaturation or annealing.

Denatured or separated DNA strands absorb more light energy than the intact DNA double strand. The increased absorption of light energy by separated or denatured DNA strands is called hyperchromatic effect. The effect is used in knowing whether DNA is single or double stranded.

Palindromic DNA

DNA duplex possesses areas where sequence of nucleotides is the same but opposite in the two strands. These sequences are recognised by restriction endonucleases and are used in genetic engineering. Given hereunder sequence of bases in one strand ($3' \rightarrow 5'$) is GAATTC. It is same in other strand when read in $5' \rightarrow 3'$ direction. It is similar to palindrome words having same words in both forward and backward direction, e.g., NITIN, MALAYALAM.



Repetitive DNA

It is the DNA having multiple copies of identical sequences of nitrogen bases. The number of copies of the same base sequence varies from a few to millions. DNA having single copy of base sequences is called unique DNA. It is made of functional genes. rRNA genes are, however, repeated several times. Repetitive DNA may occur in tandem or inter-spersed with unique sequences.

It is of two types, highly repetitive and moderately repetitive. Highly repetitive DNA consists of short sequences of less than 10 base pairs which are repeated millions of times. They occur in preeentromeric regions, heterochromatic re-gions of Y-chromosomes and satellite regions. Moderately repetitive dna consists of a few hundred base pairs repeated at least 1000 times. It occurs in telomeres, centromeres and transposons.

Tandemly repeated sequences are especially liable to undergo misalignments during chromosome pairing, and thus the size of tandem clusters tends to be highly poly-morphic, with wide variations between individuals. Smaller clusters of such sequences can be used to characterize individual genomes in the technique of 'DNA-finger-printing'.

Satellite DNA

It is that part of repetitive DNA which has long repetitive nucleotide sequences in tandem that forms a separate fraction on density ultracentrifugation. Depending

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upon the number of base pairs involved in repeat regions, satellite DNA is of two types, microsatellite sequences (1-6 bp repeat units flanked by conserved sequences) and minisatellite sequences (11-60 bp flanked by conserved restriction sites). The latter are hyper variable and are specific for each individual. They are being used for DNA matching or finger printing as first found out by Jeffreys et al (1985).

Genetic Information

The arrangement of nitrogen bases of DNA (and its product mRNA) determines the sequence of amino acid groups in polypeptides or proteins formed over ribosomes. One amino acid is specified by the sequence of three adjacent nitrogen bases. The latter is called codon. The segment of DNA which determines the synthesis of complete polypeptide is known as cistron.

In procaryotes, a cistron has a continuous coding sequence from beginning to end. In eucaryotes a cistron contains noncoding regions which do not produce part of gene product. They are called introns. Introns are often variable. The coding parts are known as exons. Cistrons having introns are called split genes.

Coding and Noncoding DNA

Depending on the ability to form functional or non-functional products, DNA has two types of segments, coding and noncoding. In eukaryotes a greater part of DNA is noncoding since it does not form any functional product. They often possess repeated sequences or repetitive DNA. Most of them have fixed positions.

Some can move from one place to another. The mobile sequences are called jumping genes or transposons. In prokaryotes the amount of noncoding or nonfunctional DNA is small. Coding DNA consists of coding DNA sequences. These are of 2 types — protein coding sequences coding for all proteins except histone and nonprotein coding sequences for tRNA, rRNA and histones.

5.2.2 Functions of DNA

- **Genetic Information (Genetic Blue Print):** DNA is the genetic material which car-ries all the hereditary information. The genetic information is coded in the arrangement of its nitrogen bases.
- **Replication:** DNA has unique property of replication or production of carbon copies (Autocatalytic function). This is essential for transfer of genetic information from one cell to its daughters and from one generation to the next.
- **Chromosomes:** DNA occurs inside chromosomes. This is essential for equitable distribution of DNA during cell division.
- **Recombinations:** During meiosis, crossing over gives rise to new combination of genes called recombinations.

- **Mutations:** Changes in sequence of nitrogen bases due to addition, deletion or wrong replication give rise to mutations. Mutations are the fountain head of all variations and evolution.
- **Transcription:** DNA gives rise to RNAs through the process of transcription. It is heterocatalytic activity of DNA.
- **Cellular Metabolism:** It controls the metabolic reactions of the cells through the help of specific RNAs, synthesis of specific proteins, enzymes and hormones.
- **Differentiation:** Due to differential functioning of some specific regions of DNA or genes, different parts of the organisms get differentiated in shape, size and functions.
- **Development:** DNA controls development of an organism through working of an internal genetic clock with or without the help of extrinsic information.
- **DNA Finger Printing:** Hypervariable microsatellite DNA sequences of each indi-vidual are distinct. They are used in identification of individuals and deciphering their relationships. The mechanism is called DNA finger printing.
- **Gene Therapy:** Defective heredity can be rectified by incorporating correct genes in place of defective ones.
- **Antisense Therapy:** Excess availability of anti-mRNA or antisense RNAs will not allow the pathogenic genes to express themselves. By this technique failure of angioplasty has been checked. A modification of this technique is RNA interference (RNAi).

Check Your Progress

1. What is DNA?
2. What are the two strands of DNA known?
3. What is the composition of nucleotides?
4. Give few salient features of B model of DNA of Watson and Crick.

5.3 RNA: STRUCTURE, FUNCTION AND TYPES

Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes. RNA and DNA are nucleic acids, and, along with lipids, proteins and carbohydrates, constitute the four major macromolecules essential for all known forms of life. Like DNA, RNA is assembled as a chain of nucleotides, but unlike DNA it is more often found in nature as a single-strand folded onto itself, rather than a paired double-strand. Cellular organisms use messenger RNA (mRNA) to convey genetic information (using the nitrogenous bases of guanine, uracil, adenine, and cytosine,

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denoted by the letters G, U, A, and C) that directs synthesis of specific proteins. Many viruses encode their genetic information using an RNA genome.

With the discovery of the molecular structure of the DNA double helix in 1953, researchers turned to the structure of ribonucleic acid (RNA) as the next critical puzzle to be solved on the road to understanding the molecular basis of life. Ribonucleic acid (RNA) is a type of molecule that consists of a long chain of nucleotide units. Each nucleotide consists of a nitrogenous base, a ribose sugar, and a phosphate. RNA is very similar to DNA, but differs in a few important structural details: in the cell, RNA is usually single-stranded, while DNA is usually double-stranded; RNA nucleotides contain ribose while DNA contains deoxyribose (a type of ribose that lacks one oxygen atom); and RNA has the base uracil rather than thymine that is present in DNA.

RNA is transcribed from DNA by enzymes called RNA polymerases and is generally further processed by other enzymes. RNA is central to the synthesis of proteins. Here, a type of RNA called messenger RNA carries information from DNA to structures called ribosomes. These ribosomes are made from proteins and ribosomal RNAs, which come together to form a molecular machine that can read messenger RNAs and translate the information they carry into proteins. There are many RNAs with other roles – in particular regulating which genes are expressed, but also as the genomes of most viruses. Ribose Nucleic Acids Most cellular RNA is single stranded, although some viruses have double stranded RNA. The single RNA strand is folded upon itself, either entirely or in certain regions. In the folded region a majority of the bases are complementary and are joined by hydrogen bonds. This helps in the stability of the molecule. In the unfolded region the bases have no complements. Because of this RNA does not have the purine, pyrimidine equality that is found in DNA. RNA also differs from DNA in having ribose as the sugar instead of deoxyribose. The common nitrogenous bases of RNA are adenine, guanine, cytosine and uracil. Thus the pyrimidine uracil substitutes thymine of DNA. In regions where purine pyrimidine pairing takes place, adenine pairs with uracil and guanine with cytosine. In addition to the four bases mentioned above, RNA also has some unusual bases.

Chemical Structure of Rna

An important structural feature of RNA that distinguishes it from DNA is the presence of a hydroxyl group at the 2' position of the ribose sugar. The presence of this functional group causes the helix to adopt the A-form geometry rather than the B-form most commonly observed in DNA. This results in a very deep and narrow major groove and a shallow and wide minor groove. A second consequence of the presence of the 2'-hydroxyl group is that in conformationally flexible regions of an RNA molecule (that is, not involved in formation of a double helix), it can chemically attack the adjacent phosphodiester bond to cleave the backbone. Most cellular RNA molecules are single stranded. They may form secondary structures such as stem-loop and hairpin (Refer Figure 5.4).

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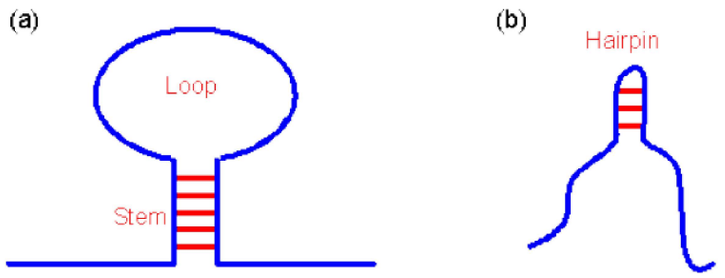


Fig. 5.4 *Secondary Structure of RNA - Stem-loop and Hairpin.*

There are more unusual bases in RNA than in DNA. All normal RNA chains either start with adenine or guanine: Three types of cellular RNA have been distinguished:

1. Messenger RNA (mRNA) or template RNA
2. Ribosomal RNA (rRNA) and
3. Soluble RNA (sRNA) or transfer RNA (tRNA)

Ribosomal and transfer RNA comprise about 98% of all RNA. All three forms of RNA are made on a DNA template. Transfer RNA and messenger RNA are synthesized on DNA templates of the chromosomes, while ribosomal RNA is derived from nucleolar DNA. The three types of RNA are synthesized during different stages in early development. Most of the RNA synthesized during cleavage is mRNA. Synthesis of tRNA occurs at the end or cleavage, and rRNA synthesis begins during gastrulation.

Ribosomal RNA – rRNA

Ribosomal RNA, as the name suggests, is found in the ribosomes. It comprises about 80% of the total RNA of the cell. The base sequence of rRNA is complementary to that of the region of DNA where it is synthesized. In eukaryotes ribosomes are formed on the nucleolus. Ribosomal RNA is formed from only a small section of the DNA molecule, and hence there is no definite base relationship between rRNA and DNA as a whole. Ribosomal RNA consists of a single strand twisted upon itself in some regions. It has helical regions connected by intervening single strand regions. The helical regions may show presence or absence of positive interaction. In the helical region most of the base pairs are complementary, and are joined by hydrogen bonds. In the unfolded single strand regions the bases have no complements.

Ribosomal RNA contains the four major RNA bases with a slight degree of methylation, and shows differences in the relative proportions of the bases between species. Its molecules appear to be single polynucleotide strands which are unbranched and flexible. At low ionic strength rRNA behaves as a random coil, but with increasing ionic strength the molecule shows helical regions produced by base pairing between adenine and uracil and guanine and cytosine.

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Hence rRNA does not show purine-pyrimidine equality. The rRNA strands unfold upon heating and refold upon cooling. Ribosomal RNA is stable for at least two generations. The ribosome consists of proteins and RNA. The 70S ribosome of prokaryotes consists of a 30S subunit and a 50S subunit. The 30S subunit contains 16S rRNA, while the 50S subunit contains 23S and 5S rRNA. The 80S eukaryote ribosome consists of a 40S and a 60S subunit. In vertebrates the 40S subunit contains 18S rRNA, while the 60S subunit contains 28- 29S, 5.8S and 5S rRNA. In plants and invertebrates the 40S subunit contains 16- 18S RNA, while the 60S subunit contains 25S and 58 and 5.8S rRNA.

There are three types of ribosomal RNA on the basis of sedimentation and molecular weight. Two of these classes are high molecular weight RNAs, while the third is a low molecular weight RNA. The three classes are:

(1) high molecular weight rRNA with molecular weight of over a million, for example 21s-29s RNA,

(2) high molecular weight rRNA with molecular weight below a million, for example 12-8-188 rRNA,

(3) low molecular weight rRNA, for example 58 rRNA.

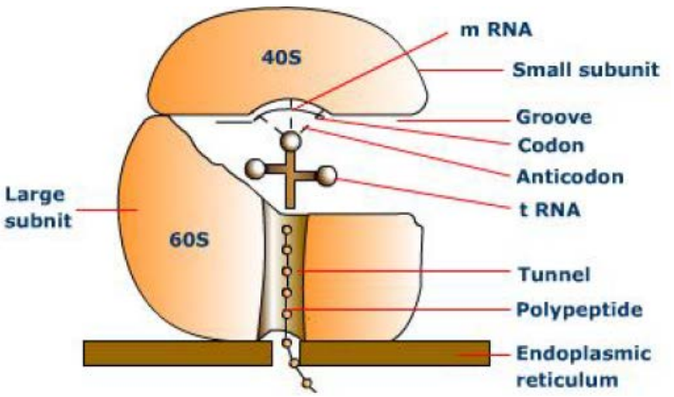


Fig. 5.5

Messenger RNA - mRNA

Jacob and Monod (1961) proposed the name messenger RNA for the RNA carrying information for protein synthesis from the DNA (genes) to the sites of protein formation (ribosomes). It consists of only 3 to 5% of the total cellular RNA.

Size of Messenger RNA - mRNA

The molecular weight of an average sized mRNA molecule is about 500,000, and its sedimentation coefficient is 8S. It should be noted however, that mRNA varies greatly in length and molecular weight. Since most proteins contain at least a hundred amino acid residues, mRNA must have at least 100 X 3= 300 nucleotides on the basis of the triplet code.

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Stability of Messenger RNA - mRNA

The cell does not contain large quantities of mRNA. This is because mRNA, unlike other RNAs is constantly undergoing breakdown. It is broken down to its constituent ribonucleotides by ribonucleases.

Structure of Messenger RNA

mRNA Messenger RNA is always single stranded. It contains mostly the bases adenine, guanine, cytosine and uracil. There are few unusual substituted bases. Although there is a certain amount of random coiling in extracted mRNA, there is no base pairing. In fact base pairing in the mRNA strand destroys its biological activity. Since mRNA is transcribed on DNA (genes), its base sequence is complementary to that of the segment of DNA on which it is transcribed. This has been demonstrated by hybridization experiments in which artificial RNA, DNA double strands are produced. Hydrization takes place only if the DNA and RNA strands are complementary.

Usually each gene transcribes its own mRNA. Therefore, there are approximately as many types of mRNA molecules as there are genes. There may be 1,000 to 10.000 different species of mRNA in a cell. These mRNA types differ only in the sequence of their bases and in length. When one gene (cistron) codes for a single mRNA strand the mRNA is said to be monocistronic. In many cases, however, several adjacent cistrons may transcribe an mRNA molecule, which is then said to be polycistronic or polygenic.

The mRNA molecule has the following structural features:

1. **Cap:** At the 5' end of the mRNA molecule in most eukaryote cells and animal virus molecules is found a 'cap'. This is blocked methylated structure, m7Gpp Nmp Np or m7Gpp Nmp Nmp Np. where: N = any of the four nucleotides and Nmp = 20 methyl ribose. The rate of protein synthesis depends upon the presence of the cap. Without the cap mRNA molecules bind very poorly to the ribosomes.
2. **Noncoding Region 1 (NC1):** The cap is followed by a region of 10 to 100 nucleotides. This region is rich in A and U residues, and does not translate protein.
3. **The Initiation Codon:** It is AUG in both prokaryotes and eukaryotes.
4. **The Coding Region:** It consists of about 1,500 nucleotides on the average and translates protein It is made up of 73-93 nucleotides (Rich and RajBhandary, 1976). Each bacterial cell probably contains about a hundred or more different types of tRNA. The function of tRNA is to carry amino acids to mRNA during protein synthesis. Each amino acid is carried by a specific tRNA. Since 20 amino acids are coded to form proteins, it follows that there must be at least 20 types of tRNA.

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It was formerly thought that only 20 tRNA molecular types exist, one for each amino acid. It has, however, been shown that in several cases there are at least two types of tRNA for each amino acid. Thus there are many more tRNA molecules than amino acid types. These are probably coded by one gene.

Transfer RNA is synthesized in the nucleus on a DNA template. Only 0.025% of DNA codes for tRNA. Synthesis of tRNA occurs near the end of cleavage stages. Transfer RNA is an exception to other cellular RNAs in that a part of its ribonucleotide sequence (-CCA) is added after it comes off the DNA template. Like rRNA, tRNA is also formed from only a small section of the DNA molecule. Therefore, it does not show any obvious base relationships to DNA. The tRNA molecule consists of a single strand looped about it self. The 3' end always terminates in a -C-C-A (cytosine-cytosine-adenine) sequence. The 5' end terminates in G (guanine) or C (cytosine). Many of the bases are bonded to each other, but there are also unpaired bases.

Transfer RNA - tRNA OR Soluble RNA

sRNA After rRNA the second most common RNA in the cell is transfer RNA. It is also called soluble RNA because it is too small to be precipitated by ultracentrifugation at 100,000 g. It constitutes about 10-20% of the total RNA of the cell. Transfer RNA is a relatively small RNA having a molecular weight of about 25,000 to 30,000 and the sedimentation coefficient of mature eukaryote tRNA is 3.8S.

Structure of Transfer RNA – tRNA

The nucleotide sequence (primary structure) of tRNA was first worked out by Holley et al (1965) for yeast alanine tRNA. Since then the sequence of about 75 different tRNAs, ranging from bacteria to mammals, has been established. The different tRNAs are all minor variants of the same basic type of structure. Several models of the secondary structure of tRNA have been proposed, and of these the cloverleaf model of Holley is the most widely accepted.

Transfer RNA (tRNA) is an essential component of the protein synthesis reaction. There are at least twenty different kinds of tRNA in the cell1 and each one serves as the carrier of a specific amino acid to the site of translation. tRNA's are L-shaped molecules.

The amino acid is attached to one end and the other end consists of three anticodon nucleotides. The anticodon pairs with a codon in messenger RNA (mRNA) ensuring that the correct amino acid is incorporated into the growing polypeptide chain. The L-shaped tRNA is formed from a small single-stranded RNA molecule that folds into the proper conformation. Four different regions of double-stranded RNA are formed during the folding process (Refer Figure 5.6).

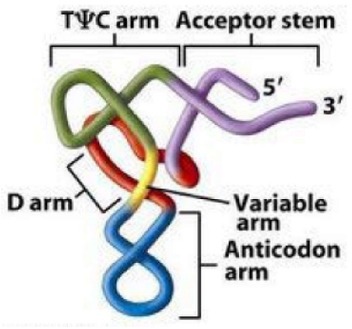


Fig. 5.6 *Double-stranded RNA*

The two ends of the molecule form the acceptor stem region where the amino acid is attached. The anticodon is an exposed single-stranded region in a loop at the end of the anticodon arm. The two other stem/loop structures are named after the modified nucleotides that are found in those parts of the molecule. The D arm contains dihydrouridylate residues while the TψC arm contains a ribothymidylate residue (T), a pseudouridylate residue (ψ) and a cytidylate (C) residue in that order. All tRNA's have a similar TψC sequence. The variable arm is variable, just as you would expect. In some tRNA's it is barely noticable while in others it is the largest arm. tRNA's are usually drawn in the 'cloverleaf' form (below) to emphasize the base-pairs in the secondary structure (Refer Figure 5.7)

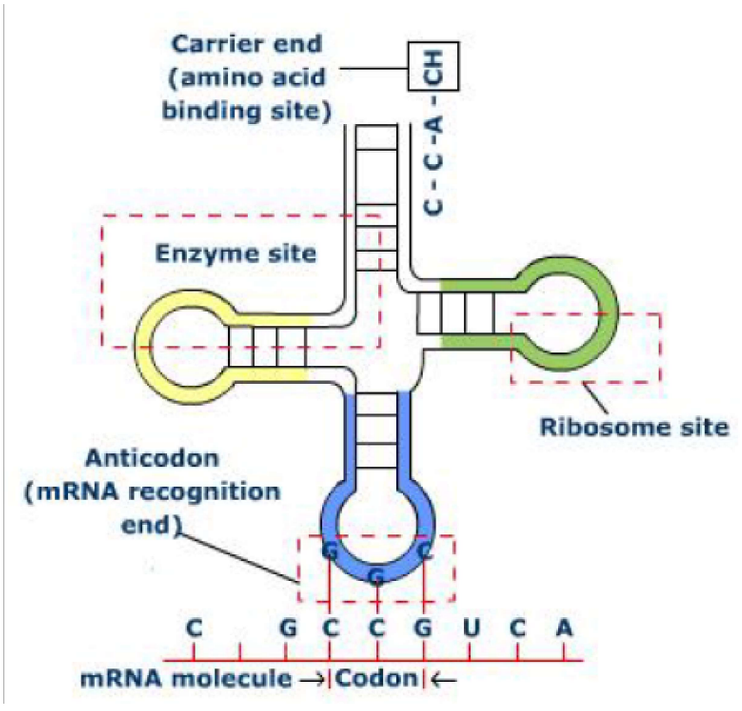


Fig. 5.7 *Cover Leaf Model of tRNA*

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Unusual Bases in tRNA

In addition to the usual bases A, U, G and C, tRNA contain a number of unusual bases, and in this respect differs from mRNA and rRNA. The unusual bases of tRNA account for 15-20% of the total RNA of the cell. Most of the unusual bases are formed by methylation (addition of -CHa or methyl group to the usual bases), for example cytosine and guanine on methylation yield methylcytosine and methyl/ guanine, respectively. Precursor tRNA molecules transcribed on the DNA template contains the usual bases. These are then modified to unusual bases.

The unusual bases are important because they protect the tRNA molecule against degradation by RNase. This protection is necessary because RNA is found floating freely in the cell. Some of the unusual bases of tRNA are methyl guanine (GMe), dimethylguanine(GMe2), methylcytosine (Me), ribothymine (T), pseudouridine (ψ), dihydrouridine (DHU, H2U, UH2), inosine (I) and methylinosine (IMe, MeI). In general, organisms high in the evolutionary scale contain more modified bases than lower organisms.

Classification of tRNA

A Study of different tRNAs shows that the structure of the acceptor stem, the anticodon arm and the TøC arm are constant. The differences in the tRNAs lie in the D arm and the variable arm. Based on the differences in these two variable regions, three classes of tRNA have been recognized.

Class I (D4-V4-5), with 4 base pairs in the D stem and 4-5 bases in the variable loop.

Class II (DS-V4-5), with 3 base pairs in the D stem and 4-5 bases pairs in the variable loop.

Class III (D3-VN), with 3 base pairs in the D stem and a large variable arm.

A simpler classification based only on the variable arm recognizes two types of tRNA.

Class I with 4-5 bases in the variable loop

Class II with a large variable arm of 13-21 bases.

Tertiary Structure of Transfer - tRNA

Electron density maps have revealed that tRNA has a tertiary structure. This structure is due to hydrogen bonds

(i) between bases,

(ii) between bases and ribose phosphate backbone and

(iii) between the backbone residues. (The hydrogen bonding in the double helical stem regions of the tRNA molecular are considered to be in the secondary structure).

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Initiator Transfer RNA - tRNA

The starting amino acid in eukaryote protein synthesis is methionine, while in prokaryotes it is N-formyl methionine. The tRNA molecule3 specific for these two amino acids are methionyl tRNA (tRNAmet) and N-formyl- methionyl IRNA (tRNAfmet) respectively. These tRNAs are called initiator tRNAs, because they initiate protein synthesis. Initiator tRNAs have certain features which distinguish them from other tRNAs, and the initiator tRNAs of prokaryotes' and eukaryotes also differ. In most prokaryotes the 5' terminal nucleoside is C. It has opposite it (i.e. in the fifth position from the 3' end) an A nucleotide. There is no Watson-Crick base pairing between the two. In the blue green 'alga' Anacystis nidulans, however, the fifth nucleotide from the 3' end is C.

In eukaryotes there is an A.U base pair at the acceptor stem. As noted previously, prokaryotes use tRNAf-met for initiation of protein synthesis, while eukaryotes use tRNAmet. The prokaryote Halo bacterium cutirubrum is, however, reported to initiate protein synthesis with tRNA met and has an A.U base pair at the end of the accept or stem. In these respects it resembles eukaryotes The D loop of prokaryote initiator tRNAs contains an A11, U24 base pair. All other tRNAs have a Y11, R24 base pair. Eukaryotic cytoplasmic initiator tRNAs have AU or AU instead of Tψ in the TψC loop. Also, in eukaryotes instead of a pyrimidine nucleotide (Y) there is A at the 3' end of the TψC loop. In some eukaryotic cytoplasmic initiator tRNAs the anticodon sequence CAU is preceeded by C instead of U as in all other tRNAs.

In prokaryotes the purine nucleotide following C in the TψC loop is A, while in eukaryotes it is G. In tRNA fmet the nucleotide adjacent to the 3' side of the anticodon triplet is adenosine while in tRNA met it is alkylated adenosine.

Specificity of Tranfer RNA - tRNA

Two important steps in translation during protein synthesis are the activation of amino acids and the transfer of amino acids to tRNAs. Each amino acid has a specific activating enzyme tRNA aminoacyl synthetase. Thus there are 20 different tRNA aminoacyl synthetases for the 20 common amino acids found in proteins.

Some tRNA synthetases can activate more than one amino acid, i.e. they show only a limited substrate specificity. Thus isoleucine tRNA synthetase can also activate L valine, and valine tRNA synthetase can also react with threonine. The enzymes, however, recognize only a specific set of, tRNAs as substratesL isolecine tRNA synthetase recognizes only tRNAileu and valine tRNA synthetase recognizes only tRNAval. Thus specificity is involved at two stages, activation of the amino acid and transfer of the amino acid to tRNA. Another group of enzymes, the tRNA aminoacyl transferases catalyse the transfer of an amino acid from the amino acid - tRNA complex to specific acceptor molecules.

NOTES

Summary of Differences Between DNA and RNA

- DNA contains the sugar deoxyribose, while RNA contains the sugar ribose. The only difference between ribose and deoxyribose is that ribose has one more -OH group than deoxyribose, which has -H attached to the second (2') carbon in the ring.
- DNA is a double-stranded molecule while RNA is a single stranded molecule.
- DNA is stable under alkaline conditions while RNA is not stable.
- DNA and RNA perform different functions in humans. DNA is responsible for storing and transferring genetic information while RNA directly codes for amino acids and as acts as a messenger between DNA and ribosomes to make proteins.
- DNA and RNA base pairing is slightly different since DNA uses the bases adenine, thymine, cytosine, and guanine; RNA uses adenine, uracil, cytosine, and guanine. Uracil differs from thymine in that it lacks a methyl group on its ring.

Comparison of DNA and RNA

While both DNA and RNA are used to store genetic information, there are clear differences between them. This table 5.2 summarizes the key points:

Table 5.2 *Difference between DNA and RNA*

Comparison	DNA	RNA
Name	DeoxyriboNucleic Acid	RiboNucleic Acid
Function	Long-term storage of genetic information; transmission of genetic information to make other cells and new organisms.	Used to transfer the genetic code from the nucleus to the ribosomes to make proteins. RNA is used to transmit genetic information in some organisms and may have been the molecule used to store genetic blueprints in primitive organisms.
Structural Features	B-form double helix. DNA is a double-stranded molecule consisting of a long chain of nucleotides.	A-form helix. RNA usually is a single-strand helix consisting of shorter chains of nucleotides.
Composition of Bases and Sugars	deoxyribose sugar phosphate backbone adenine, guanine, cytosine, thymine bases	ribose sugar phosphate backbone adenine, guanine, cytosine, uracil bases
Propagation	DNA is self-replicating.	RNA is synthesized from DNA on an as-needed basis.
Base Pairing	AT (adenine-thymine) GC (guanine-cytosine)	AU (adenine-uracil) GC (guanine-cytosine)
Reactivity	The C-H bonds in DNA make it fairly stable, plus the body destroys enzymes that would attack DNA. The small grooves in the helix also serve as protection, providing minimal space for enzymes to attach.	The O-H bond in the ribose of RNA makes the molecule more reactive, compared with DNA. RNA is not stable under alkaline conditions, plus the large grooves in the molecule make it susceptible to enzyme attack. RNA is constantly produced, used, degraded, and recycled.
Ultraviolet Damage	DNA is susceptible to UV damage.	Compared with DNA, RNA is relatively resistant to UV damage.

Check Your Progress

5. Define RNA.
6. How is RNA transcribed from DNA?
7. What makes RNA different from DNA?
8. Name the types of cellular RNA.

5.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Deoxyribonucleic acid is a molecule composed of two chains that coil around each other to form a double helixcarrying the geneticinstructions used in the growth, development, functioning, are reproductionof all known living organismsand many viruses.
2. The two DNA strands are also known as polynucleotidesas they are composed of simpler monomericunits called nucleotides.
3. Each nucleotide is composed of one of four nitrogen-containing nucleobases (cytosine[C], guanine[G],adenine[A] or thymine[T]), a sugarcalled deoxyribose, and a phosphate group.
4. Salient Features of Â model of DNA of Watson and Crick are:
   - DNA is the largest biomolecule in the cell.
   - DNA is negatively charged and dextrorotatory.
   - Molecular configuration of DNA is 3D.
   - DNA has two polynucleotide chains.
   - The two chains of DNA have antiparallel polarity, $5' \rightarrow 3'$ in one and $3' \rightarrow 5'$ in other.
   - Backbone of each polynucleotide chain is made of alternate sugar-phosphate groups. The nitrogen bases project inwardly.
5. Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes.
6. RNA is transcribed from DNA by enzymes called RNA polymerases and is generally further processed by other enzymes. RNA is central to the synthesis of proteins.
7. An important structural feature of RNA that distinguishes it from DNA is the presence of a hydroxyl group at the 2' position of the ribose sugar. The presence of this functional group causes the helix to adopt the A-form geometry rather than the B-form most commonly observed in DNA.

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8. Three types of cellular RNA have been distinguished:
   - Messenger RNA (mRNA) or template RNA
   - Ribosomal RNA (rRNA) and
   - Soluble RNA (sRNA) or transfer RNA (tRNA).

5.5 SUMMARY

- Deoxyribonucleic acid is a molecule composed of two chains that coil around each other to form a double helixcarrying the geneticinstructions used in the growth, development, functioning, are reproductionof all known living organismsand many viruses.
- DNA and ribonucleic acid(RNA) are nucleic acids; alongside proteins, lipidsand complex carbohydrates (polysaccharides), nucleic acids are one of the four major types of macromoleculesthat are essential for all known forms of life.
- The two DNA strands are also known as polynucleotidesas they are composed of simpler monomericunits called nucleotides.
- Each nucleotide is composed of one of four nitrogen-containing nucleobases(Cytosine[C], Guanine[G],Adenine[A] or Thymine[T]), a sugarcalled deoxyribose, and a phosphate group.
- The nucleotides are joined to one another in a chain by covalent bondsbetween the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone.
- The nitrogenous bases of the two separate polynucleotide strands are bound together, according to base pairing rules (A with T and C with G), with hydrogen bonds to make double-stranded DNA.
- Nucleic acids were first isolated by Friedrich Miescher (1869) from pus cells.They were named nuclein. Hertwig (1884) proposed nuclein to be the carrier of hereditary traits. Because of their acidic nature they were named nucleinic acids and then nucleic acids (Altmann, 1899).
- Fisher (1880s) discovered the presence of purine and pyri-midine bases in nucleic acids. Levene (1910) found deoxyribose nucleic acid to contain phosphoric acid as well as deoxyribose sugar.
- The two chains are twisted helically just as a rope ladder with rigid steps twisted into a spiral. Each turn of the spiral contains 10 nucleotides. This double helix or duplex model of DNA with antiparallel polynucleotide chains having complementary bases has an implicit mechanism of its replication and copying.

- DNA duplex model proposed by Watson and Crick is right handed spiral and is called B-DNA (Balanced DNA). In the model the base pairs lie at nearly right angles to the axis of helix. Another right handed duplex model is A-DNA (Alternate DNA). Here, a single turn of helix has 11 base pairs.
- The base pairs lie 20° away from perpendicular to the axis. C-DNA has 9 base pairs per turn of spiral while in D-DNA the number is only 8 base pairs. Both are right handed. Z-DNA (Zigzag DNA) is left-handed double helix with zigzag back-bone, alternate purine and pyrimidine bases, single turn of 45 A length with 12 base pairs and a single groove.
- B-DNA is more hydrated and most frequently found DNA in living cells. It is physiologically and biologically active form. However, it can get changed into other forms. Right handed DNA is known to change temporarily into the left handed form at least for a short distance. Such changes may cause changes in gene expression.
- In many prokaryotes the two ends of a DNA duplex are covalently linked to form circular DNA. Circular DNA is naked, that is, without association with histone proteins, though polyamines do occur. In linear DNA the two ends are free. It is found in eukaryotic nuclei where it is associated with histone proteins.
- Linear DNA, without association with histone proteins, also occurs in some prokaryotes, for example Myco-plasma. In semi-autonomous cell organelles (mitochondria, plastids) DNA is circular, less commonly linear. It is always naked.
- DNA or deoxyribonucleic acid is a helically twisted double chain polydeoxyribonucleotide macromolecule which constitutes the genetic material of all organisms with the exception of rhinoviruses. In prokaryotes it occurs in nucleoid and plasmids. This DNA is usually circular. In eukaryotes, most of the DNA is found in chromatin of nucleus.
- A deoxyribonucleotide of DNA is formed by cross-linking of three chemicals ortho- phosphoric acid (H_3PO_4), deoxyribose sugar ($C_5H_{10}O_4$) and a nitrogen base. Four types of nitrogen bases occur in DNA. They belong to two groups, purines (9-membered double rings with nitrogen at 1,3,7 and 9 positions) and pyrimidines (six membered rings with nitrogen at 1 and 3 positions). DNA has two types of purines (adenine or A and guanine or G) and two types of pyrimidines (cytosine or C and thymine or T).
- Ribonucleic acid (RNA) is a polymeric molecule essential in various biological roles in coding, decoding, regulation and expression of genes.
- RNA and DNA are nucleic acids, and, along with lipids, proteins and carbohydrates, constitute the four major macromolecules essential for all known forms of life.

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- Like DNA, RNA is assembled as a chain of nucleotides, but unlike DNA it is more often found in nature as a single-strand folded onto itself, rather than a paired double-strand.
- Cellular organisms use messenger RNA (mRNA) to convey genetic information (using the nitrogenous bases of guanine, uracil, adenine, and cytosine, denoted by the letters G, U, A, and C) that directs synthesis of specific proteins.
- DNA to structures called ribosomes. These ribosomes are made from proteins and ribosomal RNAs, which come together to form a molecular machine that can read messenger RNAs and translate the information they carry into proteins. There are many RNAs with other roles – in particular regulating which genes are expressed, but also as the genomes of most viruses.
- Ribose Nucleic Acids Most cellular RNA is single stranded, although some viruses have double stranded RNA. The single RNA strand is folded upon itself, either entirely or in certain regions. In the folded region a majority of the bases are complementary and are joined by hydrogen bonds. This helps in the stability of the molecule.
- An important structural feature of RNA that distinguishes it from DNA is the presence of a hydroxyl group at the 2' position of the ribose sugar. The presence of this functional group causes the helix to adopt the A-form geometry rather than the B-form most commonly observed in DNA.
- Ribosomal and transfer RNA comprise about 98% of all RNA. All three forms of RNA are made on a DNA template. Transfer RNA and messenger RNA are synthesized on DNA templates of the chromosomes, while ribosomal RNA is derived from nucleolar DNA.
- The three types of RNA are synthesized during different stages in early development. Most of the RNA synthesized during cleavage is mRNA. Synthesis of tRNA occurs at the end or cleavage, and rRNA synthesis begins during gastrulation.
- Ribosomal RNA contains the four major RNA bases with a slight degree of methylation, and shows differences in the relative proportions of the bases between species. Its molecules appear to be single polynucleotide strands which are unbranched and flexible.
- At low ionic strength rRNA behaves as a random coil, but with increasing ionic strength the molecule shows helical regions produced by base pairing between adenine and uracil and guanine and cytosine.

5.6 KEY WORDS

NOTES

- **DNA:** Deoxyribonucleic acid is a molecule composed of two chains that coil around each other to form a double helixcarrying the geneticinstructions used in the growth, development, functioning, are reproductionof all known living organismsand many viruses.
- **Cytosine:** Cytosine is one of the four main bases found in DNA and RNA, along with adenine, guanine, and thymine (uracil in RNA).
- **Adenine:** Adenine is one of the two purine nucleobases (the other being guanine) used in forming nucleotides of the nucleic acids.
- **Guanine:** Guanine is one of the four main nucleobases found in the nucleic acids DNA and RNA, the others being adenine, cytosine, and thymine (uracil in RNA).
- **Denaturation:** Denaturation is a process in which proteins or nucleic acids lose the quaternary structure, tertiary structure, and secondary structure which is present in their native state, by application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent.
- **Codon:** A codon is a sequence of three DNA or RNA nucleotides that corresponds with a specific amino acid or stop signal during protein synthesis.
- **Cistron:** The segment of DNA which determines the synthesis of complete polypeptide is known as cistron.
- **Histones:** Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes.
- **RNA:** Ribonucleic acid, a nucleic acid present in all living cells. Its principal role is to act as a messenger carrying instructions from DNA for controlling the synthesis of proteins, although in some viruses RNA rather than DNA carries the genetic information.

5.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Write a short note on DNA.
2. Write in brief about types of DNA.
3. Brief a note on Chargaff's Rules.

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4. Write some of the salient features of Â model of DNA of Watson and Crick.
5. What is repetitive DNA?
6. Write a short note on RNA.
7. What is rRNA?
8. Write a note on structure of mRNA.

Long Answer Questions

1. Give a detailed note on DNA and its history.
2. Explain about the types of DNA.
3. Write a detailed note on structure of DNA.
4. Write about the function of DNA.
5. Write a detailed note on RNA.
6. Explain about chemical structure of RNA.
7. Discuss about the three types of cellular RNA.
8. Write a note on structure of mRNA.
9. Distinguish between RNA and DNA.

5.8 FURTHER READINGS

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UNIT 6 ENZYMES INVOLVED IN MOLECULAR BIOLOGY

Structure

6.0 Introdcution
6.1 Objectives
6.2 Molecular Biology
- 6.2.1 DNA Polymerases
- 6.2.2 RNA Polymerase
- 6.2.3 DNA Helicases
- 6.2.4 Primase
- 6.2.5 Restriction Enzyme (Restriction Endonuclease)
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6.3 Answers to Check Your Progress Questions
6.4 Summary
6.5 Key Words
6.6 Self Assessment Questions and Exercises
6.7 Further Readings

6.0 INTRODCUTION

Molecular biology is the study of living things at the level of the molecules which control them and make them up. While traditional biology concentrated on studying whole living organisms and how they interact within populations, molecular biology strives to understand living things by examining the components that make them up. Both approaches to biology are equally valid, although improvements to technology have permitted scientists to concentrate more on the molecules of life in recent years.

Molecular biology is a specialised branch of biochemistry, the study of the chemistry of molecules which are specifically connected to living processes. Of particular importance to molecular biology are the nucleic acids (DNA and RNA) and the proteins which are constructed using the genetic instructions encoded in those molecules. Other biomolecules, such as carbohydrates and lipids may also be studied for the interactions they have with nucleic acids and proteins. Molecular biology is often separated from the field of cell biology, which concentrates on cellular structures, organelles and the like, molecular pathways within cells and cell life cycles.

In this unit, you will study about enzymes involved in molecular biology, DNA polymerase, helicase, primase, ligase, exonuclease and endonuclease in detail.

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6.1 OBJECTIVES

After going through this unit, yow will be able to:

- Understand what is molecular biology
- Analyse about the enzymes used in molecular biology
- Discuss about different enzymes like DNA polymerase, helicase, primase, ligase
- Describe about exonuclease and endonuclease

6.2 MOLECULAR BIOLOGY

Molecular biology is the study of the structure function, and makeup of the molecular building blocks of life. It focuses on the interactions between the various systems of a cell, including the interrelationship of DNA, RNA and protein synthesis and how these interactions are regulated. The youngest of the bio-sciences, molecular biology is closely interrelated with the fields of biochemistry, genetics and cell biology.

Molecular biology traces its origins to the 1930s, when scientists focused on explaining the phenomena of life by studying the macromolecules that generate life. The chief discoveries of molecular biology took place in a period of only about 25 years, starting in 1940, when George Beadle and Edward Tatum established the existence of a precise relationship between genes and proteins (they shared the 1958 Nobel Prize in Medicine). Another 15 years were required before new and more sophisticated technologies, united today under the name genetic engineering, would permit the isolation and characterization of genes.

Enzymes are macromolecular biological catalysts. Enzymes accelerate chemical reactions. The molecules upon which enzymes may act are called substrates and the enzyme converts the substrates into different molecules known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life. Metabolic pathways depend upon enzymes to catalyze individual steps. The study of enzymes is called enzymology and a new field of pseudoenzyme analysis has recently grown up, recognising that during evolution, some enzymes have lost the ability to carry out biological catalysis, which is often reflected in their amino acid sequences and unusual 'pseudocatalytic' properties.

Enzymes are known to catalyze more than 5,000 biochemical reaction types. Most enzymes are proteins, although a few are catalytic RNA molecules. The latter are called ribozymes. Enzymes specificity comes from their unique three-dimensional structures.

The various enzymes used in molecular biology are DNA polymerase, helicase, primase, ligase, exonuclease and endonuclease which are explained below:

6.2.1 DNA Polymerases

DNA polymerase is an enzyme that synthesizes DNA molecules from deoxyribonucleotides, the building blocks of DNA. DNA molecules are the troves of genetic information of an organism. DNA is the basis of life and is transferred from parent to offspring's. The DNA content of the parent is doubled by means of replication mechanism aided by a specific enzyme, DNA polymerases. DNA polymerase plays a central role in process of life and carries a weighty responsibility of making an accurate copy of the cell's genome. The DNA polymerases are enzymes molecules by assembling nucleotides, the building blocks of DNA. The first evidence of the existence of an enzymatic activity capable of synthesizing DNA came in 1958 with the discovery of E. coli Pol I by A. Kornberg and colleagues. DNA polymerase moves along the old strand in the 3'-5' direction, creating a new strand having a 5'-3' direction.

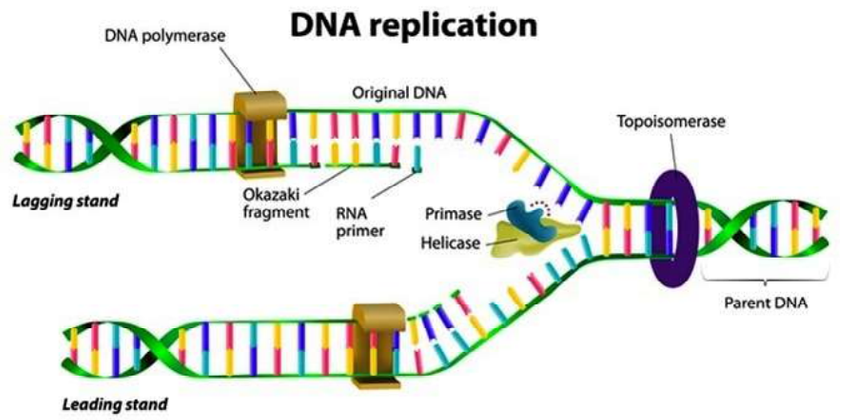


Fig. 6.1 *DNA Replication*

Types and Roles of DNA Polymerases (Replicative and Repairative)

1. Prokaryotic DNA polymerase

Prokaryotes contain five different types of DNA polymerase. These are described below:

Pol I

- Polymerase I is a DNA repair enzyme from the family A polymerases that has a 5' to 3' and 3' to 5' activity.
- Pol I accounts for more than 95% of polymerase activity in *coli*, although cells that lack this polymerase have been found and its activity can be replaced by the other.

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- four types of polymerase.
- This DNA polymerase has a poor processivity rate, adding around 15 to 20 nucleotides per second.
- This repair polymerase is involved in excision repair with 3'-5' and 5'-3' exonuclease activity and processing of Okazaki fragments.

Pol II

- DNA polymerase II, a Family B polymerase. Polymerase II is a DNA repair enzyme with a 3' to 5' exonuclease activity.
- When DNA acquires damage in the form of short gaps, which block Pol III activity, Pol II helps to remedy this problem by restarting DNA synthesis downstream of these gaps.
- Pol II has 3'-5' exonuclease activity and participates in DNA repair, replication restart to bypass lesions, and its cell presence can jump from ~30-50 copies per cell to ~200-300 during SOS induction.

Pol III

- This holoenzyme is the main polymerase in *coli* DNA replication and is one of the family C polymerases.
- Polymerase III is the exonucleolytic proofreader, and can process of both the leading and lagging DNA strands.

Pol IV

- This enzyme belongs to the Y family of DNA polymerases.
- Pol IV is an error-prone polymerase that has no 3' to 5' proofreading activity and is involved in mutagenesis or the altering of DNA to give rise to a mutation.

Pol V

- Pol V also belongs to the Y family of polymerases and allows DNA damage to be bypassed in order for replication to continue.
- It is involved inSOS response and translation synthesis DNA repair mechanisms.

2. Eukaryotic DNA polymerase

POL α

- POL α is a members of Family B Polymerases and are the main polymerases involved with nuclear DNA replication.
- This unique enzyme has two distinct polymerase activities: a 5'- 3' DNA-dependent DNA polymerase, and a 5'- 3'.

- DNA-dependent RNA polymerase. The RNA polymerase activity is a primase. Because of this, the enzyme is often referred to as Pol a: primase. It is the only enzyme known to have both DNA polymerase and primase activities, and the only one capable of selfprimed DNA synthesis on a previously unprimed ssDNA.
- Pol α does not have an intrinsic 3'- 5' exonuclease activity and also lacks a 5'- 3' exonuclease activity. In vivo, the primary function of Pol a: primase is to make short RNA/DNA primers for replicative DNA synthesis.

D NA polymerase β

- Belongs to family X polymerases are found mainly in vertebrates, and a few are found in plants and fungi.
- Pol β is required for short-patch base excision repair, a DNA repair pathway that is essential for repairing alkylated or oxidized bases as well as a basic sites.
- This is the smallest and simplest of the classical eukaryotic polymerases; it is composed of a single ~40-48 kDa protein.
- Pol β is not highly active and is not very processive. It has no intrinsic exonuclease activities.
- Its preferred template is duplex DNA with short gaps, although it can bind a nicked duplex and is capable of some limited displacement synthesis. Pol β is primarily involved in DNA repair.

Polymerases λ, σ and μ (lambda, sigma, and mu)

- Family X polymerases also contain the well-known eukaryotic polymerasesuch as Pol σ (sigma), Pol λ (lambda)
- Pol σ and Pol μ, are involved innon-homologous end-joining, a mechanism for rejoining DNA double-strand breaks due to hydrogen peroxide and ionizing radiation, respectively.

Polymerase	Function	Exonuclease Actvity
α	Synthesizes the RNA primer, initiations DNA synthesis and the lagging strand	None
β	Repair DNA	None
γ	Replicate mitochondrial DNA	3′ to 5′
δ	Synthesizes the leading strand, filling DNA gaps after removal of primer	3′ to 5′
ε	Repair DNA	3′ to 5′

Polymerases η, ι and κ (eta, iota, and kappa)

- Pol η (eta), Pol ι (iota), and Pol κ (kappa), are Family Y DNA polymerases involved in the DNA repair by translation synthesis.

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- Polymerases in Family Y are low-fidelity polymerases, but have been proven to do more good than harm as mutations that affect the polymerase can cause various diseases, such a skin cancer and Xeroderma Pigmentosum Variant (XPS).
- Pol η is particularly important for allowing accurate translesion synthesis of DNA damage resulting from ultraviolet radiation
- Pol κ is thought to act as an extender or an inserter of a specific base at certain DNA lesions.

Polymerases ζ (zeta)

- Pol ζ another B family polymerase, is involved in translesion synthesis.
- Pol ζ lacks 3' to 5' exonuclease activity, is unique in that it can extend primers with terminal mismatches.

Polymerases γ and θ (gamma and theta)

- Pol γ (gamma) and Pol θ (theta) are Family A polymerases.
- Pol γ, is the only mtDNA, polymerase and therefore replicates, repairs, and has proofreading 3'-5' exonuclease.
- Any mutation that leads to limited or non-functioning Pol γ has a significant effect on mtDNA and is the most common cause of autosomal inherited mitochondrial disorders.
- Pol θ, found in eukaryotes, its function is not clearly understood. Pol θ belongs to Family A polymerase.
- Pol θ extends mismatched primer termini and can bypass abasic sites by adding a nucleotide.

3. Reverse transcriptase (RT)

Retrovirus reverse transcriptase

1. Retroviruses package their genomes as ssRNA but replicate this RNA through a dsDNA intermediate. To do this, they employ an RNA dependent DNA polymerase (reverse transcriptase).
2. RT is a typical DNA polymerase in the 5'- 3' direction of synthesis, requirement for a template primer with a 3'-OH terminus, and requirements for dNTPs and Mg2+.
3. RT does not have a detectable DNA specific exonuclease activity, and therefore has no proofreading function. As a result, its error rate is relatively high. This is reflected in a high mutation rate; virus variants are generated at high frequency. This is an important aspect of pathogenesis: retroviruses are adept at evading host immunosurveillance because of high mutation frequency.

RT is unique among DNA polymerases in at least two respects:

- o It can use primer, natural ssRNAs as template. It can also use a primed ssDNA as template.
- o It has intrinsic RNase H activity. RNase H is a processive exonuclease that specifically degrades the RNA strand of a DNA-RNA hybrid beginning from either the 5' or 3' end
- o It can also act as an endonuclease. RNase H hydrolyzes phosphodiester bonds to leave products with 3' hydroxyl and 5' phosphate ends.
- o The enzyme is relatively processive and can replicate the 8 kb retrovirus genome without a processivity factor.

Telomerase

Telomerase is a ribonucleoprotein recruited to replicate ends of linear chromosomes because normal DNA polymerase cannot replicate the ends, or telomere. Telomerase acts like other DNA polymerases by extending the 3' end, but, unlike other DNA polymerases, telomerase does not require a template.

The protein and RNA components make up an active enzyme of ~200 kDa. The RNA component (~1.3 kb in yeast) contains the template sequence that is used for DNA synthesis. (So telomerase carries its own template.) In vitro, telomerase from a given species synthesizes the G-rich strand sequence characteristic of the species. However, telomerase activity is present in actively dividing cells, including immortalized (transformed) cells in culture, and in most cancer cells. So telomerase may be useful for cancer diagnostics, and is a possible target for therapeutics.

Significance of DNA Polymerases in Molecular Biology

DNA polymerases also play central roles in modern molecular biology and biotechnology, enabling techniques including DNA cloning, the polymerase chain reaction (PCR), DNA sequencing, single nucleotide polymorphism (SNP) detection, whole genome amplification (WGA), synthetic biology, and molecular diagnostics.

Thermostable DNA Polymerase

Taq DNA polymerase. The original report of this enzyme, purified from the hot springs bacterium *Thermus aquaticus*, was published in 1976. Later, the polymerase chain reaction was developed and shortly thereafter 'Taq' became a household word in molecular biology circles.

The thermophilic DNA polymerases, like other DNA polymerases, catalyze template-directed synthesis of DNA from nucleotide triphosphates. A primer having a free 3' hydroxyl is required to initiate synthesis and magnesium ion is necessary. In general, they have maximal catalytic activity at 75 to 80C, and substantially reduced activites at lower temperatures. At 37æ%C, Taq polymerase has only about 10% of its maximal activity. In addition to Taq DNA polymerase, several other

thermostable DNA polymerases have been isolated and expressed from cloned genes. Three of the most-used polymerases are described in the following Table 6.1

NOTES

Table 6.1 *Most Used Polymerases*

Polymerase	3'->5' Exonuclease	Source and Properties
Taq	No	From *Thermus aquaticus*. Halflife at 95C is 1.6 hours.
Pfu	Yes	From *Pyrococcus furiosus*. Appears to have the lowest error rate of known thermophilic DNA polymerases.
Vent	Yes	From *Thermococcus litoralis*; also known as Tli polymerase. Halflife at 95 C is approximately 7 hours.

6.2.2 RNA Polymerase

A RNA polymerase (RNAP), or ribonucleic acid polymerase, is a multi sub-unit enzyme that catalyzes the process of transcription where an RNA polymer is synthesized from a DNA template. The sequence of the RNA polymer is complementary to that of the template DNA and is synthesized in a 5′→3′ orientation. This RNA strand is called the primary transcript and needs to be processed before it can be functional inside the cell.

RNA polymerases interact with many proteins in order to accomplish their task. These proteins help in enhancing the binding specificity of the enzyme, aid in unwinding the double helical structure of DNA, modulate the activity of the enzyme based on the requirements of the cell and alter the speed of transcription. Some RNAP molecules can catalyze the formation of a polymer over four thousand bases in length every minute. However, they have a dynamic range of velocities and they can occasionally pause, or even stop at certain sequences in order to maintain fidelity during transcription.

Functions of RNA Polymerase

Traditionally, the central dogma of molecular biology has looked at RNA as a messenger molecule, that exports the information coded into DNA out of the nucleus in order to drive the synthesis of proteins in the cytoplasm: DNA → RNA → Protein. The other well known RNAs are transfer RNA (tRNA) and ribosomal RNA (rRNA) which are also intimately connected with the protein synthetic machinery. However, over the past two decades, it has become increasingly clear that RNA serves a range of functions, of which protein coding is only one part. Some regulate gene expression, others act as enzymes, some are even crucial in the formation of gametes. These are called non-coding or ncRNA.

Since RNAP is involved in the production of molecules that have such a wide range of roles, one of its main functions is to regulate the number and kind of RNA transcripts formed in response to the cell's requirements. A number of different proteins, transcription factors and signaling molecules interact with the enzyme,

especially the carboxy-terminal end of one subunit, to regulate its activity. It is believed that this regulation was crucial for the development of eukaryotic plants and animals, where genetically identical cells show differential gene expression and specialization in multicellular organisms.

In addition, the optimal functioning of these RNA molecules depends on the fidelity of transcription – the sequence in the DNA template strand must be represented accurately in the RNA. Even a single base change in some regions can lead to a completely non-functional product. Therefore, while the enzyme needs to work quickly and complete the polymerization reaction in a short span of time, it needs robust mechanisms to ensure extremely low error rates. The nucleotide substrate is screened at multiple steps for complementarity to the template DNA strand. When the correct nucleotide is present, it creates an environment conducive to catalysis and the elongation of the RNA strand. Additionally, a proofreading step allows incorrect bases to be excised.

Finally, RNA polymerases are also involved in post-transcriptional modification of RNAs to make them functional, facilitating their export from the nucleus towards their ultimate site of action.

Types of RNA Polymerase

There is remarkable similarity in the RNA polymerases found in prokaryotes, eukaryotes, archea and even some viruses. This point to the possibility that they evolved from a common ancestor. Prokaryotic RNAP is made of four subunits, including a sigma-factor that dissociates from the enzyme complex after transcription initiation. While prokaryotes use the same RNAP to catalyze the polymerization of coding as well as non-coding RNA, eukaryotes have five distinct RNA polymerases.

Eukaryotic RNAP I is a workhorse, producing nearly fifty percent of the RNA transcribed in the cell. It exclusively polymerizes ribosomal RNA, which forms a large component of ribosomes, the molecular machines that synthesize proteins. RNA Polymerase II is extensively studied because it is involved in the transcription of mRNA precursors. It also catalyzes the formation of small nuclear RNAs and micro RNAs. RNAP III transcribes transfer RNA, some ribosomal RNA and a few other small RNAs and is important since many of its targets are necessary for normal functioning of the cell. RNA polymerases IV and V are found exclusively in plants, and together are crucial for the formation of small interfering RNA and heterochromatin in the nucleus.

Process of Transcription

Transcription begins with the binding of the RNAP enzyme to a specific part of the DNA, also known as the promoter region. This binding requires the presence of a few other proteins – the sigma factor in prokaryotes and various transcription factors in eukaryotes. One set of proteins called general transcription factors are necessary for all eukaryotic transcriptional activity and include Transcription Initiation

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Factor II A, II B, II D, II E, II F and II H. These are supplemented by specific signaling molecules that modulate gene expression through stretches of non-coding DNA located upstream. Often initiation is aborted multiple times before a stretch of ten nucleotides is polymerized. After this, the polymerase moves beyond the promoter and loses most of the initiation factors (Refer Figure 6.2).

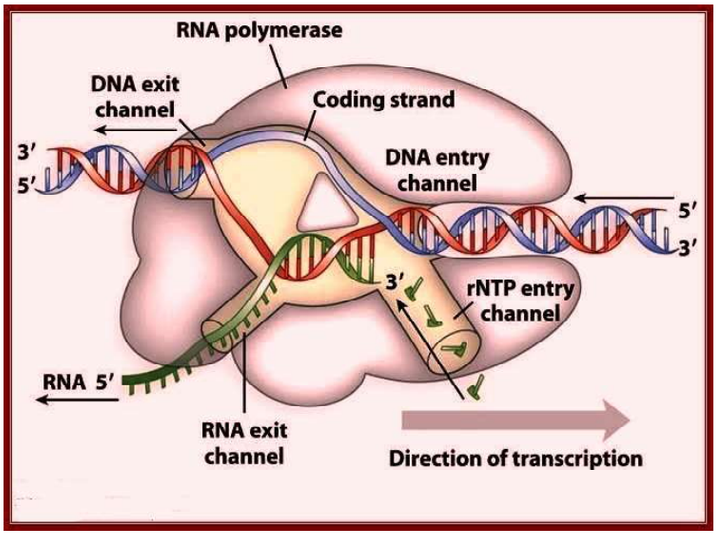


Fig. 6.2 *Process of Transcription*

This is followed by the unwinding of double stranded DNA, also known as 'melting', to form a sort of bubble where active transcription occurs. This 'bubble' appears to move along the DNA strand as the RNA polymer elongates. Once transcription is complete, the process is terminated and the RNA strand is processed. Prokaryotic RNAP and eukaryotic RNA polymerases I and II require additional transcription termination proteins. RNAP III terminates transcription when there is a stretch of Thymine bases on the non-template strand of DNA.

Comparison between DNA and RNA Polymerase

While DNA and RNA polymerases both catalyze nucleotide polymerization reactions, there are two major differences in their activity. Unlike DNA polymerases, RNAP enzymes do not need a primer to begin the polymerization reaction. They are also capable of beginning the reaction from the middle of a DNA strand and reading 'STOP' signals that cause the enzyme complex to dissociate from the template. Finally, while RNA polymerases are slightly slower than their counterparts, they have the advantage of only needing to make a complimentary copy of one strand of DNA.

6.2.3 DNA Helicases

In all cellular organisms from bacteria to humans, genetic information is locked within a double helix formed by the two antiparallel deoxyribonucleic acid (DNA) strands. Although double-stranded DNA (dsDNA) is the form most suitable for

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secure information storage, hydrogen bonds formed between complementary bases (Watson-Crick base pairing) impair readout of this information by the cellular machinery, which frequently requires a single-stranded DNA (ssDNA) intermediate as a template. The unwinding of dsDNA into ssDNA, a function critical for virtually every aspect of cellular DNA metabolism from RNA synthesis to homologous DNA recombination, is provided by a ubiquitous class of enzymes called DNA helicases. First identified in the 1970s, DNA helicases are motor proteins (often called DNA motors) that convert chemical energy into mechanical work. Chemical energy is derived from the hydrolysis of adenosine triphosphate (ATP) or other nucleoside triphosphates, and is coupled with mechanical work during at least two important steps within the helicase reaction cycle: (1) the unidirectional translocations along the substrate molecule and (2) the melting of the DNA duplex, which together result in the formation of the ssDNA intermediates essential for vital cellular processes.

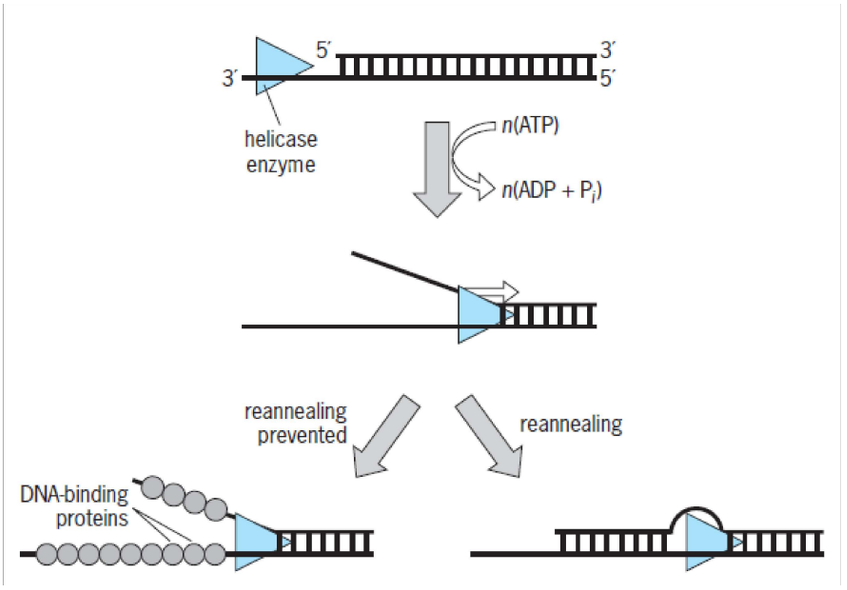


Fig. 6.3 *Schematic Representation of the Helicase Reaction*

The above figure describes about the helicase enzyme translocates along the DNA molecule and separates the strands. Energy for this unfavorable reaction is provided by the hydrolysis of adenosine triphosphates (ATP) to adenosine diphosphates (ADP) and inorganic phosphate ions (Pi). In the presence of a single-stranded DNA binding protein, reannealing of the DNA duplex is prevented. The helicase depicted here displays a 3 → 5 polarity, tracking unidirectionally along the lower of the two DNA strands in the duplex (the loading strand

Classifications

Helicases are divided into five main superfamilies based on the presence and composition of conserved amino acid (helicase signature) motifs. (It is important

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to note, however, that only a small fraction of these putative helicases have been studied biochemically and, of those proteins, not all have been shown to possess nucleic acid strand separation activity.) Biochemical and structural data have suggested that helicases function as monomers, dimers, and multimers (predominantly hexamers) and that they can also be classified based on a substrate requirement for dsDNA, dsRNA, or DNA-RNA hybrids. To unwind dsDNA efficiently, many DNA helicases need to initiate from an ssDNA region adjacent to the duplex part of the substrate molecule. Based on the requirement for an ssDNA overhang of a certain polarity, helicases are further divided into two functional groups: those that utilize a 3 - terminated ssDNA are designated as 3→5 helicases, whereas enzymes that require a 5 overhang are designated as 5→3 helicases.

Directional Translocation

It is now generally believed that the observed polarity requirement of helicases is a consequence of a directional bias in translocation on ssDNA. For example, the enzyme depicted is a 3→5 helicase. Upon binding to the ssDNA, it starts moving toward the 5 end of the loading strand, which brings the enzyme to the ssDNAdsDNA junction and subsequently through the duplex portion of the substrate Evidence for directional translocation on ssDNA was provided by two different approaches. The first examined the dependence of helicase ATPase activity on the length of the ssDNA substrate; the second, based on the ability of many helicases to create sufficient force during ssDNA translocation to disrupt the tight interaction between streptavidin and biotin (Kd = 10–15M), measured the ability of the helicase to increase the rate of streptavidin dissociation from DNA substrates biotinylated at either the 3 or 5 end. This second method was used successfully to determine the directionality of movement of several helicases on ssDNA. High-resolution structural data suggest that the helicase signature motifs are not essential for the duplex DNA separation per se, but for the ATP-dependent unidirectional motion of the helicases on either single- or doublestranded DNA lattices. Consequently, it has been proposed that the helicase signature motifs define a modular structure that functions as the DNA motor, while additional domains, which may vary from one protein to another, might be responsible for the DNA unwinding Accessory factors. Once dsDNA unwinding is achieved, spontaneous reannealing of the duplex may be avoided if the nascent ssDNA strands are trapped by single-stranded DNA binding proteins that hand off the intermediates to the next step in a reaction pathway. Although ssDNA binding proteins have frequently been shown to stimulate helicase activity in vitro, helicase activity can also be stimulated by other accessory factors that increase the rate or processivity of unwinding. The primary replicative helicase of Escherchia coli, DnaB, is a good example of a helicase that acts poorly in isolation from the accessory factors with which the enzyme is intended to operate. As part of the replisome (the DNA synthesis machinery of the cell), the role of DnaB is to separate the DNA strands at the replication fork. However, it was shown recently that the rate of movement

of the replication machinery at the fork is coordinated by an interaction between DnaB and DNA polymerase (encyme that catalyzes the addition of DNA residues) that is mediated by the ô subunit of the DNA polymerase. The ô subunit bridges the polymerase dimer and the helicase, inducing a conformational change in DnaB that enhances its translocation rate to 1000 basepairs per second. In the absence of ô, however, the replication machinery is uncoupled, and the polymerase simply follows DnaB as it unwinds DNA at approximately 35 bp/s.

6.2.4 Primase

Primase is an enzyme that synthesizes short RNA sequences called primers. These primers serve as a starting point for DNA synthesis. Since primase produces RNA molecules, the enzyme is a type of RNA polymerase. Primase functions by synthesizing short RNA sequences that are complementary to a single-stranded piece of DNA, which serves as its template. It is critical that primers are synthesized by primase before DNA replication can occur. This is because the enzymes that synthesize DNA, which are called DNA polymerases, can only attach new DNA nucleotides to an existing strand of nucleotides. Therefore, primase serves to prime and lay a foundation for DNA synthesis. The enzyme is active only in the presence of other proteins (including a helicase), which create a complex called the primosome

6.2.5 Restriction Enzyme (Restriction Endonuclease)

Restriction enzyme, also called restriction endonuclease, is a protein produced by bacteria that cleaves DNA at specific sites along the molecule. Restriction endonucleases cut the DNA double helix in very precise ways. It cleaves DNA into fragments at or near specific recognition sites within the molecule known as restriction sites. They have the capacity to recognize specific base sequences on DNA and then to cut each strand at a given place. Hence, they are also called as 'molecular scissors'.

Source of Restriction Enzymes

The natural sources of restriction endonucleases are bacterial cells. These enzymes are called restriction enzymes because they restrict infection of bacteria by certain viruses (i.e., bacteriophages), by degrading the viral DNA without affecting the bacterial DNA. Thus, their function in the bacterial cell is to destroy foreign DNA that might enter the cell. The restriction enzyme recognizes the foreign DNA and cuts it at several sites along the molecule. Each bacterium has its own unique restriction enzymes and each enzyme recognizes only one type of sequence.

Recognition Sites

The DNA sequences recognized by restriction enzymes are called palindromes. Palindromes are the base sequences that read the same on the two strands but in opposite directions.

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For example, if the sequence on one strand is GAATTC read in 5′→3′ direction, the sequence on the opposite strand is CTTAAG read in the 3′→5′ direction, but when both strands are read in the 5′→3′ direction the sequence is the same. The palindrome appears accordingly —

5′ GAATTC 3′

3′ CTTAAG 5′

In addition, there is a point of symmetry within the palindrome. In the example, this point is in the centre between the AT/AT.

The value of restriction enzymes is that they make cuts in the DNA molecule around this point of symmetry.

Some enzymes cut straight across the molecule at the symmetrical axis producing blunt ends.

Of more value, however, are the restriction enzymes that cut between the same two bases away form the point of symmetry on two strands, thus, producing a staggered break.

Mechanism of Cleavage of Restriction Enzymes

When a restriction endonuclease recognizes a particular sequence, it snips through the DNA molecule by catalyzing the hydrolysis (splitting of a chemical bond by addition of a water molecule) of the bond between adjacent nucleotides. To cut DNA, all restriction enzymes make two incisions, once through each sugar-phosphate backbone (i.e., each strand) of the DNA double helix.

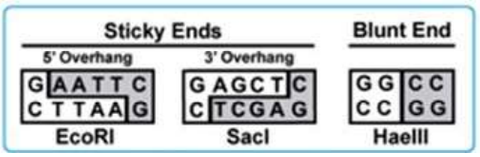


Fig. 6.4 *Sticky and Blunt Ends*

Types of Restriction Enzymes

Traditionally, four types of restriction enzymes are recognized, designated I, II, III, and IV, which differ primarily in structure, cleavage site, specificity, and cofactors.

Type I enzymes cleave at sites remote from a recognition site; require both ATP and S-adenosyl-L-methionine to function; multifunctional protein with both restriction and methylase activities.

Type II enzymes cleave within or at short specific distances from a recognition site; most require magnesium; single function (restriction) enzymes independent of methylase.

Type III enzymes cleave at sites a short distance from a recognition site; require ATP (but do not hydrolyse it); S-adenosyl-L-methionine stimulates the reaction but is not required; exist as part of a complex with a modification methylase.

Type IV enzymes target modified DNA, for example methylated, hydroxymethylated and glucosyl-hydroxymethylated DNA.

Nomenclature of Restriction Enzymes

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Since their discovery in the 1970s, many restriction enzymes have been identified while Type II restriction enzymes have been characterized.

Each enzyme is named after the bacterium from which it was isolated, using a naming system based on bacterial genus, species and strain. For example, the name of the EcoRI restriction enzyme was derived as:

E – Escherichia : Genus

co- coli: specific species

R- RY13 : strain

I- First identified: order of identification in the bacterium

Applications of Restriction Enzymes

Restriction enzymes can be isolated from bacterial cells and used in the laboratory to manipulate fragments of DNA, such as those that contain genes; for this reason they are indispensible tools of recombinant DNA technology (genetic engineering).

The most useful aspect of restriction enzymes is that each enzyme recognizes the same unique base sequence regardless of the source of the DNA. It means that these enzymes establish fixed landmark along an otherwise very regular DNA molecule. This allows dividing a long DNA molecule into fragments that can be separated from each other by size with the technique of gel electrophoresis.

Each fragment, thus generated, are also available for further analysis, including the sequencing. One value of cutting DNA molecule up into discrete fragments is being able to locate a particular gene on the fragment where it resides which is done by the general technique of Southern blotting.

6.2.6 Exonucleases

Secondary Exonucleases are a broad class of enzymes that cleave off nucleotides one at a time from the 3' or 5' ends of DNA and RNA chains. This activity contrasts with endonucleases, which hydrolyze internal phosphodiester bonds. Biological functions of exonucleases include RNA degradation and turnover, DNA proofreading, and transcriptional regulation. In molecular biology, commercial exonucleases are available for a variety of applications: Exonuclease I can be used for degrading excess single-stranded primers from a mixture containing double stranded products. Exonuclease III can be used for unidirectional nested deletions and site-directed mutagenesis. Lambda Exonuclease can be used for the removal of single nucleotides from the 5' ends in duplex DNA. Discover and compare exonucleases from different manufacturers in our enzyme search tool.

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6.2.7 DNA Ligase

Ends of DNA strands may be joined by the enzyme polynucleotide ligase, called 'glue' of the recombinant DNA molecule. The enzyme catalyses the forma-tion of a phosphodiester bond between the 3'OH and 5'P terminals of two nucleotides. The enzyme is thus able to join unrelated DNA, repair nicks in single strand of DNA and join the sugar phosphate backbones of the newly repaired and resident region of a DNA strand.

The enzyme which is extensively used for covalently joining restriction fragments is the ligase from *E. coli* and that encoded by T4 phage. As the main source of DNA ligase is T4 phage, hence, the enzyme is known as T4 DNA ligase.

The ligation reaction is controlled by several factors, such as pH, temperature, concentration and kinds of sticky ends, etc. As ligase uses the ends of DNA molecules as substrates rather than the entire DNA, the kinetics of joining depend on the number of ends (concentration) available for joining.

Check Your Progress

1. Define molecular biology.
2. What is DNA polymerase ?
3. Define telomerase.
4. What is RNA polymerase ?
5. What is primase?
6. What is restriction enzyme?

6.3 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Molecular biology is the study of the structure function, and makeup of the molecular building blocks of life. It focuses on the interactions between the various systems of a cell, including the interrelationship of DNA, RNA and protein synthesis and how these interactions are regulated.
2. DNA polymerase is an enzyme that synthesizes DNA molecules from deoxyribonucleotides, the building blocks of DNA. DNA molecules are the troves of genetic information of an organism. DNA is the basis of life and is transferred from parent to offspring's. The DNA content of the parent is doubled by means of replication mechanism aided by a specific enzyme, DNA polymerases.
3. Telomerase is a ribonucleoprotein recruited to replicate ends of linear chromosomes because normal DNA polymerase cannot replicate the ends,

or telomere. Telomerase acts like other DNA polymerases by extending the 3' end, but, unlike other DNA polymerases, telomerase does not require a template.

4. A RNA polymerase (RNAP), or ribonucleic acid polymerase, is a multi subunit enzyme that catalyzes the process of transcription where an RNA polymer is synthesized from a DNA template. The sequence of the RNA polymer is complementary to that of the template DNA and is synthesized in a 5→3′ orientation. This RNA strand is called the primary transcript and needs to be processed before it can be functional inside the cell.
5. Primase is an enzyme that synthesizes short RNA sequences called primers. These primers serve as a starting point for DNA synthesis. Since primase produces RNA molecules, the enzyme is a type of RNA polymerase. Primase functions by synthesizing short RNA sequences that are complementary to a single-stranded piece of DNA, which serves as its template.
6. Restriction enzyme, also called restriction endonuclease, is a protein produced by bacteria that cleaves DNA at specific sites along the molecule. Restriction endonucleases cut the DNA double helix in very precise ways.

6.4 SUMMARY

- Molecular biology is the study of the structure function, and makeup of the molecular building blocks of life. It focuses on the interactions between the various systems of a cell, including the interrelationship of DNA, RNA and protein synthesis and how these interactions are regulated.
- Molecular biology traces its origins to the 1930s, when scientists focused on explaining the phenomena of life by studying the macromolecules that generate life.
- The chief discoveries of molecular biology took place in a period of only about 25 years, starting in 1940, when George Beadle and Edward Tatum established the existence of a precise relationship between genes and proteins (they shared the 1958 Nobel Prize in Medicine).
- Another 15 years were required before new and more sophisticated technologies, united today under the name genetic engineering, would permit the isolation and characterization of genes.
- Enzymes are macromolecular biological catalysts. Enzymes accelerate chemical reactions. The molecules upon which enzymes may act are called substrates and the enzyme converts the substrates into different molecules known as products.
- Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life. Metabolic pathways depend upon enzymes to catalyze individual steps.

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- The study of enzymes is called enzymology and a new field of pseudoenzyme analysis has recently grown up, recognising that during evolution, some enzymes have lost the ability to carry out biological catalysis, which is often reflected in their amino acid sequences and unusual 'pseudocatalytic' properties.
- Enzymes are known to catalyze more than 5,000 biochemical reaction types. Most enzymes are proteins, although a few are catalytic RNA molecules. The latter are called ribozymes. Enzymes specificity comes from their unique three-dimensional structures.
- DNA molecules are the troves of genetic information of an organism. DNA is the basis of life and is transferred from parent to offspring's. The DNA content of the parent is doubled by means of replication mechanism aided by a specific enzyme, DNA polymerases.
- DNA polymerase plays a central role in process of life and carries a weighty responsibility of making an accurate copy of the cell's genome. The DNA polymerases are enzymes molecules by assembling nucleotides, the building blocks of DNA.
- Telomerase is a ribonucleoprotein recruited to replicate ends of linear chromosomes because normal DNA polymerase cannot replicate the ends, or telomere. Telomerase acts like other DNA polymerases by extending the 3' end, but, unlike other DNA polymerases, telomerase does not require a template.
- The protein and RNA components make up an active enzyme of ~200 kDa. The RNA component (~1.3 kb in yeast) contains the template sequence that is used for DNA synthesis. (So telomerase carries its own template.) In vitro, telomerase from a given species synthesizes the G-rich strand sequence characteristic of the species.
- A RNA polymerase (RNAP), or ribonucleic acid polymerase, is a multi subunit enzyme that catalyzes the process of transcription where an RNA polymer is synthesized from a DNA template. The sequence of the RNA polymer is complementary to that of the template DNA and is synthesized in a $5' \rightarrow 3'$ orientation. This RNA strand is called the primary transcript and needs to be processed before it can be functional inside the cell.
- There is remarkable similarity in the RNA polymerases found in prokaryotes, eukaryotes, archea and even some viruses. This point to the possibility that they evolved from a common ancestor. Prokaryotic RNAP is made of four subunits, including a sigma-factor that dissociates from the enzyme complex after transcription initiation. While prokaryotes use the same RNAP to catalyze the polymerization of coding as well as non-coding RNA, eukaryotes have five distinct RNA polymerases.
- Transcription begins with the binding of the RNAP enzyme to a specific part of the DNA, also known as the promoter region. This binding requires

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the presence of a few other proteins – the sigma factor in prokaryotes and various transcription factors in eukaryotes.

- While DNA and RNA polymerases both catalyze nucleotide polymerization reactions, there are two major differences in their activity. Unlike DNA polymerases, RNAP enzymes do not need a primer to begin the polymerization reaction.
- In all cellular organisms from bacteria to humans, genetic information is locked within a double helix formed by the two antiparallel deoxyribonucleic acid (DNA) strands.
- Although double-stranded DNA (dsDNA) is the form most suitable for secure information storage, hydrogen bonds formed between complementary bases (Watson-Crick base pairing) impair readout of this information by the cellular machinery, which frequently requires a single-stranded DNA (ssDNA) intermediate as a template.
- Primase is an enzyme that synthesizes short RNA sequences called primers. These primers serve as a starting point for DNA synthesis. Since primase produces RNA molecules, the enzyme is a type of RNA polymerase.
- The natural sources of restriction endonucleases are bacterial cells. These enzymes are called restriction enzymes because they restrict infection of bacteria by certain viruses (i.e., bacteriophages), by degrading the viral DNA without affecting the bacterial DNA.
- Restriction enzymes can be isolated from bacterial cells and used in the laboratory to manipulate fragments of DNA, such as those that contain genes; for this reason they are indispensible tools of recombinant DNA technology (genetic engineering).
- Secondary Exonucleases are a broad class of enzymes that cleave off nucleotides one at a time from the 3' or 5' ends of DNA and RNA chains. This activity contrasts with endonucleases, which hydrolyze internal phosphodiester bonds.

6.5 KEY WORDS

- **Molecular biology:** Molecular biology is the study of the structure function, and makeup of the molecular building blocks of life.
- **Enzymes:** Enzymes are protein molecules in cells which work as catalysts.
- **DNA polymerase:** DNA polymerase is an enzyme that synthesizes DNA molecules from deoxyribonucleotides, the building blocks of DNA.
- **Reverse transcriptase:** A reverse transcriptase is an enzyme used to generate complementary DNA from an RNA template, a process termed reverse transcription.

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- **Telomerase:** Telomerase, also called terminal transferase, is a ribonucleoprotein that adds a species-dependent telomere repeat sequence to the 3' end of telomeres.
- **RNA polymerase:** RNA polymerase is an enzyme that is responsible for copying a DNA sequence into an RNAsequence, duyring the process of transcription.
- **Helicases:** Helicases are enzymes that bind and may even remodel nucleic acid or nucleic acid protein complexes.
- **Primase:** Primase is an enzyme that synthesizes short RNA sequences called primers. These primers serve as a starting point for DNA synthesis.
- **Exonucleases:** Exonucleases are enzymes that work by cleaving nucleotides one at a time from the end (exo) of a polynucleotide chain.

6.6 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Write a short note on molecular biology.
2. What is DNA polymerase?
3. Write a brief note on reverse transcriptase.
4. Brief a note on telomerase.
5. What are the functions of RNA polymerase?
6. Write about process of transcription.
7. Distinguish between DNA and RNA polymerase.
8. Write a brief note on primase.

Long Answer Questions

1. Give a detailed note on molecular biology mentioning about the enzymes that are involved in it.
2. Explain in detail about DNA polymerase and its types.
3. Discuss about RNA polymerase in detail mentioning about its functions and types.
4. Explain about DNA helicases, its classification and directional translocation.
5. Give a detailed note on primase.
6. Explain about exonucleus and endonucleus in detail.
7. Discuss about DNA ligase in detail.

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UNIT 7 MECHANISM OF PROKARYOTIC AND EUKARYOTIC DNA REPLICATION

Structure

7.0 Introduction
7.1 Objectives
7.2 Mechanism of Prokaryotic and Eukaryotic DNA Replication
  7.2.1 DNA Replication in Prokaryotes
  7.2.2 DNA Replication in Eukaryotes
  7.2.3 Okazaki Fragments
7.3 Difference between Prokaryotic DNA Replication and Eukaryotic DNA Replication
7.4 Answers to Check Your Progress Questions
7.5 Summary
7.6 Key Words
7.7 Self Assessment Questions and Exercises
7.8 Further Readings

7.0 INTRODUCTION

DNA replication is the biological process of producing two identical replicas of DNA from one original DNA molecule. This process occurs in all living organisms and is the basis for biological inheritance. The cell possesses the distinctive property of division, which makes replication of DNA essential.

Prokaryotes do not have nucleus and other membrane-bound organelles, like mitochondria, endoplasmic reticulum, and Golgi bodies. The prokaryotic DNA is present as a DNA-protein complex called nucleoid. The replication occurs in the cytoplasm of the cell.

In case of eukaryotes, the organisms that contain a membrane-bound nucleus, the DNA is sequestered inside the nucleus. Hence, the nucleus is the site for DNA replication in eukaryotes.

In prokaryotes, DNA replication is the first step of cell division, which is primarily through binary fission or budding whereas in eukaryotes, cell division is a comparatively complex process, and DNA replication occurs during the synthesis (S) phase of the cell cycle.

In this unit, you will study about mechanism of prokaryotes and eukaryotic replication, machinery for replication, synthesis of leading and lagging strands, Okazaki fragments, difference between prokaryotic and eukaryotic replication in detail.

7.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand about the mechanism of prokaryotes and eukaryotic replication
- Discuss about machinery for replication
- Explain the synthesis of leading and lagging strands
- Discuss about Okazaki fragments
- Understand the difference between prokaryotic and eukaryotic replication

7.2 MECHANISM OF PROKARYOTIC AND EUKARYOTIC DNA REPLICATION

Comparing and Contrasting DNA Replication in Prokaryotes and Eukaryotes

Replication of DNA – deoxyribonucleic acid – happens before a cell divides to ensure that both cells receive an exact copy of the parent's genetic material. While there are many similarities in how prokaryotic and eukaryotic cells replicate their DNA, there are several distinctions between them, due to the different size and complexity of the molecules, including the time it takes to complete the process.

Differences Between Eukaryotic and Prokaryotic Cells

Prokaryotic cells are quite simple in structure. They have no nucleus, no organelles and a small amount of DNA in the form of a single, circular chromosome. Eukaryotic cells on the other hand, have a nucleus, multiple organelles and more DNA arranged in multiple, linear chromosomes.

Steps in DNA Replication

DNA replication begins at a specific spot on the DNA molecule called the origin of replication. At the origin, enzymes unwind the double helix making its components accessible for replication. Each strand of the helix then separates from the other, exposing the now unpaired bases to serve as templates for new strands. A small segment of RNA – ribonucleic acid – is added as a primer, then new nucleotide bases that complement the unpaired bases can be assembled to form two daughter strands next to each parent strand. This assembly is accomplished with enzymes called DNA polymerases. When the process is complete, two DNA molecules have been formed identical to each other and to the parent molecule.

Similarities Between Prokaryotic and Eukaryotic DNA Replication

The steps for DNA replication are generally the same for all prokaryotic and eukaryotic organisms. Unwinding the DNA is accomplished by an enzyme named DNA helicase. Manufacturing new DNA strands is orchestrated by enzymes called polymerases.

Both types of organisms also follow a pattern called semi-conservative replication. In this pattern, the individual strands of DNA are manufactured in different directions, producing a leading and a lagging strand. Lagging strands are created by the production of small DNA fragments called Okazaki fragments that are eventually joined together. Both types of organisms also begin new DNA strands with a small primer of RNA.

Differences Between Prokaryotic and Eukaryotic DNA Replication

Differences between prokaryotic and eukaryotic DNA replication are largely related to contrasts in size and complexity of the DNA and cells of these organisms. The average eukaryotic cell has 25 times more DNA than a prokaryotic cell.

In prokaryotic cells, there is only one point of origin, replication occurs in two opposing directions at the same time, and takes place in the cell cytoplasm. Eukaryotic cells on the other hand, have multiple points of origin, and use unidirectional replication within the nucleus of the cell. Prokaryotic cells possess one or two types of polymerases, whereas eukaryotes have four or more.

Replication also happens at a much faster rate in prokaryotic cells, than in eukaryotes. Some bacteria take only 40 minutes, while animal cells such as humans may take up to 400 hours. In addition, eukaryotes also have a distinct process for replicating the telomeres at the ends of their chromosomes. With their circular chromosomes, prokaryotes have no ends to synthesize. Lastly, the short replication in prokaryotes occurs almost continuously, but eukaryotic cells only undergo DNA replication during the S-phase of the cell cycle.

DNA replication is fundamental process occurring in all living organism to copy their DNA. The process is called replication in sense that each strand of ds DNA serve as template for reproduction of complementary strand.

General Feature of DNA Replication

- DNA replication is semi conservative
- It is bidirectional process
- It proceed from a specific point called origin
- It proceed in 5'-3' direction
- It occur with high degree of fidelity
- It is a multi-enzymatic process

DNA Replication Occurs by Three Steps

1. Initiation
   - Initiation complex formation
   - Closed complex formation
   - Open complex formation

2. Elongation
   - Leading strand synthesis
   - Lagging strand synthesis
3. Termination

7.2.1 DNA Replication in Prokaryotes

1. Initiation

DNA replication begins from origin. In *E coli*, replication origin is called OriC which consists of 245 base pair and contains DNA sequences that are highly conserved among bacterial replication origin. Two types of conserved sequences are found at OriC, three repeats of 13 bp (GATRCTNTTNTTTT) and four/five repeats of 9 bp (TTATCCACA) called 13 mer and 9 mer respectively.

About 20 molecules of DNA A proteins binds with 9 mer repeats along with ATP which causes DNA to wraps around DNAA protein forming initial complex. The DNA A protein and ATP trigger the opening of 13 mer repeats froming open complex.

Two copies of DNAB proteins (helicase) binds to 13 mer repeats. This binding is facilitated by another molecule called DNAC. The DNAB-DNAC interaction causes DNA B ring to open which binds with each of the DNA strand. The hydrolysis of bound ATP release DNA leaving the DNAB bound to the DNA strand (Refer Figure 7.1).

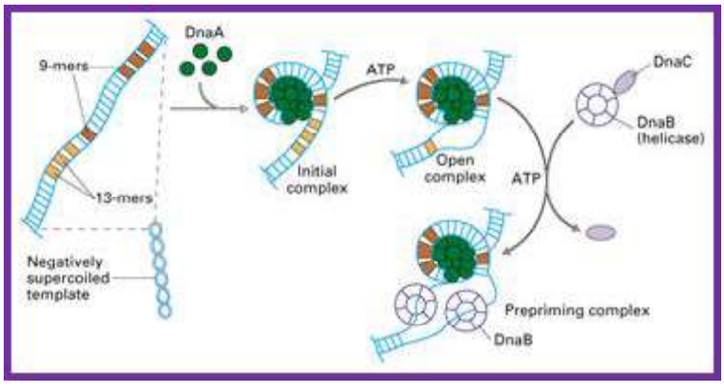


Fig. 7.1 *Initiation - DNA Replication in Prokaryotes*

The binding of helicase is key step in replication initiation. DNAB migrates along the single stranded DNA in 5'-3' direction causing unwinding of the DNA.

The activity of helicase causes the topological stress to the unwinded strand forming supercoiled DNA. This stress is relieved by the DNA topoisomerase (DNA gyrase) by negative supercoiling. Similarly, single stranded binding protein binds to th separated strand and prevents reannaeling of separated strand and stabilize the strand.

The DNA polymerase cannot initiate DNA replication. So, at first primase synthesize 10±1 nucleotide (RNA in nature) along the 5'-3' direction. In case of E.coli primer synthesized by primase starts with ppp-AG-nucleotide. Primer is closely associated with DNAB helicase so that it is positioned to make RNA primer as ssDNA of lagging strand.

2. Elongation

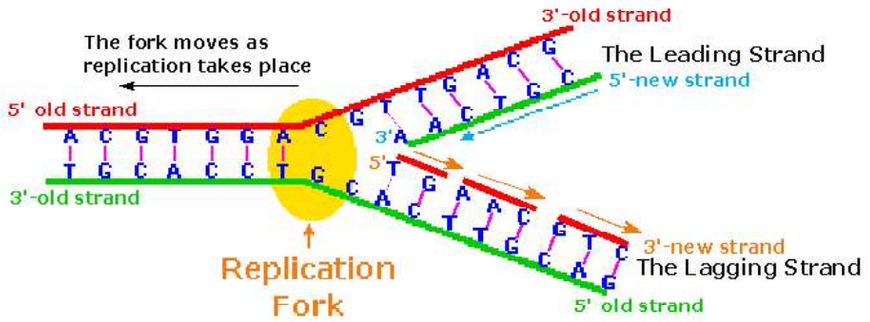


***Fig. 7.2** Elongation - Replication Fork*

i. Leading Strand Synthesis

- Leading strand synthesis is more a straight forward process which begins with the synthesis of RNA primer by primase at replication origin.
- DNA polymerase III then adds the nucleotides at 3'end. The leading strand synthesis then proceed continuously keeping pace with unwinding of replication fork until it encounter the termination sequences.

ii. Lagging Strand Synthesis

- The lagging strand synthesized in short fragments called Okazaki fragments. At first RNA primer is synthesized by primase and as in leading strand DNA polymerase III binds to RNA primer and adds dNTPS.
- On this level the synthesis of each Okazaki fragments seems straight forward but the reality is quite complex (Refer Figure 7.2).

Mechanism of Lagging strand synthesis

- The complexicity lies in the co-ordination of leading and lagging strand synthesis. Both the strand are synthesized by a single DNA polymerase III dimer which accomplished the looping of template (Refer Figure 7.3).

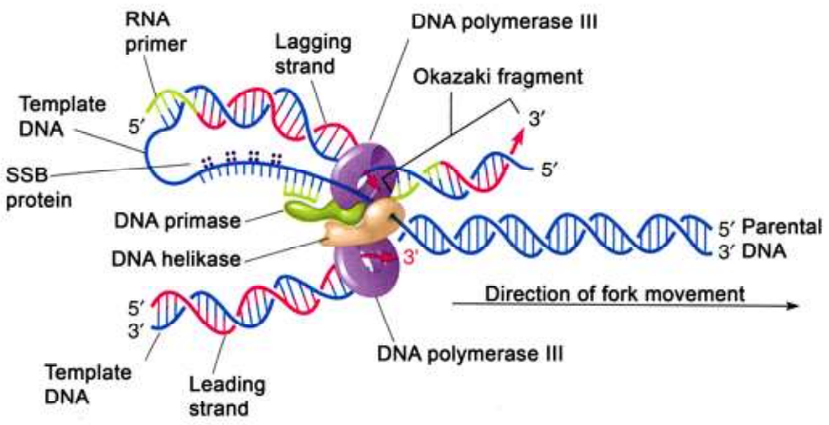


***Fig. 7.3** Mechanism of Lagging Strand Aynthesis*

- DNA of lagging strand synthesizing Okazaki fragments.Helicase (DNAB) and primase (DNAG) constitute a functional unit within replication complex called **primosome.**
- DNA pol III use one set of core sub unit (Core polymerase) to synthesize leading strand and other set of core sub unit to synthesize lagging strand.
- In elongation steps, helicasein front of primaseand pol III, unwind the DNA at the replication fork and travel along lagging strand template along 5'-3' direction.
- DNAG primase occasionally associated with DNAB helicase synthesizes short RNA primer. A new B-sliding clamp is then positioned at the primer b y
  B-clamp loading complex of DNA pol III.
- When the Okazaki fragments synthesis is completed, the replication halted and the core sub unit dissociates from their sliding clamps and associates with new clamp. This initiates the synthesis of new Okazaki fragments.
- Both leading and lagging strand are synthesized co-ordinately and simultaneously by a complex protein moving in 5'-3' direction. In this way both leading and lagging strand can be replicated at same time by a complex protein that move in same direction.
- Every so often the lagging strands must dissociates from the replicosome and reposition itself so that replication can continue.
- Lagging strand synthesis is not completes until the RNA primer has been removed and the gap between adjacent Okazaki fragments are sealed. The RNA primer are removed by exonuclease activity (5'-3') of DNA pol-I and replaced by DNA. The gap is then sealed by DNA ligase using NAD as co-factor.

Termination

- Evantually the two replication fork of circular *E. coli* chromosome meet at termination recognizing sequences (ter).
- The Ter sequence of 23 bp are arranged on the chromosome to create trap that the replication fork can enter but cannot leave. Ter sequences function as binding site for TUS protein (Refre Figure 7.4).

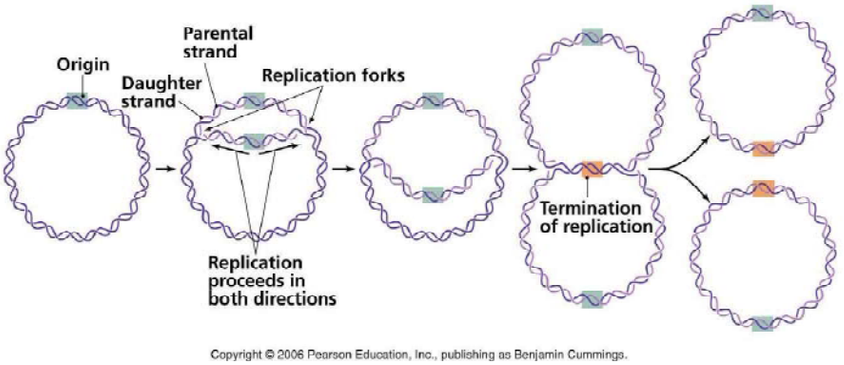


***Fig. 7.4** Termination - DNA Replication in Prokaryotes*

- Ter-TUS complex can arrest the replication fork from only one direction. Ter-TUS complex encounter first with either of the replication fork and halt it. The other opposing replication fork halted when it collide with the first one. This seems the Ter-TUS sequences is not essential for termination but it may prevents over replication by one fork if other is delayed or halted by a damage or some obstacle.
- When either of the fork encounter Ter-TUS complex, replication halted.
- Final few hundred bases of DNA between these large protein complexes are replicated by not yet known mechanism forming two interlinked (cataneted) chromosome.
- In *E. coli* DNA topoisomerase IV (type II) cut the two strand of one circular DNA and segrate each of the circular DNA and finally join the strand. The DNA finally transfer to two daughter cell.

7.2.2 DNA Replication in Eukaryotes

DNA replication in eukaryotes occur only in S-phase of cell cycle. However pre-initiation occur in G1 pahse. Due to sheer size of chromosome in eukaryotes, chromosome chromosome contains multiple origin of replication. ARS (autonomously replicating sequence) in case of yeast is origin for replication.

Steps in DNA Replication

1. Initiation

- The first steps is the formation of pre-initiation replication complex (pre-RC). It occurs in two stage. 1st stage requires, there is no CDK activities. It occur in early G1 phase. It is known as licensing but licensed pre-RC cannot initiate replication at G1 phase. 2nd stage is binding of ORC (origin recognition complex).
- The replication begins with binding of ORC to the origin. ORC is a hexamer of related protein and remains bounded even after DNA replication occurs. Furthermore ORC is analogue of prokaryotic DNAA protein.
- After binding of ORC to origin, cdc6/cdc18 and cdtl coordinate the loading of MEM (mini chromosome maintainance) to origin.
- MEM complex is thought to be major eukaryotic helicase.
- After binding of MEM complex to pre-RC, cdtl get displaced. Then DdK phosphorylates MEM, which activates its helicase activity. Again DdK and CdK recruit another protein called cdc45 which then recruit all the DNA replicating protein such that the origin get fired and replication begins (Refer Figure 7.5).

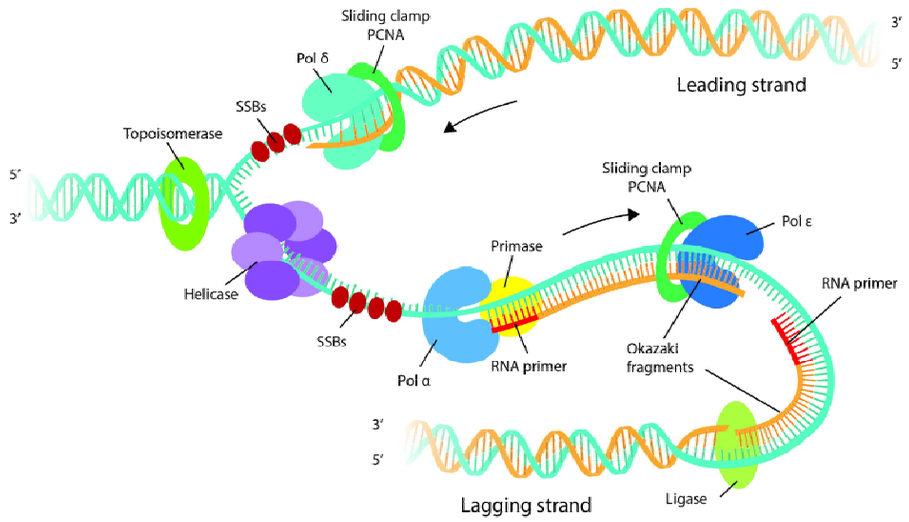


Fig. 7.5 *Initiation Phase - DNA Replication in Eukaryotes*

2. Elongation

- DNA polymerase δ synthesizes and adds dNTPs at 3' end of RNA primer.
- The leading and lagging strands are synthesized in the similar fashion as in prokaryotic DNA replication (Refer Figure 7.6).

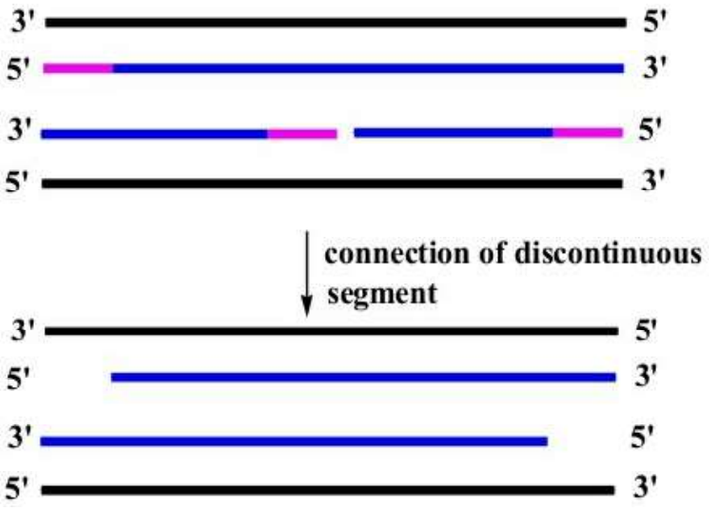


Fig. 7.6 *Elongation Phase - DNA Replication in Eukaryotes*

3. Termination

- At the end of DNA replication the RNA primer are replaced by DNA by 5'-3'exonuclease and polymerase activity of DNA polymerase ε.
- Exonuclease activity of DNA polymerase removes the RNA primer and polymerase activity adds dNTPs at 3'-OH end preceding the primer.
- In case of bacteria, with circular genome, the replacement of RNA primer with DNA is not a problem because there is always a preceding 3'-OH in a circular DNA.
- But in eukaryotic organism with linear DNA, there is a problem. When RNA primer at 5' end of daughter strand is removed, there is not a preceding 3'-OH such that the DNA polymerase can use it to replace by DNA. So, at 5' end of each daughter strand there is a gap (missing DNA). This missing DNA cause loss of information contain in that region. This gap must be filled before next round of replication.
- For solving this end replication problem;studies have found that linear end of DNA called telomere has G:C rich repeats. These sequence is known as telomere sequence. These repeats of telomere sequence is different among different organisms. Telomere in human cell consists of repeats of TTAGGG/ AATCCC. Each species has its own species specific telomere repeats. These telomere sequence donot codes anything but it is essential to fill the gap in daughter strand and maintain the integrity of DNA.

Telomere Replication: End Replication Problem in Eukaryotic DNA

- There is an enzyme found in eukaryotic cell called telomerase.

- Telomerase is a DNA polymerase (RNA dependent DNA polymerase) which adds many copies of telomere sequence at 3'-OH end of template strand. Like other DNA polymerase, terlomerase also adds deoxyribonucleotide at 3'-OH end. Unlike other DNA polymerase, telomerase adds DNA at 3'-OH end of parent strand not at the daughter strand and also it synthesizes the same sequences over and over in absence of template strand (Refer Figure 7.7).

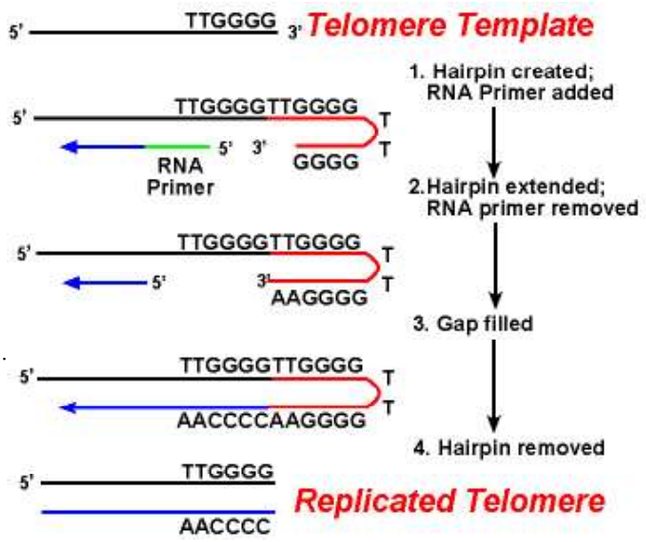


***Fig. 7.7** Telomere Replication - End Replication Problem in Eukaryotic DNA*

- First telomerase binds to 3'-OH end of parent strand by hybridization between its AACCCCAAC RNA sequences and TTGGGG DNA sequences (telomere sequences of T*etrahymena*).
- The telomerase adds TTG at 3' end of parent strand. After adding TTG sequences, telomerase translocates along 5'-3' end of parent strand. Now the telomerase adds GGGTTG to 3' end by using its CCCAAC sequence. Again telomerase translocates and adds GGGTTA sequence. This process is continued for many time. The parent strand become more longer than daughter strand. Now RNA polymerase (PRIMASE) synthesize RNA primer by copying the parent strand in 5'-3' direction using telomere sequence as template.
- The DNA polymerase can now extend the primer in 5'-3' direction by adding deoxyribonucleotide to 3' end.
- The primer is now removed and it won't be replaced because it is an extra sequence added by copying telomere sequence.
- Finally the integrity of daughter strand is maintained.

7.2.3 Okazaki Fragments

Okazaki fragments are short sequences of DNA nucleotides (approximately 150 to 200 base pairs long in eukaryotes) which are synthesized discontinuously and later linked together by the enzyme DNA ligase to create the lagging strand during DNA replication. They were discovered in the 1960s by the Japanese molecular biologists Reiji and Tsuneko Okazaki, along with the help of some of their colleagues.

DNA Replication/Duplication

For normal cell growth and division in organisms, an initial step called DNA replication is required. This process is based on semi-conservative copy of DNA in the nucleus of a growing cell.

DNA replication then begins with distortion of double helix by topoisomerase, followed by separation of two DNA strands by helicase. Separation of DNA single strands leads to formation of replication fork, where the replication machinery (protein and enzyme complex) will bind. An RNA polymerase is the constituent of this complex that adds small primers to the beginning of DNA single strand to be copied. Then another enzyme, DNA polymerase, recognizes the primers added by RNA polymerase and starts copying the DNA strands. At the end of replication there are two DNA molecules, each of the double strand containing one strand originating from cell and one copied (hence the name semi-conservative replication).

However, a major hindrance to DNA replication comes from the fact that DNA polymerase enzyme can only perform its function of nucleic acid polymerization (addition of nucleic acids to the growing strand) in $5' \rightarrow 3'$ sense. Since the two strands of DNA molecule are antiparallel and replication fork separates the two strands at the same site, strand in the $5' \rightarrow 3'$ direction is easily replicated towards the opening of replication fork. For this reason, this chain is called 'leader chain'. The problem lies in the other strand, which is oriented in $3' \rightarrow 5'$ direction, in which DNA polymerase cannot bind and perform its function because the opening of replication fork occurs in the opposite direction to replication.

Okazaki Fragments Formation

To work around this problem, cell makes copies the strand that is oriented $3' \rightarrow 6'$ in a discontinuous manner. This chain is called a 'delayed chain'.

In this process, several small fragments of the delayed chain are replicated as replication fork advances ($5' \rightarrow 3'$) and further separates the double helix from DNA molecule. The fragments resulting from this discontinuous replication are called 'Okazaki Fragments'. Thus, although backward chain is growing in the $3' \rightarrow 5'$ direction, in fact Okazaki Fragments are being synthesized in the $3' \rightarrow 5'$ direction.

After the primers are removed, nucleic acid gaps between Okazaki Fragments are filled and a final enzyme, DNA ligase, binds fragments to form a single, single strand of continuous DNA (Refer Figure 7.8) .

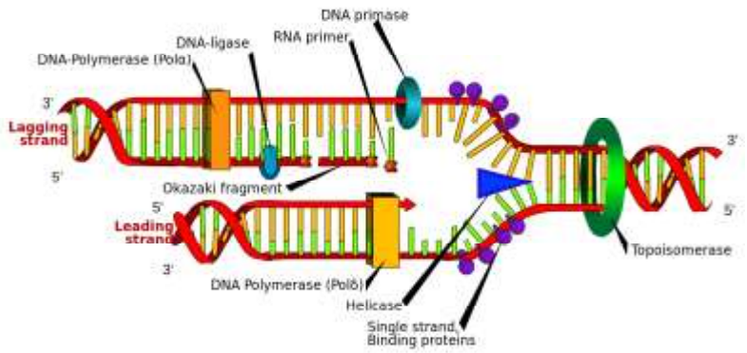


Fig. 7.8 *DNA replication and Consequent Formation of Okazaki Fragments*

Okazaki Fragments were named after the scientist who discovered them in 1969, Reiji Okazaki. In bacteria such fragments have a size of 1000 to 2000 nucleic acids; on the other hand, in eukaryotes have a size smaller than 200 nucleic acids.

Check Your Progress

1. Give a difference between Eukaryotic and Prokaryotic cell.
2. Give a differences between Prokaryotic and Eukaryotic DNA replication.
3. What are the three steps of DNA replication?
4. How does DNA replication in Eukaryotes occur?

7.3 DIFFERENCE BETWEEN PROKARYOTIC DNA REPLICATION AND EUKARYOTIC DNA REPLICATION

Some of the major Differences between Prokaryotic DNA Replication and Eukaryotic DNA Replication are as follows:

Prokaryotic DNA Replication

1. It occurs inside the cytoplasm (Refer Figure 7.9) .
2. There is single origin of replication.
3. DNA polymerase III carries out both initiation and elongation.
4. DNA repair and gap filling are done by DNA polymerase I (Refer Figure 7.10) .

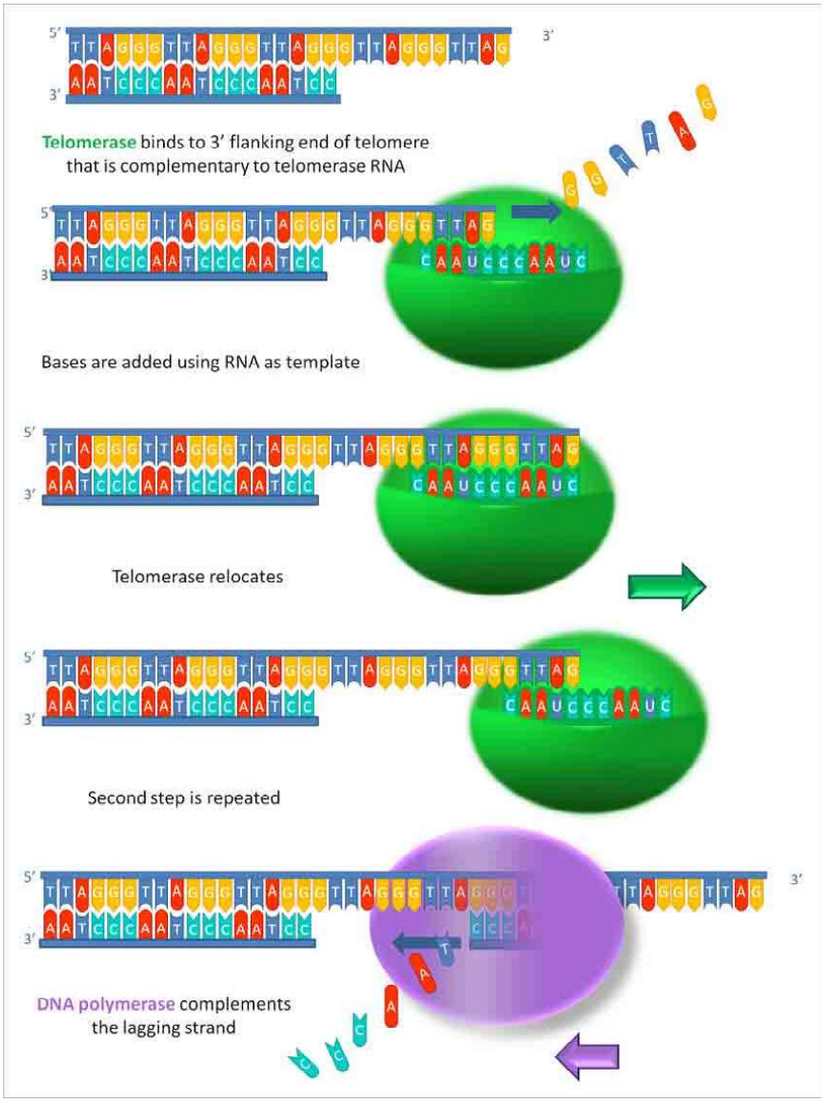


Fig. 7.9 *Prokaryotic DNA Replication*

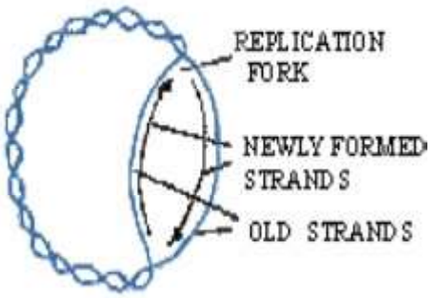


Fig. 7.10 *Replication Fork - Replication*

5. RNA primer is removed by DNA polymerase I.
6. Okazaki fragments are large, 1000-2000 nucleotides long.
7. Replication is very rapid, some 2000 bp per second.
8. DNA gyrase is needed.

Eukaryotic DNA Replication

1. It occurs inside the nucleus.
2. Origin of replications are numerous.
3. Initiation is carried out by DNA polymerase α while elongation by DNA polymerase δ and ε.

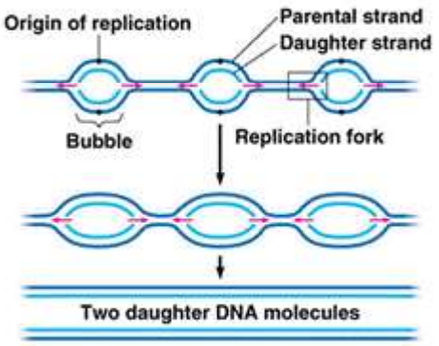


Fig. 7.11 *Eukaryotic DNA Replication*

4. The same are performed by DNA polymerase β.
5. RNA primer is removed by DNA polymerase β.
6. Okazaki fragments are short, 100-200 nucleotides long.
7. Replication is slow, some 100 nucleotides per second.
8. DNA gyrase is not needed (Refer Figure 7.11).

Check Your Progress

5. How does replication occur in Prokaryotes?
6. How does replication occur in Eukaryotes?

7.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Prokaryotic cells are quite simple in structure. They have no nucleus, no organelles and a small amount of DNA in the form of a single, circular chromosome. Eukaryotic cells on the other hand, have a nucleus, multiple organelles and more DNA arranged in multiple, linear chromosomes.

2. Differences between prokaryotic and eukaryotic DNA replication are largely related to contrasts in size and complexity of the DNA and cells of these organisms. The average eukaryotic cell has 25 times more DNA than a prokaryotic cell.

In prokaryotic cells, there is only one point of origin, replication occurs in two opposing directions at the same time, and takes place in the cell cytoplasm. Eukaryotic cells on the other hand, have multiple points of origin, and use unidirectional replication within the nucleus of the cell. Prokaryotic cells possess one or two types of polymerases, whereas eukaryotes have four or more.

3. DNA Replication occurs by three steps:

1. Initiation
   - Initiation complex formation
   - Closed complex formation
   - Open complex formation
2. Elongation
   - Leading strand synthesis
   - Lagging strand synthesis
3. Termination

4. DNA replication in eukaryotes occur only in S-phase of cell cycle. However pre-initiation occur in G1 pahse. Due to sheer size of chromosome in eukaryotes, chromosome chromosome contains multiple origin of replication. ARS (autonomously replicating sequence) in case of yeast is origin for replication.

5. Prokaryotic DNA Replication:
   - It occurs inside the cytoplasm.
   - There is single origin of replication.
   - DNA polymerase III carries out both initiation and elongation.
   - DNA repair and gap filling are done by DNA polymerase I
   - RNA primer is removed by DNA polymerase I.
   - Okazaki fragments are large, 1000-2000 nucleotides long.

6. Eukaryotic DNA Replication:
   - It occurs inside the nucleus.
   - Origin of replications are numerous.
   - Initiation is carried out by DNA polymerase á while elongation by DNA polymerase δ and ε.
   - The same are performed by DNA polymerase β.

- RNA primer is removed by DNA polymerase β.
- Okazaki fragments are short, 100-200 nucleotides long.
- Replication is slow, some 100 nucleotides per second.
- DNA gyrase is not needed

NOTES

7.5 SUMMARY

- Replication of DNA – deoxyribonucleic acid – happens before a cell divides to ensure that both cells receive an exact copy of the parent's genetic material.
- While there are many similarities in how prokaryotic and eukaryotic cells replicate their DNA, there are several distinctions between them, due to the different size and complexity of the molecules, including the time it takes to complete the process.
- Prokaryotic cells are quite simple in structure. They have no nucleus, no organelles and a small amount of DNA in the form of a single, circular chromosome. Eukaryotic cells on the other hand, have a nucleus, multiple organelles and more DNA arranged in multiple, linear chromosomes.
- DNA replication begins at a specific spot on the DNA molecule called the origin of replication.
- At the origin, enzymes unwind the double helix making its components accessible for replication.
- Each strand of the helix then separates from the other, exposing the now unpaired bases to serve as templates for new strands.
- A small segment of RNA – ribonucleic acid – is added as a primer, then new nucleotide bases that complement the unpaired bases can be assembled to form two daughter strands next to each parent strand. This assembly is accomplished with enzymes called DNA polymerases. When the process is complete, two DNA molecules have been formed identical to each other and to the parent molecule.
- The steps for DNA replication are generally the same for all prokaryotic and eukaryotic organisms. Unwinding the DNA is accomplished by an enzyme named DNA helicase. Manufacturing new DNA strands is orchestrated by enzymes called polymerases.
- Both types of organisms also follow a pattern called semi-conservative replication. In this pattern, the individual strands of DNA are manufactured in different directions, producing a leading and a lagging strand.
- Lagging strands are created by the production of small DNA fragments called Okazaki fragments that are eventually joined together. Both types of organisms also begin new DNA strands with a small primer of RNA.

NOTES

- Differences between prokaryotic and eukaryotic DNA replication are largely related to contrasts in size and complexity of the DNA and cells of these organisms. The average eukaryotic cell has 25 times more DNA than a prokaryotic cell.
- In prokaryotic cells, there is only one point of origin, replication occurs in two opposing directions at the same time, and takes place in the cell cytoplasm.
- Eukaryotic cells on the other hand, have multiple points of origin, and use unidirectional replication within the nucleus of the cell. Prokaryotic cells possess one or two types of polymerases, whereas eukaryotes have four or more.
- Replication also happens at a much faster rate in prokaryotic cells, than in eukaryotes. Some bacteria take only 40 minutes, while animal cells such as humans may take up to 400 hours. In addition, eukaryotes also have a distinct process for replicating the telomeres at the ends of their chromosomes.
- With their circular chromosomes, prokaryotes have no ends to synthesize. Lastly, the short replication in prokaryotes occurs almost continuously, but eukaryotic cells only undergo DNA replication during the S-phase of the cell cycle.
- DNA replication is fundamental process occurring in all living organism to copy their DNA. The process is called replication in sense that each strand of ds DNA serve as template for reproduction of complementary strand.
- DNA replication begins from origin.
- In *E coli*, replication origin is called OriC which consists of 245 base pair and contains DNA sequences that are highly conserved among bacterial replication origin.
- Two types of conserved sequences are found at OriC, three repeats of 13 bp (GATRCTNTTNTTTT) and four/five repeats of 9 bp (TTATCCACA) called 13 mer and 9 mer respectively.
- DNA replication in eukaryotes occur only in S-phase of cell cycle. However pre-initiation occur in G1 pahse.
- Due to sheer size of chromosome in eukaryotes, chromosome chromosome contains multiple origin of replication. ARS (autonomously replicating sequence) in case of yeast is origin for replication.
- Okazaki fragments are short sequences of DNA nucleotides (approximately 150 to 200 base pairs long in eukaryotes) which are synthesized discontinuously and later linked together by the enzyme DNA ligase to create the lagging strand during DNA replication.

- They were discovered in the 1960s by the Japanese molecular biologists Reiji and Tsuneko Okazaki, along with the help of some of their colleagues.
- For normal cell growth and division in organisms, an initial step called DNA replication is required. This process is based on semi-conservative copy of DNA in the nucleus of a growing cell.
- DNA replication then begins with distortion of double helix by to poisomerase, followed by separation of two DNA strands by helicase. Separation of DNA single strands leads to formation of replication fork, where the replication machinery (protein and enzyme complex) will bind.
- An RNA polymerase is the constituent of this complex that adds small primers to the beginning of DNA single strand to be copied. Then another enzyme, DNA polymerase, recognizes the primers added by RNA polymerase and starts copying the DNA strands.
- At the end of replication there are two DNA molecules, each of the double strand containing one strand originating from cell and one copied (hence the name semi-conservative replication).

7.6 KEY WORDS

- **Mitochondria:** Mitochondria are rod-shaped organelles that can be considered the power generators of the cell, converting oxygen and nutrients into adenosine triphosphate (ATP).
- **Endoplasmic reticulum:** The endoplasmic reticulum is a type of organelle found in eukaryotic cells that forms an interconnected network of flattened, membrane-enclosed sacs or tube-like structures known as cisternae.
- **Golgi bodies:** The Golgi apparatus, also known as the Golgi complex, Golgi body, or simply the Golgi, is an organelle found in most eukaryotic cells.
- **Nucleoid:** The nucleoid (meaning nucleus-like) is an irregularly shaped region within the cell of a prokaryote that contains all or most of the genetic material, called genophore
- **DNA polymerase:** DNA polymerase is an enzyme that synthesizes DNA molecules from deoxyribonucleotides, the building blocks of DNA.
- **Telomere:** A telomere is a region of repetitive nucleotide sequences at each end of a chromosome, which protects the end of the chromosome from deterioration or from fusion with neighboring chromosomes.
- **Replication fork:** The replication fork is a very active area where DNA replication takes place. It is created when DNA helicase unwinds the double helix structure of the DNA.

- **Exonucleases:** Exonucleases are enzymes that work by cleaving nucleotides one at a time from the end of a polynucleotide chain.
- **Okazaki fragments:** Okazaki fragments are short sequences of DNA nucleotides which are synthesized discontinuously and later linked together by the enzyme DNA ligase to create the lagging strand during DNA replication.

7.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. What are the similarities between Prokaryotic and Eukaryotic DNA replication ?
2. Give the differences between Prokaryotic and Eukaryotic DNA replication.
3. Write the general feature of DNA replication.
4. What are the phases of DNA replication?
5. Write about initiation process of DNA Replication in Eukaryotes.
6. Brief a note on Okazaki fragments.

Long Answer Questions

1. Give a detailed account on mechanism of Prokaryotic and Eukaryotic DNA replication.
2. Explain the DNA replication process in Prokaryotes in detail about its phases.
3. Discuss the mechanism of leading and lagging strands synthesis in DNA replication.
4. Give a elaborated note on DNA replication process in Eukaryotes explaining about its phases in detail.
5. Explain about Okazaki fragments in detail.
6. Distinguish between between Prokaryotic DNA replication and Eukaryotic DNA replication.

7.8 FURTHER READINGS

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NOTES

BLOCK - III
TRANSCRIPTION AND TRANSLATION

NOTES

UNIT 8 PROKARYOTIC TRANSCRIPTION

Structure

8.0 Introduction
8.1 Objectives
8.2 Prokaryotic Transcription
  8.2.1 Promoters
  8.2.2 Steps in Prokaryotic Transcription
8.3 Properties of Bacterial RNA Polymerase
8.4 Answers to Check Your Progress Questions
8.5 Summary
8.6 Key Words
8.7 Self Assessment Questions and Exercises
8.8 Further Readings

8.0 INTRODUCTION

The central region of the cell in which prokaryotic DNA resides is called the nucleoid region. Bacterial and Archaeal chromosomes are covalently-closed circles that are not as extensively compacted as eukaryotic chromosomes, but are compacted nonetheless as the diameter of a typical prokaryotic chromosome is larger than the diameter of a typical prokaryotic cell. Additionally, prokaryotes often have abundant plasmids, which are shorter, circular DNA molecules that may only contain one or a few genes and often carry traits such as antibiotic resistance.

Transcription is the process in which messenger RNA transcripts of genetic material in bacteria are produced, to be translated for the production of proteins. Unlike in eukaryotes, bacterial transcription and translation can occur simultaneously in the cytoplasm. This is impossible in eukaryotes, where transcription occurs in a membrane-bound nucleus while translation occurs outside the nucleus in the cytoplasm. In bacteria genetic material is not enclosed in a membrane-enclosed nucleus and has access to ribosomes in the cytoplasm. Transcription is carried out by RNA polymerase but its specificity is controlled by sequence-specific DNA binding proteins called transcription factors.

In this unit, you will study about transcription process in prokaryotes. Prokaryotes are mostly single-celled organisms that, by definition, lack membrane-bound nuclei and other organelles.

8.1 OBJECTIVES

NOTES

After going through this unit, you will be able to:

- Understand the process of transcription in prokaryotes
- Define promoters
- Discuss properties and functions of bacterial RNA polymerase

8.2 PROKARYOTIC TRANSCRIPTION

- The process of synthesis of RNA by copying the template strand of DNA is called transcription.
- During replication entire genome is copied but in transcription only the selected portion of genome is copied.
- The enzyme involved in transcription is RNA polymerase. Unlike DNA polymerase it can initiate transcription by itself, it does not require primase. More exactly it is a DNA dependent RNA polymerase. (Refer Figure 8.1)

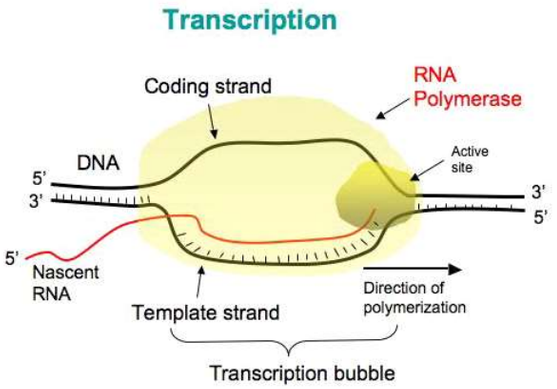


Fig. 8.1 *Transcription*

8.2.1 Promoters

In genetics, a promoter is a region of DNA that initiates transcription of a particular gene. Promoters can be about 100–1000 base pairs long.

For transcription to take place, the enzyme that synthesizes RNA, known as RNA polymerase, must attach to the DNA near a gene. Promoters contain specific DNA sequences such as response elements that provide a secure initial binding site for RNA polymerase and for proteins called transcription factors that recruit RNA polymerase. These transcription factors have specific activator or repressor sequences of corresponding nucleotides that attach to specific promoters and regulate gene expression.

NOTES

In bacteria

The promoter is recognized by RNA polymerase and an associated sigma factor, which in turn are often brought to the promoter DNA by an activator protein's binding to its own DNA binding site nearby.

In eukaryotes

The process is more complicated, and at least seven different factors are necessary for the binding of an RNA polymerase II to the promoter.

Promoters represent critical elements that can work in concert with other regulatory regions (enhancers, silencers, boundary elements/insulators) to direct the level of transcription of a given gene. A promoter is induced in response to changes in abundance or conformation of regulatory proteins in a cell, which enable activating transcription factors to recruit RNA polymerase.

8.2.2 Steps in Prokaryotic Transcription

1. Initiation

- Closed Complex Formation
- Open complex formation
- Tertiary complex formation

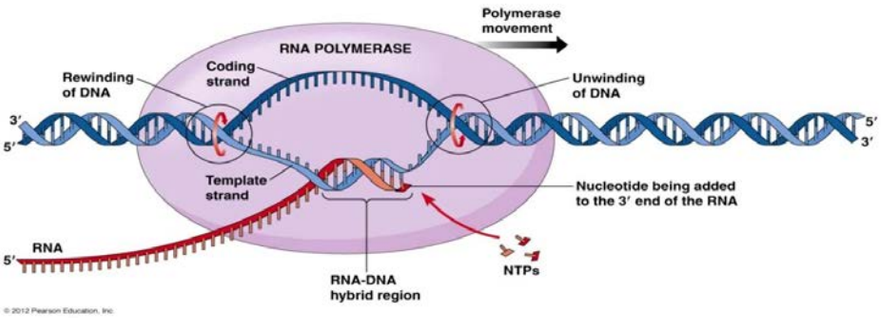
2. Elongation

3. Termination

- Rho- dependent
- Rho-independent

1. Initiation

- The transcription is initiated by RNA polymerase holoenzyme from a specific point called promoter sequence.
- Bacterial RNA polymerase is the principle enzyme involved in transcription.
- Single RNA polymerase is found in a bacteria which is called core polymerase and it consists of α, β, β' and ω sub units.
- The core enzyme bind to specific sequence on template DNA strand called promoter. The binding of core polymerase to promoter is facilitates and specified by sigma (σ) factor. (σ70 in case of *E. coli*).



- The core polymerase along with σ-factor is called Holoenzyme, i.e., RNA polymerase holoenzyme. (Refer Figure 8.2)

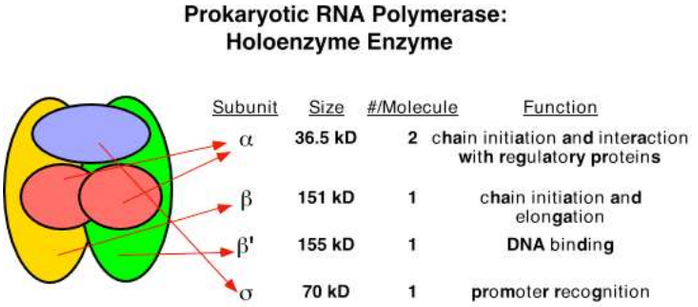


Fig. 8.2 *Holoenzyme*

- In case of E. coli, promoter consists of two conserved sequences 5'-TTGACA-3' at -35 element and 5'-TATAAT-3' at -10 element. These sequence are upstream to the site from which transcription begins. Binding of holoenzyme to two conserve sequence of promoter form close complex.
- In some bacteria, the altered promoter may exist which contain UP-element and some may contain extended -10 element rather than -35 element.

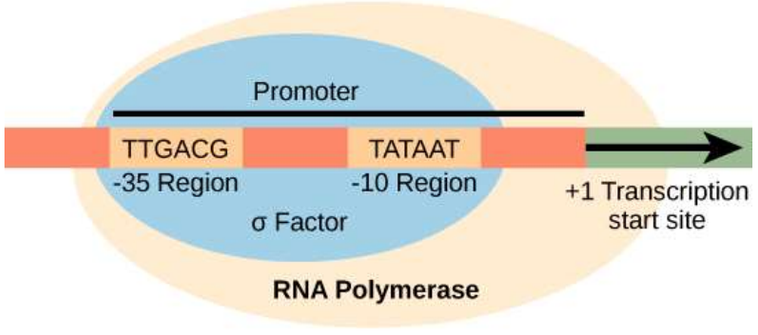


Fig. 8.3 *Promoter Site in E. coli*

σ70 of *E. coli* has Four Region.

1. Region1: it includes 1.2 and 1.1 region. Region 1.1 acts as molecular mimic of DNA

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2. Region2: it recognizes -10 element in promoter. á-helix recognizes -10 element.
3. Region 3: it recognizes extended -10 element.
4. Region 4: it recognizes -35 element in promoter by a structure called helix-turn-helix.

The UP-element is recognized by a carboxyl terminal domain of α-sub unit called αCTD (carboxyl terminal domain) which is connected to αNTD (Amino terminal domain) by flexible linker.

(i) Closed Complex Formation

Binding of RNA polymerase holoenzyme to the promoter sequence form closed complex

(ii) Open complex

After formation of closed complex, the RNA polymerase holoenzyme separates 10-14 bases extending from -11 to +3 called melting. So that open complex is formed. This changing from closed complex to open complex is called **isomerization.**

(iii) Tertiary Complex

- RNA polymerase starts synthesizing nucleotide. It does not require the help of primase.
- If the enzyme synthesize short RNA molecules of less than 10 bp, it does not further elongates which is called abortive initiation. This is because ó 3.2 acting as mimic of RNA and it lies at middle of RNA exit channel in open complex.

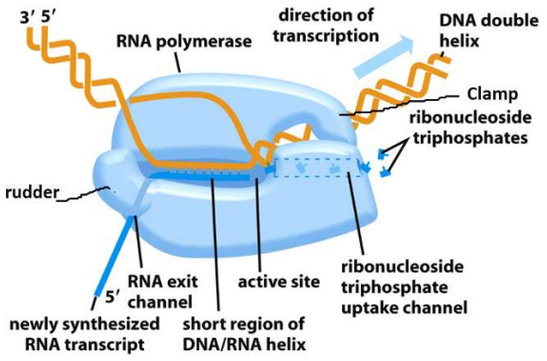


Fig. 8.4 *Tertiary Complex*

- When the RNA polymerase manage to synthesize RNA more than 10 bp long, it eject the σ 3.2 region and RNA further elongates and exit from RNA exit channel. This is the formation of tertiary complex. (Refer Figure 8.4)

2. Elongation

- After synthesis of RNA more than 10 bp long, the σ-factor is ejected and the enzyme move along 5'-3' direction continuously synthesizing RNA.
- The synthesized RNA exit from RNA exit channel.
- The synthesized RNA is proof reads by Hydrolytic editing. For this the polymerase back track by one or more nucleotide and cleave the RNA removing the error and synthesize the correct one. The Gre factor enhance this proof reading process.
- Pyrophospholytic editing another mechanism of removing altered nucleotide.

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3. Termination

There are two mechanism of termination.

(i) Rho independent:

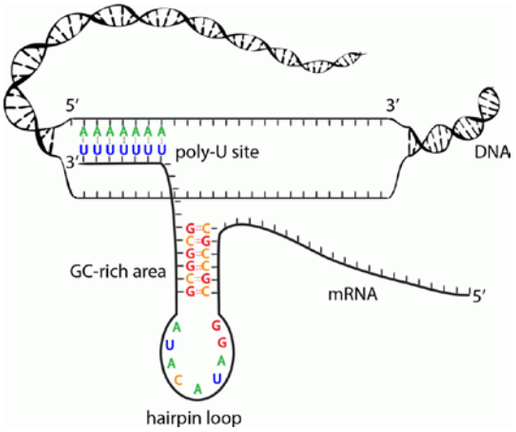


Fig. 8.5 *Rho Independent Termination*

- In this mechanism, transcription is terminated due to specific sequence in terminator DNA.
- The terminator DNA contains invert repeat which cause complimentary pairing as transcript RNA form hair pin structure.
- This invert repeat is followed by larger number of TTTTTTTT (~8 bp) on template DNA. The uracil appear in RNA. The load of hair pin structure is not tolerated by A=U base pair so the RNA get separated from RNA-DNA heteroduplex. (Refer Figure 8.5)

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(ii) Rho dependent

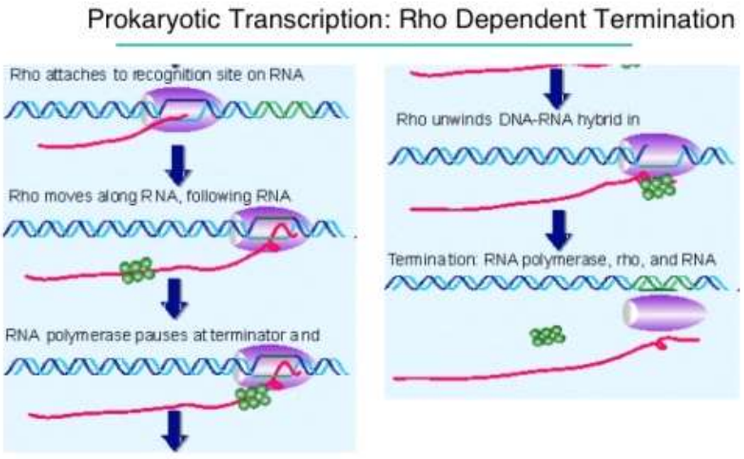


Fig. 8.6 *Rho Dependent Termination*

- In this mechanism, transcription is terminated by rho (ρ) protein.
- It is ring shaped single strand binding ATPase protein.
- The rho protein bind the single stranded RNA as it exit from polymerase enzyme complex and hydrolyse the RNA from enzyme complex.
- The rho protein does not bind to those RNA whose protein is being translated. Rather it bind to RNA after translation.
- In bacteria transcription and translation occur simultaneously so the rho protein bind the RNA after translation has completed but transcription is still ON.

Check Your Progress

1. What is transcription?
2. Which enzyme is involved in transcription?

8.3 PROPERTIES OF BACTERIAL RNA POLYMERASE

A RNA polymerase (RNAP), or ribonucleic acid polymerase, is a multi-subunit enzyme that catalyzes the process of transcription where an RNA polymer is synthesized from a DNA template. The sequence of the RNA polymer is complementary to that of the template DNA and is synthesized in a 5′→3′ orientation. This RNA strand is called the primary transcript and needs to be processed before it can be functional inside the cell.

RNA polymerases interact with many proteins in order to accomplish their task. These proteins help in enhancing the binding specificity of the enzyme, aid in unwinding the double helical structure of DNA, modulate the activity of the enzyme based on the requirements of the cell and alter the speed of transcription. Some RNAP molecules can catalyze the formation of a polymer over four thousand bases in length every minute. However, they have a dynamic range of velocities and they can occasionally pause, or even stop at certain sequences in order to maintain fidelity during transcription.

Functions of RNA Polymerase

Traditionally, the central dogma of molecular biology has looked at RNA as a messenger molecule that exports the information coded into DNA out of the nucleus in order to drive the synthesis of proteins in the cytoplasm: DNA $\rightarrow$ RNA $\rightarrow$ Protein. The other well-known RNAs are transfer RNA (tRNA) and ribosomal RNA (rRNA) which are also intimately connected with the protein synthetic machinery. However, over the past two decades, it has become increasingly clear that RNA serves a range of functions, of which protein coding is only one part. Some regulate gene expression, others act as enzymes, some are even crucial in the formation of gametes. These are called non-coding or ncRNA.

Since RNAP is involved in the production of molecules that have such a wide range of roles, one of its main functions is to regulate the number and kind of RNA transcripts formed in response to the cell's requirements. A number of different proteins, transcription factors and signaling molecules interact with the enzyme, especially the carboxy-terminal end of one subunit, to regulate its activity. It is believed that this regulation was crucial for the development of eukaryotic plants and animals, where genetically identical cells show differential gene expression and specialization in multicellular organisms.

In addition, the optimal functioning of these RNA molecules depends on the fidelity of transcription – the sequence in the DNA template strand must be represented accurately in the RNA. Even a single base change in some regions can lead to a completely non-functional product. Therefore, while the enzyme needs to work quickly and complete the polymerization reaction in a short span of time, it needs robust mechanisms to ensure extremely low error rates. The nucleotide substrate is screened at multiple steps for complementarity to the template DNA strand. When the correct nucleotide is present, it creates an environment conducive to catalysis and the elongation of the RNA strand. Additionally, a proofreading step allows incorrect bases to be excised.

Finally, RNA polymerases are also involved in post-transcriptional modification of RNAs to make them functional, facilitating their export from the nucleus towards their ultimate site of action.

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Types of RNA Polymerase

There is remarkable similarity in the RNA polymerases found in prokaryotes, eukaryotes, archaea and even some viruses. This points to the possibility that they evolved from a common ancestor. Prokaryotic RNAP is made of four subunits, including a sigma-factor that dissociates from the enzyme complex after transcription initiation. While prokaryotes use the same RNAP to catalyze the polymerization of coding as well as non-coding RNA, eukaryotes have five distinct RNA polymerases.

Eukaryotic RNAP I is a workhorse, producing nearly fifty percent of the RNA transcribed in the cell. It exclusively polymerizes ribosomal RNA, which forms a large component of ribosomes, the molecular machines that synthesize proteins. RNA Polymerase II is extensively studied because it is involved in the transcription of mRNA precursors. It also catalyzes the formation of small nuclear RNAs and micro RNAs. RNAP III transcribes transfer RNA, some ribosomal RNA and a few other small RNAs and is important since many of its targets are necessary for normal functioning of the cell. RNA polymerases IV and V are found exclusively in plants, and together are crucial for the formation of small interfering RNA and heterochromatin in the nucleus.

Process of Transcription

Transcription begins with the binding of the RNAP enzyme to a specific part of the DNA, also known as the promoter region. This binding requires the presence of a few other proteins – the sigma factor in prokaryotes and various transcription factors in eukaryotes. One set of proteins called general transcription factors are necessary for all eukaryotic transcriptional activity and include Transcription Initiation Factor II A, II B, II D, II E, II F and II H. These are supplemented by specific signaling molecules that modulate gene expression through stretches of non-coding DNA located upstream. Often initiation is aborted multiple times before a stretch of ten nucleotides is polymerized. After this, the polymerase moves beyond the promoter and loses most of the initiation factors.

This is followed by the unwinding of double stranded DNA, also known as 'melting', to form a sort of bubble where active transcription occurs. This 'bubble' appears to move along the DNA strand as the RNA polymer elongates. Once transcription is complete, the process is terminated and the RNA strand is processed. Prokaryotic RNAP and eukaryotic RNA polymerases I and II require additional transcription termination proteins. RNAP III terminates transcription when there is a stretch of Thymine bases on the non-template strand of DNA.

Comparison between DNA and RNA Polymerase

While DNA and RNA polymerases both catalyze nucleotide polymerization reactions, there are two major differences in their activity. Unlike DNA polymerases, RNAP enzymes do not need a primer to begin the polymerization reaction. They

are also capable of beginning the reaction from the middle of a DNA strand and reading 'STOP' signals that cause the enzyme complex to dissociate from the template. Finally, while RNA polymerases are slightly slower than their counterparts, they have the advantage of only needing to make a complimentary copy of one strand of DNA.

Check Your Progress

3. Which RNA polymerases is only found in plants?
4. Which RNA polymerases catalyzes the formation of messenger RNA (mRNA)?

8.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. The process of synthesis of RNA by copying the template strand of DNA is called transcription.
2. The enzyme involved in transcription is RNA polymerase.
3. RNA polymerases IV and V are found exclusively in plants.
4. RNA polymerase II.

8.5 SUMMARY

- The process of synthesis of RNA by copying the template strand of DNA is called transcription.
- In genetics, a promoter is a region of DNA that initiates transcription of a particular gene.
- Steps in Prokaryotic Transcription: 1. Initiation 2. Elongation 3. Termination.
- A RNA polymerase (RNAP), or ribonucleic acid polymerase, is a multi-subunit enzyme that catalyzes the process of transcription where an RNA polymer is synthesized from a DNA template.
- The central dogma of molecular biology has looked at RNA as a messenger molecule that exports the information coded into DNA out of the nucleus in order to drive the synthesis of proteins in the cytoplasm: DNA $\rightarrow$ RNA $\rightarrow$ Protein.
- Unlike DNA polymerases, RNAP enzymes do not need a primer to begin the polymerization reaction.

NOTES

8.6 KEY WORDS

- **Holoenzyme:** A biochemically active compound formed by the combination of an enzyme with a coenzyme.
- **Transcription:** The process of transcribing RNA, with existing DNA serving as a template, or vice versa.
- **Promoter:** A region of a DNA molecule which forms the site at which transcription of a gene starts.
- **Prokaryotic:** A microscopic single-celled organism which has neither a distinct nucleus with a membrane nor other specialized organelles, including the bacteria and cyanobacteria.
- **Base Pair:** A base pair (BP) is a unit consisting of two nucleobases bound to each other by hydrogen bonds. They form the building blocks of the DNA double helix and contribute to the folded structure of both DNA and RNA.

8.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Write a short note on promoters.
2. Describe termination in prokaryotic transcription.
3. What are the types of RNA polymerase?
4. Discuss comparison between DNA and RNA polymerase.

Long Answer Questions

1. How the process of transcription is carried out in prokaryotic?
2. Explain initiation in prokaryotic transcription.
3. Discuss properties of bacterial RNA polymerase.
4. Explain functions of RNA polymerase.

8.8 FURTHER READINGS

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UNIT 9 EUKARYOTIC TRANSCRIPTION

NOTES

Structure

9.0 Introduction
9.1 Objectives
9.2 Eukaryotic Transcription
  9.2.1 Properties of RNA Polymerases I, II and III
  9.2.2 Introduction to Transcription in Eukaryotes
9.3 Post Trancriptional Modification
9.4 Reverse Transcription in Eukaryotes
9.5 Answers to Check Your Progress Questions
9.6 Summary
9.7 Key Words
9.8 Self Assessment Questions and Exercises
9.9 Further Readings

9.0 INTRODUCTION

Unlike prokaryotic RNA polymerase that initiates the transcription of all different types of RNA, RNA polymerase in eukaryotes including humans comes in three variations, each encoding a different type of gene. A eukaryotic cell has a nucleus that separates the processes of transcription and translation. Eukaryotic transcription occurs within the nucleus where DNA is packaged into nucleosomes and higher order chromatin structures. The complexity of the eukaryotic genome necessitates a great variety and complexity of gene expression control.

Transcription in eukaryotes involves one of three types of polymerases, depending on the gene being transcribed. RNA polymerase II transcribes all of the protein-coding genes, whereas RNA polymerase I transcribes rRNA genes, and RNA polymerase III transcribes rRNA, tRNA, and small nuclear RNA genes. The initiation of transcription in eukaryotes involves the binding of several transcription factors to complex promoter sequences that are usually located upstream of the gene being copied. The mRNA is synthesized in the 5' to 3' direction, and the FACT complex moves and reassembles nucleosomes as the polymerase passes by. Whereas RNA polymerases I and III terminate transcription by protein- or RNA hairpin-dependent methods, RNA polymerase II transcribes for 1,000 or more nucleotides beyond the gene template and cleaves the excess during pre-mRNA processing.

In this unit, you will study about transcription in eukaryotes. Eukaryotic transcription is the elaborate process that eukaryotic cells use to copy genetic information stored in DNA into units of RNA replica. Gene transcription occurs in both eukaryotic and prokaryotic cells.

9.1 OBJECTIVES

NOTES

After going through this unit, you will be able to:

- Understand the process of transcription in eukaryotes
- Discuss properties RNA polymerase
- Describe reverse transcription

9.2 EUKARYOTIC TRANSCRIPTION

Eukaryotes

Prokaryotes and eukaryotes perform fundamentally the same process of transcription, with a few key differences. The most important difference between prokaryotes and eukaryotes is the latter's membrane-bound nucleus and organelles. With the genes bound in a nucleus, the eukaryotic cell must be able to transport its mRNA to the cytoplasm and must protect its mRNA from degrading before it is translated. Eukaryotes also employ three different polymerases that each transcribe a different subset of genes. Eukaryotic mRNAs are usually monogenic, meaning that they specify a single protein.

Promoters

A promoter is a regulatory region of DNA. The promoter contains specific DNA sequences that are recognized by proteins known as transcription factors. These factors bind to the promoter sequences, recruiting RNA polymerase, the enzyme that synthesizes the RNA from the coding region of the gene.

Enhancers

An enhancer is a short (50–1500 bp) region of DNA that can be bound by proteins (activators) to increase the likelihood that transcription of a particular gene will occur. These proteins are usually referred to as transcription factors. There are hundreds of thousands of enhancers in the human genome. They are found in both prokaryotes and eukaryotes.

Factors

A Transcription Factor (TF) (or sequence-specific DNA-binding factor) is a protein that controls the rate of transcription of genetic information from DNA to messenger RNA, by binding to a specific DNA sequence. The function of TFs is to regulate—turn on and off—genes in order to make sure that they are expressed in the right cell at the right time and in the right amount throughout the life of the cell and the organism. Groups of TFs function in a coordinated fashion to direct cell division, cell growth, and cell death throughout life; cell migration and organization (body plan) during embryonic development; and intermittently in response to signals from

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outside the cell, such as a hormone. There are up to 2600 TFs in the human genome.

Unlike the prokaryotic polymerase that can bind to a DNA template on its own, eukaryotes require several other proteins, called transcription factors, to first bind to the promoter region and then help recruit the appropriate polymerase.

9.2.1 Properties of RNA Polymerases I, II and III

The features of eukaryotic mRNA synthesis are markedly more complex those of prokaryotes. Instead of a single polymerase comprising five subunits, the eukaryotes have three polymerases that are each made up of 10 subunits or more. Each eukaryotic polymerase also requires a distinct set of transcription factors to bring it to the DNA template.

RNA polymerase I is located in the nucleolus, a specialized nuclear substructure in which ribosomal RNA (rRNA) is transcribed, processed, and assembled into ribosomes. The rRNA molecules are considered structural RNAs because they have a cellular role but are not translated into protein. The rRNAs are components of the ribosome and are essential to the process of translation. RNA polymerase I synthesizes all of the rRNAs except for the 5S rRNA molecule. The 'S' designation applies to 'Svedberg' units, a nonadditive value that characterizes the speed at which a particle sediments during centrifugation.

RNA polymerase II is located in the nucleus and synthesizes all protein-coding nuclear pre-mRNAs. Eukaryotic pre-mRNAs undergo extensive processing after transcription but before translation. For clarity, this module's discussion of transcription and translation in eukaryotes will use the term "mRNAs" to describe only the mature, processed molecules that are ready to be translated. RNA polymerase II is responsible for transcribing the overwhelming majority of eukaryotic genes.

RNA polymerase III is also located in the nucleus. This polymerase transcribes a variety of structural RNAs that includes the 5S pre-rRNA, transfer pre-RNAs (pre-tRNAs), and small nuclear pre-RNAs. The tRNAs have a critical role in translation; they serve as the adaptor molecules between the mRNA template and the growing polypeptide chain. Small nuclear RNAs have a variety of functions, including 'splicing' pre-mRNAs and regulating transcription factors.

Structure of an RNA polymerase II Promoter

Eukaryotic promoters are much larger and more complex than prokaryotic promoters, but both have a TATA box. For example, in the mouse thymidine kinase gene, the TATA box is located at approximately -30 relative to the initiation (+1) site (Refer Figure 9.1). For this gene, the exact TATA box sequence is TATAAAA, as read in the 5' to 3' direction on the nontemplate strand. This sequence is not identical to the *E. coli* TATA box, but it conserves the A–T rich element. The thermostability of A–T bonds is low and this helps the DNA template to locally unwind in preparation for transcription.

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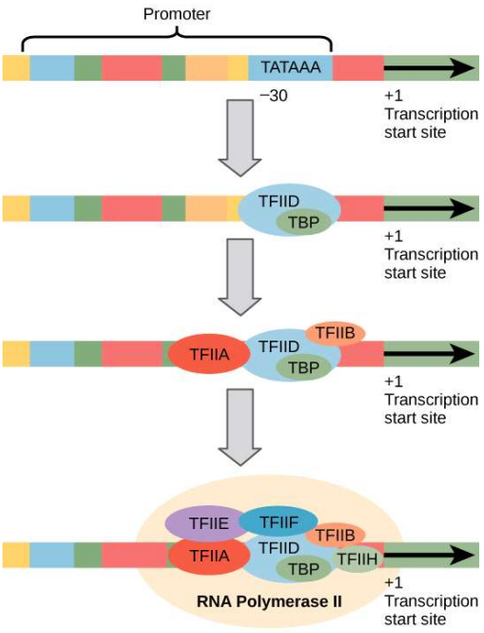


***Fig. 9.1** A Generalized Promoter of a Gene Transcribed by RNA Polymerase II*

The above figure shows a generalized is shown. Transcription factors recognize the promoter. RNA polymerase II then binds and forms the transcription initiation complex.

9.2.2 Introduction to Transcription in Eukaryotes

The synthesis of RNA from a single strand of a DNA molecule in the presence of enzyme RNA polymerase is called transcription. In other words, the process of formation of a messenger RNA molecule using a DNA molecule as a template is referred to as transcription.

The main points related to transcription in eukaryotes are briefly discussed below:

1. Synthesis

RNA is synthesized from a DNA template. The RNA is processed into messenger RNA [mRNA], which is then used for synthesis of a protein. The RNA thus synthesized is called messenger RNA (mRNA), because it carries a genetic message from the DNA to the protein- synthesizing machinery of the cell.

The main difference between RNA and DNA sequence is the presence of U, or uracil in RNA instead of the T, of thymine of DNA.

2. Template Used

The RNA is synthesized from a single strand or template of a DNA molecule. The stretch of DNA that is transcribed into an RNA molecule is called a transcription unit. A transcription unit codes the sequence that is translated into protein. It also directs and regulates protein synthesis.

NOTES

The DNA strand which is used in RNA synthesis is called template strand; because it provides the template for ordering the sequence of nucleotides in an RNA transcript. The DNA strand which does not take part in DNA synthesis is called coding strand, because, its nucleotide sequence is the same as that of the newly created RNA transcript.

3. Enzyme Involved

The process of transcription is catalyzed by the specific enzyme called RNA polymerase. DNA sequence is enzymatically copied by RNA polymerase to produce a complementary nucleotide RNA strand. In eukaryotes, there are three classes of RNA polymerases: I, II and III which are involved in the transcription of all protein genes.

4. Genetic Information Copied

In this process, the genetic information coded in DNA is copied into a molecule of RNA. The genetic information is transcribed or copied, from DNA to RNA. In other words, it results in the transfer of genetic information from DNA into RNA.

5. First Step

The expression of a gene consists of two major steps, i.e., transcription and translation. Thus transcription is the first step in the process of gene regulation or protein synthesis.

6. Direction of Synthesis

As in DNA replication, RNA is synthesized in the $5' \rightarrow 3'$ direction. The DNA template strand is read $3' \rightarrow 5'$ by RNA polymerase and the new RNA strand is synthesized in the $5' \rightarrow 3'$ direction. RNA polymerase binds to the $3'$ end of a gene (promoter) on the DNA template strand and travels toward the $5'$ end.

The regulatory sequence that is before, or $5'$, of the coding sequence is called $5'$ un-translated region ($5'$ UTR), and sequence found following, or $3'$, of the coding sequence is called $3'$ un-translated region ($3'$ UTR). Transcription has some proofreading mechanisms, but they are fewer and less effective than the controls for copying DNA; therefore, transcription has a lower copying fidelity than DNA replication.

Mechanism of Transcription in Eukaryotes

The mechanism of transcription consists of five major steps, i.e.,

(1) Pre-initiation

(2) Initiation

(3) Promoter clearance

(4) Elongation

(5) Termination

These are briefly discussed as follows:

1. Pre-Initiation

The initiation of transcription does not require a primer to start. RNA polymerase simply binds to the DNA and, along with other cofactors, unwinds the DNA to create an initiation bubble so that the RNA polymerase has access to the single-stranded DNA template. However, RNA Polymerase does require a promoter like sequence.

Proximal (core) Promoters

TATA promoters are found around -30 bp to the start site of transcription. Not all genes have TATA box promoters and there exists TATA-less promoters as well. The TATA promoter consensus sequence is TATA (A/T) A (A/T).

2. Initiation

In eukaryotes and archaea, transcription initiation is far more complex. The main difference is that eukaryotic polymerases do not recognize directly their core promoter sequences. In eukaryotes, a collection of proteins called transcription factors mediate the binding of RNA polymerase and the initiation of transcription.

Only after attachment of certain transcription factors to the promoter, the RNA polymerase binds to it. The complete assembly of transcription factors and RNA polymerase bind-to the promoter, called transcription initiation complex. Initiation starts as soon as the complex is opened and the first phosphodiester bond is formed. This is the end of Initiation.

RNA Pol II does not contain a subunit similar to the prokaryotic factor, which can recognize the promoter and unwind the DNA double helix. In eukaryotes, these two functions are carried out by a set of proteins called general transcription factors.

The RNA Pol II is associated with six general transcription factors, designated as TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH, where 'TF' stands for 'transcription factor' and 'II' for the RNA Pol II.

TFIID consists of TBP (TATA-box binding protein) and TAFs (TBP associated factors). The role of TBP is to bind the core promoter. TAFs may assist TBP in this process. In human cells, TAFs are formed by 12 subunits. One of them, TAF250 (with molecular weight 250 kDa), has the histone acetyltransferase activity, which can relieve the binding between DNA and histones in the nucleosome.

The transcription factor which catalyzes DNA melting is TFIIH. However, before TFIIH can unwind DNA, the RNA Pol II and at least five general transcription factors (TFIIA is not absolutely necessary) have to form a Pre-Initiation Complex (PIC).

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3. Promoter Clearance

After the first bond is synthesized the RNA polymerase must clear the promoter. During this time there is a tendency to release the RNA transcript and produce truncated transcripts. This is called abortive initiation and is common for both Eukaryotes and Prokaryotes.

Once the transcript reaches approximately 23 nucleotides it no longer slips and elongation can occur. This is an ATP dependent process. Promoter clearance also coincides with Phosphorylation of serine 5 on the carboxy terminal domain which is phosphorylated by TFIIH.

4. Elongation

For RNA synthesis, one strand of DNA known as the template strand or non-coding strand is used as a template. As transcription proceeds, RNA polymerase traverses the template strand and uses base pairing complementarity with the DNA template to create an RNA copy.

Although RNA polymerase traverses the template strand from $3' \rightarrow 5'$, the coding (non-template) strand is usually used as the reference point, so transcription is said to go from $5' \rightarrow 3'$.

This produces an RNA molecule from $5' \rightarrow 3'$, an exact copy of the coding strand (except that thymines are replaced with uracils, and the nucleotides are composed of a ribose (5-carbon) sugar where DNA has deoxyribose (one less oxygen atom) in its sugar-phosphate backbone).

After Pre-Initiation Complex (PIC) is assembled at the promoter, TFIIH can use its helicase activity to unwind DNA. This requires energy released from ATP hydrolysis. The DNA melting starts from about -10 bp.

Then, RNA Pol II uses Nucleoside Triphosphates (NTPs) to synthesize a RNA transcript. During RNA elongation, TFIIF remains attached to the RNA polymerase, but all of the other transcription factors have dissociated from PIC.

The Carboxyl-Terminal Domain (CTD) of the largest subunit of RNA Pol II is critical for elongation. In the initiation phase, CTD is un-phosphorylated, but during elongation it has to be phosphorylated. This domain contains many proline, serine and threonine residues.

5. Termination

In eukaryotic transcription the mechanism of termination is not very clear. In other words, it is not well understood. It involves cleavage of the new transcript, followed by template- independent addition of as at its new $3'$ end, in a process called polyadenylation.

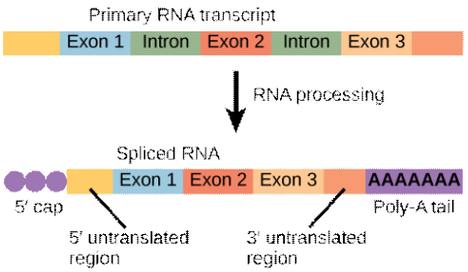


***Fig. 9.2** Eukaryotic mRNA*

Eukaryotic protein genes contain a poIy-A signal located downstream of the last exon. This signal is used to add a series of adenylate residues during RNA processing. Transcription often terminates at 0.5-2 kb downstream of the poly-A signal.

Transcription Factories in Eukaryotes

Active transcription units that are clustered in the nucleus, in discrete sites are called 'Transcription Factories'. Such sites could be visualized after allowing, engaged polymerases to extend their transcripts in tagged precursors (Br-UTP or Br-U), and immuno-labelling the tagged nascent RNA.

Transcription factories can also be localized using fluorescence in situ hybridization, or marked by antibodies directed against polymerases. There are ~10,000 factories in the nucleoplasm of a HeLa cell, among which are ~8,000 polymerase II factories and ~2,000 polymerase III factories. Each polymerase II factory contains ~8 polymerases.

As most active transcription units are associated with only one polymerase, each factory will be associated with ~8 different transcription units. These units might be associated through promoters and/or enhancers, with loops forming a 'cloud' around the factory.

Check Your Progress

1. Where are RNA polymerase I located?
2. What is the role of tRNAs in translation?
3. What is the first step in gene regulation or protein synthesis?

9.3 POST TRANCRIPTIONAL MODIFICATION

Post-transcriptional modification or Co-transcriptional modification is the process in eukaryotic cells where primary transcript RNA is converted into mature RNA. A notable example is the conversion of precursor messenger RNA into mature messenger RNA (mRNA) that occurs prior to protein translation. The process includes three major steps: addition of a 5' cap, addition of a 3' poly-adenylation

NOTES

tail, and splicing. This process is vital for the correct translation of the genomes of eukaryotes because the initial precursor mRNA produced during transcription contains both exons (coding or important sequences involved in translation), and introns (non-coding sequences). (Refer Figure 9.3)

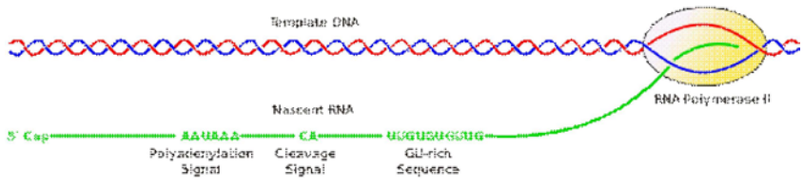


Fig. 9.3 *Signal Directing Post-Transcriptional Process*

In the above figure an example, of a signal that directs post-transcriptional processing: the conserved eukaryotic polyadenylation signal directs cleavage at the cleavage signal and addition of a poly-A tail to the mRNA transcript

mRNA Processing

The pre-mRNA molecule undergoes three main modifications. These modifications are 5' capping, 3' polyadenylation, and RNA splicing, which occur in the cell nucleus before the RNA is translated.

Capping

Capping of the pre-mRNA involves the addition of 7-methylguanosine (m7G) to the 5' end. To achieve this, the terminal 5' phosphate requires removal, which is done with the aid of a phosphatase enzyme. The enzyme guanosyl transferase then catalyses the reaction, which produces the diphosphate 5' end. The diphosphate 5' end then attacks the alpha phosphorus atom of a GTP molecule in order to add the guanine residue in a 5'5' triphosphate link. The enzyme (guanine-N7-)-methyltransferase 'cap MTase' transfers a methyl group from S-adenosyl methionine to the guanine ring. This type of cap, with just the (m7G) in position is called a cap 0 structure. The ribose of the adjacent nucleotide may also be methylated to give a cap 1. Methylation of nucleotides downstream of the RNA molecule produce cap 2, cap 3 structures and so on. In these cases the methyl groups are added to the 2' OH groups of the ribose sugar. The cap protects the 5' end of the primary RNA transcript from attack by ribonucleases that have specificity to the 3'5' phosphodiester bonds.

3' Processing Cleavage and Polyadenylation

The pre-mRNA processing at the 3' end of the RNA molecule involves cleavage of its 3' end and then the addition of about 250 adenine residues to form a poly (A) tail. The cleavage and adenylation reactions occur if a polyadenylation signal sequence (5'-AAUAAA-3') is located near the 3' end of the pre-mRNA molecule, which is followed by another sequence, which is usually (5'-CA-3') and is the site of cleavage. A GU-rich sequence is also usually present further downstream on

the pre-mRNA molecule. After the synthesis of the sequence elements, two multisubunit proteins called cleavage and polyadenylation specificity factor (CPSF) and cleavage stimulation factor (CStF) are transferred from RNA Polymerase II to the RNA molecule. The two factors bind to the sequence elements. A protein complex forms and contains additional cleavage factors and the enzyme Polyadenylate Polymerase (PAP). This complex cleaves the RNA between the polyadenylation sequence and the GU-rich sequence at the cleavage site marked by the (5'-CA-3') sequences. Poly (A) polymerase then adds about 200 adenine units to the new 3' end of the RNA molecule using ATP as a precursor. As the poly (A) tail is synthesised, it binds multiple copies of poly (A) binding protein, which protects the 3'end from ribonuclease digestion.

Splicing

RNA splicing is the process by which introns, regions of RNA that do not code for proteins, are removed from the pre-mRNA and the remaining exons connected to re-form a single continuous molecule. Exons are sections of mRNA which become 'expressed' or translated into a protein. They are the coding portions of an mRNA molecule. Although most RNA splicing occurs after the complete synthesis and end-capping of the pre-mRNA, transcripts with many exons can be spliced co-transcriptionally. The splicing reaction is catalyzed by a large protein complex called the spliceosome assembled from proteins and small nuclear RNA molecules that recognize splice sites in the pre-mRNA sequence. Many pre-mRNAs, including those encoding antibodies, can be spliced in multiple ways to produce different mature mRNAs that encode different protein sequences. This process is known as alternative splicing, and allows production of a large variety of proteins from a limited amount of DNA.

Histone mRNA Processing

Histones H2A, H2B, H3 and H4 form the core of a nucleosome and thus are called core histones. Processing of core histones is done differently because typical histone mRNA lacks several features of other eukaryotic mRNAs, such as poly (A) tail and introns. Thus, such mRNAs do not undergo splicing and their 3' processing is done independent of most cleavage and polyadenylation factors. Core histone mRNAs have a special stem-loop structure at 3-prime end that is recognized by a stem–loop binding protein and a downstream sequence, called histone downstream element (HDE) that recruits U7 snRNA. Cleavage and polyadenylation specificity factor 73 cuts mRNA between stem-loop and HDE.

Histone variants, such as H2A.Z or H3.3, however, have introns and are processed as normal mRNAs including splicing and polyadenylation.

Check Your Progress

4. Give an example of Co-transcriptional modification.
5. What is RNA splicing?

9.4 REVERSE TRANSCRIPTION IN EUKARYOTES

NOTES

Synthesis of DNA from RNA molecule in the presence of enzyme reverse transcriptase is referred to as reverse transcription. Reverse transcription was first reported by Temin and Baltimore in 1970 for which they were awarded Nobel Prize in 1975. Reverse transcription is also known as Teminism. Some viruses (such as HIV, the cause of AIDS), have the ability to transcribe RNA into DNA.

In some eukaryotic cells, an enzyme is found with reverse transcription activity. It is called telomerase. Telomerase is a reverse transcriptase that lengthens the ends of linear chromosomes. Telomerase carries an RNA template from which it synthesizes DNA repeating sequence, or 'junk' DNA. This repeated sequence of 'junk' DNA is important because every time a linear chromosome is duplicated, it is shortened in length.

With 'junk' DNA at the ends of chromosomes, the shortening eliminates some repeated, or junk sequence, rather than the protein-encoding DNA sequence that is further away from the chromosome ends.

Telomerase is often activated in cancer cells to enable cancer cells to duplicate their genomes without losing important protein-coding DNA sequence. Activation of telomerase can be part of the process that allows cancer cells to become immortal.

Role of Transcription Factors in Eukaryotes

In eukaryotes, the association between DNA and histones prevents access of the polymerase and general transcription factors to the promoter. Histone acetylation catalyzed by HATs can relieve the binding between DNA and histones. Although a subunit of TFIID (TAF250 in human) has the HAT activity, participation of other HATs can make transcription more efficient. The following rules apply to most (but not all).

(i) Binding of activators to the enhancer element recruits HATs to relieve association between histones and DNA, thereby enhancing transcription.

(ii) Binding of repressors to the silencer element recruits histone deacetylases (denoted by HDs or HDACs) to tighten association between histone and DNA.

Check Your Progress

6. Name a virus that has the ability to transcribe RNA into DNA.
7. What is telomerase?

9.5 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

NOTES

1. RNA polymerase I is located in the nucleolus, a specialized nuclear substructure in which ribosomal RNA (rRNA) is transcribed, processed, and assembled into ribosomes.
2. The tRNAs have a critical role in translation; they serve as the adaptor molecules between the mRNA template and the growing polypeptide chain.
3. Transcription is the first step in the process of gene regulation or protein synthesis.
4. A notable example is the conversion of precursor messenger RNA into mature messenger RNA (mRNA) that occurs prior to protein translation.
5. RNA splicing is the process by which introns, regions of RNA that do not code for proteins, are removed from the pre-mRNA and the remaining exons connected to re-form a single continuous molecule.
6. HIV, the cause of AIDS, has the ability to transcribe RNA into DNA.
7. Telomerase is a reverse transcriptase that lengthens the ends of linear chromosomes. Telomerase carries an RNA template from which it synthesizes DNA repeating sequence

9.6 SUMMARY

- The most important difference between prokaryotes and eukaryotes is the latter's membrane-bound nucleus and organelles.
- A promoter is a regulatory region of DNA located upstream (towards the 5' region) of a gene, providing a control point for regulated gene transcription.
- An enhancer is a short (50–1500 bp) region of DNA that can be bound by proteins (activators) to increase the likelihood that transcription of a particular gene will occur.
- A transcription factor (TF) (or sequence-specific DNA-binding factor) is a protein that controls the rate of transcription of genetic information from DNA to messenger RNA, by binding to a specific DNA sequence.
- RNA polymerase I is located in the nucleolus, a specialized nuclear substructure in which ribosomal RNA (rRNA) is transcribed, processed, and assembled into ribosomes.
- RNA polymerase II is located in the nucleus and synthesizes all protein-coding nuclear pre-mRNAs.

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- RNA polymerase III is also located in the nucleus. This polymerase transcribes a variety of structural RNAs that includes the 5S pre-rRNA, transfer pre-RNAs (pre-tRNAs), and small nuclear pre-RNAs.
- Eukaryotic promoters are much larger and more complex than prokaryotic promoters, but both have a TATA box.
- The process of formation of a messenger RNA molecule using a DNA molecule as a template is referred to as transcription.
- The mechanism of transcription consists of five major steps, viz: (1) Pre-initiation, (2) Initiation, (3) Promoter clearance, (4) Elongation and (5) Termination.
- Active transcription units that are clustered in the nucleus, in discrete sites are called 'Transcription Factories'.
- Post-transcriptional modification or Co-transcriptional modification is the process in eukaryotic cells where primary transcript RNA is converted into mature RNA. A notable example is the conversion of precursor messenger RNA into mature messenger RNA (mRNA) that occurs prior to protein translation.
- Synthesis of DNA from RNA molecule in the presence of enzyme reverse transcriptase is referred to as reverse transcription.

9.7 KEY WORDS

- **Transcription:** The process of transcribing RNA, with existing DNA serving as a template, or vice versa.
- **Promoter**: A region of a DNA molecule which forms the site at which transcription of a gene starts.
- **Eukaryotes**: An organism consisting of a cell or cells in which the genetic material is DNA in the form of chromosomes contained within a distinct nucleus. Eukaryotes include all living organisms other than the eubacteria and archaea.

9.8 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Define promoters, enhancers and transcription factors.
2. Discuss structure of an RNA polymerase II promoter.
3. What are transcription factories in eukaryotes?
4. Discuss reverse transcription in eukaryotes.

Long Answer Questions

1. How is transcription process carried out in eukaryotes? Explain.
2. Explain properties of RNA Polymerases I, II AND III.
3. Discuss about transcription in Eukaryotes.
4. Give a detailed note on mechanism of transcription in Eukaryotes.
5. Explain post-transcriptional modification.

NOTES

9.9 FURTHER READINGS

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UNIT 10 PROTEIN SYNTHESIS

NOTES

Structure

10.0 Introduction
10.1 Objectives
10.2 Protein Synthesis
  10.2.1 Components of Protein Synthesis
  10.2.2 Composition of Genetic Code
10.3 Mechanisms of Protein Synthesis
  10.3.1 Role of Ribosomes in Protein Synthesis
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  10.4.4 Modification of Folding of Released Polypeptides
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10.0 INTRODUCTION

Protein synthesis is the process whereby biological cells generate new proteins; it is balanced by the loss of cellular proteins via degradation or export. Translation, the assembly of amino acids by ribosomes, is an essential part of the biosynthetic pathway, along with generation of messenger RNA (mRNA), aminoacylation of transfer RNA (tRNA), co-translational transport, and post-translational modification. Protein biosynthesis is strictly regulated at multiple steps. They are principally during transcription (phenomena of RNA synthesis from DNA template) and translation (phenomena of amino acid assembly from RNA).

In protein synthesis, a succession of tRNA molecules charged with appropriate amino acids are brought together with an mRNA molecule and matched up by base-pairing through the anti-codons of the tRNA with successive codons of the mRNA. The amino acids are then linked together to extend the growing protein chain, and the tRNAs, no longer carrying amino acids, are released. This whole complex of processes is carried out by the ribosome, formed of two main chains of RNA, called ribosomal RNA (rRNA), and more than 50 different proteins. The ribosome latches onto the end of an mRNA molecule and moves along it, capturing loaded tRNA molecules and joining together their amino acids to form a new protein chain.

In this unit, you will study about protein synthesis, mechanism of proteen synthesis, formation of initiation complex, polypeptide chain along elongation, translocation in detail.

10.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the concept of protein synthesis
- Know about translocation, chain elongation and chain termination in protein synthesis
- Discuss post-translational modifications

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10.2 PROTEIN SYNTHESIS

Proteins are giant molecules formed by polypeptide chains of hundreds to thousands of amino acids. These polypeptide chains are formed by about twenty kinds of amino acids. An amino acid consists of a basic amino group ($-NH_2$) and an acidic carboxyl group (-COOH). Different arrangement of amino acids in a polypeptide chain makes each protein unique. Proteins are fundamental constituents of protoplasm and building material of the cell.

They take part in the structural and functional organization of the cell. Functional proteins like enzymes and hormones control the metabolism, biosynthesis, energy production, growth regulation, sensory and reproductive functions of the cell. Enzymes are catalysts in most of the biochemical reactions. Even the gene expression is controlled by enzymes. The replication of DNA and transcription of RNA is controlled by the proteinous enzymes.

10.2.1 Components of Protein Synthesis

Protein synthesis is governed by the genetic information carried in the genes on DNA of the chromosomes.

The main components of the protein synthesis are:

- DNA
- Three types of RNAs
- Amino acids
- Ribosomes
- Enzymes.

DNA is the master molecule which possesses the genetic information about the sequence of amino acids in a polypeptide chain. Structure and properties of DNA regulate and control the synthesis of proteins. DNA present in the nucleus sends out information in the form of messenger RNA into the cytoplasm, which is the site of the protein synthesis in eukaryotes. The messenger RNA carries the information regarding the sequence of amino acids of the polypeptide chain to be synthesized. This message or information is in the form of a genetic code. This genetic code specifies the language of amino acids to be assembled in a polypeptide.

The genetic code is deciphered or translated into a sequence of amino acids.

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10.2.2 Composition of Genetic Code

DNA molecule has three components. They are sugar, phosphates and nitrogen bases. Only nitrogen base sequence varies in different DNA molecules. Thus, the sequence of nitrogen bases or nucleotides in a DNA segment is the code or language in which the DNA sends out the message in the form of messenger RNA (mRNA).

The mRNA carries the genetic message (genetic code) in the form of nucleotide sequence. It has been found that there is co-linearity between nucleotide sequence of mRNA and amino acid sequence of the polypeptide chain synthesized.

The genetic code is the language of nitrogen bases. There are four kinds of nitrogen bases and twenty kinds of amino acids. Therefore four-letter language of nitrogen bases specifies the twenty letter language of amino acids.

Check Your Progress

1. What are proteins?
2. What are the main components of protein synthesis?
3. How many kinds of nitrogen bases are there?

10.3 MECHANISMS OF PROTEIN SYNTHESIS

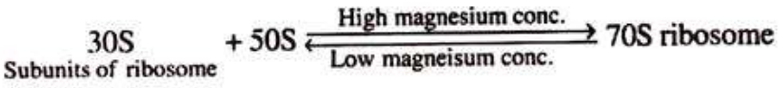
In prokaryotes, the RNA synthesis (transcription) and protein synthesis (translation) take place in the same compartment as there is no separate nucleus. But in eukaryotes, the RNA synthesis takes place in the nucleus while the protein synthesis takes place in the cytoplasm. The mRNA synthesized in the nucleus is exported to cytoplasm through nucleopores.

First, Francis Crick in 1955 suggested and later Zemecnik proved that prior to their incorporation into polypeptides, the amino acids attach to a special adaptor molecule called tRNA. This tRNA has a three nucleotide long anticodon which recognizes three nucleotide long codon on mRNA.

10.3.1 Role of Ribosomes in Protein Synthesis

Ribosome is a macromolecular structure that directs the synthesis of proteins. A ribosome is a multicomponent, compact, ribonucleoprotein particle which contains rRNA, many proteins and enzymes needed for protein synthesis. Ribosome brings together a single mRNA molecule and tRNAs charged with amino acids in a proper orientation so that the base sequence of mRNA molecule is translated into amino acid 1 sequence of polypeptides.

Ribosome is a nucleoprotein particle having two subunits. These two subunits lie separately but come together for the synthesis of polypeptide chain. In *E. coli* ribosome is a 70S particle having two subunits of 30S and 50S. Their association and dissociation depends upon the concentration of magnesium.



NOTES

Small subunit of ribosome contains the decoding centre in which charged tRNAs decode o the codons of mRNA. Large subunit contains peptidyl transferase centre, which forms the peptide bonds between successive amino acids of the newly synthesized peptide chain.

Both 30S and 50S subunits consist of ribosomal RNA (rRNA) and proteins.

The mRNA binds to the 16S rRNA of smaller subunit. Near its 5′ -end mRNA binds to the 3′-end of 16S rRNA.

The main role of ribosome is the formation of peptide bond between successive amino acids of the newly synthesized polypeptide chain. The ribosome has two channels in it. The linear mRNA enters and escapes through one channel, which has the decoding centre. This channel is accessible to the charged tRNAs. The newly synthesized polypeptide chain escapes through the other channel.

Direction of Translation

Each protein molecule has an -NH2 end and -COOH end. Synthesis begins at amino end and ends at carboxyl end. The mRNA is translated in 5′ → 3′ direction from amino to carboxyl end. Synthesis of mRNA from DNA transcription also occurs in 5′ → 3′ direction.

Check Your Progress

4. What is a ribosome?
5. What is the main role of ribosome?

10.4 FORMATION OF INITIATION COMPLEX

First of all 30S subunit of the 70S ribosome starts initiation process. The 30S subunit, mRNA and charged tRNA combine to form pre-initiation complex. Formation of pre-initiation complex involves three initiation factors IF1, IF2 and IF3 along with GTP (guanosine triphosphate). Later 50S subunit of ribosome joins 30S subunit to form 70S initiation complex.

Information for protein synthesis is present in the form of three nucleotide codons on mRNA. Protein coding regions on mRNA consist of continuous, non-overlapping triplet codons. The protein coding region on mRNA is called open reading frame which has a start codon 5′ -AUG-3′ and a stop codon in the end. Each open reading frame specifies a single protein. Prokaryote mRNA has many open reading frames, therefore encode multiple polypeptides and are called polycistronic mRNAs.

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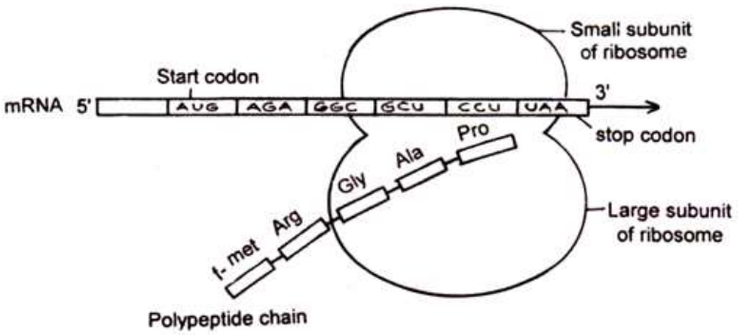


Fig. 10.1 *A Ribosome Translates an mRNA Molecule*

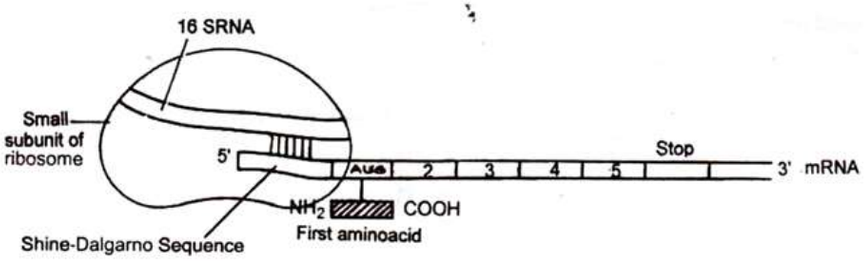


Fig. 10.2 *mRNA Binds to Small Subunit of Ribosome*

Near the 5′ -end of mRNA lies the start codon which is mostly 5′ -AUG-3′ (rarely GUG) in both prokaryotes and eukaryotes. Ribosome binding site (RBS) in prokaryotes lies near the 5′ - end of mRNA ahead (upstream) of AUG codon.

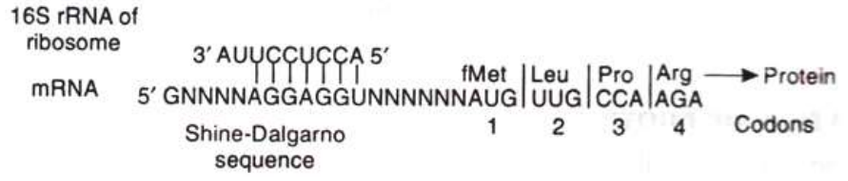


Fig. 10.3

Between 5′ -end and AUG codon there is a sequence of 20-30 bases. Of these, there is a sequence 5′ -AGGAGGU-3′ . This purine rich sequence is called Shine-Dalgarno sequence and lies 4-7 bases ahead (upstream) of AUG codon.

The 3′ -end region of 16S rRNA is 30S subunit has a complementary sequence 3′ -AUUCCUCCA-5′. This sequence forms base pairs with Shine-Dalgarno sequence for binding of mRNA to ribosome. Shine-Dalgarno sequence is the ribosome binding site (RBS). It positions the ribosome correctly with respect to the start codon.

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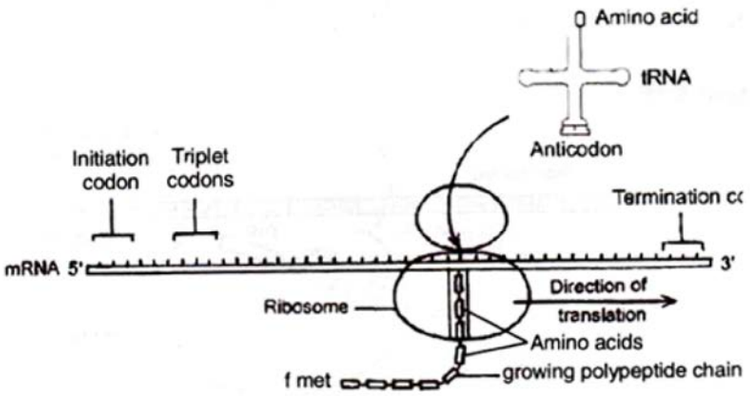


Fig. 10.4 *Components of Proteen Synthesis*

In the above figure, components of Protein Synthesis, Ribosome translates mRNA in 5′ → 3′ direction Codons on mRNA are recognized by tRNA which acts as an adaptor molecule bringing in amino acids for polypeptide chain.

There are two tRNA binding sites on ribosome covering 30S and 50S subunits. The first site is called 'P' site or peptidyl site. The second site is called 'A' site or aminoacyl site. Only the initiator tRNA enters the 'P' site. All other tRNAs enter the 'A' site

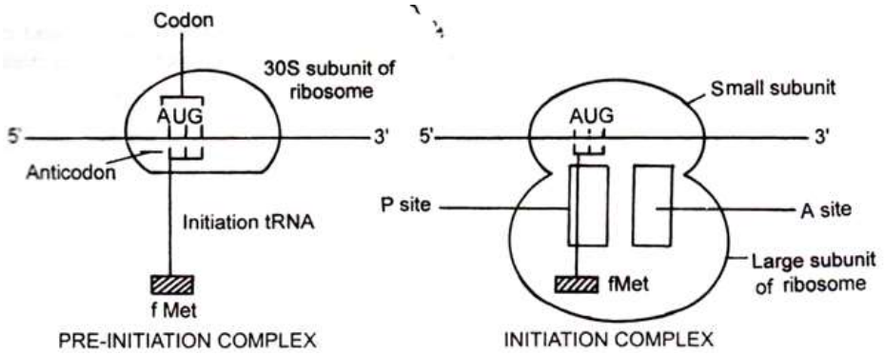


Fig. 10.5 (a) *Pre-initiation Complex (b) Initiation Complex*

For every amino acid, there is a separate tRNA. The identity of a tRNA is indicated by superscript, such as tRNAArg (specific for amino acid Arginine). When this tRNA is charged with amino acid Arginine, it is written as Arginine-tRNAArg or Arg-tRNAArg. Charged tRAN is called aminoacylated tRNA.

In bacteria, the first amino acid starting the protein is always formyl methionine (fMet). When AUG appears as the start codon on mRNA only fMet is incorporated. The tRNA molecule carrying formyl methionine is called tRNA™61. Therefore the first initiator charged aminoacyl tRNA is always fMet-tRNAfMet. When AUG codon is encountered in the internal location (other than the start codon), methionine is not formylated and tRNA carrying this methinine is tRNAmMet.

NOTES

First of all the charged initiator tRNA called tMet-tRNAfMet occupies the 'P' site on ribosome. This position brings its anticodon and start codon AUG of mRNA together in such a way that the anticodon of charged tRNA and codon of mRNA form base pair with each other. Thus reading or translation of mRNA begins.

The 'A' site is available to the second incoming charged tRNA whose anticodon forms base pairs with the second codon on mRNA.

Charging of tRNA

Attachment of amino acids to tRNAs is called charging of tRNA. All tRNAs at their 3′ -terminus have a sequence 5′ -CCA-3′. At this site amino acids bind with the help of enzyme aminoacyl tRNA synthatase. Charging of tRNA occurs in two steps.

1. Activation of amino acids

Energy molecule ATP activates the amino acids. This step is catalysed by specific activating enzymes called aminoacyl tRNA synthatases. Every amino acid has a separate enzyme AA-RNA synthatase enzyme.

$$AA + ATP \xrightarrow{\text{AA-RNA synthatase}} (AA - AMP)\ \text{enzyme} + PP$$

2. Transfer of amino acids to tRNA

AA-AMP enzyme complex reacts with a specific tRNA and transfers the amino acid to tRNA, as a result of which AMP and enzyme are set free.

$$(AA - AMP)\ \text{enzyme} + tRNA \longrightarrow AA - tRNA + AMP + \text{enzyme}$$

This first AA-tRNA is fMet-tRNAfmet which is amino acid formyl methionine bound to tRNA. This fixes itself to 'P' site on ribosome. After this the second AA-tRNA attaches itself to 'A' site on ribosome. In this way polypeptide chain elongation begins.

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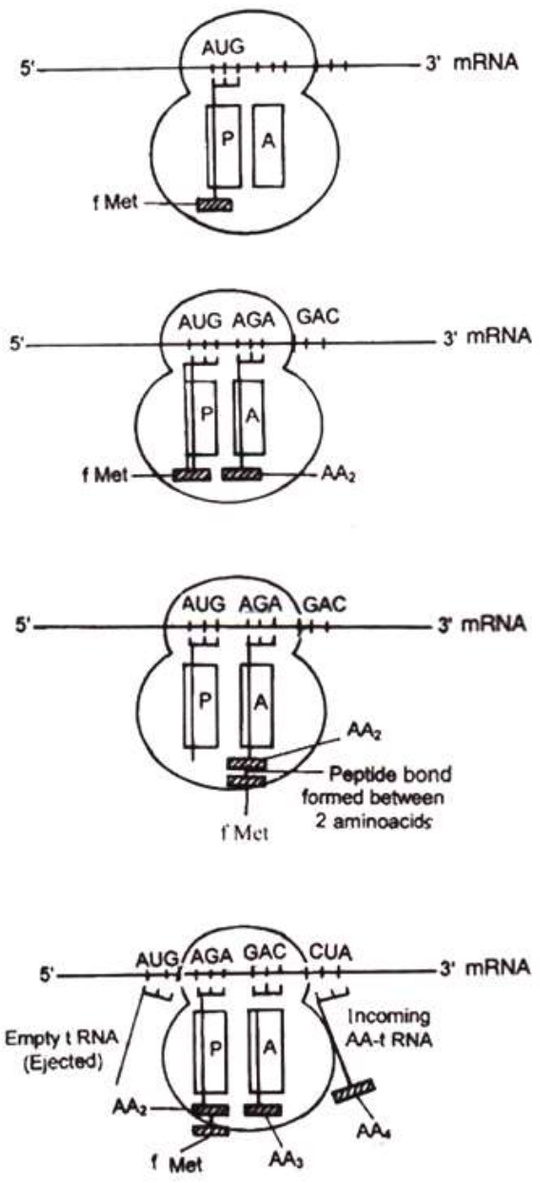


Fig. 10.6 *Polypeptide Chain Formation, Binding of Charged tRNA, Peptide Bond Formation, and Translocation*

10.4.1 Polypeptide Chain Elongation

Polypeptide chain elongation requires some elongation factors. These elongation factors are Tu and G.

EF-Tu forms a complex with AA2-tRNA and GTP and brings it to the 'A' site of ribosome. Once the AA2-tRNA is in place at 'A' site, the GTP is hydrolysed to GDP and EF- Tu is released from the ribosome. EF-Tu-GTP complex is regenerated with the help of another factor Ts.

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Formation of Peptide Bond

The main role of ribosome is to catalyse the formation of peptide bonds between successive amino acids. In this way amino acids are incorporated into protein.

Now both 'P' site and 'A' site on ribosome are occupied by charged tRNAs having amino acids. Peptide bond is formed between two successive amino acids at 'A' site. It involves cleavage of bond between f-Met and tRNA. This is catalysed by the enzyme tRNA deacylase.

Peptide bond is formed between the free carboxyl group (-COOH) of the first amino acid and the free amino group (- NH_2) of the second amino acid at the 'A' site. The enzyme involved in this reaction is peptidyl transferase. After the formation of peptide bond, between two amino acids, the tRNA at 'P' site becomes uncharged or deacylated and tRNA at 'A' site now carries a – ill protein chain having two amino acids. This occurs in 50S subunit of ribosome.

The peptidyltransferase which catalyzes the peptide bond formation between successive amino acids consists of several proteins and molecule of 23S rRNA in the ribosome. This 23S rRNA is a ribozyme.

10.4.2 Translocation

The peptidyl tRNA carrying two amino acids present at 'A' site is now translocated to 'P' site. This movement is called translocation. Elongation factor called EF-G control translocation. This factor G is called translocase. Hydrolysis of GTP provides energy for translocation and release of deacylated tRNA (free of amino acid).

Translocation also involves movement of ribosome along mRNA towards its 32 -end by a distance of one codon from first to second codon. This movement shifts the dipeptidyl tRNA (carrying two amino acids) from 'A' to 'P' site.

In addition to these two sites P and A, a third site 'E' (exit site) on 50 S ribosome is present. Deacylated tRNA (deprived of amino acid) moves for 'P' site to 'E' site from where it is ejected out.

Then the third amino acid (next amino acid) charged on tRNA comes to lie in now empty site 'A'. Then dipeptidyl chain having two amino acids present on P site form peptide bond with the third amino acid at 'A' site. Then the three amino acid chain is translocated to 'P' site. Now the polypeptide chain has three amino acids. This elongation process goes on and on. At each step a new amino acid is added to the polypeptide chain. After each elongation, ribosome moves by one codon in $5' \rightarrow 3'$ direction.

10.4.3 Chain Termination

The presence of termination codons or stop codons on mRNA causes the polypeptide chain to be terminated. Synthesis stops when elongation chain comes across stop codons on 'A' site. The stop codons are UAA, UGA and UAG.

There is no tRNA which can bind these codons.

There are three release factors in prokaryotes, which help in chain termination. They are RF1, RF2 and RF3.

NOTES

Polyribosome or Polysome

A single mRNA molecule can be read simultaneously by several ribosomes. A polyribosome or polysome consists of several ribosomes attached to the same RNA. The number of ribosomes in a polysome depends upon the length of mRNA.

A fully active mRNA has one ribosome after every 80 nucleotides. There may be about 50 ribosomes in a polycistronic mRNA of prokaryotes. Ribosomes move along mRNA in $5' \rightarrow 3'$ direction. There is a gradual increase in the size of polypeptide chain as the ribosomes move along mRNA towards its 3′-end. Polypeptide chain starts near the 5′-end and is completed near the 3′-end.

Polyribosome

The ribosomes closest to the 5′-end of mRNA have the smallest polypeptide chain, while ribosomes nearest to the 3′-end have longest chain. Polysome increases the rate of protein synthesis tremendously. In bacteria protein is synthesized at the rate of about 20 amino acids per second.

Simultaneous Transcription and Translation in Prokaryotes:

In prokaryotes, all components of transcription and translation are present in the same compartment. The mRNA molecule is synthesized in $5' \rightarrow 3'$ direction and protein synthesis also occurs in $5' \rightarrow 3'$ direction. In this way mRNA molecule while still under synthesis has a free 5′-end whose other end is still under synthesis.

Ribosomes bind at free 5′-end and start protein synthesis. In this way the free end (5′-end) of mRNA starts the process of protein synthesis while still attached to DNA. This is called Coupled Transcription and Translation. This increases the speed of protein synthesis. After the protein synthesis is completed, the degradation of mRNA molecule by nucleases also starts at 5′-end and proceeds in $5' \rightarrow 3'$ direction.

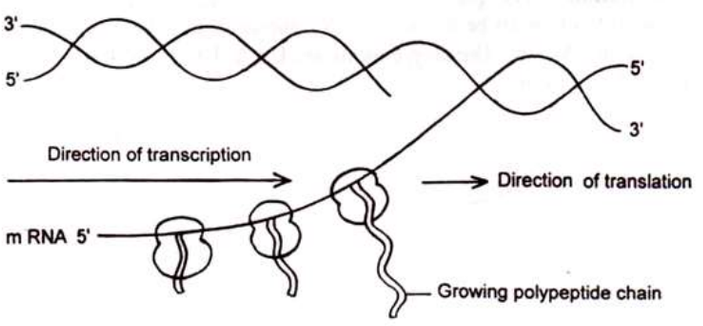


Fig. 10.7 *Coupled Transcription and Translation*

Protein Synthesis in Eukaryotes

Protein synthesis in eukaryotes is basically similar to that of prokaryotes except some differences.

NOTES

The ribosomes in eukaryotes are of 80S having 40S and 60S subunits. In eukaryotes the initiating amino acid is methionine and not f-methionine as in the case of prokaryotes. A special tRNA binds methionine to start codon AUG. This tRNA is called tRNAiMet. This is distinct from tRNAMet which binds amino acid methionine to any other internal position in the polypeptide.

There is no Shine-Dalgarno sequence in eukaryotic mRNA to function as ribosome binding site. Between 5′-end and AUG codon of mRNA there is a sequence of bases called cap. Small subunit of ribosome scans the mRNA in 5′ $\rightarrow$ 3′ direction until it comes across 5′AUG-3′ codon. This process is called scanning. Initiation factors also closely associated with 3′-end of mRNA through its poly-A tail. Initiation factors circularize mRNA by its poly-A tail. In this way poly-A tail also contributes to the translation of mRNA. Eukaryotic mRNAs are monocistsonic and encode a single polypeptide, therefore have a single open reading frame.

There are ten initiation factors in eukaryotes. They are elF (eukaryotic initiation factors) are elFI, eIF2, eIF3, eIF4A, eIF4B, eIF4C, eIF4D, eIF4F, eIF5, eIF6.

There are two elongation factors in eukaryotes like prokaryotes. They are eEFl (similar to EF-Tu) and eEF2 (similar to EF-G).

Eukaryotes have only one release factor eRF which requires GTP termination of protein synthesis. It recognizes all the three stop codons.

In eukaryotes the mRNA is synthesized in the nucleus, then processed, modified and passed on into the cytoplasm through nucleopores. The protein synthesis takes place in the cytoplasm. The mRNA in prokaryotes is very unstable and its life span is of a few minutes only. The mRNA of eukaryotes is quite stable and has a longer life span extending up to several days.

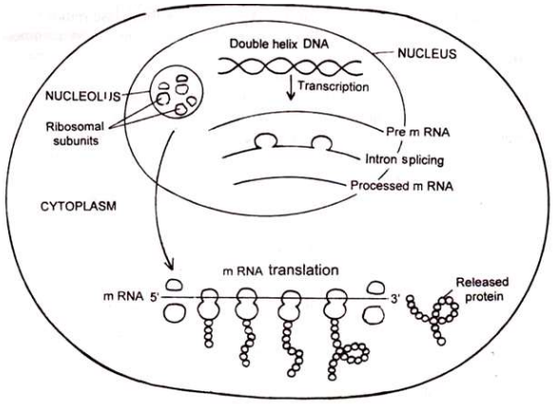


Fig. 10.8 *Transcription, mRNA processing and mRNA Translation*

Protein Synthesis on Bound Ribosomes

NOTES

Ribosomes occur in free state in the cytoplasm as well as bound to the outer surface of endoplasmic reticulum called Rough Endoplasmic Reticulum (RER). The attachment of ribosomes to ER occurs after the protein synthesis starts. Whether the ribosomes synthesize protein on free or attached state depends upon the type of proteins to be synthesized by ribosomes. Most of the proteins which remain in free state in the cytoplasm are synthesized by free ribosomes.

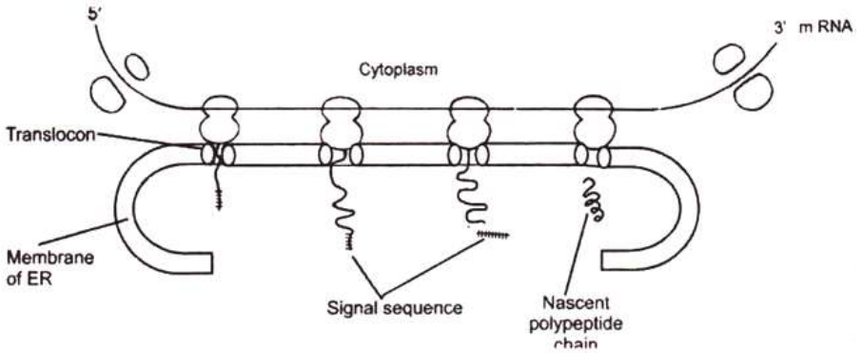


Fig. 10.9 *Protein Synthesis on Ribosome bound on ER (RER)*

Proteins synthesized by ribosomes on ER enter into the lumen of cisternae of ER from where they may enter into golgi apparatus where they are glycosylated and form secretary granules and many of them enter lysosomes.

10.4.4 Modification of Folding of Released Polypeptides

DNA molecule specifies only the primary structure while folding and other modifications controlled by proteins themselves.

The newly synthesized polypeptide is not always a functional protein. The newly released polypeptide may undergo various modifications. An enzyme deformylase removes the formyl group of first amino acid methionine. The cleavages of proteins are most common. Some enzymes like exo-amino-peptidases remove some amino acids either from N-terminus end or from C-terminus end or both ends.

Internal amino acids may also be removed as in the case of insulin. Polyproteins are cleaved to generate individual proteins. The polypeptide chain singly or in association with other chains may fold up to form tertiary or quaternary structures. Prosthetic groups join many proteins. Some proteins assist in folding up of polypeptides. They are called chaperone proteins or chapronin proteins. Examples are Bacterial gro EL (*E. coli*), mitochondrial hsp 60 mitonin.

Various chemical common modifications of newly released proteins are glycosylation, phosphorylation, methylation, acetylation etc.

NOTES

Protein Sorting or Protein Trafficking or Protein Targeting

The proteins synthesized in the cell have to be translocated to the nucleus or other target organelles. Newly synthesized polypeptides have a signal sequence (which is a polypeptide) consisting of 13-36 amino acids. It is known as leader sequence. This signal sequence is recognized by receptors located within the membranes of the target organelles.

When proteins are synthesized on free ribosomes, the transfer takes place after the translation. When the protein synthesis takes place on ribosomes attached to the Endoplasmic Reticulum (Rough ER), the transfer takes place simultaneously with translation and is called co-translational transfer. The proteins which enter into the lumen of Rough ER may enter into Golgi apparatus, from where they may enter secretary lysosomes. The signal sequence is degraded by protease enzymes.

Once all these proteins are assembled into their proper place, they provide the proper biochemical machinery, which keeps the cell feeding, locomoting, multiplying and alive.

Inhibitors of Protein Synthesis

There are many chemicals, both synthetic as well as those obtained from different sources like fungi, which bind to the components of translation machinery and arrest the translation process. Most of them are antibacterial agents or antibiotics that act exclusively on bacteria and are thus powerful tools in the hands of man to combat various infectious diseases. Most of antibiotics are inhibitors of translation machinery.

Puramycin: It binds at 'A' site on ribosome. This causes pre-mature termination of polypeptide chain.

Kirromycin: It inhibits the elongation factor EF-Tu.

Fusidic acid: It inhibits the elongation factor EF-G.

Tetracycline: It attacks 'A' site on ribosome and prevents the binding of aminoacyl-tRNA.

Chloramphenicol: It blocks the peptidyl transfer reaction.

Erythromycin: It binds the polypeptide exit channel of ribosome, therefore blocks the exit of growing polypeptide chain, and thus stops the translation process.

Streptomycin and Neomycin: These inhibit the binding of tRNAfMet to the 'P' site.

Check Your Progress

6. What is the first amino acid to start the protein in bacteria?
7. What is charging of tRNA?
8. What are inhibitors of protein synthesis?

10.5 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

NOTES

1. Proteins are giant molecules formed by polypeptide chains of hundreds to thousands of amino acids.
2. The main component of proteen synthesis are:
   - DNA
   - Three types of RNAs
   - Amino acids
   - Ribosomes
   - Enzymes.
3. There are four kinds of nitrogen bases.
4. A ribosome is a multicomponent, compact, ribonucleoprotein particle which contains rRNA, many proteins and enzymes needed for protein synthesis.
5. The main role of ribosome is the formation of peptide bond between successive amino acids of the newly synthesized polypeptide chain.
6. In bacteria, the first amino acid starting the protein is always formyl methionine (fMet).
7. Attachment of amino acids to tRNAs is called charging of tRNA.
8. There are many chemicals, both synthetic as well as those obtained from different sources like fungi, which bind to the components of translation machinery and arrest the translation process.

10.6 SUMMARY

- Proteins are giant molecules formed by polypeptide chains of hundreds to thousands of amino acids.
- Protein synthesis is governed by the genetic information carried in the genes on DNA of the chromosomes.
- DNA is the master molecule which possesses the genetic information about the sequence of amino acids in a polypeptide chain.
- The genetic code is deciphered or translated into a sequence of amino acids.
- DNA molecule has three components. They are sugar, phosphates and nitrogen bases.
- In prokaryotes, the RNA synthesis (transcription) and protein synthesis (translation) take place in the same compartment as there is no separate nucleus. But in eukaryotes, the RNA synthesis takes place in the nucleus while the protein synthesis takes place in the cytoplasm.

NOTES

- Ribosome is a macromolecular structure that directs the synthesis of proteins.
- First of all 30S subunit of the 70S ribosome starts initiation process. The 30S subunit, mRNA and charged tRNA combine to form pre-initiation complex.
- Polypeptide chain elongation requires some elongation factors. These elongation factors are Tu and G.
- The main role of ribosome is to catalyse the formation of peptide bonds between successive amino acids. In this way amino acids are incorporated into protein.
- The peptidyl tRNA carrying two amino acids present at 'A' site is now translocated to 'P' site. This movement is called translocation.
- The presence of termination codons or stop codons on mRNA causes the polypeptide chain to be terminated. Synthesis stops when elongation chain comes across stop codons on 'A' site.
- Various chemical common modifications of newly released proteins are glycosylation, phosphorylation, methylation, acetylation etc.

10.7 KEY WORDS

- **Synthesis:** The production of chemical compounds by reaction from simpler materials.
- **Translocation:** A chromosome translocation is a chromosome abnormality caused by rearrangement of parts between nonhomologous chromosomes.
- **Protein:** Any of a class of nitrogenous organic compounds which have large molecules composed of one or more long chains of amino acids and are an essential part of all living organisms, especially as structural components of body tissues such as muscle, hair, etc., and as enzymes and antibodies.

10.8 SELF ASSESSEMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. What are the components of protein synthesis?
2. Explain composition of genetic code.
3. Discuss chain termination.
4. What are the inhibitors of protein synthesis?

Long Answer Questions

1. What is the role of ribosomes in protein synthesis? Explain.
2. Explain formation of initiation complex.
3. How are peptide bonds formed in protein synthesis?
4. What do you understand by translocation?
5. Discuss modification of folding of released polypeptides.

NOTES

10.9 FURTHER READINGS

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UNIT 11 CELL FREE PROTEIN SYNTHESIS

NOTES

Structure

11.0 Introduction
11.1 Objectives
11.2 Cell Free Protein Synthesis
  11.2.1 Application of Cell Free Proteins
11.3 Comparison of Protein Synthesis in Prokaryotes and Eukaryotes
11.4 Answers to Check Your Progress Questions
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11.8 Further Readings

11.0 INTRODUCTION

During early days of molecular biology, cell-free protein synthesis played an essential role in deciphering the genetic code and contributed to our understanding of translation of protein from messenger RNA. Owning to several decades of major and incremental improvements, modern cell-free systems have achieved higher protein synthesis yields at lower production costs. Commercial cell-free systems are now available from a variety of material sources, ranging from 'traditional' E. coli, rabbit reticulocyte lysate and wheat germ extracts to recent insect and human cell extracts to defined systems reconstituted from purified recombinant components. Though each cell-free system has certain advantages and disadvantages, the diversity of the cell-free systems allows in vitro synthesis of a wide range of proteins for a variety of downstream applications. In the post-genomic era, cell-free protein synthesis has rapidly become the preferred approach for high throughput functional and structural studies of proteins and a versatile tool for in vitro protein evolution and synthetic biology.

In this unit, you will study about the history of cell-free protein synthesis, applications of this important technology, and comparison of protein biosynthesis in prokaryotes and eukaryotes.

11.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand cell free protein synthesis
- Know about applications of cell free protein synthesis
- Discuss comparison between protein biosynthesis in prokaryotes and eukaryotes

11.2 CELL FREE PROTEIN SYNTHESIS

NOTES

Cell-free protein synthesis is one of widely used methods in molecular biology. Production of proteins using cell-free protein synthesis usually takes a few hours, in contrast to production of proteins in cells, which typically takes days if not weeks. In fact, even first-time users can often obtain newly synthesized proteins on the same day he or she begins to use a commercial system. But, even though many users will use commercial systems rather than making them in their own laboratories, users should understand the processes and mechanisms underlying cell-free protein synthesis.

Early History of Cell-Free Protein Synthesis

At the dawn of molecular biology, many laboratories used cell-free protein synthesis to address the question of how amino acids are incorporated into proteins. Researchers conducted cell-free protein synthesis using the extracts of rat liver cells, bacteria, human and rabbit reticulocytes, ascites cells and wheat germ. They discovered protein synthesis occurred in the microsome fraction that requires ATP and GTP. In 1961, Nirenberg and Matthaei found the first correspondence between nucleotide triplets and the amino acids they encoded, demonstrating cell-free synthesis of polyphenylalanine from synthetic polyuridylic acid in the *E. coli* extract. In the years that followed, cell-free synthesis continued to play an important part in identifying the amino acids encoded by the remaining triplets, that is, in breaking the remaining characters in the genetic code, and establishing the 'central dogma of molecular biology'.

Landmark Advances in the Development of Cell-Free Protein Synthesis Systems

Since the preparation processes did not remove messenger RNA (mRNA), the cell extracts that comprised early cell-free systems synthesized mostly native proteins from endogenous mRNA. These extracts were essentially supernatants obtained by high speed centrifugation of lysates from disrupted cells for example, at 30,000*g* (the so-called S30 extract). To direct the protein synthesis machinery to translate exogenous mRNA, Nirenberg and Matthaei incubated the *E. coli* S30 extract in the presence of 20 amino acids and ATP regeneration components. Such 'incubated S30' improved the amount of the protein synthesized from exogenous mRNA, possibly by freeing ribosomes from attaching to endogenous mRNA (ribosome run-off) and allowing endogenous mRNA to be degraded by ribonucleases in the extract. Similarly, Pelham and Jackson added a Ca^{2+}-dependent micrococcal ribonuclease during the preparation of the rabbit reticulocyte extract (lysate) to degrade endogenous mRNA. The micrococcal ribonuclease can be subsequently inactivated by EGTA to prevent its digestion of exogenous mRNA. Interestingly, the wheat germ extract does not translate a significant amount of endogenous proteins, therefore, there is no need for incubation or nuclease-treatment.

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The next landmark improvement in cell-free protein synthesis was the introduction of coupled transcription-translation systems that used DNA as the template instead of mRNA. Zubay and colleagues used the *E. coli* RNA polymerase in the S30 extracts to transcribe DNA into mRNA for protein synthesis, mainly for the purpose of studying gene regulation in–vitro. To obtain higher amounts of mRNA, other researchers later used stronger phage promoters and the more efficient phage RNA polymerases (T7 or SP6) for the coupled transcription-translation from the DNA template. The coupled transcription-translation based on the phage promoters and RNA polymerases has since been adopted by most cell-free systems. In addition to using the more stable DNA template and avoiding a separate in–vitro transcription step, coupled transcription and translation in the cell-free system often result in a higher protein synthesis yield, probably due to newly synthesized mRNA is immediately translated into protein, which may minimize the adverse effects of mRNA degradation or inhibitory structures.

Cell-free protein synthesis is commonly conducted in batch format. However, the protein synthesis reactions often stop after a short period of time, in many cases in only 1 hr or so, due to depletion of ATP and GTP, degradation of mRNA and accumulation of by-products to inhibiting concentrations. Consequently, the batch cell-free protein synthesis normally yields $\mu g\ ml^{-1}$ of protein. Spirin and colleagues introduced the concept of continuous cell-free protein synthesis in both *E. coli* and eukaryotic cell-free systems. The continuous cell-free protein synthesis circumvents some of the issues of the batch format by using a flow chamber or dialysis membrane to maintain the levels of small molecules energy sources and amino acids, and at the same time removing inhibitory by-products (for example, phosphate). As a result, a continuous cell-free protein synthesis reaction can often last more than 10 hours and yield mg μg^{-1} of protein.

Due to continuous improvement by academic and industrial researchers, the cell extracts in cell free systems have improved significantly. From the 'incubated S30' first described by Nirenberg and Matthaei, Zubay, Pratt and colleagues developed the robust protocols for making the *E. coli* extract that have become the standards. Other important improvements included the use of *E. coli* strains that lacked RNase I (*rna*) (MRE600) and Exonuclease V (A19) to stabilize RNA and linear DNA templates, respectively. Recently, Swartz and colleagues pioneered the use of genome engineered *E. coli* strains to stabilize amino acids during *in vitro* reactions, *in vitro* activation of endogenous metabolic pathways to sustain high-yield protein synthesis, and systematic optimization of *E. coli* growth conditions and extraction procedures to reduce the production cost.

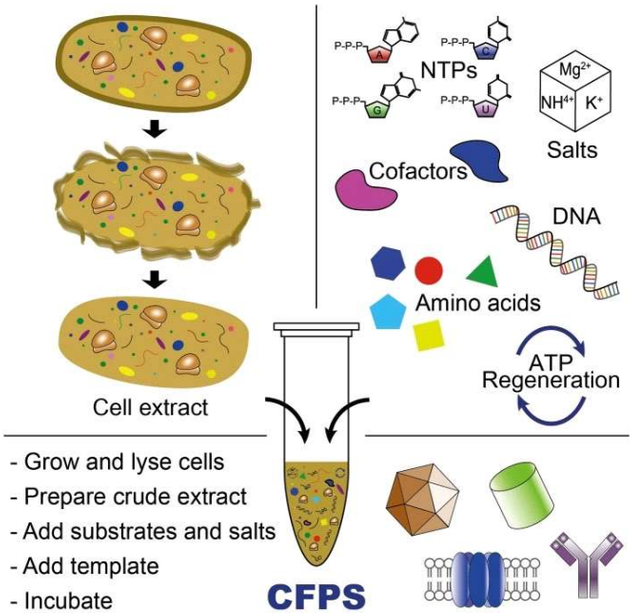


Fig. 11.1 *Cell-Free Protein Synthesis (CFPS0 for Producing Proteins*

Since their initial development, the protocols for making the 'classic' eukaryotic extracts, rabbit reticulocyte lysate and wheat germ, remain largely unchanged, and the protein synthesis yields from these eukaryotic extracts are generally lower than that from the *E. coli* extract. Recently, researchers developed improved protocols for making highly active extracts from wheat germ and mammalian cells. Endo and colleagues discovered that the remnants of endosperm on the surface of wheat germ were the sources of ribosome-inactivating proteins and other translation inhibitors, responsible for the poor stability and low yield of the conventional wheat germ cell-free system. By extensively washing isolated wheat germ, the Endo group developed a high-yield long-lasting wheat germ cell-free system. Until recently, with the exception of the rabbit reticulocyte lysate, the extracts of mammalian cells have not been widely used for cell-free protein synthesis mainly due to low yields. Imataka and colleagues at RIKEN found that the lack of functional translation initiation factors in the HeLa cell extract was a major limiting factor. By supplementing with exogenous translation initiation factors, the Imataka group developed significantly improved mammalian cell-free systems, effectively taking over the rabbit reticulocyte lysate system.

Finally, the Ueda group reconstituted the minimal protein translational machinery from *E. coli*, thereby completely circumventing the issues of inhibitory factors in cell extracts. The PURE system (Protein synthesis Using Recombinant Elements), created by the Ueda group, consists of entirely purified components with minimal nucleases, proteases, and other enzymatic activities detrimental or unrelated to protein translation. Though other laboratories had attempted to reconstitute the protein synthesis with purified components, the PURE system

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was the first robust cell-free system containing a complete set of purified translation factors and aminoacyl-tRNA synthetases. Taking the same approach, my laboratory reconstituted the minimal protein translational machinery from *Thermus thermophilus* and demonstrated cell-free protein synthesis at temperatures up to 65°C.

To make cell-free protein synthesis a protein factory rather than just an analytic tool, Swartz and colleagues pioneered chemical engineering approaches to making the *E. coli* extract. Their goal was to manufacture therapeutic proteins in the cell-free system at liter or any scale. The entire processes of cell growth, extract preparation, protein synthesis and downstream purification were subject to cost analyses and process engineering. The 'black-box' nature of the *E. coli* extract became sets of biochemical reactions amenable to pathway engineering. One of key advances was the creation of the 'Cytomim' system that can activate central metabolism and oxidative phosphorylation in the *E. coli* extract, allowing not only the use of inexpensive energy substrates, such as glutamate or glucose, but also continuous protein synthesis for more than 6 hr in the batch format. The Cytomim system was the basis for further improvements that led to the cell-free synthesis of recombinant human granulocyte-macrophage colony-stimulating factor in a 100-liter industrial stir tank.

Use of Commercially Available Cell-Free Protein Synthesis Systems

The quick and simple way to synthesize proteins in-*vitro* is to use a commercially available cell-free system. Most commercial cell-free systems provide DNA expression vectors into which the researcher clones his or her gene and allow protein production from the cloned gene. These expression vectors often contain promoter and other sequences optimized for cell-free protein synthesis. Almost all commercial cell-free systems have adopted coupled transcription and translation, which also allows PCR-generated linear DNA templates to be used for protein synthesis.

To choose an appropriate commercial cell-free system, one should consider protein size and origin, protein folding, post-translational modification, downstream assays, applications and costs. In general, the prokaryotic systems (for example, *E. coli* extracts) yield more proteins (especially small proteins, <50 kDa) than the eukaryotic systems, whereas the latter are more suited for expressing large multi-domain eukaryotic proteins. The continuous cell-free synthesis format generally produces more proteins (often >1 mg μl^{-1}) than the batch format. Systems based on cell extracts tend to provide better folding environments and post-translational modifications, whereas the reconstituted systems afford mRNA and protein stability. The reconstituted systems have minimal enzymatic activities and macromolecules other than those involved in protein translation, therefore, it is possible to carry out downstream characterization immediately after protein synthesis without purification.

11.2.1 Application of Cell Free Proteins

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High-throughout cell-free Protein Synthesis for Functional and Structural Analyses

The use of cell-free protein synthesis has made the most impact on functional and structural genomics. For the first time, researchers have been able to express and purify a large number of proteins in a short period of time for subsequent high throughput functional and structural analyses. For instance, a number of laboratories have used low-cost *E. coli* extract and wheat germ cell-free systems in high-throughput automated format to produce stable isotope-labeled proteins for nuclear magnetic resonance analyses. The advantage is that only newly synthesized proteins are labeled during cell-free protein synthesis and can be analyzed without extensive purification.

Like DNA microarrays for gene expression, protein arrays have been used for high throughput analyses of protein functions. Initially researchers had to individually purify proteins and spot each protein on solid surfaces. These protein arrays are laborious to make and surface-bound proteins can lose functions during storage. Cell-free protein synthesis circumvents these problems by allowing proteins to be synthesized and captured in the vicinity of immobilized DNA templates, effectively generating just-in-time protein arrays from more stable DNA arrays. Two such protein array technologies, protein *in situ* array (PISA) and nucleic acid programmable protein array (NAPPA) have been used for functional proteomics studies of protein-protein interactions.

Incorporation of Unnatural Amino Acids into Proteins

The translational machinery, including aminoacyl-tRNA synthetases, can tolerate a wide range of unnatural amino acids, allowing the genetic code to be expanded or even reassigned. One of the earliest applications of cell-free protein synthesis was the incorporation of unnatural amino acids into a specific position of an *in vitro* synthesized protein. Schultz and colleagues first demonstrated the incorporation of unnatural amino acids into an amber stop codon that substituted a sense codon in a protein in an *E. coli* extract. It took more than a decade for the Schultz laboratory to engineer living cells to allow such expansion of the genetic code in-vivo. And still, compared to cell-based approaches, cell-free systems are easier to manipulate for incorporation of unnatural amino acids. The reconstituted cell-free systems are especially suited for these applications. Using the reconstituted cell-free system, researchers can simply remove the competing factors, such as release factors, tRNAs or aminoacyl-tRNA synthetases, or replace them with an tRNA-synthetase pair from another organism, or an engineered aminoacyl-tRNA synthetase. Moreover, tRNAs can be pre-acylated with unnatural amino acids using natural enzymes or engineered ribozymes (so called 'Flexizymes'). The combined use of pre-charged tRNAs and the reconstituted cell-free system has

allowed unnatural peptides or peptidomimetics containing mostly unnatural amino acids to be synthesized for potential applications in therapeutics.

Directed Evolution of Proteins

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Protein functions, such as binding affinity, thermostability and catalytic activity, can often be improved by iterative rounds of mutation and selection – an approach called directed protein evolution. Directed protein evolution is critically dependent on a stable physical linkage between protein (phenotype) and nucleic acid (genotype) throughout the selection process. The cell-based approaches, for example, phage display) and yeast surface display, maintain such genotype-phenotype linkage because the different nucleic acid sequences are confined to different phage particles or cells. The use of these cell-based approaches can potentially be limited by phage packaging or cellular transformation efficiencies and growth biases as phages expressing different members of a protein library may amplify at different rates).

In comparison, in-vitro directed evolution based on cell-free protein synthesis can potentially incorporate protein libraries with higher complexities (more genes to select from) and less amplification bias. These in-vitro approaches include ribosome display and mRNA display, both of which create a physical linkage between mRNA and protein, and in-vitro compartmentalization, which encapsulates DNA, mRNA and protein in water-in-oil emulsion droplets. Although researchers have successfully used extract-based cell-free systems for ribosome display and mRNA display, they have recently shown the advantages of the reconstituted cell-free systems. Since all of their components are purified, the reconstituted cell-free systems contain minimal nuclease and protease activities, which lead to significant stabilization of the mRNA-protein linkage. A reconstituted cell-free system without the release factor 1 has been used in ribosome display, resulting in increased efficiency of ribosome stalling at the stop codon and displaying the full-length protein. When incorporation of multiple unnatural amino acids is required for in-vitro evolution and selection of drug-like molecules, the reconstituted cell-free systems become the only choice as only components necessary for in-vitro synthesis are included in the systems.

Cell-free Synthetic Biology Towards Artificial Cell

Cell-free protein synthesis is likely a key starting point for any attempt to create an artificial cell either for understanding origin of life or for practical applications. Of the 200 or so essential genes believed to the minimal set for a free-living cell, a large portion are directly involved in protein synthesis. Protein synthesis has been coupled to cell-free transcription regulation and DNA/RNA replication. In parallel, cell-free protein synthesis has been performed in liposomes, phospholipid vesicles and water-in-oil droplets. By such means, the reconstituted cell-free systems could be an important tool for bottom-up reconstruction of the minimal cell.

Check Your Progress

1. What does the correspondence between nucleotide triplets and the amino acids encoded by Nirenberg and Matthaei demonstrate?
2. What is direct protein evolution?

NOTES

11.3 COMPARISON OF PROTEIN SYNTHESIS IN PROKARYOTES AND EUKARYOTES

Protein synthesis is the process in which cells build proteins. The term is sometimes used to refer only to protein translation but more often it refers to a multistep process in which cells follow a very systematic procedure that first transcribes DNA into mRNA and then translates the mRNA into chains of amino acids. The amino acid chain then folds to form functional proteins.

Prokaryotic and eukaryotic translation are involved in the synthesis of proteins by decoding the genetic instructions carried by the mRNAs. During translation, nucleotide triplets, known as codons, on the mRNA are translated into a sequence of amino acids. Both prokaryotic and eukaryotic translation share a similar basic plan throughout the processes. However, there are several differences that can be observed in these translation processes. The main difference between prokaryotic and eukaryotic translation is that prokaryotic translation occurs synchronously with its transcription whereas eukaryotic translation occurs asynchronously with its transcription.

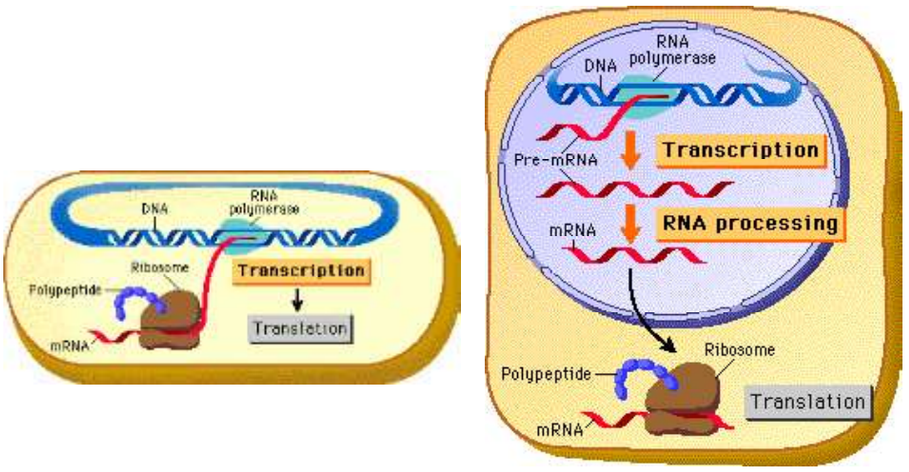


Fig. 11.2 *Prokaryotic Protein Synthesis and Eukaryotic Protein Synthesis*

Prokaryotic Translation

In prokaryotes, translation is the process of simultaneously synthesizing proteins with transcription. Translation begins just after transcribing the 5′ end of the gene

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into mRNA. Prokaryotic translation basically occurs in three steps: initiation, elongation and termination. In order to initiate the translation, the two subunits 50S and 30S are assembled. The three initiation factors, IF1, IF2 and IF3 help to assemble the initiation complex. N-formylmethionine is the first added amino acid in translation. GTP is used as the energy source for the peptide bond formation between the remaining and incoming nucleotides. The translation initiation factor is EF-P. The selection of the start codon is facilitated by the binding of the ribosome with the Shine--Dalgarno sequence. The Shine--Dalgarno sequence is a purine -rich region located upstream of the AUG start codon. This sequence is complementary to the pyrimidine- rich region on 16S rRNA. The 16S rRNA is a component of 30S subunit. These two complementary nucleotides pair together, forming a double--stranded RNA structure. This pairing brings the initiation codon into the P -site of the ribosome. The first amino acid binds with the P site. A ribosome consists of three active sites: A site, P site and E site. Incoming aminoacyl tRNAs, other than the first aminoacyl tRNA, binds with the A site. The peptide bond formation occurs at the P site. Exit site for the uncharged tRNA is the E site.

Eukaryotic Protein Synthesis vs. Prokaryotic Protein Synthesis

Eukaryotic Protein Synthesis	Prokaryotic Protein Synthesis
• mRNA molecules are **monocistronic**, containing the coding sequence only f or one polypeptide.	• mRNA molecules are **polycistronic** containing the coding sequence of several genes of a part icular metabolic pathway.
• protein synthesis occurs in the **cytoplasm**.	• protein synthesis begins even before the transcription of mRNA molecule is completed. This is cal led coupled transcription - translation.
• most of the gene have introns or non-coding sequences along with exons or coding sequences. The exons are join ed together and introns are removed during mRNA processing.	• do not have introns (Except Archaebacteria). Therefore **mRNA processing** is not required.
• The primary mRNA transcript undergoes **processing** and splicing to change into a functional mRNA.	• splicing of mRNA transcript does not occur.
• mRNA molecules are modified by the addition of a 5'G cap formed of methyla ted guanosine triphosphate.	• No such cap is formed at 5'end of bacterial mRNA.
• A poly A tail formed of about 200 adenine nucleotides is added at the 3' end of mRNA.	• No poly A tail is added to bacterial mRNA.
• 5'cap initiates translation by binding the mRNA to small ribosomal subunit us ually at the first codon AUG.	• translation begins at an AUG codon preceded by a special nucleotide se quence.
• The first amino acid methionine entering the ribosome is **not formylated**.	• The first amino acid methionine is **formylated** into N formyl methionine.
• The pre imitation complex formation is initiated by nine initiated factors.	• Only two initiating factors are involved.
• No. of initiating factors is much more than prokaryotes. About ten IFs have be en identified in reticulocytes an RBC. These are eIF1 , eIF2, eIF3, eIF4 , eIF5 , eIF6 ,eIF4B, eIF4C,eIF4D, eIF4F	• Three initiating factors found in prokaryotes. PIF-1 , PIF-2 , PIF-3
• small subunit of ribosome (40 S) gets dissociated with the initiator amino acyl tRNA (MettRNA Met) without the help of mRNA. The complex joins mRNA la ter on.	• 30 S subunit first complexes with mRNA (30S-mRNA) when then joins with f Met tRNA f-

The two elongation factors are EF-G and EF-Tu. The translation elongates until the ribosome reaches to one of the three stop codons: UAA, UGA, UAG. Release factors other than the tRNAs, recognise the stop codon. The mRNA with the termination codon at A site is referred to as the termination complex. Three release factors can be identified: RF1, RF2 and the RF3. RF1 and RF2 recognise the UAA/UAG and UAA/UGA and hydrolyze the ester bond in peptidyl-tRNA to release the nascent polypeptide chain. RF3 catalyses the releasing of RF1 and RF2. Once the new protein releases, the ribosome undergoes recycling. The Ribosome Recycling Factor and EF-G, are involved in releasing mRNA and tRNAs from the ribosome and dissociation of 70S ribosome into 30S and 50S subunits. IF3 releases the mRNA by replacing the deacylated tRNA. When bacteria enter the stationary phase, the translation is down regulated by the dimerization of ribosomes. Ribosome dimerization is facilitated by RMF, HPF and YfiA. The ribosome -dissociation factors are RsfA and HflX.

NOTES

Eukaryotic Translation

Translation is the second step of eukaryotic gene expression, a separate event from eukaryotic transcription. Transcription and translation occur in two different compartments in eukaryotes. Therefore, the two processes cannot occur simultaneously. Eukaryotic mRNAs are monocistronic and are processed in the nucleus by adding a 5′ cap, poly A tail and splicing out of introns before they are released to the cytoplasm. Ribosomal pausing also affects the translation by co--translational folding of the newly synthesising polypeptide chain on the ribosome. This process delays translation, giving time for the translation. Eukaryotic mRNAs consist of a 5′ cap and poly A tail. Therefore, the initiation of translation occurs in two different ways: cap-dependent initiation and cap-independent initiation. During cap-dependent initiation, the initiation factors bind to the 5′ end of the mRNA. These initiation factors hold the mRNA in the small subunit of the ribosome. During cap-independent initiation, internal ribosome entry sites allow the ribosome trafficking to the start site by direct binding. In eukaryotes, the first binding amino acid is methionine. 40S subunit associates with 60S subunit to form 80S ribosome. Two elongation factors are involved in eukaryotic translation: eEF-1 and eEF-2. Elongation occurs in a similar way to that of prokaryotes. Termination of the translation is also the same as in the prokaryotic system. But the universal release factor eRF1 is capable of recognising all three stop codons. The release factor, eRF3 helps eRF1 to release the polypeptide chain. Basic steps of the translation are shown in Figure 11.3.

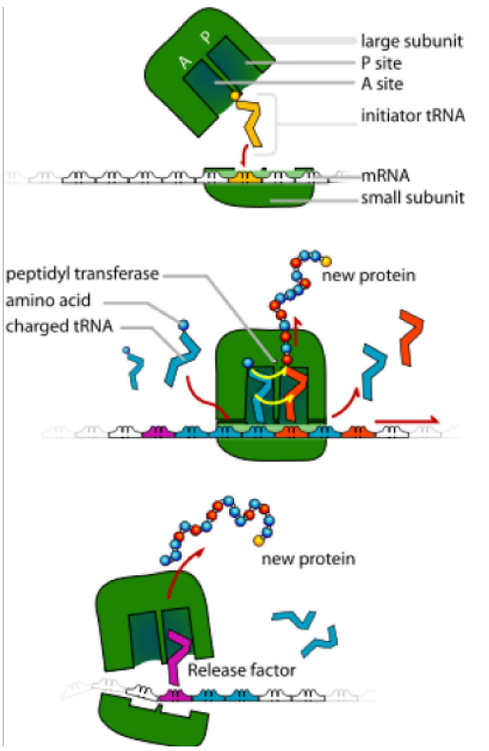


Fig. 11.3 *Generalized Translation*

NOTES

Check Your Progress

3. What is protein synthesis?
4. What are codons?

11.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. In 1961, Nirenberg and Matthaei found the first correspondence between nucleotide triplets and the amino acids they encoded, demonstrating cell-free synthesis of polyphenylalanine from synthetic polyuridylic acid in the *E. coli* extract.
2. Protein functions, such as binding affinity, thermostability and catalytic activity, can often be improved by iterative rounds of mutation and selection – an approach called directed protein evolution.
3. Protein synthesis is the process in which cells build proteins.
4. Prokaryotic and eukaryotic translation are involved in the synthesis of proteins by decoding the genetic instructions carried by the mRNAs. During translation, nucleotide triplets, known as codons, on the mRNA are translated into a sequence of amino acids.

11.5 SUMMARY

- Production of proteins using cell-free protein synthesis usually takes a few hours, in contrast to production of proteins in cells, which typically takes days if not weeks.
- In 1961, Nirenberg and Matthaei found the first correspondence between nucleotide triplets and the amino acids they encoded, demonstrating cell-free synthesis of polyphenylalanine from synthetic polyuridylic acid in the *E. coli* extract.
- Cell-free synthesis continued to play an important part in identifying the amino acids encoded by the remaining triplets, that is, in breaking the remaining characters in the genetic code, and establishing the 'central dogma of molecular biology'.
- The landmark improvement in cell-free protein synthesis was the introduction of coupled transcription-translation systems that used DNA as the template instead of mRNA.
- Cell-free protein synthesis is commonly conducted in batch format.

- Since their initial development, the protocols for making the "classic" eukaryotic extracts, rabbit reticulocyte lysate and wheat germ, remain largely unchanged, and the protein synthesis yields from these eukaryotic extracts are generally lower than that from the *E. coli* extract.
- The quick and simple way to synthesize proteins in *vitro* is to use a commercially available cell-free system. Most commercial cell-free systems provide DNA expression vectors into which the researcher clones his or her gene and allow protein production from the cloned gene.
- Prokaryotic and eukaryotic translation are involved in the synthesis of proteins by decoding the genetic instructions carried by the mRNAs. During translation, nucleotide triplets, known as codons, on the mRNA are translated into a sequence of amino acids. Both
- In prokaryotes, translation is the process of simultaneously synthesizing proteins with transcription. Translation begins just after transcribing the 5′ end of the gene into mRNA. Prokaryotic translation basically occurs in three steps: initiation, elongation and termination.
- Translation is the second step of eukaryotic gene expression, a separate event from eukaryotic transcription. Transcription and translation occur in two different compartments in eukaryotes.

11.6 KEY WORDS

- **Protein:** Any of a class of nitrogenous organic compounds which have large molecules composed of one or more long chains of amino acids and are an essential part of all living organisms, especially as structural components of body tissues such as muscle, hair, etc., and as enzymes and antibodies.
- **Biosynthesis:** The production of complex molecules within living organisms or cells.
- **Prokaryote**: A microscopic single-celled organism which has neither a distinct nucleus with a membrane nor other specialized organelles, including the bacteria and cyanobacteria.
- **Eukaryote:** An organism consisting of a cell or cells in which the genetic material is DNA in the form of chromosomes contained within a distinct nucleus. Eukaryotes include all living organisms other than the eubacteria and archaea.

11.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

NOTES

Short Answer Questions

1. Write a short note on history of cell free protein synthesis.
2. What are the advancement in the technology of cell free protein synthesis?
3. Draw a neat diagram to show biosynthesis in prokaryotes and eukaryotes.
4. What do you understand by prokaryotic translation?
5. What do you understand by eukaryotic translation?

Long Answer Questions

1. What are the applications of cell free protein? Explain.
2. What is the use of commercially available cell-free protein synthesis systems?
3. Briefly explain cell free protein synthesis.
4. Differentiate between prokaryotic protein synthesis and eukaryotic protein synthesis.

11.8 FURTHER READINGS

Hunter, L. E. 2009. *The Process of Life- An Introduction to Molecular Biology*. USA: The MIT Press.

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BLOCK - IV
REGULATION OF GENE EXPRESSION

NOTES

UNIT 12 CONCEPT OF OPERON

Structure

12.0 Introduction
12.1 Objectives
12.2 Operon: Basic Concepts
12.2.1 Repressible Versus Inducible Enzymes
12.3 Concept of Operon
12.3.1 Lac Operon
12.3.2 Tryptophan Operon
12.4 Answers to Check Your Progress Questions
12.5 Summary
12.6 Key Words
12.7 Self Assessment Questions and Exercises
12.8 Further Readings

12.0 INTRODUCTION

In biology, an operon is a functioning unit of DNA containing a cluster of genes under the control of a single promoter. The genes are transcribed together into an mRNA strand and either translated together in the cytoplasm, or undergo splicing to create monocistronic mRNAs that are translated separately, i.e. several strands of mRNA that each encode a single gene product. The result of this is that the genes contained in the operon are either expressed together or not at all. Several genes must be co-transcribed to define an operon.

Originally, operons were thought to exist solely in prokaryotes, but since the discovery of the first operons in eukaryotes in the early 1990s, more evidence has arisen to suggest they are more common than previously assumed. In general, expression of prokaryotic operons leads to the generation of polycistronic mRNAs, while eukaryotic operons lead to monocistronic mRNAs.

In this unit, you will study about operons in detail.

12.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the concept of operons
- Know about lac operon and trp operon

- Discuss positive and negative control
- Describe repressor and inducer

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12.2 OPERON: BASIC CONCEPTS

Operon

- The Basic Concept Structural gene = Gene that codes for a polypeptide.
- A regulated cluster of adjacent structural genes with related functions.
- Has a single promoter region, so an RNA polymerase will transcribe all structural genes on an all-or-none basis.
- Transcription produces a single polycistronic mRNA with coding sequences for all enzymes in a metabolic pathway.

Polycistronic mRNA

- A large mRNA molecule that is a transcript of several genes. Contains stop and start codons for the translation of each polypeptide.

Operator

- A DNA segment between an operon's promoter and structural genes, which controls access of RNA polymerase to structural genes.
- Acts as an on/off switch for movement of RNA polymerase and transcription of the operon's structural genes.

Repressor

- Specific protein that binds to an operator and blocks transcription of the operon.
- Blocks attachment of RNA polymerase to the promoter.
- Repressors are encoded by regulatory genes.

Regulatory genes

- Genes that code for repressor or regulators of other genes.
- Are often located some distance away from the operons they control.
- Are involved in switching on or off the transcription of structural genes.

Co-repressor

- A molecule, usually a metabolite that binds to a repressor protein, causing the repressor to change into its active conformation.
- Only the repressor-corepressor complex can attach to the operator and turn off the operon.

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Inducer

- An inducer is a molecule that regulates gene expression. An inducer can bind to protein repressors or activators.
- Inducers function by disabling repressors.
- The gene is expressed because an inducer binds to the repressor.
- The binding of the inducer to the repressor prevents the repressor from binding to the operator. RNA polymerase can then begin to transcribe operon genes.

12.2.1 Repressible Versus Inducible Enzymes

Repressible enzymes

Enzymes which have their synthesis inhibited by a metabolite (for example, tryptophan).

Inducible enzymes

Enzymes which have their synthesis stimulated or induced by specific metabolites (for example, lac operon).

Repressible Enzymes	Inducible Enzymes
● Their genes are switched on until a specific metabolite activates the repressor.	● Their genes are switched off until a specific metabolite inactivates the repressor.
● Generally function in anabolic pathways.	● Function in catabolic pathways.
● Pathway end product switches off its own production by repressing enzyme synthesis.	● Enzyme synthesis is switched on by the nutrient the pathway uses.

Positive control of a regulatory system occurs only if an activator molecule interacts directly with the genome to turn on transcription.

CRP (cAMP receptor protein)

- A protein that binds within an operon's promoter region and enhances the promoter's affinity for RNA polymerase.
- When glucose is missing, the cell accumulates cyclic AMP (cAMP), a nucleotide derived from ATP. cAMP activates CAP so that it can bind to the lac promoter.
- When glucose concentration rises, glucose catabolism decreases the intracellular concentration of cAMP. Thus, cAMP releases CRP.

Catabolite repression

- Repression of a variety of unrelated catabolic enzymes when cells are grown in a medium containing glucose.

Enhancer/Silencer

- Regulatory regions on eukaryotic DNA that bind activator/repressor proteins controlling single gene expression.

NOTES

Check Your Progress

1. What is an operator?
2. What is co-repressor?
3. What are repressible enzymes?

12.3 CONCEPT OF OPERON

An operon is a cluster of functionally-related genes that are controlled by a shared operator. Operons consist of multiple genes grouped together with a promoter and an operator. Operons are present in prokaryotes (bacteria and archaea), but are absent in eukaryotes. In some situations multiple operons are controlled by the same regulatory protein; in these cases the operons form a regulon. Operons were first identified as a mode of gene expression control in 1961 by François Jacob and Jacques Monod.

Operon Structure

Operons are regions of DNA that contain clusters of related genes. They are made up of a promoter region, an operator, and multiple related genes. The operator can be located either within the promoter or between the promoter and the genes. RNA polymerase initiates transcription by binding to the promoter region. The location of the operator is important as its regulation either allows or prevents transcription of the genes into mRNA.

Operon Function

An operon is a complete package for gene expression and synthesis of polypeptides. By combining the related genes, all polypeptides required for a specific function are synthesized in response to a single stimulus. For example, the bacterium Escherichia coli contains a number of genes clustered into operons and regulons: the Lac operon which is involved in lactose degradation, the Trp operon which is involved in tryptophan biosynthesis, and the His operon which is involved in histidine biosynthesis. These operons are turned on when the gene products are needed.

Positive and Negative Control

Operons can be under negative or positive control. Negative control involves turning off the operon in the presence of a repressor; this can be either repressible or inducible. A repressible operon is one that is usually on but which can be repressed in the presence of a repressor molecule. The repressor binds to the

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operator in such a way that the movement or binding of RNA polymerase is blocked and transcription cannot proceed. An inducible operon is one that is usually off. In the absence of an inducer the operator is blocked by a repressor molecule. When the inducer is present it interacts with the repressor protein, releasing it from the operator and allowing transcription to proceed. Repressible operons are generally involved in anabolic pathways, or the synthesis of an essential component, while inducible operons are generally involved in catabolic pathways, or the breakdown of a nutrient. Positive control of an operon is when gene expression is stimulated by the presence of a regulatory protein.

12.3.1 Lac Operon

The Lac operon is the classic operon example, and is responsible for the degradation of the milk protein lactose. The Lac operon is an inducible operon; in the absence of lactose the operator is blocked by a repressor protein. The operon is made up of a promoter with operator, and three genes (lacZ, lacY, and lacA) which encode β-galactosidase, permease, and transacetylase. The three genes are involved in the breakdown of lactose into its metabolites: β-galactosidase breaks lactose down into glucose and galactose, while the other two proteins aid in the metabolic process. The expression of the Lac operon is controlled by the regulatory gene lacI, located immediately adjacent to the promoter region. LacI encodes an allosteric repressor protein that keep the Lac operon 'off'.

In order for the Lac operon to be turned on, an inducer molecule must inactivate the repressor protein. The inducer molecule in this system is allolactose, an isomer of lactose. When lactose and its isomer are present in the cell, allolactose will bind to allosteric sites on the repressor protein, changing its conformation and rendering it inactive. As the repressor protein detaches from the operator, RNA polymerase can bind to the promoter, transcription can occur, and the three lactose degradation genes can be synthesized (Refer Figure 12.1).

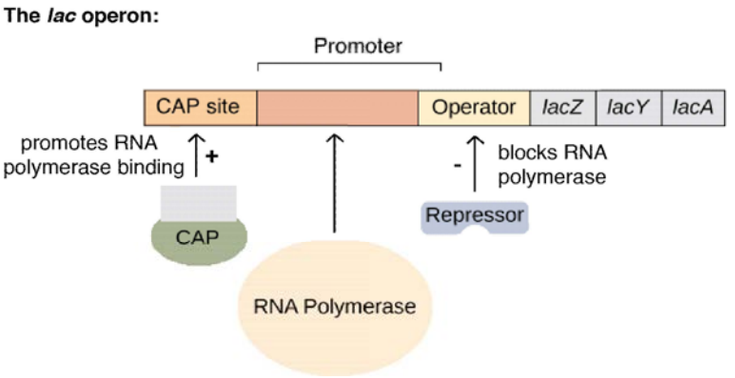


Fig. 12.1 *Structure of the Lac operon and the adjacent lacR Repressor gene.*

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The Lac operon is also under positive gene regulation. While the removal of the repressor protein in the presence of lactose is required for synthesis of the lacZ, lacY, and lacA genes, the gene expression will remain low. The level of gene expression is controlled by the amount of the preferred energy source, glucose, in the cell. This control is regulated by an allosteric regulatory protein, catabolite activator protein (CAP). When glucose levels in the cell are low, the organic molecule cyclic AMP is in high concentration. Cyclic AMP activates CAP by binding to the allosteric sites, causing CAP to attach to the Lac operon promoter. Unlike the repressor proteins, binding of CAP to the Lac operon stimulates gene expression. When the cell glucose levels increase, the cyclic AMP levels in the cell decrease, and the activator protein will disassociate from the promoter. Transcription will return to low levels, or will turn off if the repressor protein reattaches (Refer Figure 12.1).

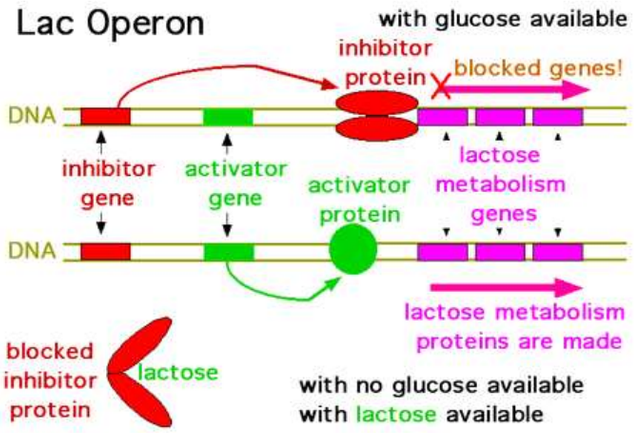


Fig. 12.2 *Lac Operon When Both Glucose and Lactose are Present*

The Figure 12.3 depicts the Lac operon and how its gene expression is under both positive and negative control.

The lac operon exhibits both systems. It is a negative control system because expression is typically blocked by an active repressor (the lac repressor) that turns off transcription. The lac repressor binds to the operator region and negatively controls (prevents) transcription.

However, when CAP (Catabolite Gene Activating Protein) binds upstream of this operator region near the promoter and transcription increases, this is an example of a positive control system. We see this positive control of transcription happen when glucose levels decline.

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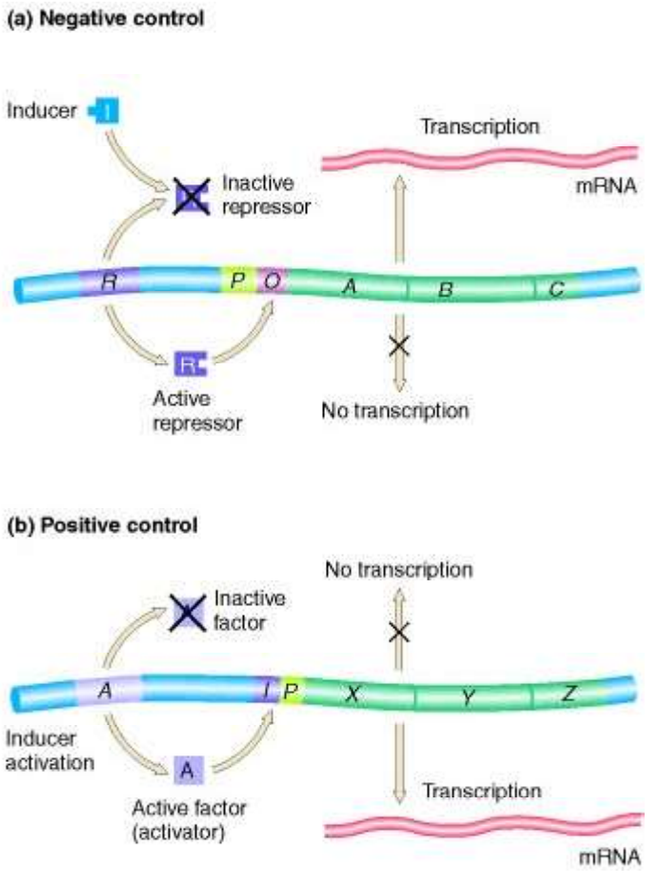


Fig. 12.3 *Positive and Negative Control in Lac Operon*

Positive Regulation of the lac Operon

- A bacterium's environment is too complex for its genes to be controlled by one signal.
- Other factors besides lactose affect the expression of the lac genes, such as the availability of glucose.
- Glucose, is *E. coli's* preferred energy source. Other sugars can serve as the main or sole nutrient, but extra steps are required to prepare them for entry into glycolysis, necessitating the synthesis of additional enzymes. Clearly, expressing the genes for proteins that metabolize sugars such as lactose or arabinose is wasteful when glucose is abundant.

NOTES

Expression of the Lac Operon when Both Glucose and Lactose are Present

- A regulatory mechanism known as catabolite repression restricts expression of the genes required for catabolism of lactose, arabinose, and other sugars in the presence of glucose, even when these secondary sugars are also present.
- The effect of glucose is mediated by cAMP, as a co-activator, and an activator protein known as cAMP receptor protein, or CRP (the protein is sometimes called CAP, for catabolite gene activator protein).
- CRP is a homodimer (subunit Mr 22,000) with binding sites for DNA and cAMP.
- Binding is mediated by a helix-turnhelix motif within the protein's DNA-binding domain. When glucose is absent, CRP-cAMP binds to a site near the lac promoter and stimulates RNA transcription 50-fold.
- CRP-cAMP is therefore a positive regulatory element responsive to glucose levels
- CRP-cAMP has little effect on the lac operon when the Lac repressor is blocking transcription, and dissociation of the repressor from the lac operator has little effect on transcription of the lac operon unless CRP-cAMP is present to facilitate transcription; when CRP is not bound, the wild-type lac promoter is a relatively weak promoter.
- The open complex of RNA polymerase and the promoter does not form readily unless CRP-cAMP is present.
- CRP interacts directly with RNA polymerase through the polymerase's á subunit.
- The effect of glucose on CRP is mediated by the cAMP interaction. CRP binds to DNA most avidly when cAMP concentrations are high.
- In the presence of glucose, the synthesis of cAMP is inhibited and efflux of cAMP from the cell is stimulated. As [cAMP] declines, CRP binding to DNA declines, thereby decreasing the expression of the lac operon.
- Strong induction of the lac operon therefore requires both lactose (to inactivate the lac repressor) and a lowered concentration of glucose (to trigger an increase in [cAMP] and increased binding of cAMP to CRP.
- CRP and cAMP are involved in the coordinated regulation of many operons, primarily those that encode enzymes for the metabolism of secondary sugars such as lactose and arabinose.
- A network of operons with a common regulator is called a regulon.

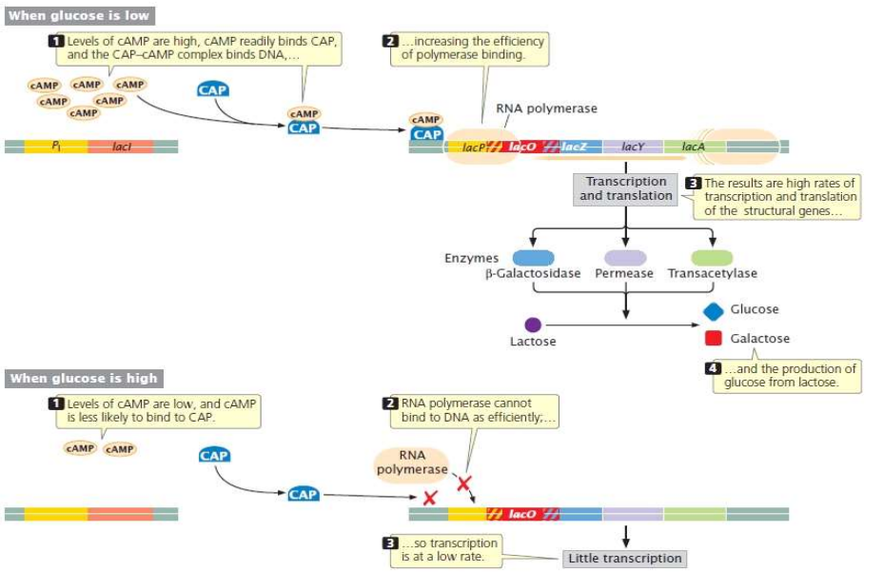


Fig. 12.4 *The Catabolite Activator Protein (CAP) Binds to the Promoter of the Lac Operon and Stimulates Transcription.*

CAP must complex cAMP before binding to the promoter of the lac operon. The binding of cAMP–CAP to the promoter activates transcription by facilitating the binding of RNA polymerase. Levels of cAMP are inversely related to glucose: low glucose stimulates high cAMP; high glucose stimulates low cAMP

12.3.2 Tryptophan operon

The tryptophan operon is the regulation of transcription of the gene responsible for biosynthesis of tryptophan from chorismate.

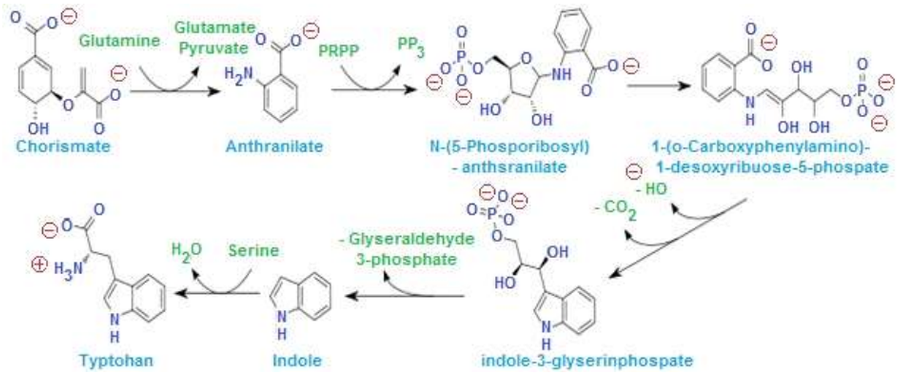


Fig. 12.5 Trp Operon

Tryptophan operon consists of structural gene and regulatory gene.

I. Regulatory gene are Promoter, Repressor, Operator, and Leader sequence.

NOTES

II. Structural gene are TrpE, TrpD, TrpC, TrpB and TrpA

1. trpE: It encodes the enzyme Anthranilate synthase I
2. trpD: It encodes the enzyme Anthranilate synthase II
3. trpC: It encodes the enzyme N-5'-Phosphoribosyl Anthranilate isomerase and Indole-3-glycerolphosphate synthase
4. trpB: It encodes the enzyme tryptophan synthase-B sub unit
5. trpA: It encode the enzyme tryptophan synthase-A sub unit

Tryptophan operon is regulated by following mechanism:

1. Repression

(i) When Tryptophan is Absent in Cell

Repressor gene (trpR) encodes the repressor protein which is originally inactive. In the absence of tryptophan, transcription of structural gene occur for the biosynthesis of tryptophan from chorismate (Refer Figure 12.6)..

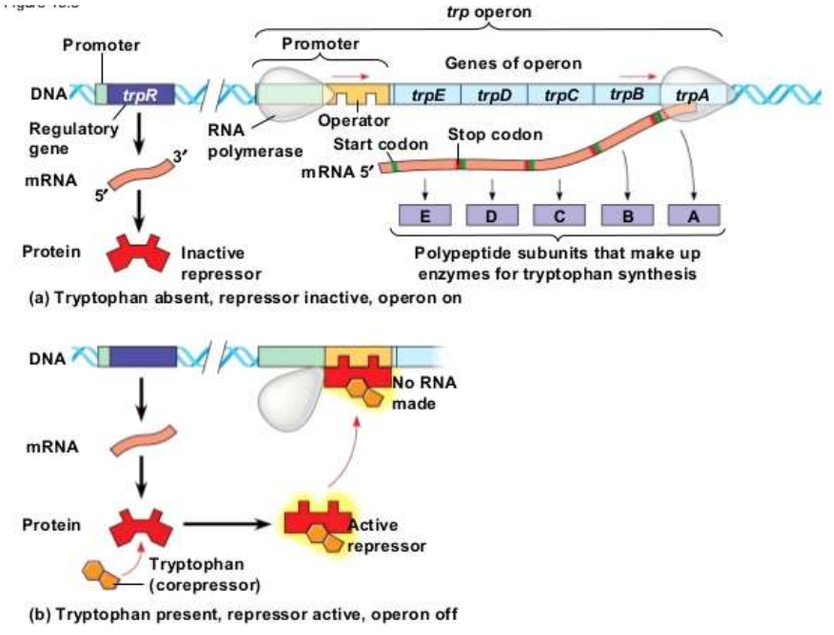


Fig. 12.6 (a) *Tryptophan Absent, Repressor Inactive, Operon on (b) Tryptophan Absent, Repressor Inactive, Operon Off*

(ii) When Tryptophan is High in Cell

When tryptophan is high in cell then it binds with repressor protein and change its confirmation so that it become active and bind to the operator near promoter.

Binding of repressor protein to operator overlaps the promoter, so RNA polymerase cannot bind to the promoter. Hence transcription is halted.

Since tryptophan is already high in cell, no transcription of structural gene is required for biosynthesis of tryptophan. This is also known as negative regulation.

2. Attenuation

NOTES

In bacteria, transcription and translation occurs simultaneously. The translation starts before transcription completes. In this attenuation mechanism, rate of translation determines whether transcription continues or terminates. Therefore the attenuation mechanism is only found in bacteria but not in eukaryotic cell.

Leader sequence (trpL) play important role in attenuation. Leader sequence contains such a nucleotide sequence that mRNA transcribed from it contains four specific region. Region 1, region 2, region 3 and Region 4. Region 3 is complementary to both region 2 and region 4.

If region 3 and region 4 base pair with each other, they form a loop like structure called attenuator and it function as transcriptional termination. If pairing occur between region 3 and region 2, then no such attenuator form so that transcription continues.

Region 1 is the most important region that determines whether to form loop between region 2-region 3 or region 3-region 4. The region 1 consists of sequence of 14 codons, out of which two codons are tryptophan codon (codon 10 and 11) (Refer Figure 12.7).

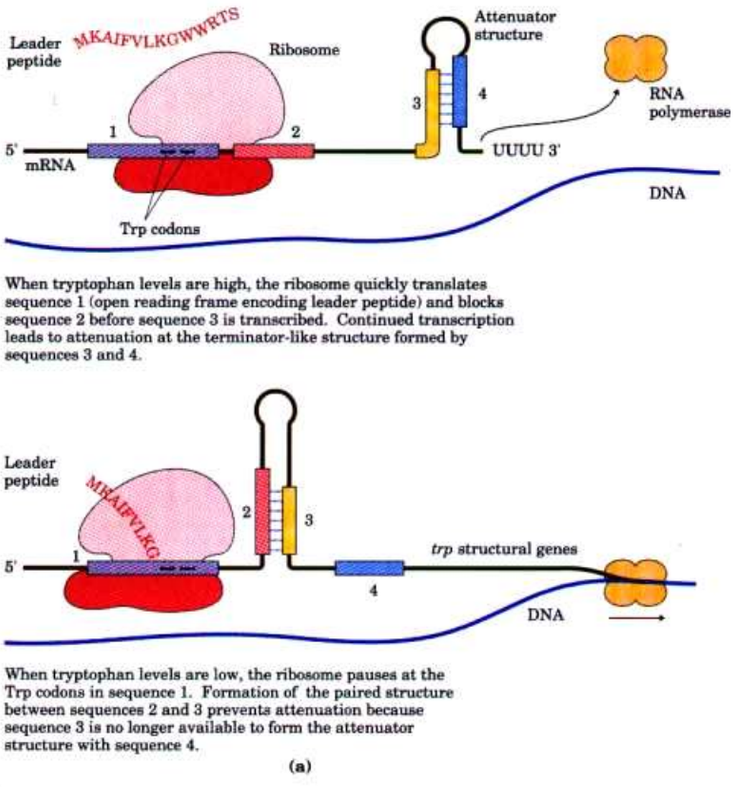


***Fig. 12.7** Attenuation*

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When tryptophan is high in cell than tRNA carrying tryptophan encodes codon 10 and 11. Such that ribosome encloses the region 2 which is near to the tryptophan codon. Hence region 3 base pair with region 4 to form attenuator as region 2 is not available for pairing. Consequently, transcription is halted.

When tryptophan is low or absent in cell, then translation stops at the position of tryptophan codon. Such that loop between region 2 and region 3 forms. Transcription continues.

Feedback Mechanism of Trp Operon

1. When tryptophan is high in cell then transcription of structural gene does not occur.
2. When tryptophan is absent or very low then transcription continues.
3. If chorismate is high in cell then it favors the transcription of structural gene.
4. If chorismate is low or absent in cell then it inhibit transcription.

Check Your Progress

4. Who identified the operons as a mode of gene expression control in 1961?
5. Which operon is involved in histidine biosynthesis?
6. What is a repressible operon?

12.4 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. It is a DNA segment between an operon's promoter and structural genes, which controls access of RNA polymerase to structural genes.
2. A DNA segment between an operon's promoter and structural genes, which controls access of RNA polymerase to structural genes.
3. Repressible enzymes are enzymes which have their synthesis inhibited by a metabolite (for example, tryptophan).
4. Operons were first identified as a mode of gene expression control in 1961 by François Jacob and Jacques Monod.
5. His operon is involved in histidine biosynthesis.
6. A repressible operon is one that is usually on but which can be repressed in the presence of a repressor molecule. The repressor binds to the operator in such a way that the movement or binding of RNA polymerase is blocked and transcription cannot proceed.

NOTES

12.5 SUMMARY

- Operon is a regulated cluster of adjacent structural genes with related functions.
- Repressor is a specific protein that binds to an operator and blocks transcription of the operon.
- An inducer is a molecule that regulates gene expression. An inducer can bind to protein repressors or activators.
- Operons are present in prokaryotes (bacteria and archaea), but are absent in eukaryotes.
- Operons are regions of DNA that contain clusters of related genes. They are made up of a promoter region, an operator, and multiple related genes.
- Operons can be under negative or positive control.
- The Lac operon is the classic operon example, and is responsible for the degradation of the milk protein lactose.
- The tryptophan operon is the regulation of transcription of the gene responsible for biosynthesis of tryptophan from chorismate.
- Tryptophan operon consists of structural gene and regulatory gene.
- Tryptophan operon is regulated by following mechanism: Repression and Attenuation.

12.6 KEY WORDS

- **Operon:** An operon is a cluster of functionally-related genes that are controlled by a shared operator. Operons consist of multiple genes grouped together with a promoter and an operator.
- **Repressor**: In molecular genetics, a repressor is a DNA- or RNA-binding protein that inhibits the expression of one or more genes by binding to the operator or associated silencers.
- **Inducer:** An inducer is a molecule that regulates gene expression. An inducer can bind to protein repressors or activators.

12.7 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short Answer Questions

1. Define the following terms: Operon, Repressor, Inducer, Polycistronic mRNA and Regulatory genes.

2. Write a short note on CRP (cAMP receptor protein).
3. Discuss tryptophan operon in brief.
4. What do you understand by positive and negative control of operons?

NOTES

Long Answer Questions

1. Differentiate between repressible enzymes and inducible enzymes.
2. Describe the structure and functions of operons.
3. Briefly discuss the lac operon.
4. What do you understand by the expression of the lac operon when both glucose and lactose are present?
5. Discuss the regulation of tryptophan operon.

12.8 FURTHER READINGS

Hunter, L. E. 2009. *The Process of Life- An Introduction to Molecular Biology*. USA: The MIT Press.

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Gupta, P.K. 1999. *Cell and Molecular Biology*. Meerut: Rastogi Publications.

UNIT 13 HARMONAL REGULATION OF GENE EXPRESSION

NOTES

Structure

13.0 Introduction
13.1 Objectives
13.2 Hormonal Regulation of Gene Expression
13.2.1 Hormonal Control of Gene Expression
13.3 Steroid Receptors
13.4 Transcription Factors
13.4.1 Function of Transcription Factors
13.5 DNA Binding Motifs in Prokaryotes and Eukaryotes
13.6 Answers to Check Your Progress Questions
13.7 Summary
13.8 Key Words
13.9 Self Assessemnt Questions and Exercises
13.10 Further Readings

13.0 INTRODUCTION

In mammals hormones can be proteins or steroids. The protein hormones do not enter the cell, but bind to receptors in the cell membrane and mediate gene expression through intermediate molecules. Steroids, though actually enter the cell and interact with steroid receptor proteins to control gene expression. Regulation of gene expression includes a wide range of mechanisms that are used by cells to increase or decrease the production of specific gene products (protein or RNA), and is informally termed gene regulation. Sophisticated programs of gene expression are widely observed in biology, for example to trigger developmental pathways, respond to environmental stimuli, or adapt to new food sources. Virtually any step of gene expression can be modulated, from transcriptional initiation, to RNA processing, and to the post-translational modification of a protein. Often, one gene regulator controls another, and so on, in a gene regulatory network.

Gene regulation is essential for viruses, prokaryotes and eukaryotes as it increases the versatility and adaptability of an organism by allowing the cell to express protein when needed. Although as early as 1951, Barbara McClintock showed interaction between two genetic loci, Activator (Ac) and Dissociator (Ds), in the color formation of maize seeds, the first discovery of a gene regulation system is widely considered to be the identification in 1961 of the lac operon, discovered by François Jacob and Jacques Monod, in which some enzymes involved in lactose metabolism are expressed by *E. coli* only in the presence of lactose and absence of glucose. The initiating event leading to a change in gene expression includes activation or deactivation of receptors.

NOTES

In this unit, you will study about hormonal regulation of gene expression. Hormones are molecules that are produced in one cellular location in an organism, and whose effects are seen in another tissue or cell type.

13.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand hormonal regulation of gene expression
- Know about transcription factors and steroid receptors
- Discuss DNA binding motifs in prokaryotes and eukaryotes

13.2 HORMONAL REGULATION OF GENE EXPRESSION

Classification of gene with respect to their expression:

Constitutive (housekeeping) Genes

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

Controllable genes

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

Several steps in the gene expression process may be modulated, including the

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

The process of alteration of gene expression been studied has been studied in detail and involves modulation of gene transcription. Transcription control can result in tissue specific gene expression and influenced by hormones, heavy metals etc. In simple terms, regulation of gene expression is of two types:

1. Negative regulation
2. Positive regulation

1. Negative regulation

When the expression of genetic information is diminished by the presence of specific regulatory element, it is called the negative regulation. The element or molecule mediating the negative regulation is called a negative regulator (Refer Figure 13.1)

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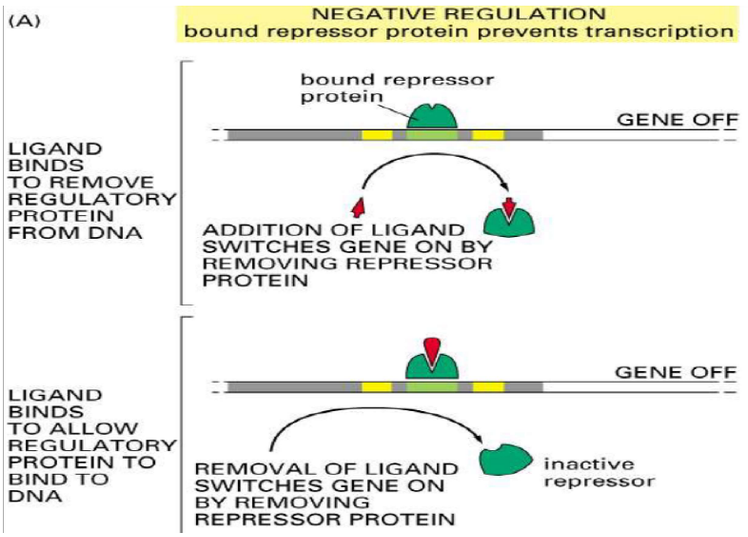


Fig. 13.1 *Negative regulation*

2. Positive Regulation

When the expression of genetic information is quantitatively increased by the presence of specific regulatory element, it is known as positive regulation. Elements or molecules modulating positive regulation are known as positive regulator (Refer Figure 13.2).

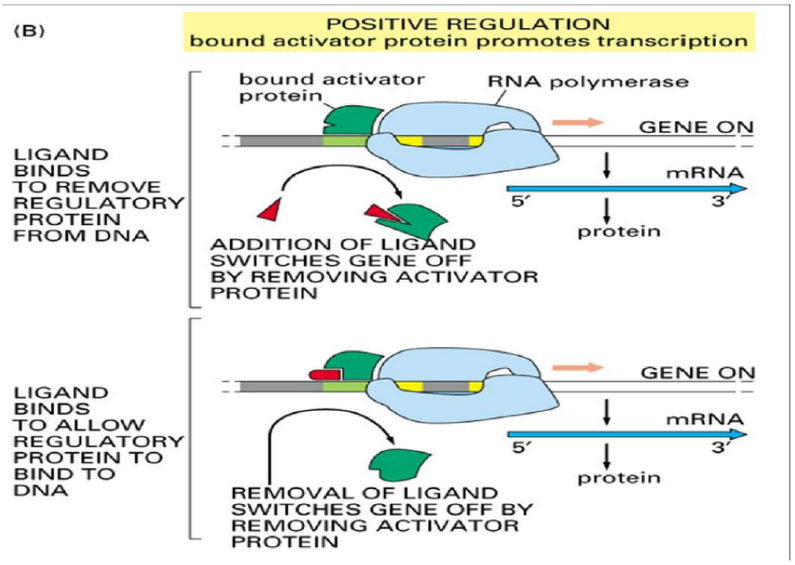


Fig. 13.2 *Positive Regulation*

13.2.1 Hormonal Control of Gene Expression

Hormones are molecules that are produced in one cellular location in the human body and whose effects are seen in another tissue or cell type. Hormones can be

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proteins or steroids for example, insulin, epinephrine estrogen, progesterone, testosterone control gene expression. The protein hormones do not enter the cell, but bind to receptors in the cell membrane and mediate gene expression through intermediate molecules. The steroid hormones enter the cell and interact with steroid receptor proteins to control gene expression.

Peptide hormone action

Hormones are synthesized in various specialized secretory cells (endocrine cells) and are released into blood stream. The peptide hormones do not normally enter the cells because of their relative large size. Their effect is mediated by receptor proteins located in target cell membranes and by the intracellular level of cAMP (called the secondary messenger). The cAMP activates a protein kinase (A-Kinase) which phosphorylates (activates) many specific enzymes (Refer Figure 13.3).

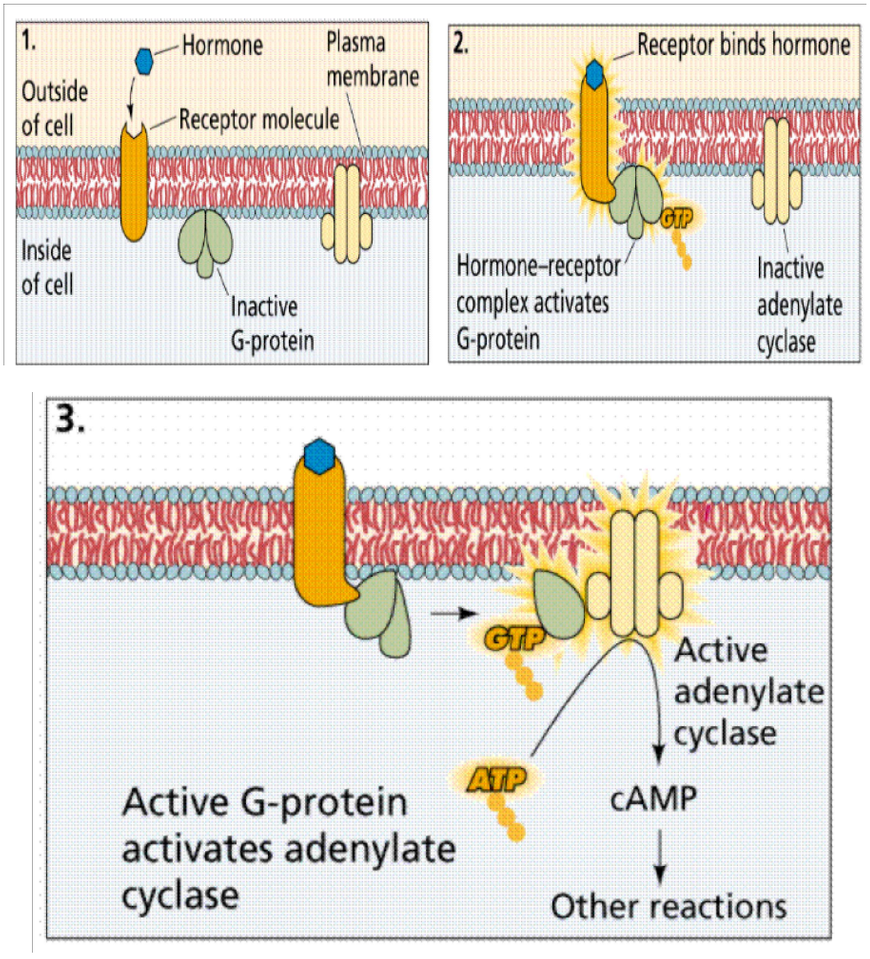


Fig. 13.3 *Peptide Hormone Action*

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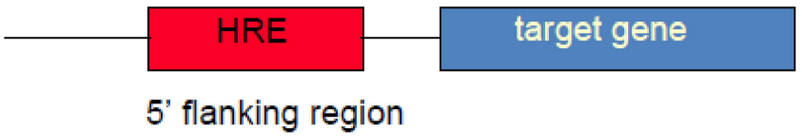
Steroid Hormone Action

Steroids, like all hormones, act to transmit information. However, the information being transmitted is not contained within the actual hormone molecule; instead, the hormone acts as a signal to activate (or deactivate) cellular processes. The precise nature of these processes depend on the cell type. Different types of hormones use different mechanisms to affect cellular functioning. The following discussion applies to steroids and related hormones; other lecturers and later chapters discuss other types of hormones and their mechanisms. A great deal has been learned about steroid hormone action recently as a result of improved techniques. All of the well characterized steroid hormone receptors are intracellular proteins. The steroid hormone enters the cell, probably by passive diffusion, and binds the receptor. The binding of hormone triggers a hormone-dependent activation of the receptor (a conformational change, and possibly phosphorylation). The activated receptor interacts with DNA and with other nuclear proteins, resulting in a change in the rate of mRNA transcription. The mRNA is then translated into new proteins which either have direct effects or return to the nucleus to alter the rate of transcription of other genes; in combination, these changes yield the biological effects associated with the steroid hormone. (Note the "±" next to the DNA; the effect of the activated receptor, and of any new proteins returning to the nucleus may be an increase or a decrease in the rate of transcription.) (Refer Figure 13.4).

Steroid hormones are small molecules that readily enter cells through plasma membrane. Once inside the appropriate target cells, the steroid hormones become tightly bound to specific receptor proteins which are present only in the cytoplasm of target cells. The hormone-receptor protein complex activate the transcription of specific genes according to following two methods:

1. The H-R PC interact with specific non-histone chromosome proteins and this interaction stimulates the transcription of correct genes.

2. H-R PC activate transcription of target genes by binding to specific DNA sequences present in the cis-acting regulatory regions of the genes.



In both of these cases, the H-R PC would function as positive regulators or activators of transcription. Glucocorticoid hormones influence nutrient metabolism in most of the body cells by promoting the metabolism of glucose, proteins and fats. The effect of glucocorticoids is to activate the transcription of specific genes. The hormone is released from the endocrine cells and secreted into the blood stream when the individual is fasting and needs to regulate its blood levels of glucose, a.a- and fats. The hormone molecules diffuse across the plasma membrane of target cells and bind to glucocorticoid receptors.

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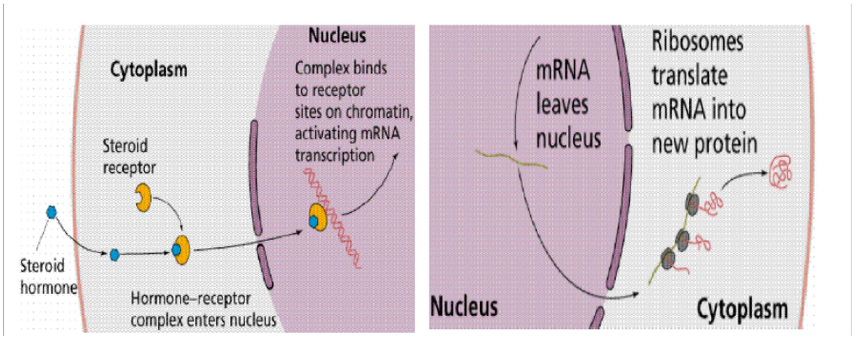


Fig. 13.4 *Steroid Hormone Action*

Check Your Progress

1. What are constitutive genes?
2. What are the types of regulation of gene expression?
3. What are hormones?

13.3 STEROID RECEPTORS

Eukaryotic cells are capable of activating gene expression in response to hormones secreted by other cells. Steroid hormones enter the cell by diffusing through the membrane. Once inside the cell, they bind to a specific receptor. The receptor complexed with the hormone is now capable of activating transcription of genes that contain a hormone-responsive element (HRE). In some ways the mechanism of action of steroid hormone regulation is similar to the mechanism of action of the arabinose operon regulation in prokaryotes. Specifically, a small external effector molecule (arabinose or the hormone) binds to a cytoplasmic transcription factor (AraC or steroid receptor) which then binds to a specific DNA element (araO or HRE) and activates transcription.

The Steroid Hormone Receptor Superfamily

All of the characterized steroid hormone receptors are members of a large evolutionarily-related superfamily of proteins. The family also includes receptors for thyroid hormone and some metabolites of Vitamins A and D. This family of proteins is also called the nuclear receptor superfamily, because the actions of these proteins are thought to occur also a result of gene transcription modulatory effects exerted within the nucleus. Members of the superfamily are thought to be present in most if not all multicellular animals. Table 13.1 shows the names of some of the human protein known to be members of the superfamily, the size of the receptor protein (in amino acids), and the chromosomal location of the gene. The receptor genes are widely scattered throughout the genome (note that the androgen receptor is on the X chromosome!), and vary in size from 427 amino

acids for the Vitamin D receptor to 984 amino acids for the mineralocorticoid receptor.

Table 13.1 Members of the Steroid Hormone Receptor Superfamily

Receptor	Size (amino acids)	Chromosome
Thyroid hormone-α	490	17
Thyroid hormone-β	456	3
Vitamin D	427	12
Retinoic Acid-α	462	17
Retinoic Acid-β	448	3
Retinoic Acid-γ	454	12
Retinoid-X-α	462	9
Retinoid-X-β	533	6
Retinoid-X-γ	454	1
Estrogen-α	595	6
Estrogen-β	477	14
Glucocorticoid (GR Type II)	777	5
Mineralocorticoid (GR Type I)	984	4
Androgen	919	X
Progesterone	934	11

NOTES

The thyroid hormones tri iodo thyronine and tetra iodo thyronine, T3 and T4 have marked effect on the growth, development and metabolic function of virtually all organ systems and tissues of human body. A significant amount of T3 is derived from T4 by 5' deiodinase in various tissues. Because of the marked alteration in the rate of oxygen consumption identified in hypothyroidism and hyperthyroidism, initial studies on mechanism of action of the thyroid hormones focused on mitochondrial function. Experimental works shows that T-H administration increased the rate of RNA synthesis, specially stimulating the accumulation of mRNAs which codes for specific proteins

The following T-H depended effects have also been analyzed:

1. Stimulation and regulation of growth hormone gene expression
2. Stimulation of malic enzyme mRNA in the liver
3. Stimulation of several other genes that encode hepatic proteins of unknown function.
4. Stimulation of the alpha-myosin heavy chain gene in the myocardium.
5. Inhibition of thyrotrophin and the beta-myosin heavy chain gene.

13.4 TRANSCRIPTION FACTORS

Transcription factors are DNA-binding proteins that play a key role in gene transcription. They are modular in structure and heterodimeric. Built within the transcription factor is a DNA-binding domain and several sites for the other transcription co-regulators to bind. Transcription factors bind to short conserved sequences located within each promoter along the strands of DNA. Ultimately, transcription factors can be thought of as the 'gatekeepers' that determine if a

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gene is expressed or not. In eukaryotic cells, this is made possible with the help of activator and repressor protein variants.

A typical transcription factor binds to DNA at a certain target sequence. Once it's bound, the transcription factor makes it either harder or easier for RNA polymerase to bind to the promoter of the gene.

Activators

Some transcription factors activate transcription. For instance, they may help the general transcription factors and/or RNA polymerase bind to the promoter, as shown in the Figure 13.5.

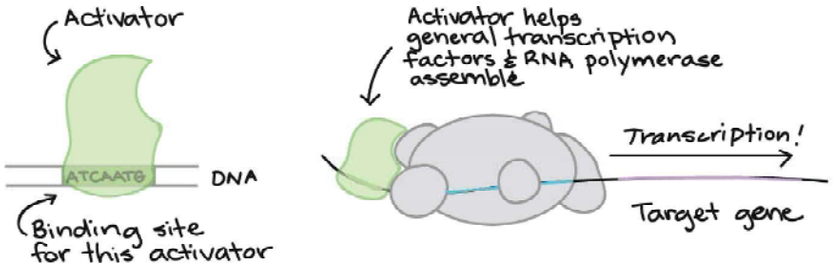


Fig. 13.5 *Activators*

Repressors

Other transcription factors repress transcription. This repression can work in a variety of ways. As one example, a repressor may get in the way of the basal transcription factors or RNA polymerase, making it so they can't bind to the promoter or begin transcription (Refer Figure 13.6)..

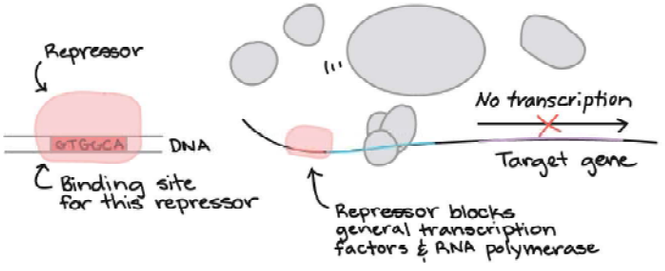


Fig. 13.6 *Repressors*

Activator proteins help to 'activate' mRNA transcription by binding to pieces of DNA called enhancers. Enhancers are essentially short (50-1500 base pair) stretches of DNA that, when bound to activators, make the transcription of a gene more likely. This causes a conformational change in the three-dimensional structure of DNA that brings them closer to a gene promoter to kick-start transcription, even if the promoter was thousands of base pairs away. This builds a transcription initiation complex that can recruit RNA polymerase to initiate transcription. The

main purpose of these elements is to bring the factors they bind, closer to the initiation complex. On the other hand, we have repressors that bind directly to the promoter and enhancer regions to physically block transcription. Repressors are physically responsive to external stimuli while corepressors help repress the start of transcription by recruiting enzyme histone deacetylase (this family of enzymes removes acetyl groups from lysine amino acids on histones, allow the histones to wrap more tightly around DNA so that it cannot unravel or allow transcription).

13.4.1 Function of Transcription Factors

The principal role transcription factors play is in allowing cells to differentiate. Through their ability to initiate or repress site-specific transcription, each cell in our bodies can differentiate into a different cell type despite containing the same exact genetic code. Turning genes on or off allows cells to potentiate into the different tissues and organs that make up our bodies. (In fact, embryonic and adult stem cells are a class of undifferentiated cells that can differentiate into the cell type that it is placed next to.)

Transcription factors also make genetic fine-tuning possible. Modulating the activity and the amount of transcription factor can upregulate (increase) or down regulate (decrease) the rates of the chosen gene's transcription. Therefore, these alterations not only allow a gene to be expressed, but they determine at which level each gene is expressed. For example, when the insulin levels in our blood are elevated, our cells trigger a down regulation in the expression of insulin receptor. So, although insulin receptors continue to be made for life-sustaining purposes, the levels of expression subside to accommodate our body's new internal conditions.

Transcription Factor Classification

Transcription factors generally fall into three categories, largely depending on their mechanism of action.

- Mechanistic transcription factors rely on their physicality to stimulate or repress the transcription of the selected gene by binding upstream of the initiation site. They are ubiquitous and transcribe all class II genes (which code for proteins via RNA polymerase II).
- Structural transcription factors are classified by sequence similarity and the resultant tertiary structure of their DNA-binding domains.

Finally, Functional transcription factors are classified by their function and contain several variants.

Constitutively active factors are present in cells at all times. Conditionally active factors require activation. Developmental types are tightly regulated until they are expressed and require no additional activation. Signal dependent types require an external influence, whether that is an extracellular (endocrine) or an intracellular (autocrine) ligand or a receptor-dependent cascade of activations.

13.5 DNA BINDING MOTIFS IN PROKARYOTES AND EUKARYOTES

NOTES

DNA binding motifs on the protein chain permit it to interlock with the major groove of the DNA helix.

1. The Helix-Turn-Helix Motif
2. Homeodomain Motif
3. The Zinc Finger Motif
4. The Leucine Zipper Motif

1. The Helix-Turn-Helix motif

This motif is constructed from two alpha-helical segments of the protein linked by a short non-helical segment, the turn. This motif has been identified in hundreds of DNA- binding proteins (Refer Figure 13.7).

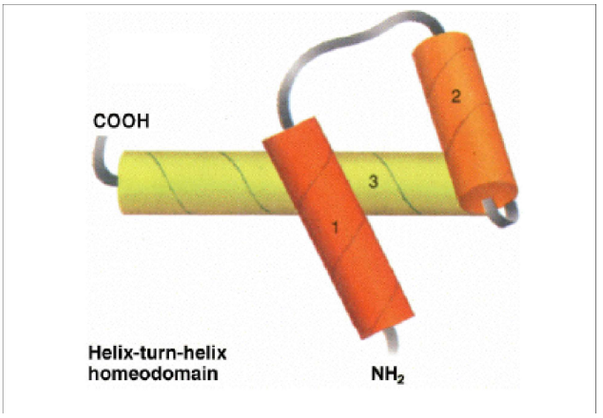


Fig. 13.7 *Helix-Turn-Helix Homeodomain*

2. Homeodomain Motif

It is a special class of Helix-Turn-Helix motif. More than 50 of these regulatory proteins have been analyzed and they all contain a nearly identical sequence of 60 amino acids, the homeodomain.

3. The Zinc Finger Motif

This uses one or more zinc atoms to coordinate its binding to DNA called zinc fingers. The more zinc fingers in the cluster, the stronger the protein binds to the DNA (Refer Figure 13.8).

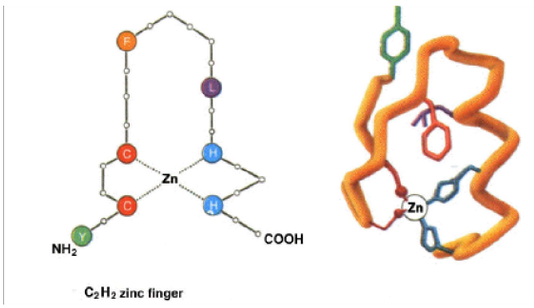


Fig. 13.8 Zinc Finger

4. The Leucine Zipper Motif

Here two different protein subunits cooperate to create a single DNA binding site. This motif is created where a region on one of the subunits containing several hydrophobic amino acids (usually Leucine) interacts with a similar regions on the other subunit (Refer Figure 13.8)..

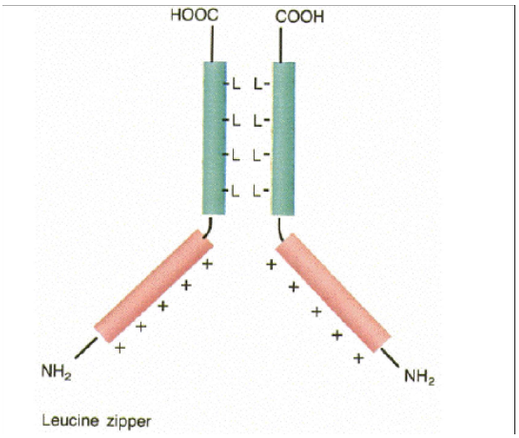


Fig. 13.9 Leucine Zipper

Check Your Progress

4. What are activators?
5. What are repressors?

13.6 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

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

NOTES

2. Regulation of gene expression is of two types: positive and negative.
3. Hormones are molecules that are produced in one cellular location in the human body and whose effects are seen in another tissue or cell type. Hormones can be proteins or steroids.
4. Activators are transcription factors that activate transcription. For instance, they may help the general transcription factors and/or RNA polymerase bind to the promoter.
5. Repressors are transcription factors that repress transcription. This repression can work in a variety of ways. As one example, a repressor may get in the way of the basal transcription factors or RNA polymerase, making it so they can't bind to the promoter or begin transcription.

13.7 SUMMARY

- There are two classes of gene with respect to their expression: Constitutive (housekeeping) genes and Controllable genes.
- Several steps in the gene expression process may be modulated, including the 1. Transcription, 2. RNA splicing, 3. Translation, and 4. Post-translational modification of a protein.
- When the expression of genetic information is diminished by the presence of specific regulatory element, it is called the negative regulation.
- When the expression of genetic information is quantitatively increased by the presence of specific regulatory element, it is known as positive regulation.
- Hormones are molecules that are produced in one cellular location in the human body and whose effects are seen in another tissue or cell type. Hormones can be proteins or steroids E.g., insulin, epinephrine estrogen, progesterone, testosterone control gene expression.
- Steroids, like all hormones, act to transmit information. However, the information being transmitted is not contained within the actual hormone molecule; instead, the hormone acts as a signal to activate (or deactivate) cellular processes.
- All of the characterized steroid hormone receptors are members of a large evolutionarily-related superfamily of proteins. The family also includes receptors for thyroid hormone and some metabolites of Vitamins A and D.
- All of the characterized steroid hormone receptors are members of a large evolutionarily-related superfamily of proteins. The family also includes receptors for thyroid hormone and some metabolites of Vitamins A and D.
- DNA binding motifs on the protein chain permit it to interlock with the major groove of the DNA helix. 1. The Helix-Turn-Helix motif, 2. Homeodomain motif, 3. The Zinc Finger motif, 4. The Leucine Zipper motif.

NOTES

13.8 KEY WORDS

- **Gene:** A gene is the basic physical and functional unit of heredity. Genes are made up of DNA
- **Steroid:** Any of a large class of organic compounds with a characteristic molecular structure containing four rings of carbon atoms (three six-membered and one five). They include many hormones, alkaloids, and vitamins.
- **DNA:** Deoxyribonucleic acid, a self-replicating material which is present in nearly all living organisms as the main constituent of chromosomes. It is the carrier of genetic information.

13.9 SELF ASSESSEMNT QUESTIONS AND EXERCISES

Short Answer Questions

1. What are the classes of gene with respect to their expression?
2. Discuss negative regulation.
3. Discuss positive regulation.
4. Write a short note on steroid hormone action.

Long Answer Questions

1. How peptide hormone bonds are formed?
2. Explain steroid receptors.
3. What are transcription factors?
4. What are the function of transcription factors?
5. Discuss DNA binding motifs in prokaryotes and eukaryotes.

13.10 FURTHER READINGS

Hunter, L. E. 2009. *The Process of Life- An Introduction to Molecular Biology*. USA: The MIT Press.

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NOTES

UNIT 14 ANALYSIS OF GENE EXPRESSION USING MOLECULAR BIOLOGY

Structure

14.0 Introduction
14.1 Objectives
14.2 Analysis of Gene Expression Using Molecular Methodology
14.3 Polymerase Chain Reaction (PCR)
- 14.3.1 Factors Affecting PCR
- 14.3.2 Types of PCR
- 14.3.3 Applications

14.4 Southern Blotting
- 14.4.1 Applications

14.5 Western Blotting
- 14.5.1 Applications

14.6 Real Time PCR
- 14.6.1 Non-specific Detection using DNA Binding Dyes
- 14.6.2 Specific Detection using Target Specific Probes
- 14.6.3 Applications
- 14.6.4 Real Time PCR VS Traditional PCR
- 14.6.5 Limitations of End-point PCR

14.7 Microarray
- 14.7.1 Microarray Technique
- 14.7.2 Types of Microarrays
- 14.7.3 Applications

14.8 Recombinant DNA Technology
- 14.8.1 Preparation of Recombinant DNA Technology

14.9 Sanger Sequencing
- 14.9.1 Method of Sanger Sequencing
- 14.9.2 Uses and Limitations

14.10 Next-Generation Sequencing
14.11 Cell Culture Methods
- 14.11.1 Significance of Cell Culture

14.12 Answers to Check Your Progress Questions
14.13 Summary
14.14 Keywords
14.15 Self Assessment Questions and Exercises
14.16 Further Peadings

14.0 INTRODUCTION

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product. These products are often proteins, but in

non-protein coding genes such as ribosomal RNA (rRNA) genes or transfer RNA (tRNA) genes, the product is a functional RNA. The process of gene expression is used by all known life - eukaryotes (including multicellular organisms), prokaryotes (bacteria and archaea) and viruses - to generate the macromolecular machinery for life. Several steps in the gene expression process may be modulated, including the transcription, RNA splicing, translation, and post-translational modification of a protein. Gene regulation gives the cell control over structure and function, and is the basis for cellular differentiation, morphogenesis and the versatility and adaptability of any organism.

Gene regulation may also serve as a substrate for evolutionary change, since control of the timing, location, and amount of gene expression can have a profound effect on the functions (actions) of the gene in a cell or in a multicellular organism. In genetics, gene expression is the most fundamental level at which genotype gives rise to the phenotype. The genetic code stored in DNA in form of nucleotide sequence is 'interpreted' by gene expression, and the properties of the expression products give rise to the organism's phenotype. A molecule which allows the genetic material to be realized as a protein was first hypothesized by François Jacob and Jacques Monod. RNA synthesis by RNA polymerase was established in vitro by several laboratories by 1965; however, the RNA synthesized by these enzymes had properties that suggested the existence of an additional factor needed to terminate transcription correctly. In 1972, Walter Fiers became the first person to actually prove the existence of the terminating enzyme. Roger D. Kornberg won the 2006 Nobel Prize in Chemistry for his studies of the molecular basis of eukaryotic transcription.

In this unit, you will study about PCR, southern blotting, western blotting, Real time PCR, microamay, recombinent DNA technology, Sanger sequencing, next generation sequencing cell culture methods in detail.

14.1 OBJECTIVES

After going through this unit, you will be able to:

- Understand the analysis of gene expression
- Know about various technologies in analysis of gene expression

14.2 ANALYSIS OF GENE EXPRESSION USING MOLECULAR METHODOLOGY

Gene expression is a highly regulated mechanism that controls the function and adaptability of all living cells including prokaryotes and eukaryotes. Several techniques exist for studying and quantifying gene expression and its regulation. Some of these techniques are old and well established while others are relatively new, multiplex techniques.

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The field of gene expression analysis has undergone major advances in biomedical research. Traditional methods focused on measuring the expression of one gene at a time and not in any particular biological context. However, today, mRNA expression techniques have led to improvements in gene identification and disease sub-classification.

Gene Expression Techniques

Analytical methods may be used to examine mRNA expression levels or differential mRNA expression. Some examples of these techniques are listed below.

Reporter Gene

A gene contains two functional segments. One is a coding DNA sequence, which contains the instructions for making a protein. The other is a DNA sequence called a promoter, which is linked to this coding region and regulates the gene's transcription, either by activating or suppressing its expression.

A reporter gene assay is used to determine the regulatory potential of a DNA sequence that is unknown. This involves a promoter sequence being linked to a detectable reporter gene such as luciferase, β-galactosidase or β-glucuronidase. Examples of methods used to determine the expressed reporter gene protein are fluorescence, absorbance and luminescence.

Northern Blotting

An adaptation of the Southern blot procedure used to detect specific sequences of RNA by hybridization with complementary DNA.

Southern Blotting

A procedure for identifying specific sequences of DNA, in which fragments separated on a gel are transferred directly to a second medium on which assay by hybridization may be carried out.

Western Blotting

Western blotting is a technique for detecting specific protein molecules within a protein mixture. This mixture might include all the proteins that are associated with a certain cell type or tissue. The technique can help to determine a protein's size, and how much of it is expressed.

Fluorescent in Situ Hybridization (FISH)

This is a cytogenetic technique that can be used to identify and locate specific gene sequences. FISH can be used to visualize copy number aberrations such as the deletion, translocation or amplification of chromosomes. The technique is used in prenatal diagnosis and also provides a useful tool in the diagnosis and predicted prognosis of various sarcomas. The technique is also used in dermatology to help evaluate atypical moles.

NOTES

Reverse Transcription Polymerase Chain Reaction (RT-PCR)

This is the most sensitive technique available for detecting and quantifying mRNA. Using RT-PCR, extremely small sample sizes can be used in the quantification of mRNA and the technique can in fact do this using just a single cell.

Serial Analysis of Gene Expression (SAGE)

SAGE is a technique used to create a library of short sequence tags which can each be used to detect a transcript. The expression level of the transcript can be determined by assessing how many times each tag is detected. This technology enables comprehensive expression analysis across the genome.

DNA Microarray

Also known of as biochip or DNA chip, a DNA microarray is a solid surface to which a collection of microscopic DNA spots are attached. The microarrays are used to determine expression levels across a large number of genes or to perform genotyping across different regions of a genome.

RNA Sequencing

This refers to methods used to measure the sequence of RNA molecules. Examples include shotgun sequencing of cDNA molecules acquired from RNA through reverse transcription and technologies used to sequence RNA molecules from a biological sample so that the primary sequence and abundance of each RNA molecule can be determined.

Tiling Arrays

A tiling array is a type of microarray chip, with labelled DNA or RNA targets hybridized to probes attached to a solid surface. However, the probes used differ to those used with traditional microarrays. Rather than known sequences or predicted genes being probed, tiling arrays probe for sequences known to be present in a contiguous region. This can provide information about regions that are sequenced but for which the local functions are largely unknown.

Analysis of Nucleic Acids

- Polymerase Chain Reaction (PCR)
- Gel electrophoresis Blotting techniques (Northern, Southern)

Gene Expression Analysis

- Real-time PCR
- Microarrays (DNA chips)
- Recombinant DNA technology (Cloning of DNA fragments)
- Sanger sequencing
- Next-generation sequencing

NOTES

Methods to study gene function

- Cell culture methods

Check Your Progress

1. What does FISH stand for?
2. What is DNA microarray?

14.3 POLYMERASE CHAIN REACTION (PCR)

Polymerase chain reaction is method for amplifying particular segments of DNA. It is an enzymatic method and carried out in vitro. PCR technique was developed by Kary Mullis in 1983. PCR is very simple, inexpensive technique for characterization, analysis and synthesis of specific fragments of DNA or RNA from virtually any living organisms.

Polymerase Chain Reaction - PCA

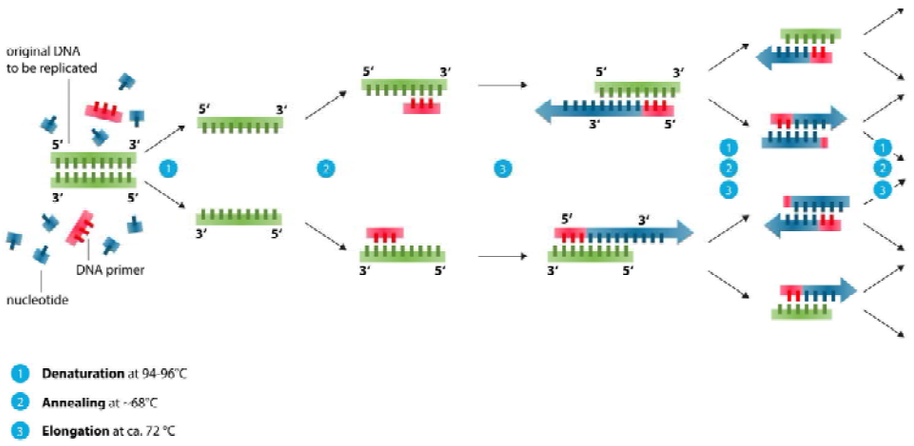


Fig. 14.1 Polymerase Chain Reaction

Procedure

PCR consists of three basic steps.

1. Denaturation

- Two strand of DNA separates (melt down) to form single stranded DNA
- This step is generally carried out at 92°-96° for 2 minutes.

2. Annealing

- Annealing of primer to each strand is carried out at 45C-55°

3. Extension

- DNA polymerase adds dNTPs complementary to templates strands at 3'end of primer. It is carried out at temperature of 72C. These three steps are repeated 20-30 times in an automated thermocycler that can heat and cool the reaction mixture in tube within very short time. This results in exponential accumulation of specific DNA fragments. The doubling of number of DNA strands corresponding to target sequences can be estimated by amplification number associated with each cycle using the formula. Amplification=2n, where n=no. of PCR cycle.
- PCR can amplify a desired DNA sequences of any origin hundred or millions time in a matter of hour, which is very short in comparison to recombinant DNA technology. PCR is especially valuable because the reaction is highly specific, easily automated and very sensitive. It is widely used in the fields like- clinical medicine for medical diagnosis, diagnosis of genetic diseases, forensic science; DNA finger printing, evolutional biology.

14.3.1 Factors Affecting PCR

(i) Primer

- PCR reaction needs two primer, a forward and a reverse primer
- Primer are synthesized oligonucleotide usually ranging from 15-30 bases long
- Primers are designed such that at 3'end they do not have more than two bases complementary to each other as this results in PRIMER-DIMER formation
- The G+C contents is in the range of 40-60%
- The melting temperature (Tm) of both forward and reverse primer is usually the same.
- Low concentration of primer results in poor yield while high concentration may results in nonspecific amplification. Hence optimal concentration of primer is needed i.e., 0.1-1μ

(ii) Amount of Template DNA

- Optimal amount of template DNA usually in nano gram. Higher concentration inhibit or results in nonspecific amplification.
- Taq DNA polymerase:
- Taq DNA polymerase is 94 KD thermostable DNA polymerase isolated from Thermus aquaticus.
- Optimal temperature for activity of Taq polymerase is 72°C but it can tolerate high temperature and do not affects by denaturating temperature of 94°C.

- Taq DNA polymerase have both 5'-3' polymerase activity and 5'-3' exonuclease activity. But it lacks 3'-5' exonuclease activity (proof reading activity).

NOTES

14.3.2 Types of PCR

1. Standard PCR

- Nested PCR
- Random amplified polymorphic DNA
- Long PCR
- Restriction fragment length polymorphism (RFLP)
- Amplified fragment length polymorphism (AFLP)
- Multiplex PCR
- Single cell PCR
- Fast cycling PCR
- In situ PCR
- High fidelity PCR
- Asymmetric PCR
- Repetitive sequence based PCR
- Overlap extension PCR
- Assemble PCR
- Mini primer PCR
- Solid phase PCR
- Touch Down PCR

2. Reverse transcriptase Polymerase chain reaction (RT-PCT): for RNA

- One step RT-PCR
- Two step RT-PCR

3. Real time PCR: for DNA or RNA

- Dye binding to ds DNA
- Fluorescent probes

14.3.3 Applications

- Forensic science: DNA finger printing, paternity testing and criminal identification
- Diagnosis: Molecular identification of microorganisms

- Evolution study: evolutionary biology
- Fossil study: paleontology
- Gene cloning and expression
- Gene sequencing
- Vaccine production by recombinant DNA technology
- Drug discovery
- Mutation study
- Human genome project

Check Your Progress

3. What are three basic steps in PCR?
4. What are the factors which affect PCR?

14.4 SOUTHERN BLOTTING

Principle

Southern blotting is an example of RFLP (restriction fragment length polymorphism). It was developed by Edward M. Southern (1975). Southern blotting is a hybridization technique for identification of particular size of DNA from the mixture of other similar molecules. This technique is based on the principle of separation of DNA fragments by gel electrophoresis and identified by labelled probe hybridization. Basically, the DNA fragments are separated on the basis of size and charge during electrophoresis. Separated DNA fragments after transferring on nylon membrane, the desired DNA is detected using specific DNA probe that is complementary to the desired DNA. A hybridization probe is a short (100-500bp), single stranded DNA. The probes are labeled with a marker so that they can be detected after hybridization (Refer Figure 14.2).

Procedure

1. Restriction digest: by RE enzyme and amplification by PCR
2. Gel electrophoresis: SDS gel electrophoresis
3. Denaturation: Treating with HCl and NaOH
4. Blotting
5. Baking and Blocking with casein in BSA
6. Hybridization using labelled probes
7. Visualization by autoradiogram

NOTES

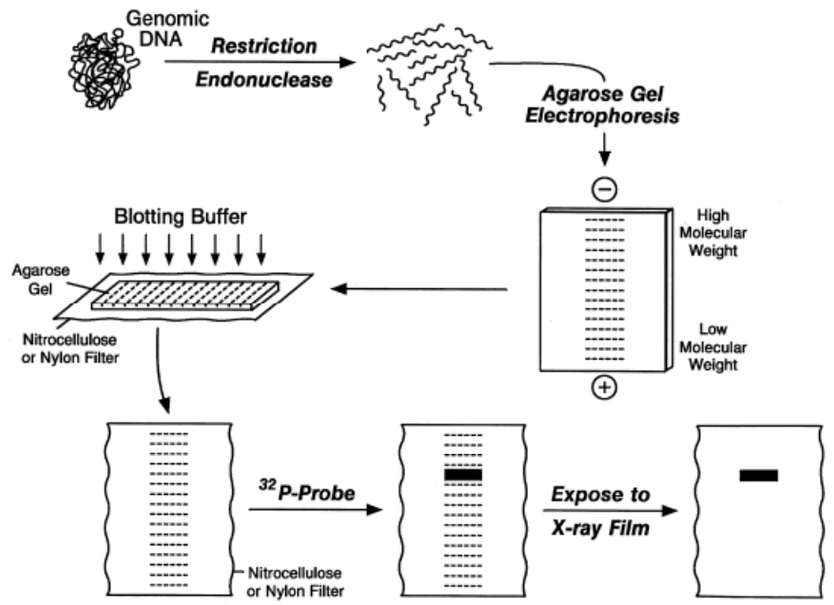


Fig. 14.2 *Southern Blotting*

Step I: Restriction Digest

- The DNA is fragmentized by using suitable restriction enzyme. RE cuts the DNA at specific site generating fragments
- The number of fragments of DNA obtained by restriction digest is amplified by PCR

Step II: Gel Electrophoresis

The desired DNA fragments is separated by gel electrophoresis

Step III: Denaturation

- The SDS gel after electrophoresis is then soaked in alkali (NaOH) or acid (HCl) to denature the double stranded DNA fragments.
- DNA strands get separated

Step IV: Blotting

The separated strands of DNA is then transferred to positively charged membrane nylon membrane (Nitrocellulose paper) by the process of blotting.

Step V: Baking and Blocking

- After the DNA of interest bound on the membrane, it is baked on autoclave to fix in the membrane.
- The membrane is then treated with casein or Bovine serum albumin (BSA) which saturates all the binding site of membrane

Step VI: Hybridization with Labelled Probes

- The DNA bound to membrane is then treated with labelled probe
- The labelled probe contains the complementary sequences to the gene of interest
- The probe bind with complementary DNA on the membrane since all other non-specific binding site on the membrane has been blocked by BSA or casein.

Step VII: Visualization by Autoradiogram

The membrane bound DNA labelled with probe can be visualized under autoradiogram which give pattern of bands.

14.4.1 Applications

1. Southern blotting technique is used to detect DNA in given sample.
2. DNA finger printing is an example of southern blotting.
3. Used for paternity testing, criminal identification, victim identification
4. To isolate and identify desire gene of interest.
5. Used in restriction fragment length polymorphism.
6. To identify mutation or gene rearrangement in the sequence of DNA.
7. Used in diagnosis of disease caused by genetic defects.
8. Used to identify infectious agents.

14.5 WESTERN BLOTTING

Principle

- Western blotting technique is used for identification of particular protein from the mixture of protein.
- In this method labelled antibody against particular protein is used identify the desired protein, so it is a specific test. Western blotting is also known as immuno blotting because it uses antibodies to detect the protein (Refer Figure 14.3).

Procedure

1. Extraction of protein
2. Gel electrophoresis: SDS PAGE
3. Blotting: electrical or capillary blotting
4. Blocking: BSA
5. Treatment with primary antibody

6. Treatment with secondary antibody(enzyme labelled anti Ab)
7. Treatment with specific substrate; if enzyme is alkaline phosphatase, substrate is p-nitro phenyl phosphate which give color.

NOTES

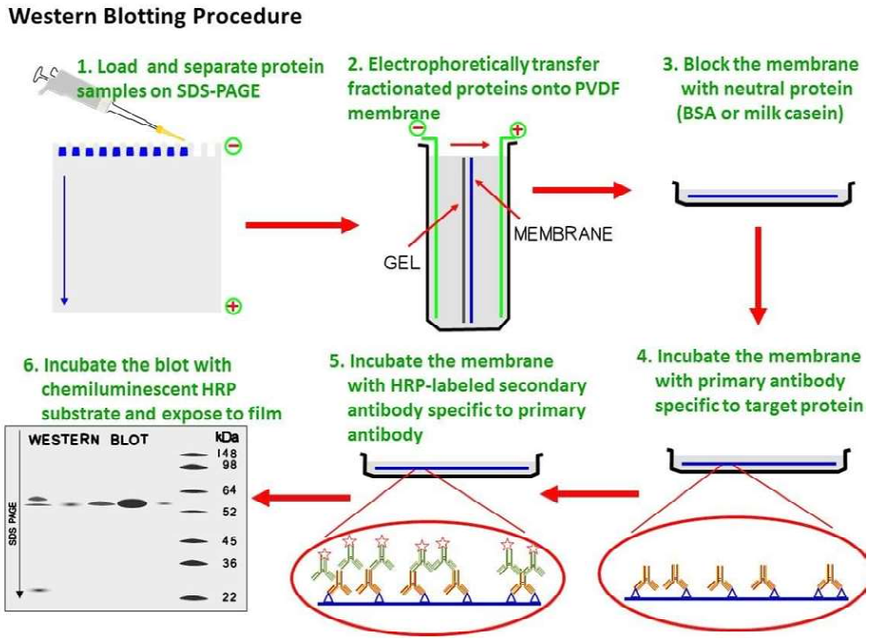


Fig. 14.3 *Western Blotting Procedure*

Step I: Extraction of Protein

- Cell lysate is most common sample for western blotting.
- Protein is extracted from cell by mechanical or chemical lysis of cell. This step is also known as tissue preparation.
- To prevent denaturing of protein protease inhibitor is used.
- The concentration of protein is determined by spectroscopy.
- When sufficient amount of protein sample is obtained, it is diluted in loading buffer containing glycerol which helps to sink the sample in well.
- Tracking dye (bromothymol blue) is also added in sample to monitor the movement of proteins.

Step II: Gel electrophoresis

- The sample is loaded in well of SDS-PAGE Sodium dodecyl sulfate- poly-acrylamide gel electrophoresis.
- The proteins are separated on the basis of electric charge, isoelectric point, molecular weight, or combination of these all.
- The small size protein moves faster than large size protein.

- Protein are negatively charged, so they move toward positive (anode) pole as electric current is applied.

Step III: Blotting

- The nitrocellulose membrane is placed on the gel. The separated protein from gel get transferred to nitrocellulose paper by capillary action. This type of blotting is time consuming and may take 1-2 days
- For fast and more efficient transfer of desired protein from the gel to nitrocellulose paper electro-blotting can be used.
- In electro-blotting nitrocellulose membrane is sandwich between gel and cassette of filter paper and then electric current is passed through the gel causing transfer of protein to the membrane.

Step IV: Blocking

- Blocking is very important step in western blotting.
- Antibodies are also protein so they are likely to bind the nitrocellulose paper. So before adding the primary antibody the membrane is non-specifically saturated or masked by using casein or Bovine serum albumin (BSA).

Step V: Treatment with Primary Antibody

- The primary antibody (1° Ab) is specific to desired protein so it form Ag-Ab complex.

Step VI: Treatment with secondary antibody

- The secondary antibody is enzyme labelled. For example, alkaline phosphatase or Horseradish peroxidase (HRP) is labelled with secondary antibody.
- Secondary antibody (2° Ab) is antibody against primary antibody (anti-antibody) so it can bind with Ag-Ab complex.

Step VII: Treatment with suitable substrate

- To visualize the enzyme action, the reaction mixture is incubated with specific substrate.
- The enzyme convert the substrate to give visible colored product, so band of color can be visualized in the membrane.
- Western blotting is also a quantitative test to determine the amount of protein in sample.

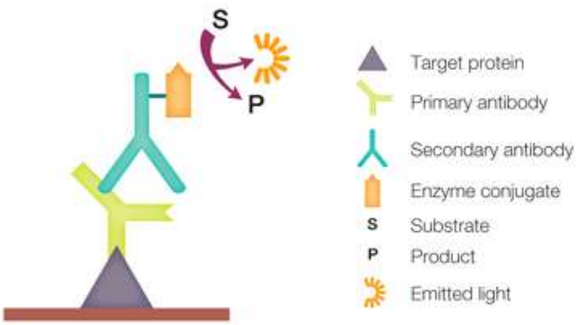


Fig. 14.4 *Western Blotting*

14.5.1 Applications

- To determine the size and amount of protein in given sample.
- Disease diagnosis: detects antibody against virus or bacteria in serum.
- Western blotting technique is the confirmatory test for HIV. It detects anti HIV antibody in patient's serum.
- Useful to detect defective proteins. For example, Prions disease.
- Definitive test for Creutzfeldt-Jacob disease, Lyme disease, Hepatitis B and Herpes.

Check Your Progress

5. Write one application of Southern blotting.
6. Who developed the technique of Southern blotting?
7. Write one application of Western blotting.

14.6 REAL TIME PCR

Real-time PCR is a PCR technique used to quantify starting amounts of nucleic acid template by analysing the amount of DNA produced during each cycle of PCR. It is a form of quantitative PCR (Q-PCR). Real-time PCR is often preceded by reverse transcription to quantify RNA via their cDNA. In fact, mRNA quantification is one of the most frequent uses of real-time PCR. The sub-technique is sometimes called qRT-PCR for quantitative reverse transcription PCR.

Principle

Amplification of DNA is exponential in the early and middle cycles of a PCR (i.e., it is linear on a logarithmic scale). This property can be exploited to infer the starting amount of PCR template (see diagram in Hunt tutorial). During the exponential or log phase each copy of DNA is being amplified, and thus can be a better measure than in endpoint PCR, where reagents such a nucleotides may

become exhausted and result in inefficient amplification, resulting in inaccurate quantification of the gene of interest.

Real-time PCR is more precise than previously used reverse transcription PCR (RT PCR) because the generation of product is continuously monitored during the PCR run (this is where the term "real time" comes in), rather than at the end of a PCR reaction ("endpoint" PCR) (Refer Figure 14.5).

Generation of product is detected in one of two ways. First, the amount of double stranded DNA in the tube can be measured using fluorescent dyes which intercalate double-stranded DNA (like the DNA binding dye SYBR Green I). The intensity of fluorescence is proportional to the quantity of DNA present in the reaction. Second, the amount of PCR product can be measured by monitoring the hybridization of a set concentration of fluorescently labeled probe oligonucleotide. The Oligo probe provide selectivity and only monitors the concentration of PCR product with a particular sequence. In contrast, SYBR green I will bind even nonspecific PCR products.

Procedure

In a real time PCR protocol, a fluorescent reporter molecule is used to monitor the PCR as it progresses. The fluorescence emitted by the reporter molecule manifolds as the PCR product accumulates with each cycle of amplification. Based on the molecule used for the detection, the real time PCR techniques can be categorically placed under two heads:

1. Non-specific Detection using DNA Binding Dyes
2. Specific Detection Target Specific Probes

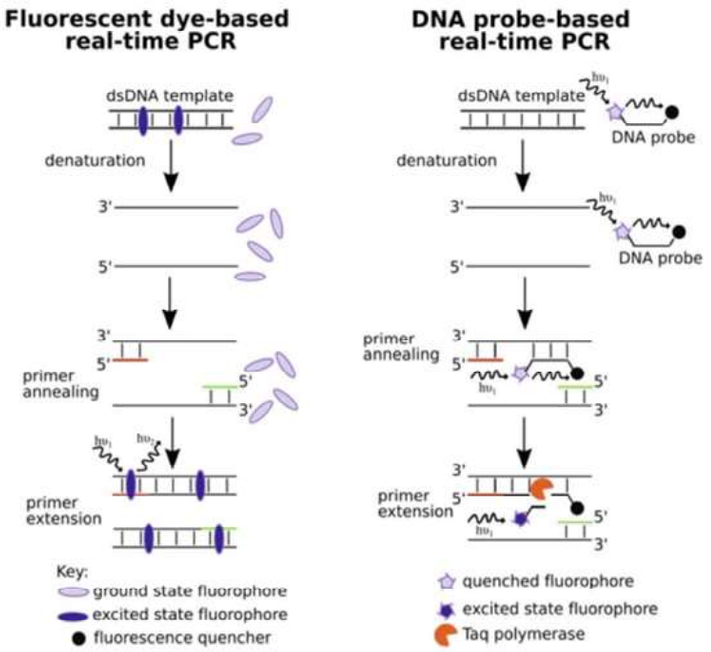


Fig. 14.5 *Real-Time PCR*

14.6.1 Non-specific Detection using DNA Binding Dyes

In real time PCR, DNA binding dyes are used as fluorescent reporters to monitor the real time PCR reaction. The fluorescence of the reporter dye increases as the product accumulates with each successive cycle of amplification. By recording the amount of fluorescence emission at each cycle, it is possible to monitor the PCR reaction during exponential phase. If a graph is drawn between the log of the starting amount of template and the corresponding increase the fluorescence of the reporter dye fluorescence during real time PCR, a linear relationship is observed.

SYBR Green is the most widely used double-strand DNA-specific dye reported for real time PCR. SYBR Green binds to the minor groove of the DNA double helix. In the solution, the unbound dye exhibits very little fluorescence. This fluorescence is substantially enhanced when the dye is bound to double stranded DNA. SYBR Green remains stable under PCR conditions and the optical filter of the thermocycler can be affixed to harmonize the excitation and emission wavelengths. Ethidium bromide can also be used for detection but its carcinogenic nature renders its use restrictive. Although these double-stranded DNA-binding dyes provide the simplest and cheapest option for real time PCR, the principal drawback to intercalation based detection of PCR product accumulation is that both specific and nonspecific products generate signal.

14.6.2 Specific Detection using Target Specific Probes

Specific detection of real time PCR is done with some oligonucleotide probes labeled with both a reporter fluorescent dye and a quencher dye. Probes based on different chemistries are available for real time detection, these include:

- Molecular Beacons
- TaqMan Probes
- FRET Hybridization Probes
- Scorpion Primers

14.6.3 Applications

- Quantitative mRNA expression studies.
- DNA copy number measurements in genomic or viral DNAs.
- Allelic discrimination assays or SNP genotyping.
- Verification of microarray results.
- Drug therapy efficacy.
- DNA damage measurement.

14.6.4 Real Time PCR VS Traditional PCR

Real time PCR allows for the detection of PCR product during the early phases of the reaction. This ability of measuring the reaction kinetics in the early phases of

PCR provide a distinct advantage over traditional PCR detection. Traditional methods use gel electrophoresis for the detection of PCR amplification in the final phase or at end-point of the PCR reaction.

14.6.5 Limitations of End-point PCR

In a PCR reaction as the reaction progresses, the reagents are being consumed as a result of amplification. Now the PCR product is no longer being doubled at each cycle due to this reagent constraint. This depletion will occur at different rates for each replicate. Thus, the samples begin to diverge in their quantities. This diminished amplification is the linear phase of the reaction. The plateau for each tube will differ due to the different reaction kinetics for each sample. It is in this phase where traditional PCR takes its measurement, also known as the end-point. This End-Point Detection has some problems such as low resolution, poor precision, low sensitivity and the need for post PCR processing. Also, the results of this detection are not expressed in numbers and there is no scope for automation.

14.7 MICROARRAY

Molecular Biology research evolves through the development of the technologies used for carrying them out. It is not possible to research on a large number of genes using traditional methods. DNA Microarray is one such technology which enables the researchers to investigate and address issues which were once thought to be non-traceable. One can analyze the expression of many genes in a single reaction quickly and in an efficient manner. DNA Microarray technology has empowered the scientific community to understand the fundamental aspects underlining the growth and development of life as well as to explore the genetic causes of anomalies occurring in the functioning of the human body.

A typical microarray experiment involves the hybridization of an mRNA molecule to the DNA template from which it is originated. Many DNA samples are used to construct an array. The amount of mRNA bound to each site on the array indicates the expression level of the various genes. This number may run in thousands. All the data is collected and a profile is generated for gene expression in the cell (Refer Figure 14.6).

14.7.1 Microarray Technique

An array is an orderly arrangement of samples where matching of known and unknown DNA samples is done based on base pairing rules. An array experiment makes use of common assay systems such as microplates or standard blotting membranes. The sample spot sizes are typically less than 200 microns in diameter usually contain thousands of spots.

Thousands of spotted samples known as probes (with known identity) are immobilized on a solid support (a microscope glass slides or silicon chips or nylon

membrane). The spots can be DNA, cDNA, or oligonucleotides. These are used to determine complementary binding of the unknown sequences thus allowing parallel analysis for gene expression and gene discovery. An experiment with a single DNA chip can provide information on thousands of genes simultaneously. An orderly arrangement of the probes on the support is important as the location of each spot on the array is used for the identification of a gene.

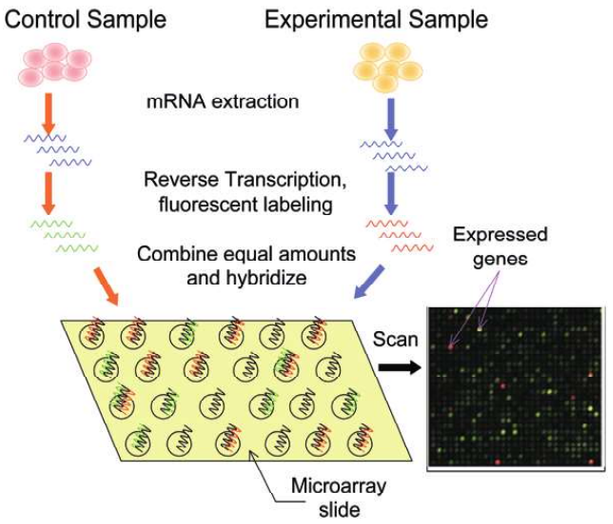


Fig. 14.6 *Microarray*

14.7.2 Types of Microarrays

Depending upon the kind of immobilized sample used construct arrays and the information fetched, the Microarray experiments can be categorized in three ways:

1. **Microarray Expression Analysis:** In this experimental setup, the cDNA derived from the mRNA of known genes is immobilized. The sample has genes from both the normal as well as the diseased tissues. Spots with more intensity are obtained for diseased tissue gene if the gene is over expressed in the diseased condition. This expression pattern is then compared to the expression pattern of a gene responsible for a disease.
2. **Microarray for Mutation Analysis:** For this analysis, the researchers use gDNA. The genes might differ from each other by as less as a single nucleotide base. A single base difference between two sequences is known as Single Nucleotide Polymorphism (SNP) and detecting them is known as SNP detection.
3. **Comparative Genomic Hybridization:** It is used for the identification in the increase or decrease of the important chromosomal fragments harboring genes involved in a disease.

14.7.3 Applications

- Gene Discovery: DNA Microarray technology helps in the identification of new genes, know about their functioning and expression levels under different conditions.
- Disease Diagnosis: DNA Microarray technology helps researchers learn more about different diseases such as heart diseases, mental illness, infectious disease and especially the study of cancer. Until recently, different types of cancer have been classified on the basis of the organs in which the tumors develop. Now, with the evolution of microarray technology, it will be possible for the researchers to further classify the types of cancer on the basis of the patterns of gene activity in the tumor cells. This will tremendously help the pharmaceutical community to develop more effective drugs as the treatment strategies will be targeted directly to the specific type of cancer.
- Drug Discovery: Microarray technology has extensive application in Pharmacogenomics. Pharmacogenomics is the study of correlations between therapeutic responses to drugs and the genetic profiles of the patients. Comparative analysis of the genes from a diseased and a normal cell will help the identification of the biochemical constitution of the proteins synthesized by the diseased genes. The researchers can use this information to synthesize drugs which combat with these proteins and reduce their effect.
- Toxicological Research: Microarray technology provides a robust platform for the research of the impact of toxins on the cells and their passing on to the progeny. Toxicogenomics establishes correlation between responses to toxicants and the changes in the genetic profiles of the cells exposed to such toxicants.

Check Your Progress

8. What are the two categories of Real-time PCR?
9. Write one application of microarrays.

14.8 RECOMBINANT DNA TECHNOLOGY

In nature, exchange of information between two genetic materials resulting in acquisition of new segments of DNA by a genome is very common, but it occurs only among genetic materials of the same or closely related species.

In the last several decades, thousands of scientists working in genetics, biochemistry, cell biology and physical chemistry have yielded techniques to construct recombinant molecules contai-ning foreign DNA into cells of unrelated organism. A specific characteristic of one organism would then be visible in a different organism resulting in a living chimera. Thus things have happened that were not even dreamt of.

NOTES

The deliberate modification of an organism's genetic information by directly changing its nucleic acid genome is called genetic engineering. Genetic engineering is accomplished by a collection of methods known as recombinant DNA technology. First the DNA responsible for a particular phenotype is identified and isolated.

Once purified, the gene or genes are fused with other pieces of DNA to form recombinant DNA molecules or rDNA. The term recom-binant DNA usually refers to the creation of new combination of DNA molecules that are not found together naturally.

Although processes such as crossing over technically produce recombinant DNA, the term is gene-rally reserved for DNA molecules produced by joining molecules derived from different biological sources. These rDNAs are then propagated or cloned by insertion into an organism that need not even be in the same kingdom as the original gene donor.

Techniques for DNA cloning — either by cell- based method or cell-free in vitro method — have opened hitherto unimaginable oppor-tunity to identify and study the genes involved in almost every known biological process.

Recombinant DNA technology or gene-tic engineering has opened up totally new areas of research and applied biology. Thus, it is an essential part of biotechnology which is now experiencing a stage of exceptionally rapid development and progress.

Procedure

Recombinant DNA technology is a powerful tool for the isolation of poten-tially unlimited quantities of a gene.

The basic procedure involves the following steps:

(i) Purification of DNA from cells or tissues or construction of cDNA (comple-mentary DNA) from total RNA or poly(A)+ messenger RNA (mRNA) using the enzyme reverse transcriptase.

(ii) Production of DNA fragments (for genomic DNA, not for cDNA) using the enzymes called restriction endonucleases that recognise and cut DNA molecules at specific nucleotide sequences.

(iii) Joining of DNA fragments or cDNA using the enzyme ligase to other DNA molecules that serve as vectors or carrier molecules, having capacity of independent replication. For joining genomic DNA fragments, cutting the target DNA and the vector will be done by the same restriction enzyme. The vector with an inserted DNA segment is a recombinant DNA molecule.

(iv) Transformation of the recombinant DNA molecule, carrying an inserted DNA fragment into a host cell (often bacterial or yeast cells) in which the chosen molecule can undergo replication, independently of the host cell chromosomes and produce dozens of identical cells known as clone.

As host cells replicate, the recombinant DNA molecules within them are passed on to all their progeny cells, creating a population of host cells, each of which carries copies of a cloned DNA sequence.

(v) Isolation of the recombinant DNA clones by harvesting the expanded cell cul-tures; subsequent purification and analysis of that target DNA. Potentially, the cloned DNA can be transcribed, its mRNA translated and the gene product isolated and used for research or sold commercially.

14.8.1 Preparation of Recombinant DNA Technology

Before describing the methods of prepa-ration of recombinant DNA molecules, concepts about three key elements viz, restric-tion endonuclease, DNA ligase and vectors that are essential for recombinant DNA tech-nology are given below:

Restriction Enzymes

The corner stone of genetic engineering or recombinant DNA technology is a special class of enzymes called restriction enzymes. These are bacterial enzymes that recognise specific nucleotide sequences, called restric-tion sites, and cut both strands of DNA at this site.

Since these enzymes cleave DNA within the molecule, they are called restric-tion endonucleases to distinguish them from exonuclease enzymes which digest or cleave nucleic acids from an end (Refer Figure 14.7).

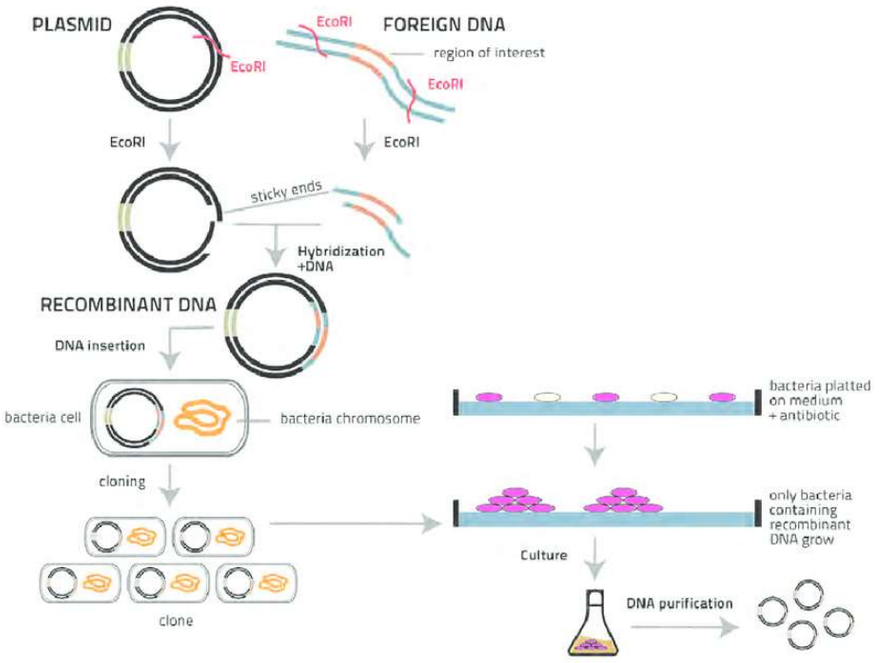


Fig. 14.7 *Cloning into a Plasmid*

These enzymes got the name restriction because these enzymes naturally occur in bacteria where they prevent or restrict viral infection by degrading the invading viral DNA. The term restriction endonuclease was coined by Lederberg and Meselson (1964). In 1978, Werner Arber, Hamilton Smith and Daniel Nathans received Nobel Prize for their work on these enzymes.

To date, about 350 restric-tion enzymes have been identified and puri-fied from several hundred different species of bacteria. In genetic engineering technolo-gy, restriction endonucleases are popularly called as molecular knives or molecular scissors or molecular scalpels due to their ability to reproducibly cut DNA into frag-ments.

Naming of restriction endonucleases According to Smith and Nathans (1973) naming of the restriction enzymes is based on following rules:

- Each enzyme-name should have a three-letter code.
- The first letter of the code name is derived from the first epithet of the genus name of the bacteria from which it has been obtained.
- The second and third letters are from the species name of the bacteria.
- The three-letter code name is followed by the strain number. If one strain has more than one restriction enzymes, these will be identified by Roman numerals as I, II, III, etc.
- A general name endonuclease R may be added, for example, EcoRI enzyme which is derived from E. coli carrying antibiotic resis-tance plasmid (RI).

Types of Restriction Enzymes

1. Type I Restriction Enzymes

Some restriction enzymes interact with an unmodi-fied recognition sequence in double strand DNA and then attach to long DNA molecule. After travelling far distance between 1,000 to 5,000 nucleotides, the enzyme cleaves only one strand of the DNA at an apparently ran-dom site, and creates a gap of about 75 nucleotides in length.

Since, the site of clea-vage of these enzymes are not specific, these enzymes are not useful for gene manipula-tion. The type I enzymes can hold two diffe-rent sites on DNA, simultaneously creating a loop in nucleic acid. These enzymes consist of three subunits, for example, the EcoK has R2M2S sub- units. The R subunit is responsible for restric-tion, the M subunit for methylation and the S subunit helps in binding of enzyme to DNA.

2. Type II Restriction Enzymes

Unlike type I enzymes these restriction enzymes recognise a particular sequence in the double- stranded DNA molecule and cleave the chain within or near that sequence to give rise to discrete DNA fragments of defined length and sequence. These enzymes require Mg2+ ions for their function e.g., EcoRI.

3. Type III restriction enzymes:

These enzymes possess intermediate properties between type I and type II enzymes. They cleave double stranded DNA at specific sites. They require ATP, Mg^{2+} and partially S- adenosylmethionine for their activities for example, EcoPI, Eco15 etc.

DNA Ligase

It is the key enzyme that seals single-stranded nicks between adja-cent nucleotides in a duplex DNA chain. Ligase enzymes are isolated from E. coli and T4 phage. The catalytic action of both ligase enzymes is very similar, but they differ in their cofactor requirements. T4 ligase requires ATP, while E. coli ligase requires NAD+.

Mechanism of action

ATP in case of T4 DNA ligase and NAD+ in case of E. coli ligase first split and form an enzyme-AMP complex (Refer Figure 14.9). The complex then binds to the nick that must expose a 5′ phosphate and 3'OH group. The AMP then reacts with the phos-phate group. The 3'OH group on the moiety then generates a new phosphodiester bond which seals the nick.

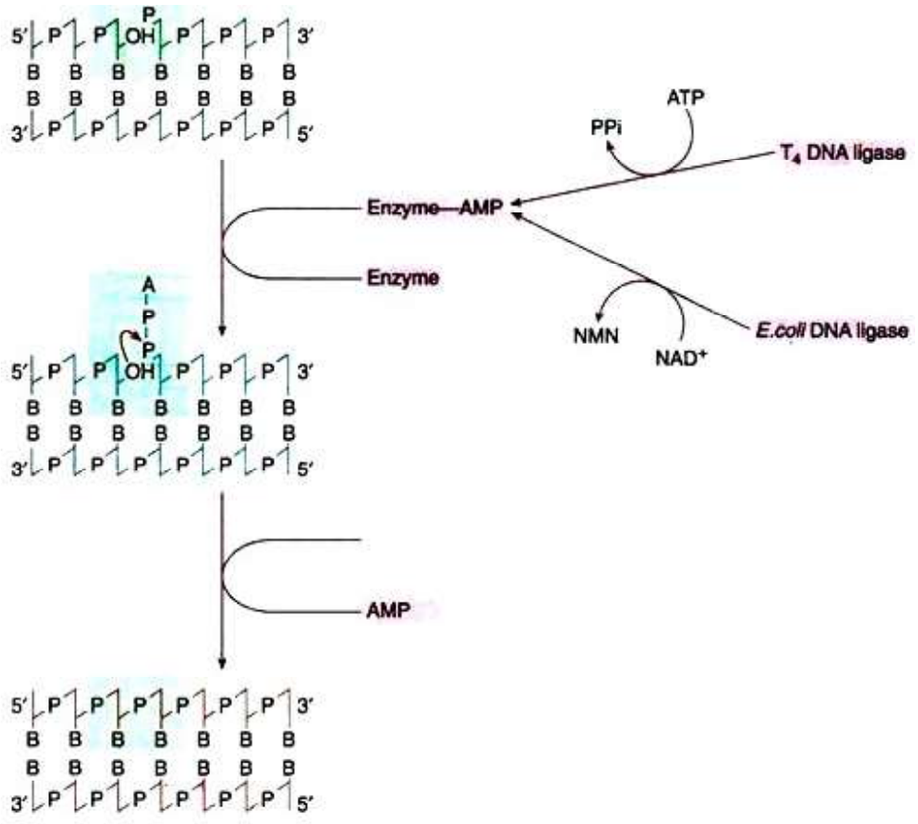


Fig. 14.9 *Mechanism of action of DNA Ligase*

Action of DNA Ligase

Cohesive ends created by restriction enzymes have nicks a few base pairs apart in opposite strands. DNA ligase can then repair these nicks to form an intact duplex (Refer Figure 14.10). The optimum temperature for this ligation is 37°C, but at this temperature, the hydrogen- bonded between the sticky ends is unstable.

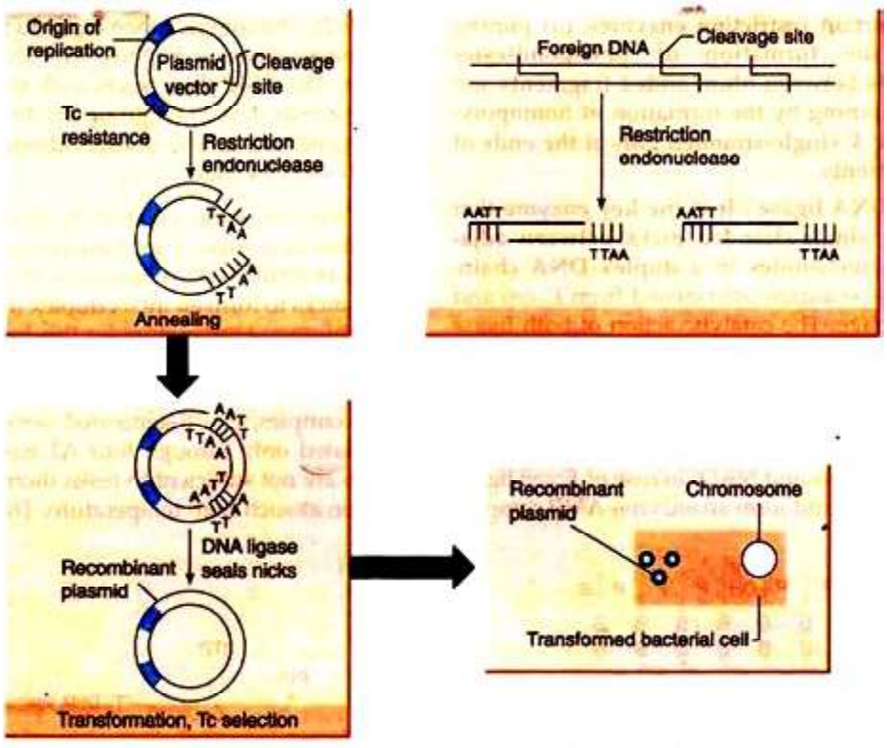


Fig. 14.10 *Use of DNA Ligase to generate a covalent DNA recombinant*

For examples, EcoRI-generated termini get associated only through four AT base pairs which are not sufficient to resist thermal dis-ruption at such high temperature. Therefore, the ligating temperature is a compromise between the rate of enzyme action and asso-ciation of the termini. It has been found to be in the range of 4-15°C.

Joining of blunt ended DNA molecules is mainly done by annealing complementary homopolymer sequences. Thus, by adding Oligo (dA) sequences to the 3′ ends of one population of DNA molecules and Oligo (dT) blocks to the 3′ ends of another population, the two types of molecules can be annealed to form mixed dimeric circles (Refer Figure 14.11).

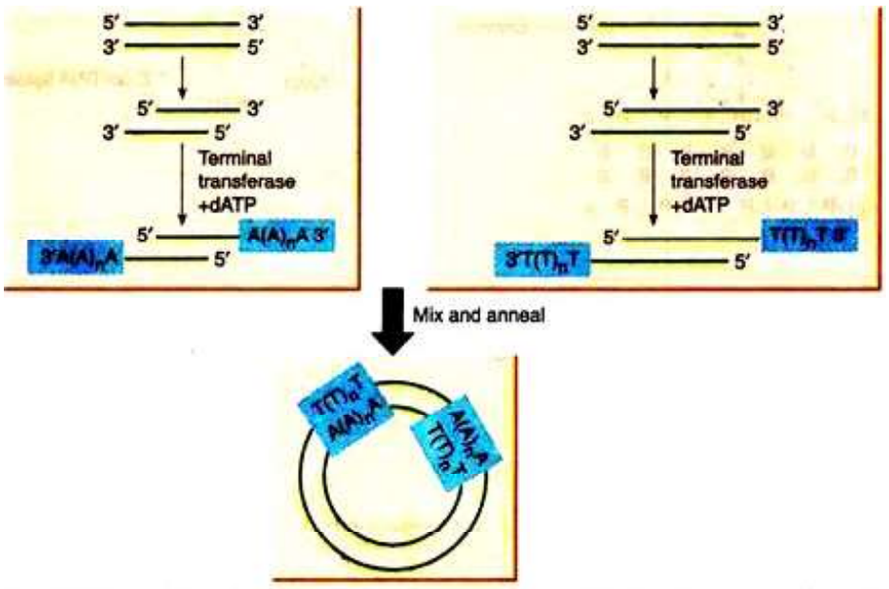


Fig. 14.11 *Use of Terminal Deoxynucleotidyl Transfer to Add Complimentary Homopolymer Tails to Two DNA Molecules*

Use of Terminal Deoxynucleotidyl

The enzyme terminal deoxynucleotidyl transferase, purified from calf thymus, pro-vides the means by which the homo-polymeric extensions can be synthesised. It can repeatedly add nucleotides to the 3′OH ter-mini of a DNA molecule.

DNA with exposed 3′OH can be generated by pretreatment with phage exonuclease or restriction enzyme Pestle. In recent years, homopolymer tailing has been largely replaced as a result of the availability of a much wider range of restric-tion endonuclease and other DNA modifying enzymes. However, it is still important for cDNA cloning.

Prevention of recircularisation

During cloning, the vector DNA molecule following cutting should be treated with alkaline phos-phatase. It will remove 5′ terminal phosphate groups, thus preventing recircularisation and plasmid dimer formation (Refer Figure 14.12).

In this case, circularization of the vector can occur only by insertion of non-phosphatase treated foreign DNA which has one 5′ terminal phos-phate at each join. One nick at each join remains un-ligated, but after transformation of host bacteria, cellular repair mechanisms reconstitute the intact duplex.

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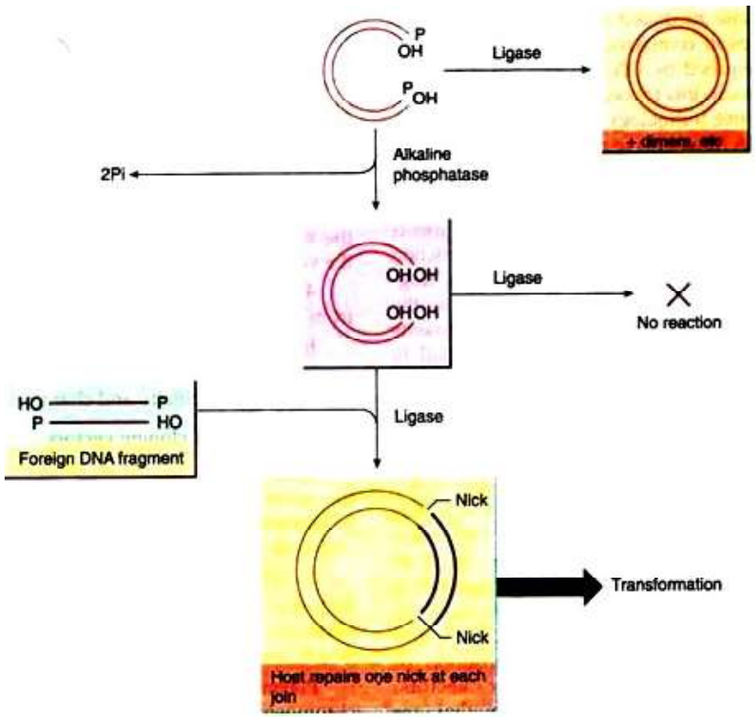


Fig. 14.12 *Application of Alkaline Phosphate Treatment to Prevent Recirculation of Vector Plasmid Without Insertion of Foreign DNA*

Joining DNA molecules without ligase

The enzyme vaccinia topoisomerase can alone cause cleavage and joining of DNA molecules. When CCCTT cleavage motif is placed near the end of a duplex DNA, the enzyme generates a stable, highly recombinogenic protein DNA adduct that can only relegate to acceptor DNAs, that contain complementary single-strand extensions.

Thus, linear DNAs containing CCCTT cleavage sites at both ends can be activated by topoisomerase and inserted into a plasmid vector. Topo-isomerase mediated ligation requires only 5 minutes in comparison to overnight incuba-tion required by DNA ligase. For these pro-perties vaccinia topoisomerase can be used for ligase-free technology for the covalent joining of DNA fragments to plasmid vectors.

Vectors

Cutting of DNA molecules into fragments is possible with the help of specific restriction endonuclease enzymes. These DNA frag-ments can be readily joined together by the activities of DNA ligase enzymes. If fragments of DNA are inserted into cells, they fail to replicate except in those rare cases where they integrate into the chromosome. To enable such fragments to be propagated, they are inserted into DNA molecules (vectors) that are capable of extra-chromosomal replication.

Originally, the purpose of vectors was the propagation 'of cloned DNA but today vectors fulfill many other roles, such as facilitating DNA sequencing, promoting expression of cloned genes, facilitating purification of cloned gene products and reporting the activity and localisation of proteins. There are two basic vectors, cloning and expression vectors that are most widely required for recombinant DNA technology.

Cloning Vectors

These are auto-nomously replicating genetic elements used to carry a cDNA or fragment of genomic DNA into a host cell for the purpose of gene cloning. DNA fragments produced by restriction enzyme digestion cannot directly enter the bacterial cells, but when a DNA fragment is joined to a vector, however, it can gain entry to a host cell. Vectors are, therefore, carrier of DNA molecules.

However, to serve as a vector, a DNA molecule must possess the following properties:

1. Vector must be able to independently replicate itself and the carried DNA segment.
2. Vector should contain several restric-tion enzyme cleavage sites that are present only once in the vector. The restriction enzyme that cleaves this site should also be used to cut the DNA fragment to be inserted into the vec-tor. That means the same restriction enzyme/ enzymes will be used to cleave both vector as well as DNA fragments to be inserted.
3. Vector should carry suitable marker (usually in the form of antibiotic resistance genes or genes for enzymes missing in the host cell) to distinguish host cells that carry the vectors from host cells that do not carry the vectors.
4. It should be easy to obtain vectors from their host cells.

In recent years many vectors are being used in recombinant DNA technology, each with its own specificity and characteristics.

14.9 SANGER SEQUENCING

DNA sequencing enables us to perform a thorough analysis of DNA because it provides us with the most basic information of all: the sequence of nucleotides. With this knowledge, for example, we can locate regulatory and gene sequences, make comparisons between homologous genes across species and identify mutations. Scientists recognized that this could potentially be a very powerful tool, and so there was competition to create a method that would sequence DNA. Then in 1974, two methods were independently developed by an American team and an English team to do exactly this. The Americans, led by Maxam and Gilbert, used a "chemical cleavage protocol", while the English, led by Sanger, and designed a procedure similar to the natural process of DNA replication. Even though both teams shared the 1980 Nobel Prize, Frederick Sanger's method became the standard because of its practicality (Speed, 1992). Sanger's method, which is

also referred to as dideoxy sequencing or chain termination, is based on the use of dideoxynucleotides (ddNTP's) in addition to the normal nucleotides (NTP's) found in DNA. Dideoxynucleotides are essentially the same as nucleotides except they contain a hydrogen group on the 3' carbon instead of a hydroxyl group (OH). These modified nucleotides, when integrated into a sequence, prevent the addition of further nucleotides. This occurs because a phosphodiester bond cannot form between the dideoxynucleotide and the next incoming nucleotide, and thus the DNA chain is terminated (Refer Figure 14.13).

14.9.1 Method of Sanger Sequencing

The DNA sample to be sequenced is combined in a tube with primer, DNA polymerase, and DNA nucleotides (dATP, dTTP, dGTP, and dCTP). The four dye-labeled, chain-terminating dideoxy nucleotides are added as well, but in much smaller amounts than the ordinary nucleotides.

The mixture is first heated to denature the template DNA (separate the strands), then cooled so that the primer can bind to the single-stranded template. Once the primer has bound, the temperature is raised again, allowing DNA polymerase to synthesize new DNA starting from the primer. DNA polymerase will continue adding nucleotides to the chain until it happens to add a dideoxy nucleotide instead of a normal one. At that point, no further nucleotides can be added, so the strand will end with the dideoxy nucleotide.

This process is repeated in a number of cycles. By the time the cycling is complete, it's virtually guaranteed that a dideoxy nucleotide will have been incorporated at every single position of the target DNA in at least one reaction. That is, the tube will contain fragments of different lengths, ending at each of the nucleotide positions in the original DNA (Refer Figure 14.14). The ends of the fragments will be labeled with dyes that indicate their final nucleotide.

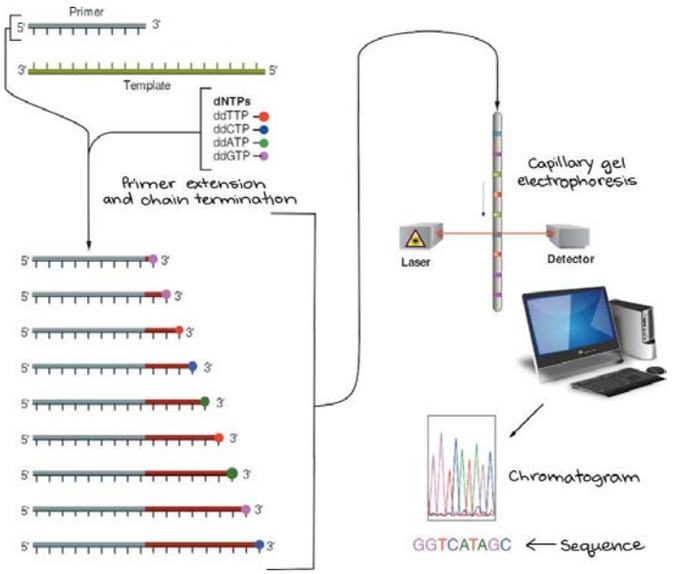


Fig. 14.13 Sanger sequencing

After the reaction is done, the fragments are run through a long, thin tube containing a gel matrix in a process called capillary gel electrophoresis. Short fragments move quickly through the pores of the gel, while long fragments move more slowly. As each fragment crosses the "finish line" at the end of the tube, it's illuminated by a laser, allowing the attached dye to be detected.

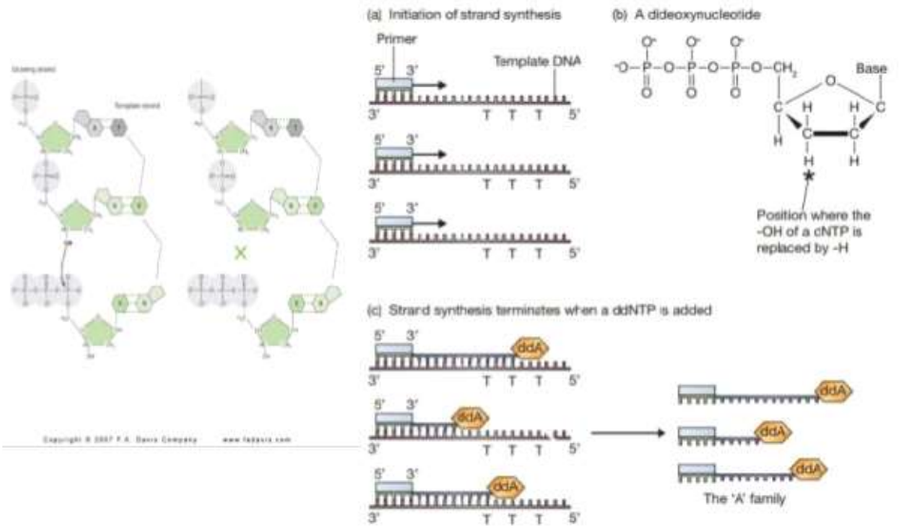


Fig. 14.14 *Sanger's Method*

The smallest fragment (ending just one nucleotide after the primer) crosses the finish line first, followed by the next-smallest fragment (ending two nucleotides after the primer), and so forth. Thus, from the colors of dyes registered one after another on the detector, the sequence of the original piece of DNA can be built up one nucleotide at a time. The data recorded by the detector consist of a series of peaks in fluorescence intensity, as shown in the chromatogram above. The DNA sequence is read from the peaks in the chromatogram.

14.9.2 Uses and Limitations

Sanger sequencing gives high-quality sequence for relatively long stretches of DNA (up to about 900900 base pairs). It's typically used to sequence individual pieces of DNA, such as bacterial plasmids or DNA copied in PCR.

However, Sanger sequencing is expensive and inefficient for larger-scale projects, such as the sequencing of an entire genome or metagenome (the "collective genome" of a microbial community). For tasks such as these, new, large-scale sequencing techniques are faster and less expensive.

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14.10 NEXT-GENERATION SEQUENCING

The name may sound like Star Trek, but that's really what it's called! The most recent set of DNA sequencing technologies are collectively referred to as next-generation sequencing.

There are a variety of next-generation sequencing techniques that use different technologies. However, most share a common set of features that distinguish them from Sanger sequencing:

- Highly parallel: many sequencing reactions take place at the same time
- Micro scale: reactions are tiny and many can be done at once on a chip
- Fast: because reactions are done in parallel, results are ready much faster
- Low-cost: sequencing a genome is cheaper than with Sanger sequencing
- Shorter length: reads typically range from 5050 -700700 nucleotides in length

Check Your Progress

10. What are restriction enzymes?
11. What is DNA ligase?
12. What is the use of Sanger sequencing?

14.11 CELL CULTURE METHODS

Essentially, cell culture involves the distribution of cells in an artificial environment (in-vitro) which is composed of the necessary nutrients, ideal temperature, gases, pH and humidity to allow the cells to grow and proliferate.

In-vivo - When the study involves living biological entities within the organism.

In-vitro - When the study is conducted using biological entities (cells, tissue etc.) that has been isolated from their natural biological environment. For example, tissue or cells isolated from the liver or kidney.

When the cells obtained directly from the organism/animal tissue (or even plant tissue) through enzymatic or mechanical techniques, such cells are referred to as primary cells. However, cells that continue to proliferate indefinitely (after the first subculture) under special conditions are referred to as cell lines. These particular cells tend to have been passaged for a long period of time, which causes them to acquire homogenous (similar) genotypic and phenotypical traits.

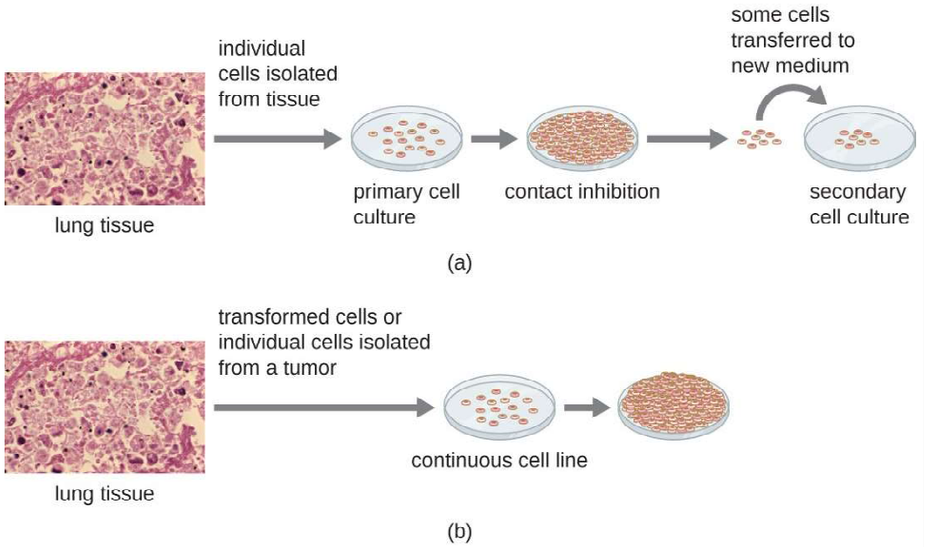


Fig. 14.15 *Cell Culture*

Based on their appearance, cells in culture can be categorized in to three main groups:

- **Fibroblastic:** This includes cells that tend to be bipolar/multipolar with elongated shapes. These cells are attached to the substrate as they grow.
- **Epithelial:** Epithelial like cells attain a polygonal shape with regular dimensions. Although they tend to grow in discrete patches, these cells also grow attached to the substrate.
- **Lymphoblast:** These cells are usually spherical in shape and do not attach to the surface of the substrate. As a result, they are grown in suspension.

14.11.1 Significance of Cell Culture

Cell culture is an important technique in both cellular and molecular biology given that it provides the best platform for studying the normal physiology and biochemistry of cells. A cell is the basic structural, functional and biological unit of all living things.

In order to understand an organism or given tissues, it is important to understand how its cells work. Through cell culture, this becomes possible especially due to the fact the primary cells resemble the parental cells from the organism/tissue. Therefore, whatever is learnt about the cells in vitro is representative of what is happening to the organism/tissue. This makes cell culture significantly important for vaccine development, screening (drugs etc.) and diagnosis of given diseases/conditions.

Given that different types of cells require different environments for proliferation, there are different types of media used for culture such as serum-free media and serum containing media among others.

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Once the right requirements have been provided, the cells will increase in numbers and may form colonies, which can then be easily seen and identified. However, all this requires that the purpose of the procedure be understood.

Having a good understanding of what the procedure is meant to achieve, it becomes easier to prepare the culture with the right components. For instance, by understanding what the procedure is aimed for, the researcher will know whether to prepare a selective media (which allow for specific cells to grow) or differential media (allowing for different types of cells to grow).

Cell culture is a process where cells (animal or plant cells) are removed from the organism and introduced in to an artificial environment with favorable conditions for growth. This allows for researchers to study and learn more about the cells. There are three major types of cell culture, which include;

- Primary cell culture
- Secondary cell culture, and
- Cell line

There are two types of primary cells

Adherent cells: Also referred to as anchorage dependent cells, these are the type of cells that require attachment for growth. Adherent cells are immobile, and obtained from such organs as kidney.

Suspension cells: These are the type of cells that do not require attachment in order to grow. They are therefore also referred to as anchorage independent cells, and include such cells as lymphocytes found in the blood system.

In primary cell culture, cells obtained from such parental tissues (living tissues) as the liver and kidney, are introduced into suitable media for growth. Once the cells have been obtained, they can either be cultured as explants culture, suspension or monolayer.

In cell culture techniques, cells (or tissues) are removed from a plant or an animal and introduced into a new, artificial environment that can support their proliferation (survival and growth). Some of the requirements of such an environment for the proliferation of the cells include;

- A substrate (source of nutrition)
- Ideal temperature range (controlled)
- Growth medium, and
- Ideal pH among others

Here, we shall focus on the medium (cell culture media)

Although there are different types of culture media (for different types of cells) they are typically composed of;

- Glucose
- Amino acids

- Vitamins
- Inorganic salts
- Attachment factors

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There are two major types of culture media. These include:

Natural media: Natural culture media is composed of biological fluids that are naturally occurring. Although this type of media can be used for a range of cells, its biggest disadvantage is that it may lack the exact components required by given cells, which can greatly affect reproducibility.

Artificial media: Also referred to as synthetic media, artificial media refers to the type of media that is produced by adding such nutrients as vitamins, gases (oxygen and carbon dioxide) and protein among others. These organic and inorganic nutrients are added so as to meet the specific needs of given cells, and thus provide the ideal environment for their growth. As such, they can be used for a number of purposes including:

- Providing immediate survival of the cells
- Allowing for prolonged survival of the cells
- Allowing for indefinite growth of the cells
- Providing for specialized functions

On the other hand, culture media may be categorized as:

Selective media: This is a special type of media that only allows for certain cells to grow. For instance, blood agar (used to isolate Streptococcus & Moraxella species) can be turned in to a selective media by adding antibiotics.

Differential media: This type of media allows for different types of cells/ microorganisms to grow depending on their metabolism.

As mentioned above, different types of synthetic media are prepared in a manner that will provide the ideal proliferation environment for given cells. For this reason, synthetic media can be divided in to four major categories. These include;

Serum containing media: In these types of media, serum (fetal bovine serum) is used as a carrier for nutrients and growth factors among others that tend to be water insoluble.

Serum-free media: These types of media is typically produced for the purposes of supporting single cell type of culture. As such, it provides specified nutrients and other factors required by the cell type. In this media, serum is absent because it present some disadvantages and can result in misinterpretation of immunological results.

Chemically defined media: Like the name suggests, this type of media is composed of contamination- free pure organic and inorganic ingredients. Constituents of this type of media are typically produced through genetic engineering in bacteria/yeast.

Protein-free media: Protein- free media are typically lacking of any type of protein. It is largely used to promote superior growth of the cells as well as protein expression in addition to facilitating for the purification of any expressed product.

Some of the major components of cell culture media include

Nutrients: provided for by peptides and amino- acids, which are the building blocks of proteins

Carbohydrates for energy: Essential minerals such as calcium, magnesium, phosphates and iron among others buffering agents such as acetates to stabilize the culture media

pH: Change indicators such as phenol red

Cell culture media are used for the proliferation of cells, which can then be identified and studied. As such, it can be used for various purposes including for education, diagnosis and treatment of a disease among others.

Cell Suspension

In culture methods, cell suspension refers to a type of culture where cells are suspended in a liquid medium.

To obtain single cells, a friable callus (small tissue that falls apart easily) is put in agitated liquid medium (agitation allows for gaseous exchange unlike solid medium), breaking it up. This allows for single cells to be released, which are then transferred to another fresh medium.

Cell suspension cultures have a big advantage over the stationary ones given that it allows for the cells to be uniformly bathed. Moreover, given that the medium tends to be agitated, it allows for aeration of the medium, providing gases to the cells. Given that the medium is a suspension, it also becomes easy to manipulate the contents of the culture.

Like any other culture, suspension cell culture has to be under controlled conditions, proving the cells with an ideal environment to proliferate. Once they reach about 80 percent confluence, it is time to subculture in order to ensure continued proper growth.

80 percent confluence refers to the state where 80 percent of the culture surface is covered with the growing cells.

In some cases, the cells in suspension may adhere on to the plastic surface of the culture flask or even form clumps. In such cases, a pipette can be used to pick these cells and expel them on to the surface of the flask and therefore away from the plastic surface. This helps obtain single cells given that they are no adhere on to the plastic surface

Counting Cells

Counting the number of cells in a suspension is a process that involves the use of a stain. For instance, when trypan blue is used, it penetrates the cell membrane of the dead cells, but not the living cells. The cells are then gently expelled into a haemocytometer (contains the counting chamber) under the cover slip and observed under a microscope. Cells are then counted within a given number of squares for calculations.

Significance

This method is largely preferred due to the fact that it allows for cells to be suspended in a solution rather than being held in a solid media. Here, therefore, it becomes easier to manipulate the contents thereby preventing them from forming clusters. With cell suspensions, it is also easier to observe single cells under the microscope.

In this case, it becomes possible to not only study the structure of the cells, but also get to observe how well they have differentiated; viewing dead and living cells under the microscope.

Cell Culture Protocol

Cell culture protocols are meant to ensure that culture procedures are carried out to the required standards. This is not only meant to prevent the contamination of the cells, but to also ensure that the researchers themselves are protected from any form of contamination.

Moreover, the nature of the work is expected to conform to the appropriate ethical guidelines. Therefore, before anything else, it is essential to ensure that the entire procedure conforms to both medical-ethical and animal- experiment guidelines. This is because going against such legislation and guidelines can result in heavy penalties and even shutting down of the laboratory.

Before any work starts, carry out the following procedure:

- Ensure that the working are is sanitized (using 70 percent ethanol).
- Always use a new pair of gloves. If a pair of gloves has to be used for another cell culture procedure, they should be sanitized using 70 percent ethanol and allowed to air dry.
- Any equipment that had been taken out of the cabinet should also be sanitized to prevent any contamination.
- Such equipment as pipette, glass jars and plastics to be used for the procedure should be autoclaved.
- Although there are a wide range of culture media for cells, it is important to keep in mind that cell cultures, and particularly primary cell cultures are easily prone to contamination in addition to the risk of containing undetected viruses. For this reason, all material should be handled as potentially infectious in order to avoid any infections.

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- In addition, for safety purposes, work on cell culture should be carried out in the appropriate laminar flow hood, where air is directed away from the researcher.

Protocols for cell culture preparation

- Always check the information on the container to ensure that the medium is appropriate for the cell to be cultured.
- Once prepared, the cell culture should be maintained under the recommended temperature range.
- Monitor the culture every 30- 48 hours and check for confluency (when cells completely cover the surface of the culture) - However, this is largely dependent on the type of cells.
- Once the procedure is completed and the cells have been analyzed, the culture should be appropriately discarded. Here, it is important to take a lot of caution given that by this time, cells have already proliferated and increased in numbers. Moreover, there are high chances that the specimen has been contaminated, which increase the risks of causing infections to the researcher if not handled appropriately.

Disposal

- For such items as scalpels, glass slides and cover slips among others, de-contamination may involve autoclaving at 121°C Celsius and 15 psi (pressure) for at least 30 minutes. However, if they are to be discarded, it is important that they are put into a plastic bag and into the appropriate container to be incinerated later.
- With liquid waste, chemical disinfection is one of the best methods through which the waste product can be deactivated. Such chemicals as bleach can be used for this purpose before pouring the liquid down the sink drain.
- For solid waste products, they are collected into a plastic bag for incineration. On the other hand, they can first be autoclaved before being incinerated.

Conclusion

- In general, cell culture, whether it involves using a suspension or a stationary media, involves the growth of cells in an artificial environment with favorable conditions. Whereas enzymatic action can be used to obtain cells for culture, it is the mechanical disaggregation method that is most preferred given that it provides a simpler and less traumatic way of obtaining cells.
- This method simply involves the slicing of a tissue into smaller pieces from which the spill out cells is then collected. On the other hand, primary explants technique can be used to obtain the cells. However, this method is mostly useful for the disaggregation of smaller quantities of tissue.

- While any cells can be used in cultures to observe their behavior, embryonic tissues are preferred (for primary cells) as compared to adult cells because they provide cells that are more viable and can rapidly proliferate.
- Here, it is also important to ensure that that the cells are of higher quantity given that their survival rate tends to be lower in comparison to the sub-cultures. In order to enhance the success of cultures, it is also important that ensure that damage to the cells is minimized both during cell collection and processing. This is in addition to using the appropriate medium for the cells in question.

Check Your Progress

13. What is natural culture media?
14. What is serum containing media?

14.12 ANSWERS TO CHECK YOUR PROGRESS QUESTIONS

1. Fluorescent ln-Situ Hybridization.
2. DNA microarray is a solid surface to which a collection of microscopic DNA spots are attached. The microarrays are used to determine expression levels across a large number of genes or to perform genotyping across different regions of a genome.
3. Denaturation, annealing and extension.
4. Primer and amount of template DNA.
5. Southern blotting technique is used to detect DNA in given sample.
6. It was developed by Edward M. Southern (1975).
7. Western blotting technique is the confirmatory test for HIV. It detects anti HIV antibody in patient's serum.
8. Based on the molecule used for the detection, the real time PCR techniques can be categorically placed under two heads: 1. Non-specific Detection using DNA Binding Dyes 2. Specific Detection Target Specific Probes.
9. Gene Discovery: DNA Microarray technology helps in the identification of new genes, know about their functioning and expression levels under different conditions.
10. The corner stone of genetic engineering or recombinant DNA technology is a special class of enzymes called restriction enzymes.
11. It is the key enzyme that seals single-stranded nicks between adja-cent nucleotides in a duplex DNA chain.

12. Sanger sequencing gives high-quality sequence for relatively long stretches of DNA (up to about 900900 base pairs). It's typically used to sequence individual pieces of DNA, such as bacterial plasmids or DNA copied in PCR.
13. Natural culture media is composed of biological fluids that are naturally occurring. Although this type of media can be used for a range of cells, its biggest disadvantage is that it may lack the exact components required by given cells, which can greatly affect reproducibility.
14. In these types of media, serum (fetal bovine serum) is used as a carrier for nutrients and growth factors among others that tend to be water insoluble.

14.13 SUMMARY

- Gene expression is a highly regulated mechanism that controls the function and adaptability of all living cells including prokaryotes and eukaryotes.
- Gene Expression Techniques: Real-time PCR, Microarrays (DNA chips), Recombinant DNA, technology (Cloning of DNA fragments), Sanger sequencing and Next-generation sequencing.
- Analysis of Nucleic Acids is done in two ways: Polymerase Chain Reaction (PCR) and Gel electrophoresis Blotting techniques (Northern, Southern).
- Methods to study gene function: Cell culture methods
- Polymerase chain reaction is method for amplifying particular segments of DNA.
- Southern blotting is a hybridization technique for identification of particular size of DNA from the mixture of other similar molecules.
- Western blotting technique is used for identification of particular protein from the mixture of protein.
- Real-time PCR is a PCR technique used to quantify starting amounts of nucleic acid template by analysing the amount of DNA produced during each cycle of PCR.
- Recombinant DNA technology is a powerful tool for the isolation of poten-tially unlimited quantities of a gene.
- DNA sequencing enables us to perform a thorough analysis of DNA because it provides us with the most basic information of all: the sequence of nucleotides.
- Cell culture involves the distribution of cells in an artificial environment (in vitro) which is composed of the necessary nutrients, ideal temperature, gases, pH and humidity to allow the cells to grow and proliferate.

NOTES

14.14 KEY WORDS

- **Gene:** A unit of heredity which is transferred from a parent to offspring and is held to determine some characteristic of the offspring.
- **Blotting:** A blot, in molecular biology and genetics, is a method of transferring proteins, DNA or RNA, onto a carrier.
- **Cell:** The smallest structural and functional unit of an organism, which is typically microscopic and consists of cytoplasm and a nucleus enclosed in a membrane.

14.15 SELF ASSESSMENT QUESTIONS AND EXERCISES

Short-Answer Questions

1. What is the principle of Southern blotting?
2. What are the limitations of end-point PCR?
3. Write a short note on recombinant DNA technology.
4. What are the uses ad limitations of Sanger's method?
5. Write a short note on next generation sequencing.
6. Discuss the types of primary cells.
7. What are the major types of culture media?

Long-Answer Questions

1. Write applications of PCR.
2. Describe the procedure of Western blotting.
3. Write applications of real-time PCR.
4. What are the types of microarrays?
5. Describe the mechanism of action of DNA ligase.
6. Discuss the types of restriction enzymes.
7. Explain the method of Sanger sequencing.
8. What is the significance of cell culture? Discuss.
9. Explain cell culture protocol.

NOTES

14.16 FURTHER READINGS

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