M.Sc. Previous Year

Botany, MB-01

CELL AND MOLECULAR BIOLOGY OF PLANTS

मध्यप्रदेश भोज (मुक्त) विश्वविद्यालय – भोपाल

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Published by Registrar, MP Bhoj (Open) University, Bhopal in 2020

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VIKAS® PUBLISHING HOUSE PVT. LTD.
E-28, Sector-8, Noida - 201301 (UP)
Phone: 0120-4078900 • Fax: 0120-4078999
Regd. Office: A-27, 2nd Floor, Mohan Co-operative Industrial Estate, New Delhi 1100 44
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INTRODUCTION

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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.

Both the 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 of Plants*, has been designed keeping in mind the Self-Instruction Mode (SIM) format and follows a simple pattern, wherein each Unit of the book begins with the Introduction followed by the Unit Objectives. The content is then presented in a simple and easy-to-understand manner, and is interspersed with Check Your Progress questions to reinforce the student's understanding of the topic. A list of Questions and Exercises is also provided at the end of each Unit. The Summary, Key Terms and Further Reading further act as useful tools for students and are meant for effective recapitulation of the text.

UNIT 1 ELECTRON MICROSCOPE AND CELL BIOLOGY

Structure

1.0 INTRODUCTION

Microscopy is the technical field of using microscopes to view objects and areas of objects that cannot be seen with the naked eye. Microscopy plays an integral role in the study of microorganisms and can provide extremely useful information about them. Microscope as the name suggests are instruments that help to enlarge minute organisms or their parts. A microscope not only enlarges or magnifies the object but also 'resolves' it, that is makes it possible to differentiate between two points present close together in the objects being viewed. The first microscope was constructed by Anton Van Leeuwenhoek (1632-1723). This microscope consisted of a single biconvex lens fitted in a small window of a 'board' and the object was viewed through it. This was a simple microscope. Next stage was that of a very primitive compound microscope in which two lenses were used. Improvements continued, newer and newer microscopes were designed and are still being improved.

The nucleus is an organelle found in eukaryotic cells. The nuclear membrane is fully enclosed and enclosed inside it is the majority the cell's genetic material. This material is organized as DNA molecules, along with a variety of proteins, to form chromosomes. Eukaryotic cells have intracellular membranes around organelles and vacuoles called as cell organelles or organoids. These are highly organized subcellular protoplasmic structures that have shape, composition and definite function which can be carried out by them even outside cytoplasm provided they are supplied with substance which is normally provided by the cell. Nucleus is extra cytoplasmic organelle while others are cytoplasmic organelles. A few examples include endoplasmic reticulum, Golgi apparatus, lysosomes, mitochondria, plastids, microbodies, cytoskeletal structures, etc. The membranes compartmentalize the cell. Neither

the cell nor the compartments in it are totally isolated from surrounding medium. The membranes allow continuous flow of selected material across them as required from time to time. This helps the cell and organelles to have content different from those of surrounding medium. The prokaryotic cells lack intracellular membranes.

In this unit, you will study about the tools and techniques in cell biology with special reference to electron microscopy, fluorescence microscopy microdensitometry, flow cytometry and autoradiography, ultrastructure, function, biogenesis and special aspects of plasma membrane, nucleus, Endoplasmic reticulum, Golgi apparatus, mitochondria, chloroplasts, lysosomes, ribosome in detail.

1.1 UNIT OBJECTIVES

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

- Discuss about the tools and techniques in cell biology with special reference to different microscopes
- Discuss the structure and functioning of a nucleus
- Explain the function of endoplasmic reticulum
- Analyse the process of photosynthesis in chloroplasts
- Explain the structure and functioning of a lysosome

1.2 TOOLS AND TECHNIQUES IN CELL BIOLOGY

Progress of science depends not only on the scientists ability to observe and infer but also on the tools and techniques available to them for study. More precise and sophisticated tools and techniques enable the scientists to give detailed and accurate information of the object studied. Biology was confined to the study of plants and animals or their parts visible to the naked eye till 1665 when an English scientist Robert Hooke discovered cells in a thin slice of cork examined under a primitive microscope assembled by him. From 1675 to 1680, a Dutch worker Anton van Leeuwenhoek discovered human sperms, red corpuscles, bacteria and protozoans with self-designed simple microscope.

New tools and techniques were invented which helped in the study of finer structure of various kinds of organisms and their parts. Microscope not only revealed a world of minute organisms but also minute details of internal structure of organisms. In the course of history of biology, various new tools and techniques have developed, like microscopy, paper chromatography, etc.

Microscope as the name suggests are instruments that help to enlarge minute (micro = very small) organisms or their parts. A microscope not only enlarges or magnifies the object but also 'resolves' it, that is makes it possible to differentiate between two points present close together in the objects being viewed.

The first microscope was constructed by Anton Van Leeuwenhoek (1632-1723). This microscope consisted of a single biconvex lens fitted in a small window of a 'board' and the object was viewed through it. This was a simple microscope. Next stage was that of a very primitive compound microscope in which two lenses were used.

There are different types of microscopes which are used in studying the various structures and activities inside a cell. Some of these are as follows:

- Simple Microscope
- Compound Microscope
- Transmission Electron Microscope (TEM)
- Phase Contrast Microscope
- Scanning Electron Microscope (SEM)

Resolving Power: It is the ability of a microscope to show two closely lying points as two separate points.

Magnification: It is the ratio of the size of the image to that of the object.

Simple Microscope: A simple microscope is one which uses a single lens for magnification, such as a magnifying glass (Refer Figure 1.1). These are of two types:

- **Hand Lens:** It consists of a biconvex lens, mounted on a handle. The lens is of different sizes and different magnifying powers. It is commonly used to magnify an entire object.
- **Dissecting Microscope:** It consists of a biconvex lens which is moved up and down by an adjustment screw, to bring the object in sharp focus. The light is focused with the help of a concave mirror fitted below. A magnified image of the full object can be seen through it.

Fig. 1.1 *Simple Microscope*

Compound Microscopes: It is commonly used in the laboratories to view extremely minute organisms and parts and sections of larger organisms. Apart from the lenses, it also has a condenser, having a simple mirror on one side and concave mirror on the other. The object is placed first below the objective lens over the stage. The objective lens forms an image of the object. This image is further magnified by the eye piece (Refer Figure 1.2).

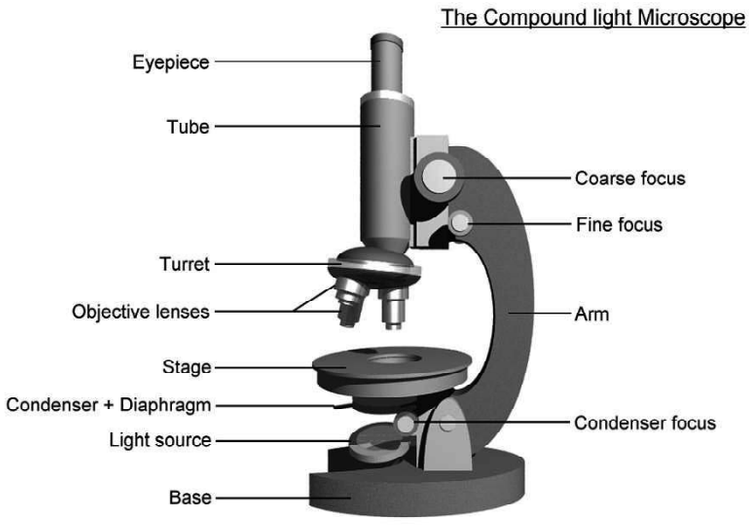

Fig. 1.2 *Compound Microscopes*

Transmission Electron Microscope (TEM): The organelles of the cell became known after the electron microscope was invented. The magnification and resolution of the electron microscope are much higher than that of the compound microscope. A Transmission Electron Microscope produces a high-resolution, black and white image from the interaction that takes place between prepared samples and energetic electrons in the vacuum chamber. Air needs to be pumped out of the vacuum chamber, creating a space where electrons are able to move.

The electrons then pass through multiple electromagnetic lenses. These solenoids are tubes with coil wrapped around them. The beam passes through the solenoids, down the column, makes contact with the screen where the electrons are converted to light and form an image. The image can be manipulated by adjusting the voltage of the gun to accelerate or decrease the speed of electrons as well as changing the electromagnetic wavelength via the solenoids. The coils focus images onto a screen (Refer Figure 1.3).

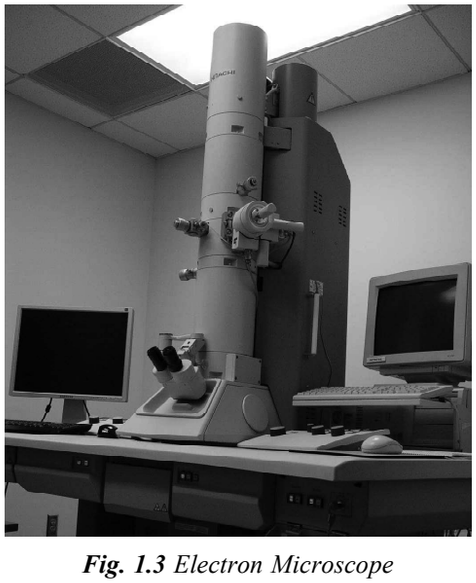

Fig. 1.3 *Electron Microscope*

Phase-Contrast Microscope: It has an annular diaphragm located below the condenser an objective having a phase plate. When light is transmitted through lenses, some of its rays pass directly while others are diffracted laterally (Refer Figure 1.4). The diffracted light

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rays are thus separated from the direct light and an image of strong contrast is produced. Mainly it is used to:

- Examine living cells.
- Observe the nuclear and cytoplasmic changes taking place during mitosis.
- Study phagocytosis and pinocytosis.
- Observe the effect of different chemicals inside the living cells.

Phase Contrast is one of the best methods for viewing transparent or translucent specimens without staining the specimens and without a loss of resolution. The use of **Phase Contrast Microscopes** has proven to be a valuable tool for **medical researchers** in the study of living cells, cultures, human chromosomes/genes. Prior to the introduction of phase contrast, living cells other semi-transparent specimens were viewed in bright-field microscopy by various staining techniques.

Fig. 1.4 *Phase Contrast Microscope*

Scanning Electron Microscope (SEM): In this type of microscope, three dimensional images are developed. It gives more detailed and clear structure of surface of cells. It is particularly used for the study of the surface of an object. Although there are similarities in principle between the layout of optical and electron microscopes, in practice the two are very different. The conventional electron microscope requires that the electron beam be in a vacuum, because electrons cannot ordinarily travel an appreciable distance in air at atmospheric pressure. The column of the electron microscope is evacuated by pumps, and the specimens and any other necessary apparatus are introduced into the vacuum by means of air locks. Unlike the optical microscope, in which the lenses are of fixed focus and the distance between specimen and objective lens is varied, the electron microscope has variable-focus lenses, and the distance between specimen and objective lens and the separation of the lenses remain constant (Refer Figure 1.5).

The magnification is determined mainly by the value of the current (for magnetic lenses) through the intermediate and projector lens coils. The image is focused by changing the current through the objective lens coil. Another difference is that the optical microscope is usually operated so that the image is a virtual one, while in the electron microscope the final image is invariably real and is visualized on a fluorescent screen or recorded for study on a photographic plate in traditional instruments or more usually in today's laboratory on a digital imaging system.

In the optical microscope the image is formed by absorption of light in the specimen; in the electron microscope the image results from a scattering of electrons by atoms in the

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specimen. A heavy atom is more effective in scattering than one of low atomic number, and the presence of heavy atoms will increase the image contrast. The electron microscopist may incorporate more heavy atoms into the specimen for this purpose.

Early microscopes relied on electrostatic lenses, but modern instruments use electromagnetic lenses. These consist of a solenoid of wire together with a magnetic pole piece that creates and concentrates a magnetic field. The lenses used for the condenser and projector system of the microscope differ from the objective lens only in details. For example, the manufacturing and performance tolerances for a condenser or projector lens are less demanding than for an objective lens.

Efforts to improve the resolution of the electron microscope have tended toward production of a single-field condenser-objective lens of low aberrations. In such a lens, the upper part acts as a condenser and the lower as the objective; the specimen is inserted into the centre of the lens, where the axial magnetic field (the field along the axis of the instrument) is at a maximum.

All electron lenses show spherical aberration, distortion, coma, astigmatism, curvature of field, and chromatic aberration due to variations in the wavelengths within the electron beam. Such changes of electron velocity may be either due to variations in the high-voltage supply to the electron gun or due to energy losses from collisions of electrons with atoms in the specimen. The first effect may be minimized by careful stabilization of the high-voltage supply; and for the very thin specimens and the high electron energies commonly used, the second effect may usually be neglected. The resolving power of the microscope is ultimately limited by the spherical aberration of the objective lens. It is not possible to correct this aberration by adding a second lens of opposite characteristics, as can be done for the optical microscope, because magnetic electron lenses are always convergent. Computer-aided lens design has led to great improvements in performance, but electron lenses still require much smaller numerical apertures than do optical lenses in order to function optimally.

Astigmatism in the electron microscope is largely due to deviations from cylindrical symmetry in the radial components of the magnetic field of the lens and is the result of imperfect construction of the lens. The interaction of the electron beam with residual gas molecules in the column may also lead to deposits along the beam path that charge up under the influence of the beam and introduce asymmetries. Astigmatism may usually be completely corrected by use of the stigmators fitted to the objective lens.

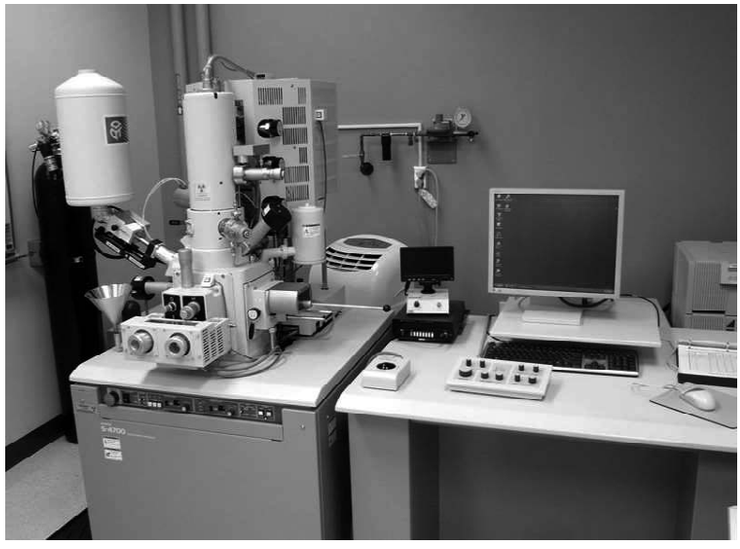

Fig. 1.5 *Scanning Electron Microscope*

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Some Other Techniques

There are other types of tools and techniques that have been developed which helped in the progress of biology as a subject. Some of them are given below:

- **Cytochemical Methods:** These methods are used to locate specific chemical constituents within the cells by differentiating a particular part from other parts by colouring them with a specific stain or dye. It is done either by the use of certain dyes or by using the substrates of enzymes, for example, Schiff's reagent used in Feulgen staining, is used to localize the presence of DNA in a cell.
- **Autoradiography:** This technique is used for study the steps and location of synthesis of molecules and to trace metabolic events in the cells. The radiolabelled compounds are injected into the organism. Then various tissues are investigated to find out where the radioactivity is located. This is done by using photosensitive film of silver bromide. Whenever in the cell or tissue or the organism, the radio labelled substance is present, silver gets reduced by radiation and is seen as black patches in the autoradiographs.
- **Ultracentrifugation:** By rotation at a high speed, particles/organelles of different sizes and shape separate, according to their density. Since the rotation is at very high speed, friction with air produces heat, so has to run under refrigeration and vacuum. Nucleus, mitochondria etc. separate out at different speeds.
- **Cell Fractionation:** By this method different organelles of cells such as nucleus, mitochondria, ribosomes etc. having different particle size and weight are separated by rotating them in a centrifuge at different speeds.
- **Paper Chromatography:** In this method the chemical substances present in a mixture can be separated. A drop of the mixture is put on one end of a long strip of the Whatman filter paper. The filter paper is hung in a manner that the end with the drop of the mixture dips into the solvent mixture kept in the tray/jar. As the liquid is drawn up on the paper, different substances in the mixture begin to separate according to their molecular weight, size and solubility in the solvent and rise up to different heights on the paper. It is then analysed by using certain chemicals for further investigation.

  The cells are first homogenised or broken down by a special method. The homogenate (crushed cells) is then put into tubes and tubes are placed in a centrifuge. The centrifuge is rotated at a high speed. By doing so under the influence of centrifugal force, organelles separate according to their particle density and sizes. The lighter particles settle at the top and the heaviest particles settle at the bottom. The layers are then studied separately and the structure in details gets to be known.
- **Tissue Culture:** This technique involves growing living cells outside the organism by providing all necessary conditions for their survival and growth. The cells from an organism are grown in the laboratory on a nutritive medium at a suitable temperature. Using this technique it has been possible to develop a whole organism from a single cell. Some new fully grown plants have been developed in this way

1.2.1 Tools and Techniques Used in the Modern Study

Some of the tools and techniques being used in the modern study of the cell are described as follows:

Microscopy

Microscopy is the technical field of using microscopes to view objects and areas of objects that cannot be seen with the naked eye (objects that are not within the resolution range of the normal eye).

I. Role of Microscope

Microscope is the instrument that is used to see objects not visible to the naked eye. The study of fine structure of an object under a microscope is called microscopy, and the person who studies it is termed microscopist. The microscope magnifies as well as resolves the objects seen through it.

- **Magnification:** Magnification (M) brought about by a microscope is defined as the ratio of the visible size of an object to its actual size.

$$M = \frac{\text{Size image seen with microscope}}{\text{Size of image seen with normal naked eye}}$$

It is calculated by multiplying the magnification of the objective lens by that of eyepiece. It is possible to increase the magnification of the microscope by increasing the power of the objective or eyepiece or both.

- **Resolving Power:** Human eye is unable to see objects smaller than 100 microns. This means that two points less than 100 micrometer appear as one point to our eyes. The ability to see two close points as separate points is called resolving power. Thus the resolving power of the human eye is 100 micrometer. The resolving power of a microscope depends on the kind of illumination used. It is equal to one half of the wavelength of the illuminating light. The wavelength of the visible light is 3900 to 7800 Å. Taking the average as 5850Å, the resolving power of the light microscope is about 3000Å. We cannot see objects smaller than 0.3 micrometer even with light microscope. Many cell organelles are smaller than 0.3 micrometer. In the electron microscope, a stream of high speed electrons is used for illumination. The wavelength of the electrons depends on the voltage at which they are generated. Standard electron microscope operate with a voltage range of 10,000 to 1,00000 volts. However the resolving power below 10Å is rarely achieved because of certain technical difficulties, mainly inefficient nature of magnetic lenses needed to focus the electron beams.

II. Types of Microscopes

There are four kinds of microscope according to source of light used to illuminate the object:

- Light Microscope
- Ultraviolet Microscope
- X-Ray Microscope
- Electron Microscope

Many modifications of light microscope have been prepared for specific purposes. These include phase contrast microscope, fluorescence microscope, polarizing microscope and interference microscope.

Light or Compound Microscope: The first compound microscope was assembled by Zacharias Janssen and J. Janssen. It was simply a long tube with only two lenses. Various accessories were added by different men. The compound microscope is the oldest and most commonly used microscope for studying the structure of organisms and cells. Its essential parts are:

- **Reflector:** It illuminates the object with visible light.
- **Condenser Lens:** It concentrates the rays of light on the object.
- **Stage:** It provides space for the object mounted on a glass slide in a fluid medium and often covered with a thin glass coverslip.

- **Objective Lens:** It is near the object and produces an initial magnified image of the object.
- **Ocular Lens (Eyepiece):** It is near the observer's eye, and further magnifies the initial or primary aerial images and produces the field image.
- **Adjustment Screws:** These move the body tube up and down to focus the object, i.e., to obtain its sharp image.

The condenser was first added in 1635, two lens eyepiece in 1660, the adjustment screws in 1668 and the reflector in 1712.

Electron Microscope: It was invented by Knoll and Buska in 1931. It has electromagnetic lenses which are coils of wire enclosed in a soft iron case. Its essential parts are:

- **Metal (Tungsten) Filament:** It is heated in a vacuum tube to emit a beam of electrons for illumination. The electrons follow a straight path like the light rays. Vacuum is kept in the tube so that the electrons may not collide with oxygen or nitrogen atoms of the air. Such collisions would scatter the beam, reducing the number of electrons that might pass through the object and contribute to an image.
- **Electromagnetic Condenser Lens:** It collects and focuses the beam of electrons on the object.
- **Electromagnetic Projector Lens:** It magnifies the image, and projects it on to a florescent viewing screen or a photographic plate. The photographs produced by electron microscope are called electron micrographs. The image results from the differential scattering of electrons form the cell components. Denser the material, greater is the scattering of electrons irrespective of chemical composition. The poor penetrative power of electrons requires that ultra-thin sections of the specimens must be prepared for the conventional electron microscope. Such sections are obtained with ultra-microtome using a cut glass or diamond knife.

Microdensitometry

Microdensitometry, or microspectrophotometry, is the measurement of the concentration or mass of a chromophore in microscopically defined regions, and is governed by well-established laws of physics. Initially it proved of value in Feulgen cytophotometry of the relative amounts of DNA in individual nuclei of isolated cells. It has now achieved wide applicability to the measurement of cellular biochemical activity by means of stoichiometric chromogenic reactions. The validity of some of these measurements has been confirmed by comparative biochemical and microdensitometric assays. Thus microdensitometry, even of heterogeneously distributed chromophores, can be precise, provided that the technique is operated with due regard to its limitations within the laws of physics. The potential errors include: variation in thickness of tissue sections (path-length); scatter; glare; diffraction; occlusion of light by optically dense particles; and the inhomogeneity error. However, under correct conditions for the cytochemical reactions and for operating the microdensitometer, these potential errors become small or negligible. Thus the highly sensitive cytochemical bioassay of thyrotropin exemplifies the precision that can be achieved by controlled use of microdensitometry.

A **microdensitometer** is an optical instrument used to measure optical densities in the microscopic domain. A well-known microdensitometer, used in the photographic industry, is a granularity instrument or granularity machine. The granularity measurement involves the use of an optical aperture, 10-50 micrometers in diameter, and in the recording of thousands of optical density readings. The standard deviation of this series of measurements is known as the **granularity** of the measured transmission surface, optical film, or photographic film, in particular.

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An alternative version to the traditional point-by-point microdensitometer is the beam expanded laser microdensitometer. This instrument can illuminate simultaneously an area a few centimeters wide with an ultrathin height, in the micrometer regime. Advantages include increased depth of focus, significant increases in data collection speed, and superior signal to noise ratios. In microscopy applications, this type of ultrathin beam-expanded illumination can also be known as light sheet illumination or selective plane illumination.

This measurement technique, using ultra-thin expanded laser beams, is particularly useful to detect microscopic imperfections in optical coatings or transmission optical surfaces.

Flow cytometry is a technique used to detect and measure physical and chemical characteristics of a population of cells or particles. A sample containing cells or particles is suspended in a fluid and injected into the flow cytometer instrument. The sample is focused to ideally flow one cell at a time through a laser beam and the light scattered is characteristic to the cells and their components. Cells are often labeled with fluorescent markers so that light is first absorbed and then emitted in a band of wavelengths. Tens of thousands of cells can be quickly examined and the data gathered are processed by a computer.

Flow cytometry is routinely used in basic research, clinical practice, and clinical trials. Uses for flow cytometry include:

- Cell Counting
- Cell Sorting
- Determining Cell Characteristics and Function
- Detecting Microorganisms
- Biomarker Detection
- Protein Engineering Detection
- Diagnosis of Health Disorders, such as Blood Cancers

A flow cytometry analyzer is an instrument that provides quantifiable data from a sample. Other instruments using flow cytometry include cell sorters which physically separate and thereby purify cells of interest based on their optical properties (Refer Figure 1.6).

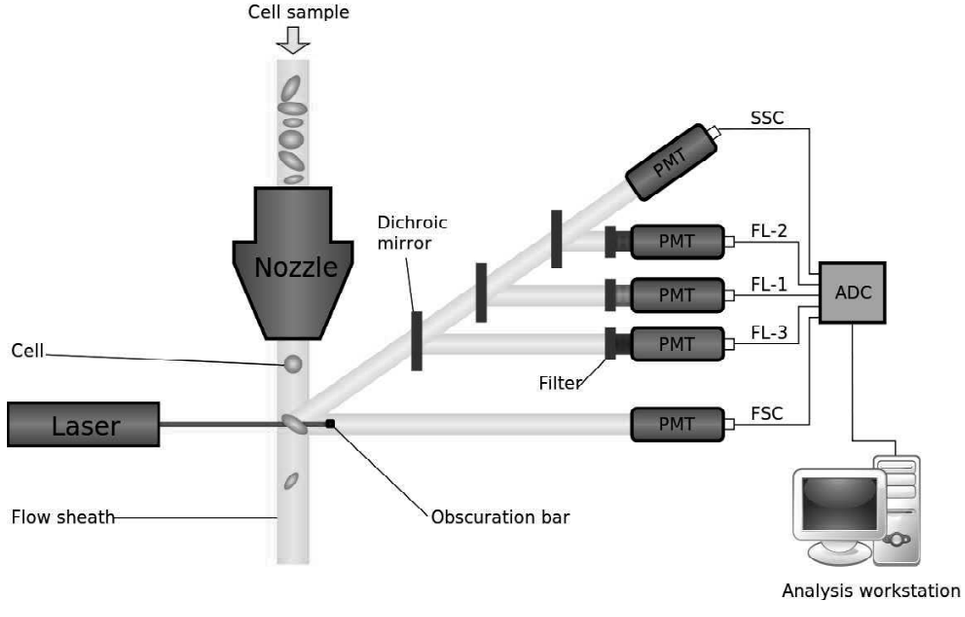

Fig. 1.6 *Flow Cytometry*

Modern flow cytometers are able to analyze many thousand particles per second, in 'real time', and, if configured as cell sorters, can actively separate and isolate particles with specified optical properties at similar rates. A flow cytometer is similar to a microscope, except that, instead of producing an image of the cell, flow cytometry offers high-throughput, automated quantification of specified optical parameters on a cell-by-cell basis. To analyze solid tissues, a single-cell suspension must first be prepared.

A flow cytometer has five main components, as follows:

- Flow Cell
- Measuring System
- Detector
- Amplification System
- Computer for Analysis of the Signals

The flow cell has a liquid stream (sheath fluid), which carries and aligns the cells so that they pass single file through the light beam for sensing. The measuring system commonly use measurement of impedance or conductivity and optical systems - lamps (mercury, xenon); high-power water-cooled lasers (argon, krypton, dye laser); low-power air-cooled lasers (argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet) resulting in light signals. The detector and Analog-To-Digital Conversion (ADC) system converts analog measurements of Forward-Scattered Light (FSC) and Side-Scattered Light (SSC) as well as dye-specific fluorescence signals into digital signals that can be processed by a computer. The amplification system can be linear or logarithmic.

The process of collecting data from samples using the flow cytometer is termed 'acquisition'. Acquisition is mediated by a computer physically connected to the flow cytometer, and the software which handles the digital interface with the cytometer. The software is capable of adjusting parameters, for example voltage, compensation, for the sample being tested, and also assists in displaying initial sample information while acquiring sample data to ensure that parameters are set correctly. Early flow cytometers were, in general, experimental devices, but technological advances have enabled widespread applications for use in a variety of both clinical and research purposes. Due to these developments, a considerable market for instrumentation, analysis software, as well as the reagents used in acquisition such as fluorescently labeled antibodies has developed.

Modern instruments usually have multiple lasers and fluorescence detectors. The current record for a commercial instrument is ten lasers and 30 fluorescence detectors. Increasing the number of lasers and detectors allows for multiple antibody labeling, and can more precisely identify a target population by their phenotypic markers. Certain instruments can even take digital images of individual cells, allowing for the analysis of fluorescent signal location within or on the surface of cell.

Principle: The instrument, flow cytometer, is based on the principle that single cells in suspensions axe passed through a field of illumination and each cell is quantitatively assayed by staining cells with fluorochromes or noting the scattering of light by each cell.

Procedure: Cell suspensions are placed at first in a flow cell fitted with a device to form a liquid jet. Cells will travel through the center of a liquid jet at the rate of 5-10 m/second. Cells are then passed through the area of intense light. If the cells contain some fluorochromes, fluorescence emission can be detected using lens, beam splitters and photomultiplier tubes

Different fluorochromes used will show different types of fluorescence emission. These signals are then quantified and are kept in the memory of computers as histograms. During the flow, droplet formation is done using piezoelectric crystal in the flow cell.

At the end of the instrument there is a droplet deflector assembly. Giving a definite quantitative value to the computer for collecting a specific cell, desirable will then be collected in a microtiter plate or tube and undesirable cells will be collected in the waste. Different fluorochromes are used for different types of cells which will then help to sort the cells having two types of nuclei (heterokaryons).

Applications of Flow Cytometer and Cell Sorter: This instrument has a great importance in selecting somatic hybrid and cybrid cells using fluorescence labelling technique. It can also be applied to know the physical and physiological characteristics of plant protoplasts which has a great importance in molecular biology and in the manipulation of plant protoplasts. This instrument is also useful in the study of the cell cycle and in the isolation of chromosomes.

This is also suitable for cell sorting. In this case, the fluid-containing cells is charged temporarily and the charged cells of desirable particles axe deflected in the electric field. These are collected in the tube for the collection of mitochondria, nuclei, chromosomes besides cells, protoplasts and pollen.

Flow cytometer is also used to measure the DNA content of cell, chromosomes etc. for the measurement of the ploidy level in short time. In case of cell-fusion studies heterokaryons can be rapidly sorted out in flow cytometer. In Genetic Engineering studies, transformed or hybrid cells can be sorted out with this instrument.

Autoradiography

Autoradiography is a photographic technique used to localize a radioactive substance within a solid specimen; also known as radioautography.

A photographic emulsion is placed in contact with the object to be tested and is left for several hours, days, or weeks depending on the suspected concentration of the radioactive material to be measured. The emulsion, which is a gel containing silver halide, is then developed, fixed, and washed as in the usual photographic process. At sites where theemulsion was close enough to the radioactive substance, it appears dark because of the presence of silver grains. When thenumber of grains is insufficient to darken the film to the unaided eye, the film may be examined with the aid of a microscope.The individual silver grains may then be seen. The pattern formed by the grains depends on the type of radiation and the nature of the photographic emulsion. Alpha particles produce short, straight rows or tracks of grains. Beta particles as well as x-rays and gamma rays, which affect film by producing beta particles, produce tortuous tracks whose lengths and graindensities depend on the energy of the beta particles. Low-energy particles produce shorter tracks with higher grain densities. Very low energy particles like those from tritium (3-hydrogen) may produce only a single grain very close to the site of decay.

Autoradiography (Refer Figure 1.7) can be used to detect, and measure semi-quantitatively, the radioactive materials in almost any object that can be placed in contact with film or photographic emulsion in some form. However, in biological research the object may be:

- A whole plant or animal that can be flattened against a film.
- The cut surface of a plant or animal, or one of its organs.
- Thin sections of tissues or cells.
- Squashed or otherwise flattened cells.
- Surface films produced by spreading onwater the protein monolayers containing DNA or RNA that are picked up on grids for electron microscopy.

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- Sheets of paper or other materials on which radioactive substances have been separated by chromatography or electrophoresis.
- Acrylamide gels in which DNA, RNA, or proteins have been separated by electrophoresis.

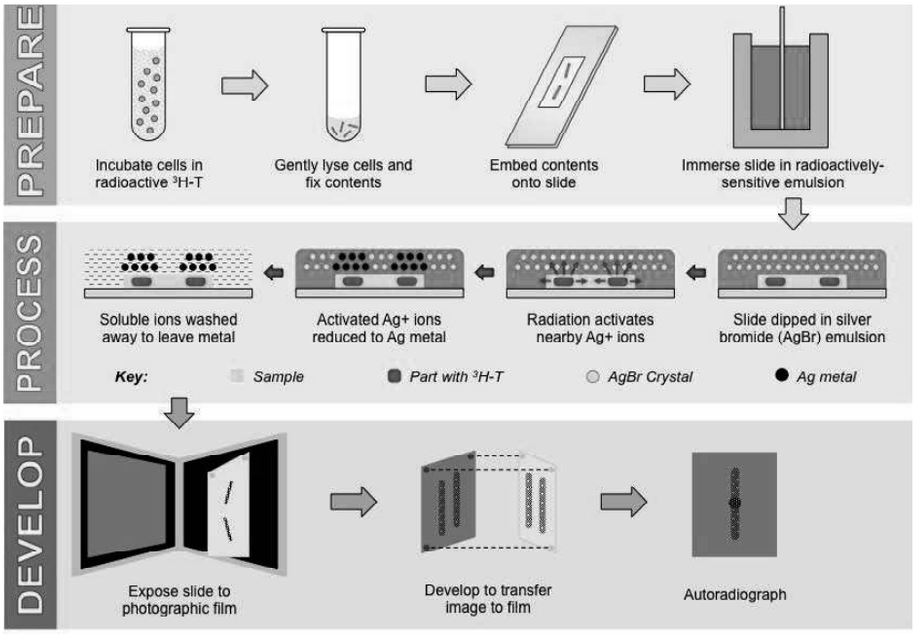

***Fig. 1.7** Autoradiography*

1.2.2 Cell Biology Techniques

The following points highlight the top techniques used in cell biology. Some of the techniques are:

- Immunofluorescence Microscopy
- Ion-Exchange Chromatography
- Affinity Chromatography
- Partition and Adsorption Chromatography
- Gel Filtration Chromatography
- Radioactive Tracer Technique
- Radioimmunoassay (RIA)
- Enzyme Immunoassay
- Spectroscopy

Immunofluorescence Microscopy: Immunological technique is the method to locate an antigen to a particular position of the cell of specific antibody for a particular protein to be studied. Immunofluorescence is another slightly modified technique used to study cells under fluorescence microscopy to locate the distribution of the antigen in the cells.

In this case the first antibody is un-labelled and the second antibody is made against IgG of the organisms in which the first antibody is made. This secondary antibody is coupled to some fluorochrome such as Fluorescein Isothiocyanate, Rhodamine, etc. This method is used to study cellular architecture, subcellular localisation and the localisation of specific proteins during cellular activities and cell cycle events.

The microfilaments, microtubules and intermediate filaments can be studied to have an idea about the cytoskeletal structure of the cell .This technique is useful to study many structures at higher resolution in the electron microscope top.

The main instrument needed for the immunofluorescence studies is the fluorescence microscope with automatic photomicrograph system. Confocal microscopy is being recently used for detailed studies of the structures in or near the nucleus and also for round cells to obtain greater resolutions at different levels in the cell.

Ion-Exchange Chromatography: In this method, molecules are separated on the basis of differences in charge. Many biological macromolecules, such as amino acids and proteins, have ionisable groups. They may carry positive or negative charge. The charge showed by these compounds depend on the pH of the solution. The ion-exchange separations are performed in columns packed with ion-exchanger. Two types of ion-exchangers are present like Cation and Anion exchangers. Cation exchangers are negatively charged and so they can attract positively charged molecules (Refer Figure 1.8).

Anion exchangers are positively charged, so they can bind negatively charged molecules. Tire commercial ion-exchangers are made of porous polystyrene beads. The co-polymerisation of styrene is made with varying proportions of divinylbenzene and styrene.

The most common example is Dowex 50, Sephadex, etc. Sometimes chemically modified celluloses are used instead of polystyrene-based exchangers, such as DEAE-cellulose, Carboxymethyl cellulose, etc.

When the samples to be separated are passed through a column, molecules with opposite charge will bind while the other molecules with the same charge of the medium used in the column and some unwanted materials will pass through.

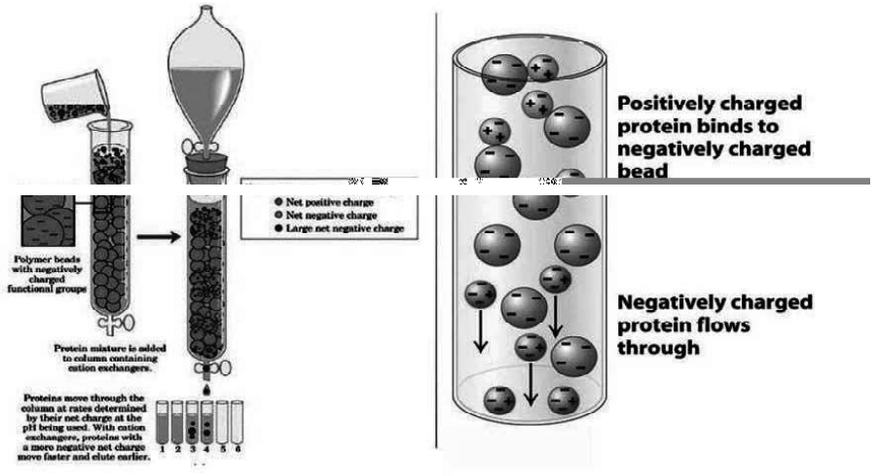

Fig. 1.8 *Ion-Exchange Chromatography Principle*

As the desired compound is retained in the column, this technique is sometimes known as Sorption chromatography. The bind molecules can be eluted by increasing the concentration of the buffer or by changing the pH of the buffer. Sometimes the unwanted molecules are also retained within the column thus eluting the desired substances.

Ion-exchange chromatography has application in separating amino acids and proteins. The selection of strong and weak exchanger depends on the stability over pH and the effect of pH on charge. Generally, cationic buffers like Tris, Pyridine and Alkyl amines are used with anion exchangers and anionic buffers like Acetate, Barbiturate and Phosphate are used with cation exchangers.

Two types of elution can be made, i.e., by passing the single buffer throughout the separation which is known as Isocratic separation. When the gradient of buffer is passed through the column it is called Gradient elution.

The separation of amino acids is generally performed using strong acid cation exchanger. Gradient elution method helps in the sequential elution of amino acids. The acidic amino acids come out first followed by the neutral amino acids like Glycine and Valine.

This is followed by basic amino acids like Arginine and Lysine. This principle is also followed in the Amino acid Analyser. Proteins are separated generally through weakly acidic or basic exchangers. The elution of proteins takes place on the basis of their isotonic points.

Affinity Chromatography: In this method, the property of biological interactions between the molecules is used in order to get separation and purification. In this case some ligand molecule, i.e., substrate of an enzyme or some antibody is bound to the matrix of the column.

On passing the molecules through the column, only those materials that form a specific bond or complex with the ligand will be retained and all others will pass through the column

The main principle of this method is that the compound to be purified is passed through the column containing some immobilised ligand, and the desired compound will bind to the ligand. The ligand is the substrate in case of enzymes.

Instead of column, this method can be utilised on nitrocellulose membranes for the purification of single-stranded DNA molecules. When the enzyme is to be purified, the ligand used is generally the substrate and, for the separation of proteins, specific antibodies are used (Refer Figure 1.9).

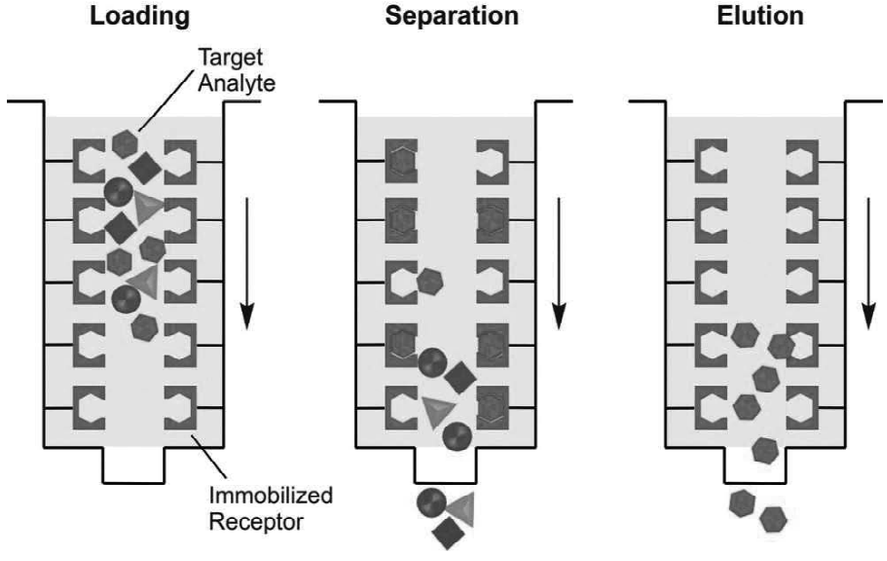

Fig. 1.9 *Affinity Chromatography Principle*

The bound-molecules can be eluted by increasing the ionic strength of the buffer. Thus this type of chromatography is highly specific and versatile. This affinity chromatography is very useful for the purification and separation of macromolecules of very small amount.

The mRNA is purified and isolated from various types of RNA through Affinity chromatography. Immobilised single-stranded DNA is used to isolate complementary RNA or DNA.

Partition and Adsorption Chromatography: It is a common practice to separate many substances by shaking the substance in two immiscible liquid phases in a separating funnel. When a substance is shaken in the solvent it will partition with the formation of two phases. If one phase is allowed to move the substance will also move on the basis of its partition coefficient.

The substance will move rapidly if it likes the mobile phase while, if it prefers the stationary phase, it will move slowly. Now the mobile phase may be liquid or gas and, if the substance is adsorbed on the stationary phase and starts to move along with the stationary phase, then it is called Adsorption chromatography.

The substance will move at a varying speed depending on the intensity and characteristics of adsorption and solubility in the solvent used for separation. Adsorption chromatography can be performed either in the column or on the thin layer of the matrix. Adsorbents may be Silica gel, Aluminum oxide, Calcium carbonate, Magnesium carbonate, cellulose, etc. which are used as the stationary phase.

Hydroxyapatite (Calcium phosphate) is used in the column to separate proteins, nucleic acids etc. It has the unique property of binding double-stranded DNA and not single- stranded DNA. For mobile phases, different organic solvents may be used, depending on the polarity of the compounds to be resolved.

The basic principle of Adsorption and partition chromatography is used in Paper chromatography, Thin-layer chromatography and Gas chromatography. In case of paper chromatography, the paper is the support. Water is used to moist the paper and this hydrated phase is the stationary phase. The organic solvent which is used in the chromatography is the mobile phase which moves rapidly through the aqueous phase.

In Gas chromatography, the partitioning takes place between a liquid and a gas phase. Here the gas is the mobile phase and the non-volatile liquid that coats the matrix substances of a column is the stationary phase (Refer Figure 1.10).

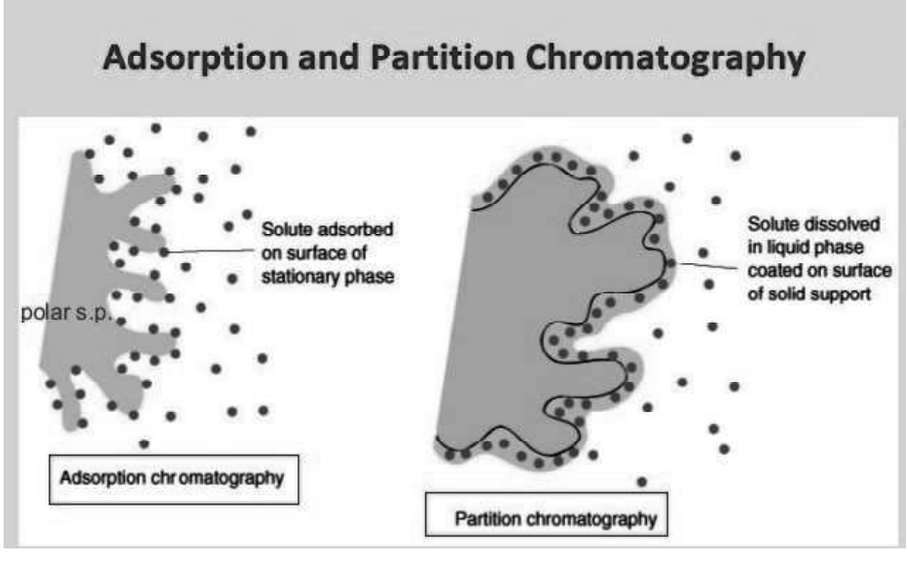

Fig. 1.10 *Adsorption and Partition Chromatography*

This partitioning depends on the temperature and the gradual increase in temperature helps more and more of the substance to come out in the mobile gas phase from the stationary phase. This method is widely used for the qualitative and quantitative analyses of large number of compounds.

The principle for the separation is the difference in the partitioning of the volatilized compounds between the liquid and gas phases. During the passage of the substance through the column a detector is attached with a chart recorder, which scan the peak as the substance passes through the detector.

Gel Filtration Chromatography: This type of chromatographic separation takes place on the basis of the size and shape of molecules utilizing the porosity of the gel materials. This method is also known as exclusion or permeation chromatography.

This is done by using a column full of matrix consisting of Sephadex, Agarose, Sepharose, Bio Gel A, Bio Gel P, Polystyrenes (Bio-beads S), etc. Varying properties of the matrix axe obtained by crosslinking the poly-dextran with other compounds.

Most of the gel compounds are hydrophilic in nature, thus causing very little-denaturation and adsorption of sensitive biochemical substance. Sephadex and polyacrylamide gels are used as swollen beads. Many gels are available in the market as Superfine, Fine, Medium and Coarser.

The coarser bead shows fast flow rate but with poorer resolution. Fine and Superfine beads are used for analytical work and the coarse one for preparative work.

When the molecules of different sizes and pores are passed through a column, molecules larger than pores of gel will pass through it. Molecules of small pore size will enter the beads and flows are retarded in the column (Refer Figure 1.11).

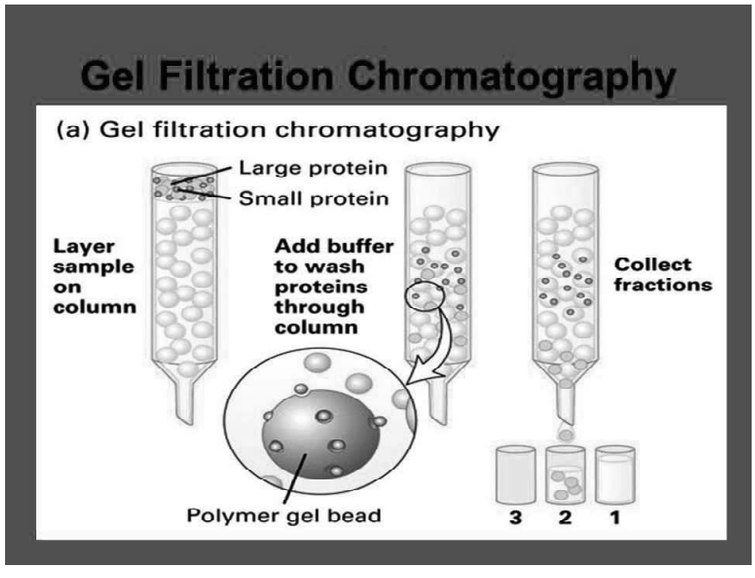

Fig. 1.11 *Gel Filtration Chromatography*

The main use of this procedure is in the purification of macromolecules, viruses, proteins, enzymes, hormones, antibodies, nucleic acids, amino acids etc. It is also used for the isolation of ribosomal proteins.

Radioactive Tracer Technique: The easiest method for monitoring cellular events and their localisation in the cell is the use of radioactive isotopes. The most commonly used isotopes in the biological research are H3, C14, P32, S35, I125, I131. They release high energy electron or beta particles during their radioactive decay.

Radioactive decay is a spontaneous process and its rate varies with the source. The number of atoms disintegrating at any time is proportional to the number of atoms present in the isotope at that time. Conveniently, it is expressed as half-life which is defined as the time taken for the activity to fall from any value to half that value.

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According to SI system, the unit of radioactivity is Becquerel (Bq), which is one disintegration per second. But the most commonly used unit is the curie (Ci) which is measured as the number of nuclear disintegrations per second as compared to that of 1 gm of Radium, i.e., 3.7×10^{10} per second.

In case of biological materials, the microcurie (μCi) and millicurie (mCi) are used. The disintegrations measured by the Counter are referred to as Counts.

For any biological research, macromolecules of the cell are made radioactive by administering the radioactive compounds to tissues or cells and then the fate of the radioactive compound can be monitored. For the study of DNA synthesis in the various types of cells or tissues, the use of H_3 is necessary.

Many organic and inorganic compounds labelled with Tritium are available of Bhabha Atomic Energy Research Centre, Mumbai. Tritiated Thymidine and Uridine are used for the study of DNA and RNA, respectively.

The radioactive substance is added in the solution where cells or tissues are exposed for a certain period of time, either cells are fixed at regular intervals or aliquots are removed at various time intervals and the radioactivity is noted.

The radioactivity may be measured through Liquid Scintillation Counter or the location of the radioactivity in different positions of the cell, can be noted through Radioactive Tracer Technology, called as Autoradiography. In this method labelled cells are fixed and squashed on a slide or can be spread on a slide.

Then a thin layer of Special Auto radiographic stripping film is placed over it and is kept in the dark for exposure. Instead of film, sometimes the slide is coated with photographic emulsions for autoradiography and is kept in the dark.

During storage of slides in the dark, the emission of beta particles from the radioactive substance activates Silver halide crystals of film or emulsion. After a few weeks, the slides are developed like the photographic film, which show the activated silver crystals as black spots under the light microscope.

The auto radiographic technique is also applicable to electron microscopy where cells or tissues are placed on a grid instead of a slide. Here Silver grains are found to be opaque and electron dense against the electron transparent background.

The important use of this radioactive tracer technique is to note the metabolic pathway in the cell using Pulse-chase experiment through some radioactive precursor. When tissues or cells are exposed to some radioactive compounds for a certain period of time, it is called Pulse and then cells are kept in a radioactive free medium after washing is called Chase.

By fixing cells or taking aliquots from cell fractionation in different times, biochemical transformations and movement of the precursor (labelling) can be observed in the cell through autoradiography or through Scintillation Counter.

Radioimmunoassay (RIA): This method is widely used in Biochemistry and also in clinical fields for diagnostic purposes. The quantitative analysis of hormones, steroids and drugs can be done with the help of this method. As the name implies, it combines the method of immunology as well as the radioisotope labelling techniques.

In this method labelled antigen, unlabelled antigen and fixed quantity of antibody are mixed together and a calibration curve is plotted with percentage of labelled antigen against the unlabelled antigen added (Refer Figure 1.12).

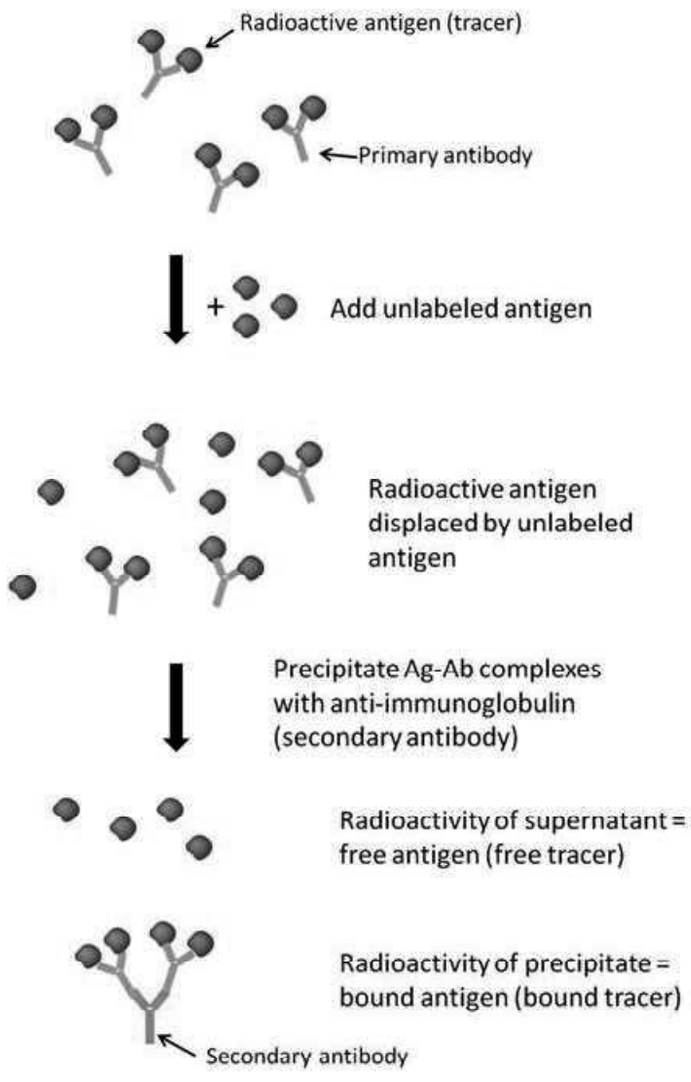

Fig. 1.12 *Radioimmunoassay*

Unlabelled antigen is treated as samples. Then antibody-bound antigen is separated from free antigens through ion-exchange or adsorption chromatography. Sometimes the mixture of these antigens and antibodies are passed through Sephadex column, when the bound antibodies remain attached to the Sephadex beads and the unbound antigens can be washed out.

Then the bound labelled antigens are eluted from the column and can be quantitatively arranged through Liquid Scintillation counter. Antibodies are generally labelled with H3, C14 or I131. When cells or tissues axe used, radiolabeled antibodies can be used with the help of autoradiography to localize the various components within the cell.

Enzyme Immunoassay: This method is also known as Enzyme-Linked Immuno Sorbent Assay or ELISA it combines the principle of Antibody-antigen reactions as well as the spectrophotometric enzyme assays by using antibodies or antigens conjugated with some enzymes like Alkaline phosphatase, Alcoholic dehydrogenase, etc. ELISA is easy to operate and the cost is also less. Hence ELISA is gradually replacing RIA.

In this method, specific antibodies are attached to solid phase (filter paper, polystyrene micro titration plates, etc.) then a limited amount of labelled antigen (with a specific enzyme) and excess amount of unlabelled antigen were added, incubated and washed.

Some un-labelled antigens will also attach with antibodies. The enzyme substrate is then added and the enzyme activity is measured through spectrophotometer. The amount of enzyme activity is found to be directly proportional to the amount of antigen present. The sensitivity of ELISA is greatly enhanced through enzyme amplification technique using double antibody method.

ELISA is used in clinical fields to measure any antigen.

Immunoglobulin, haematological factor, hormone, etc. It is also used in the detection of bacterial toxins, viruses, Hepatitis B surface antigen, etc., and in the assay of different antibodies like antiviral and antifungal antibodies.

Instead of enzymes, sometimes some fluorochrome is tagged with antibody to assay the-antigens and antibodies using fluorescence system. Then this method is known as Fluorescence ImmunoAssays (FIA).

Spectroscopy: Variety of techniques are developed with the principle of spectroscopy, i.e., study of interaction between electromagnetic radiation and the substance. Light, heat, microwaves, infra-red, X-rays, etc. have electromagnetic waves with average speed of 3×10^8m/sec.

These waves are composed of two components, such as electric field and magnetic field which are oscillating as perpendicular to each other. Light has both properties, such as waves and particles.

Electrons of an atom remain in the lowest energy level in the ground state. When an atom is treated with light, then its electrons move from the ground state to the excited state with the absorption of light or electromagnetic radiation. When an excited electron returns to the ground state, it will emit radiation of certain specific wavelength (energy) which is utilised in spectroscopic analysis.

In some substance, emission of energy takes place spontaneously, i.e., without application of any external radiation. Besides absorption or emission of light, atoms of certain substances undergo some changes in electronic or nuclear properties when the molecule is exposed to electromagnetic radiation.

The study of absorption or emission properties as well as some changes in the nuclear structure gives many information of different macromolecules.

Nuclear Magnetic Resonance (NMR) Spectroscopy: It is a method for detecting interaction between the nuclei of an atom with the magnetic field of the electromagnetic radiation. It is known that protons and neutrons of the atom have spin properties. When protons and neutrons of an atom are present in pairs in the nucleus, there will be no net spin.

But if there is any unpaired protons, these protons will impart a magnetic moment which can interact with an applied magnetic field, i.e., the nuclei will absorb the energy and may lie either in a low energy state (nuclear spin parallel with the field) or in a higher energy state (antiparallel to the field).

This interaction of unpaired proton with the magnetic field is the main principle of NMR spectroscopy. In a magnetic field, these nuclei absorb radiation of radio wave length showing a phenomenon known as nuclear magnetic resonance.

For NMR study, unpaired nuclei like H1, C13, N14, O17, P31, etc. are commonly used in case of biological materials. NMR spectra are plotted as energy absorbed against the magnetic field strength. 40 MHz radio wave frequency is used to obtain resonance of H^1 nucleus. The NMR spectrometer scales in tan (T) units.

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The basic NMR Instrument Includes:

- Source of Radiation
- Receiver to Detect the Absorption of Energy
- Magnetic Field
- Oscilloscope or Recorder

NMR is used for the study of molecular structure of some organic molecules, action of different antibiotics and drugs on living systems, any alteration in the structure of molecules in the plasma membrane, effect of cholesterol on erythrocyte membranes, etc. High field NMR instruments have been developed to explore the structure and dynamic properties of proteins in solution.

Optical Rotatory Dispersion (ORD) and Circular Dichroism (CD): The three-dimensional structure of macromolecules in solution can be studied by noting their properties of absorption of polarised light. The plane polarised light is a type of light consisting of waves oscillating in a single plane. This is obtained by passing a beam of light through a Nicol prism or a polarizing screen.

When a plane polarised light is passed through a substance, the polarised light will rotate to a certain angle depending on its structure. It has also been found that this depends on the wavelength of light. Hence, the rate of change of rotation is measured with wavelength of light which is known as Optical Rotatory Dispersion (ORD).

Certain optically active substances have been found to absorb polarised light differently, i.e., differential absorption of Right (R) and Left (L) circularly polarised light. Thus another spectroscopy Circular Dichroism Spectroscopy (CD) has been developed to investigate the interaction of polarised light and the samples.

Both CD and ORD are almost same but, due to the relative simplicity of CD spectra, CD analysis has gained its superiority. The resolution of CD bands is also superior.

Circularly polarised lights is obtained by superimposing two plane polarised light of the same wavelength which come through the monochromator and Nicol prism. This superimposed light can be resolved into Right (R) and Left (L) waves. Certain substances absorb differentially R and L waves and show refraction with elliptically polarised beam.

ORD and CD are useful in the study of the secondary structure of macromolecules, particularly protein and amino acids in solution. CD spectra is also useful for the study of binding of substrate and inhibitor to the enzyme.

The helical structure of DNA and protein can be studied with the CD spectra. CD spectrum, is very sensitive to any structural changes of the macromolecule. So, any interaction of protein with nucleic acids can be studied by observing changes in the CD spectra. The transitions between double-stranded and single- stranded nucleic acids can be studied with CD spectrophotometer.

Infra-Red (IR) Spectrophotometry: Infra-red (10^3-10^4 nm) shows vibrational spectra, so molecules under infra-red show different vibrational levels. These vibrational levels change with the bonding characteristics of the compound. For example, vibrations of C – H,-CH_2 and CH_3 will differ.

Similarly, various functional groups like methyl, carbonyl, amide groups, etc. will show different IR spectra. Hence the IR spectra is useful in biochemistry, particularly for the study of macromolecules and membranes, for the identification of drugs, for the study of secondary structure of proteins, such as the number of helical structures present in protein etc.

Atomic Absorption/ Flame Spectrometry: When vitalization of atoms of any compound occurs either in a flame or electro thermally, then it absorbs or emits atoms of specific wavelength. Emission flame spectrophotometer measures the emission of specific wavelength of atoms in a flame which is used to assay different elements present in any biological sample.

Atomic absorption spectrophotometer detects the absorption of a particular wavelength by atoms of a sample when it is heated either in a flame or otherwise. The flameless method is more sensitive than flame spectrophotometer. This atomic absorption spectrophotometer is useful in measuring amount of heavy metals or other toxic metals present in any biological sample.

The main components of the flame are:

- Nebulizer or Atomizer which makes five drops of the sample in solution, and then pass these drops with a forced air pressure to the burner (flame).
- Monochromator is used to select the wavelength.
- Detector containing a photocell.

In case of Atomic absorption spectrophotometer, a source of white light (cathode discharge lamp) and a double monochromator are used in addition to a detector

Non-Invasive Scanning of Soft Tissues: Several methods are now developed to examine the soft tissue of the body, particularly the brain, without injecting any colored substance in the body.

These include:

- Computer Tomography Scan (CT Scan).
- Magnetic Resonance Imaging (MRI).
- Microscopic Magnetic Resonance Imaging (mMRI).
- Positron Emission Tomography (PET).

In CT scan, differences in the absorption of X-ray by the brain tissue are used to construct a three dimensional image (3D image). Here the dose of X-ray needed is very low.

The principle of MRI is to use magnetic field which shows differences in vibration of Protons (HT) of the water molecules present in the tissues of body. This vibration of water molecule depends on the chemical surroundings of the tissue. Besides protons of water molecules, the vibration of other atoms like Sodium, Phosphorus and Nitrogen can also be detected.

Through MRI studies, many things of the metabolic processes of brain and other organs may be investigated. Both these types of scanning help to detect the location of tumours, sites of hemorrhage, intracranial bleeding, etc. MRI is better than CT scan because it does not require X-ray. Again, all these modern techniques are possible only with the help of computer.

mMRI is the most technically complex of all techniques. It requires placing the specimen inside a strong magnetic field. The advantage of mMRI is that it can image specimen which are too large and opaque and it can also image living specimen. It can also provide digitally recording anatomical information from intact specimen. Unique contrast mechanism can be applied to highlight the different features of specimens.

PET scan is based on the use of positron, which is like an electron except that it has positive (+) charge. It also gives 3D image where the molecules are located. It helps to measure the blood flow in the brain, glucose utilisation and oxygen consumption, and for the

diagnosis of psychiatric disorders, brain tumours, epilepsy and regenerative changes due to Alzheimer's disease.

'Check Your Progress'

1. Define phase contrast.
2. Where are cytochemical methods used?
3. What is the use of cell fractionation method?
4. What is flow cytometry? Give its uses.
5. What is immunological technique and immunofluorescence?

NOTES

1.3 ULTRASTRUCTURE, FUNCTION, BIOGENESIS AND SPECIAL ASPECTS OF CELL ORGANELLES

1.3.1 Nucleus

Nucleus is a specialized double membrane bound protoplasmic structure which carries all the genetic information for controlling cell metabolism and transmitting the information to next generation. Nucleus is the largest cell organelle, and it was first studied by Robert brown in orchid root cell. It is present in all the eukaryotic cells except mature sieve cells of higher plants, RBCs of mammals, and blood platelets. Experimental evidence of role of nucleus in transmission of hereditary information comes from the work of Boveri on sea urchins. Nucleus is generally the most conspicuous organelle of a eukaryotic cell. It is noticeable with light microscope but its fine structure can be revealed by electron microscope. Nucleus was observed by a Dutch microscopist, Anton van Leewenhoek, in the RBCs of fishes. A Scotch botanist Robert Brown was the first to describe the nucleus in orchid cells in 1831. Nucleus is present in all eukaryotic cells, but certain mature cells do not have nucleus. The mammalian red blood cells lose their nuclei at maturity and survive for few months only. The food conducting phloem cells called sieve tubes also lose their nuclei at maturity but remain functional for several years. Hence it was known that the nucleus is essential for the survival of cell. The cells deprived of nuclei cannot divide and differentiate. Prokaryotic cells don't have an organized nucleus having nuclear envelope. They have one or more nucleoid each having circular DNA molecule without membrane (Refer Figure 1.13).

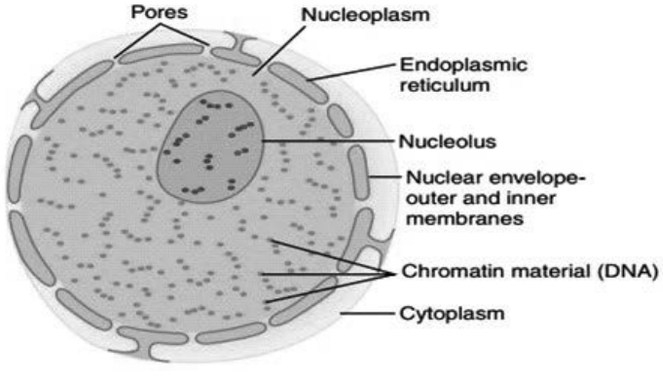

Fig. 1.13 *Structure of a Nucleus*

Number: Commonly cells are uninucleate. The protozoan Paramecium is binucleate one for controlling metabolic activity and other for possessing hereditary information. Multinucleate

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or polynucleate condition is found in cells of bone marrow (upto 100 nuclei), latex vessels. Multinucleate animal cells are called syncytial cells while in plants it is known as coenocyte cells.

Position: Nucleus is found in peripheral position in plant cell due to development of large central vacuole, also in adipose cells. In glandular cells, it is present towards the base. Nucleus is generally found in the region of maximum metabolic activity in the cytoplasm

Shape: It is generally oval in plant cells, elongated in muscle cells, kidney shaped in paramecium, variously lobed in basophil, neutrophil, monocyte and lymphocyte of WBCs.

Chemical Composition: DNA – 10-12%, RNA- 5%, Lipids-3%, Basic proteins-15%, acid proteins, neutral proteins and enzymes -65%. Minerals like Calcium, Magnesium, Sodium in traces.

Ultrastructure: Interphase nucleus is differentiated into 5 parts, namely- nuclear envelope, nucleoplasm, nuclear matrix, chromatin and nucleolus.

Nuclear Envelope (Karyotheca)

It separates the nucleus from the cytoplasm. The separation of a cell genetic material from surrounding cytoplasm is the single most important feature that distinguishes eukaryotes from prokaryotes.

- It is made up of two membranes both made of lipoprotein. Inner membrane is smooth and provides sites for attachment to the chromatin fibers.
- Outer membrane may be smooth or may bear ribosomes.
- The two membranes are separated by electron transparent perinuclear space which is 100-700 Å in width. The inner surface of the nuclear envelope is bound by integral membrane protein to a thin filamentous meshwork called nuclear lamina. It provides support to the nuclear envelope; serve as a site for attachment for chromatin fibers.
- Outer membrane is connected to endoplasmic reticulum due to which perinuclear space contains fluid similar to the one present in spaces of Endoplasmic Reticulum.

Functions

Following are the functions of a nuclear envelope:

- It maintains the shape of nucleus.
- It protects the genetic material from enzymes and other biochemicals present in the cytoplasm.
- Inner membrane provides attachments sites to telomeres of elongated chromosomes.
- Outer membrane is the seat of attachment of microfilaments and microtubules.

Nuclear Matrix

- Network of fibrils of acid proteins which function as scaffold for chromatin.
- It provides sites for attachment to chromatin.
- Just below nuclear envelope, matrix form dense layer called nuclear lamina.
- Nuclear lamina performs three functions, i.e., provide mechanical strength to envelope, provide components for nuclear pore complex formation and attachment sites to parts of chromatin.

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Nucleoplasm, Nuclearsap, Karyolymph; Strasburger, 1882)

- It is transparent, jelly like colloidal complex.
- It is similar in composition to cytosol as it contain nucleosides, various enzymes (DNA polymerase, RNA polymerase and nucleoside phosphorylase).
- Proteins present in nucleoplasm are essential for spindle formation.

Nucleolus

- Discovered by Fontana (1781). It is darkly staining naked, round irregular structure attached to chromatin at region called nucleolar organizer region.
- Up to 1600 nucleoli have been reported in the oocytes of Xenopus.
- Cells having small nucleoli produce little protein synthesis.
- Covering membrane is absent in them though calcium is essential for maintaining its configuration.

Functions

- Essential for spindle formation during nuclear division.
- The proteins are associated with r RNA to produce ribosomes.
- Transcription and early processing of t RNA occur in nucleolus.
- Principal site for synthesis of ribosomal RNAs.

Chromatin

- It is DNA protein hereditary complex named due to its ability to get stained with basic dyes.
- Chromatin is differentiated into two parts: Euchromatin and heterochromatin.
- Euchromatin: It forms majority of chromatin.
- It shows normal cycle of crossing over, replication and gene activation.
- It is influenced by changes in pH temperature, hormones and chemicals.
- Heterochromatin is condensed, granular part which shows late replication, high condensation , little gene activity and crossing over.
- Larger heterochromatin granules are called chromocentres or false nucleoli.
- Heterochromatin is of two types, constitutive and facultative.
- Constitutive heterochromatin is present in all the cells, it contain DNA having repeated sequences and provide strength to delicate regions.
- Facultative heterochromatin develops at particular stage of life, it is meant for inactivating genes not required in a set of cells.
- One X chromosome of female becomes heterochromatic during embryogenesis.
- Heterochromatin is generally found in areas having fewer genes like centromere, satellite and telomere.

Functions

The functions of chromatin are listed below:

- It is carrier of hereditary information.

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- It give rise to chromosomes passed to the next generation during reproduction
- It controls biosynthetic activity of the cell through the formation of m RNAs.

Functions of Nucleus

Herein below are the functions of a nucleus:

- It controls metabolism of cell and other activities through formation of RNAs (m RNA, r RNA , t RNA) which controls synthesis of enzymes.
- It contain hereditary information called chromatin which is DNA protein complex made of fibers that condense to form chromosomes.
- It possess all the genetic information required for growth and development of organism, metabolism and behaviour.
- It directs cell differentiation by allowing only particular sets of genes to function.
- The nucleolus part of nucleus forms the ribosomes.
- Nucleus directs the synthesis of structural proteins and chemicals required for growth and maintenance.
- All the variations caused by changes in genetic material is present in nucleus.

1.3.2 Endoplasmic Reticulum

Discovered by **Porter** and **Thomson** in **1945**. It is a three dimensional, interconnected system of membrane lined channels that run through the cytoplasm. ER is connected with plasma membrane (cortical ER) as well as nuclear envelope (perinuclear ER). The RER is composed of network of flattened sacs and is continuous with the outer membrane of nuclear envelope which bears ribosomes on its cytosolic surface. In contrast, the membranous elements of SER are tubular and form interconnecting system through the cytoplasm ER forms 50-90% of membrane system of cell which increases the internal surface 40 times compared to external surface. It is noticeable only with electron microscope. In 1945, Porter, Claude and Fullman noted with the help of electron microscope a delicate membranous network in the cytoplasm. It was called endoplasmic reticulum by Keith Porter in 1953.

Location: ER is extensive in metabolically active cells, i.e., pancreas, liver. Simple in storage cells (tubules in adipose tissue) reduced in spermatocytes, absent in eggs, RBCs, prokaryotic cells.

Structure: It is the largest membrane in a cell. It represents 30-60% of total membrane in a cell. It comprises three types of elements: cisternae, tubules and vesicles

Cisternae: Flattened, sac like, un-branched, lie parallel to but interconnected with one another. They are found in bundles where they lie parallel to one another. It is involved in synthetic activity. They bear ribosomes on the surface

Tubules: Irregular, branching elements often free of ribosomes. Tube like extensions connected with cistenae and vesicles to form reticular system.

Vesicles: Oval, vacuole like which occur isolated in cytoplasmic matrix and free of ribosomes. They are also called as microsomes.

All the elements freely communicate with one another and contains fluid called endoplasmic matrix in their lumina. The membrane bounding cisternae, tubules and vesicles of ER is similar to the cell membrane (Refer Figure 1.14).

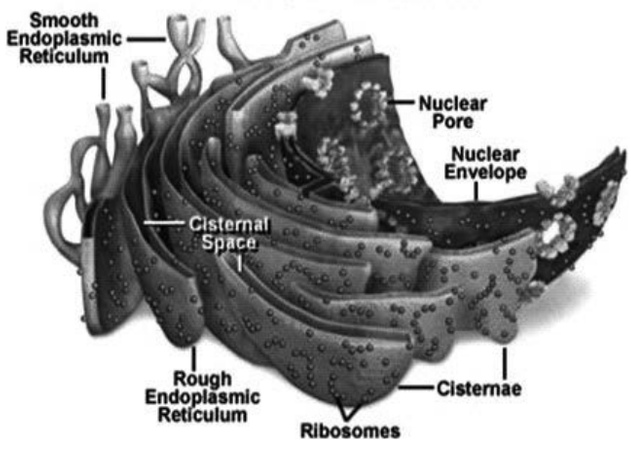

Fig 1.14 *Endoplasmic Reticulum*

Ultrastructure: The membranes are composed of two layers of phospholipid sandwiched by two layers of proteins. As many as 30-40 enzymes are present along with ER membranes for various synthetic activities. The membranes have high protein to lipid ratio.

Types: It is of two types, smooth and rough. In liver both types of Endoplasmic Reticulum is found. In others, only one type is found. Sarcoplasmic reticulum of muscle cell form plexus around myofibrils. A third type called annulate ER is also found sometimes. All the three types are continuous with one another, nucleus and plasma membrane.

Smooth Endoplasmic Reticulum: It is well developed in skeletal muscle, adipose cell, spermatocytes, leucocytes, glycogen storing liver cells, cells that synthesize and secrete steroids. Smooth ER of muscle cells is called sarcoplasmic reticulum. It releases and reabsorbs calcium. SER of retinal pigment cells form tightly packed vesicles and tubules called as myeloid bodies.

Rough Endoplasmic Reticulum: It has rough membranes due to presence of ribosomes on its outer surface, also called granular endoplasmic reticulum. As it is basophilic, area could be stained so is called ergastoplasm. It is highly developed in cells that synthesize and secrete proteins. These include liver cells, pancreatic cells, salivary gland cells, cartilage cells, plasma cells and endocrine cells that secrete peptide hormones. The rough ER is more sparsely distributed in plant cells. The membrane bears number of gated channels or translocons in area of attached ribosome to pass polypeptide into channel of ER for transport. Rough ER is sparsely distributed in plant cells compared to animal cells.

Transitional Endoplasmic Reticulum: It is part of smooth endoplasmic reticulum that lies towards the face of goligi apparatus. It is also called as ER-Golgi intermediate compartment also called as transition vesicles.

Annulate Endoplasmic Reticulum: It is believed to be formed by protusion from the nuclear envelope. It has storage sites of huge nuclear pore complexes. It takes part in synthesis of new nuclear envelope after telophase.

Functions

The functions of a rough endoplasmic reticulum are disussed below:

- The RER provides a large surface for attachment of ribosomes.
- RER offers extensive surface on which protein synthesis can be carried on by ribosomes. The newly formed protein enters the ER membranes, becoming part of membrane structure or pass into the ER lumen. The proteins becoming a part of ER

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membrane move from ER via membranes of other cell organelles, namely Golgi apparatus, to become permanent plasma membrane proteins. The proteins entering ER lumen are packed for export. RER provides a large surface area to ribosomes.

- The proteins in ER lumen are processed and enclosed in spherical membrane bound vesicle which pinches off from the ER. Some remain in cytoplasm as storage vesicles; others migrate to plasma membrane and expel their content by exocytosis. The process of directing proteins to their final destination is called protein sorting or protein trafficking. There is a coordinated system of protein synthesis, transport and secretion in the cell. It synthesizes membrane proteins, serum proteins and other proteins.
- It provide enzyme precursor for formation of lysosomes by Golgi complex.
- Proteins synthesized by ribosomes enter the channels of RER both as intracellular and extracellular transport.
- It contain single receptor protein or ribophorins for providing attachment to ribosomes.

Smooth Endoplasmic Reticulum (SER)

The SER provides surface for the synthesis of fatty acids, phospholipids, glycolipids, steroids and visual pigments. A few features and functions of SER are listed below.

- Sequestering calcium ions with in cytoplasm of skeletal and cardiac muscle cells. The regulated release of Ca^{2+} from the SER triggers contraction.
- Detoxification in a liver of many organic compounds leads to proliferation of SER in liver cells. It is carried out by a system of oxygen transferring enzyme like cytochrome P450 family. The harmless compound benzopyrene is formed when meat is charred on a grill is converted.
- SER carries enzymes for glycogen metabolism in liver cells. Granules of glycogen are attached in larger numbers to outside of SER membranes in liver cells. When body needs energy, glycogen is hydrolysed under hormonal control by enzyme phosphorylase to glucose 1 phosphate, which is converted to glucose 6 phosphate in the cytoplasm. Glucose 6 phosphate cannot leave the liver cell as membranes are impermeable to it. The enzyme glucose 6 phosphatase of SER membrane catalyzes the dephosphorylation of glucose 6 phosphate to glucose and transfers the glucose formed into the SER lumen. From here glucose enters the blood which carries it to needy cell for use in energy release.
- The SER produces Golgi apparatus, lysosomes, microbodies (peroxisomes, glyoxisomes, etc.) and vacuoles. The protein shift from RER through SER to Golgi apparatus for further processing.
- The sarcoplasmic reticulum in skeletal muscle cells releases Ca^{2+} ions to cause contraction and absorbs Ca^{2+} ions to bring about relaxation.
- The SER has enzymes that bring about detoxification in the liver i.e. converts harmful materials such as pesticides, carcinogens into harmless ones for excretion by the cell.
- The SER membranes carry out the initial reaction in the oxidation of fats. Synthesis of fats inside the adipose tissue.
- Formation of visual pigments from vitamin A.
- Synthesis of ascorbic acid.
- Synthesis of glycogen and glycogenolysis in liver cells.

- It contains cytochrome P450 and related enzymes that take part in detoxification of toxins. These enzymes change lipid soluble toxins to water soluble state so that it can be excreted out of the body.
- SER produces Golgi apparatus, lysosomes, microbodies and vacuoles.
- SER membrane carry out the reaction in oxidation of fats.

1.3.3 Golgi Complex

The Golgi apparatus also called Golgi complex is system of membranes which takes part in membrane transformation, secretion and production of biochemicals which is noticeable with both light and electron microscope. Golgi apparatus was discovered by Italian Scientist Camillo Golgi in 1898 in nerve cells of barn owl and cat by metallic impregnation method. It is also named as Golgisome, Golgi membranes, Golgi body. Though Golgi complex remained a centre of controversy for decades between those who believed that organelle existed in living cells and thought it as artificial structure formed during microscopy preparation. It consists of flattened disc like, membranous cisternae and tubules. The Golgi stacks in mammalian cell are interconnected by membranous tubules and form large ribbon like complex situated next to the cell nucleus (Refer Figures 1.15 and 1.16).

Location: It is present in all eukaryotic cell except mammalian RBCs, sperm cells of bryophytes and pteridophytes and sieve tubes of plants. In secretory and absorptive cells, Golgi lies between the nucleus and cell surface. In invertebrate and plant cells, Golgi complex consists of isolated units called dictyosomes or Golgi stacks. Dictyosomes are capable of changing position with the help of ATP dependent motors.

The cytoplasm containing Golgi complex has no organelles and glycogen granules and called as zone of exclusion. In enucleated Amoeba, the Golgi complex becomes reduced and disappears, but redevelops after re-nucleation of the organism. This shows that nucleus is necessary for maintaining a healthy Golgi complex.

Structure: Golgi apparatus varies in size and form in different cell types but has similar organization for any kind of cells, for example, it is well developed in secretory and nerve cells, but small in muscle cells.

Usually a compact Golgi apparatus is made up of four parts- Cisternae, Tubules, Vesicles and Vacuoles. Tubules, vacuoles and vesicles are more on the outer side of apparatus.

Cisternae: Membrane lined flat which occur in stack of 4-8 in lower organisms. The cisternae are curved with convex forming, cis face towards endoplasmic reticulum and concave maturing face, trans face towards plasma membrane. Cisternae in between cis and trans ones is medial cisternae. Much of processing and elaboration of biochemicals occur in medial cisternae.

Tubules: Short branched hollow filament which form complicated network. They are quite active in elaboration of secretory products.

Vesicles: Small sacs of 70-80 nm diameter which develop as protusion from tubules, cisternae and ER. All of them are coated with proteins. Protein covering of coated vesicles is of two types- clathrin and cytosolic coat protein. Clathrin coated vesicles take part in transport of storage proteins. They take part in endocytosis by forming receptor over cell membrane. Some secretory vesicles pass to plasma membrane and release content to the outside.

Golgian Vacuoles: They function as lysosome precursors. They are expanded part of cisternae which gets modified to form vacuoles.

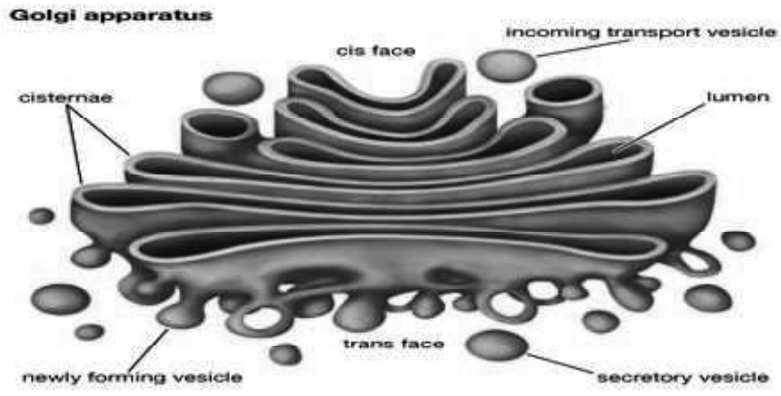

Fig 1.15 *Golgi Apparatus*

Chemical Composition: Protein content is 60-75% while lipid content is 20-35%. Important enzymes present are adenosine diphosphatase, ATPase, CTPase, Glucose 6 phosphatase, Cytochrome c reductase, thiamine pyrophosphatase. The membranes of the Golgi apparatus resemble the cell membrane in molecular structure. They consist of a phospholipid bilayer sandwiched by two protein monolayers. A variety of enzymes are associated with the Golgi membranes. These include ATPase, thiamine pyrophoaphatase, glycosyl transferase, glucose 6 phophatase, etc. The cis and trans regions of the Golgi complex are different in their protein and lipid composition. The cis region resembles the ER in chemistry and trans region resembles plasma membrane. This shows that molecular change occurs in the membranes of the Golgi complex.

Origin

The Golgi apparatus originates from the smooth Endoplasmic Reticulum.

Functions

Following are the functions of Golgi apparatus listed in detail.

- Golgi apparatus synthesizes mucopolysaccharides from sugars.
- Golgi apparatus brings about membrane transformation, that is converting one type of membrane (i.e., that of ER) into other types (i.e., selectively permeable plasma membrane, differentiated membrane of lysosome).
- Golgi apparatus links the sugars with proteins coming from rough ER to form glycoproteins. N linked glycoproteins synthesized in the lumen of RER are passed into the lumen of Golgi apparatus. Here certain sugars (i.e., mannose) are removed while others are added. Glycosylation of OH groups of certain amino acids also take place so that each protein become specific and carries marker which specifies its ultimate destination. Glycoproteins are passed out to cell wall and other places through vesicles. Here they control biosynthetic activities. Mucoproteins are components of mucus and matrix of solidified connective tissue of animals (cartilage and bone).
- Golgi complex give rise to lysosomes by budding.
- The production of hormones by endocrine glands is mediated through Golgi apparatus.
- In chick embryo, the retinal pigment has been synthesized by Golgi apparatus
- Acrosome is an important constituent of tip of animal sperms which help in digesting away the covering sheath of the egg. After the formation of acrosome the rest of Golgi complex degenerates so that mature sperm is devoid of the apparatus.

- The Golgi complex to store cell secretion such as proteins and lipids.
- It gives rise to nematocysts in coelenterates.
- It brings about membrane transformation, i.e., changing one type of membrane to another.
- A variety of enzymes are localized in Golgi complex to help in biochemical reactions.
- The Golgi apparatus produces yolk and cortical in eggs. Formation of yolk is called vitellogenesis.
- In some algae, cellulose plates for cell wall are synthesized in Golgi complex. In plant cell, Golgi complex synthesize pectin and carbohydrates necessary for cell wall formation and produce secretion such as mucilage.
- The formation of root hair from their mother cells take place through agency of Golgi apparatus.
- Most of the complex carbohydrates are synthesized inside Golgi apparatus for example, hemicellulose, mucopolysaccharide, pectic compound. Therefore also called as carbohydrate factory.

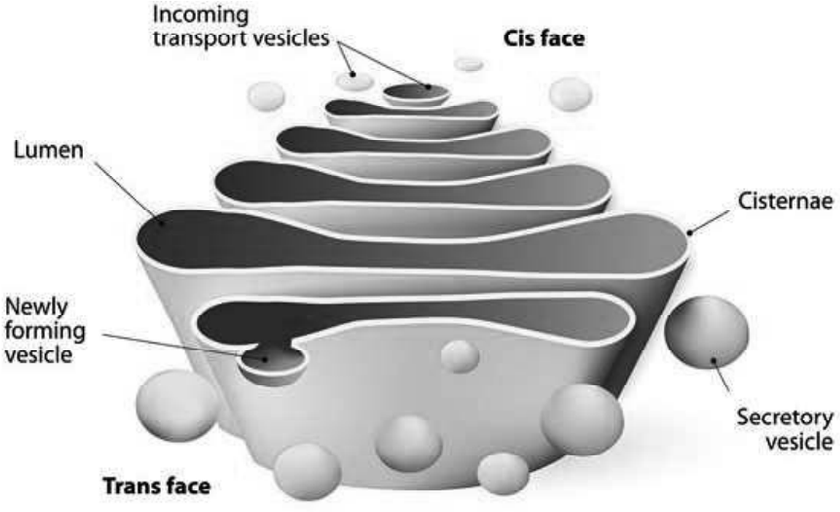

Fig 1.16 *Representation of a Golgi Apparatus and its Sub Components*

1.3.4 Mitochondria

The name Mitochondria was given by Benda in 1898. It was first seen in 1880 by Kolliker who isolated them from insect muscle cells. Mitochondria is known by variety of names such as parabasal bodies, chondriosomes, and plasmosomes.

Cell organelles of aerobic eukaryotes which take part in oxidative phosphorylation and Kreb cycle of aerobic respiration therefore called as power house of cell. Michaelis in 1900, found mitochondria to be respiratory organelle as it can oxidize Janus Green B. Its ultrastruture can be studied under electron microscope. Mitochondria is absent in prokaryotes and anerobic eukaryotes. They are secondarily lost in RBC of mammals. Their number varies from one in some algae (Chlorella), 25 in sperm cell, 300-400 in kidney cell, 30,000 in some oocytes and 5 lacs in flight muscle cells. Cell of dormant seeds have few mitochondria. In general green plant cell contain less number of mitochondria as compared to non-green plant cells and animal cells. The position of mitochondria in a cell depends upon the requirement of energy and amino acids (Refer Figure 1.17).

Shape and Size: Mitochondria differ in shape. These can either be spherical, cylindrical, tubular or filamentous. In chorella the single mitochondria is tubular and branched. The shape is controlled by physiological condition of the cells.

Structure: Both the inner and outer membrane resemble plasma membrane in molecular structure. Mitochondrial envelope is asymmetrical to both structure and function.

Membranes: Outer membrane is smooth and permeable to small molecules having channels formed by protein porin. The outer membrane consists of 50% lipid. It contain enzymes but is poor in proteins.

Inner membrane is selectively permeable and permeable to only some metabolites. It is rich in double phospholipid called cardiolipin (having four fatty acids) which make membrane impermeable to ions. It contain no of enzymes and carrier proteins. Protein content of the inner membrane is highest for any membrane. It regulates the entry of material into and out of mitochondria. Protein content of the inner membrane is the highest for any membrane being 70-75% of the total component. The inner membrane is infolded variously to form involutions called cristae which are meant for increasing the physiologically active area of inner membrane. The cristae are arranged like baffles at right angle to the longitudinal axis of the mitochondria. They are tubular (most plant cells) or plate like (most animal cells). A crista encloses a space that is in continuation of the outer chamber. The density of cristae indicates the intensity of respiration. The inner membrane as well as cristae possess small tennis racket like particles called elementary particles or oxysomes. Each elementary particle function as ATP synthetase. It is differentiated into three parts- head, stalk and base.

Cristae: Cristae extend inwards to varying degrees. They are arranged in characteristic ways in different cells. They run at right angles to the long axis of mitochondria. In protozoans, insect flight muscle cell cristae are tubular. The active cells have many cristae whereas inactive cells have few. Heart and muscle cells have 3 times as many cristae as in liver, mitochondria

Matrix: The space between cristae is called inner chamber, filled with gel like material termed mitochondrial matrix. It contains proteins mainly in the form of enzymes concerned with energy producing activity, DNA is circular, active in transcription and has more guanine and cytosine. Ribosomes are 70 S in size. All the three RNAs are present in mitochondrial matrix.

Oxysomes: Inner membrane bears minute spaced particles known as elementary particles or oxysomes. An oxysome consists of 3 parts- rounded head piecejoined by short stalk located in inner membrane. The oxysome complex represent ATPase or ATP synthetase which is concerned with ATP formation.

Outer Chamber: It is the space that lies between the outer and inner membrane of the mitochondrial envelope. It extends into the spaces of the cristae. The chamber conatins a fluid having few enzymes.

Inner Chamber: It contains a semi fluid matrix. pHof matrix is higher than cytoplasm. The matrix has protein particles, ribosomes, RNA, DNA, amino acid synthesis and fatty acid metabolism, crystals of calcium phosphate. DNA is naked, circular.

Chemical Composition: Proteins- 65-70%, Lipids- 25-30% (mostly phospholipid) such as cephalin and lecithin. About 60 different enzymes are found to exist in the mitochondria.

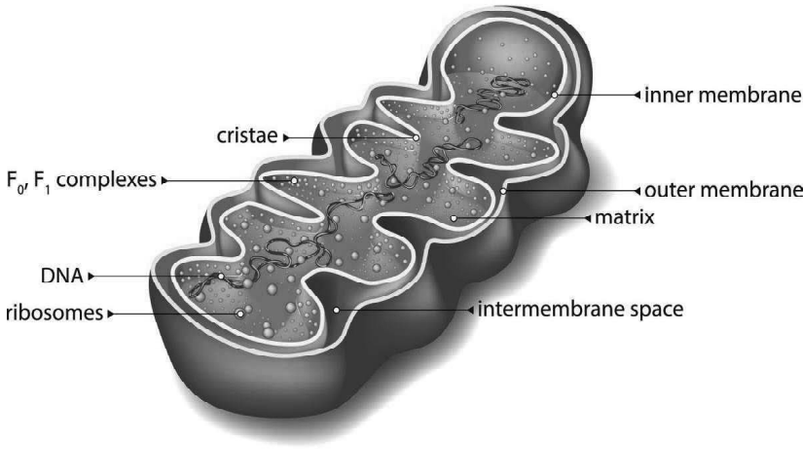

***Fig. 1.17** Diagrammatic Representation of Mitochondria*

Functions

The functions of Mitochondria are listed below in detail:

- They provide intermediates for synthesis of biochemicals like chlorophyll, cytochromes, steroids, alkaloids, etc.
- Synthesis of many amino acids occurs in mitochondria. The first formed amino acid is glutamic acid and asparatic. They are synthesized from á ketoglutaric acid and oxaloacetic acid, respectively.
- Mitochondria may store and release calcium when required. They contain K^+, Mg^{2+} and phosphate in cells.
- Organism receives mitochondria from mother and takes part in maternal inheritance.
- They are mini biochemical factories where food is oxidized to carbon dioxide and water. They undergo oxidation and form energy rich ATP. ATP performs various energy requiring processes like muscle contraction, nerve impulse conduction, cell division, movement. Because of formation of ATP the mitochondria are called power house of cell.

1.3.5 Chloroplast

Plastids are the semiautonomous organelles having DNA and double membrane envelope which synthesizes various types of organic compounds like fatty acids, amino acids, purines, pyrimidines, etc., found in plant cells and certain protists. Plastids develop from colorless precursors called proplastids. Proplastids are small spherical, colorless structures which occur in meristematic cells. It is covered by double membrane envelope. Its internal membrane is shown to develop lamellae. Lamellae occur free in the interior. They have some starch and possess circular nucleoid and are of two types: leucoplasts and chromoplasts. Leucoplasts are colorless, while chromoplast is coloured and occurs in cells exposed to sunlight. Chromoplast with green pigment or chlorophyll is known as chloroplast (Refer Figure 1.18).

The chloroplast are greenish plastids that possess photosynthetic pigments, chlorophylls, carotenoids and take part in the synthesis of food from inorganic raw material in the presence of sunlight. Chloroplast of algae other than green ones are called chromatophores. Chloroplast is the most common type of plastids as they provide food to all organism through photosynthesis. Like mitochondria, the chloroplasts don't have fixed position and shift from place to place.

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Bacteria, blue green algae, protist, fungi and animals lack chloroplast. All plastids develop from small rounded pigmented bodies called proplastids. The proplastid grows and in presence of light, its lamellae develop to form mature chloroplast.

Shape: They are spherical, lens shaped, disc like. In lower plants, they may be cup shaped (Chlamydomonas), ribbon like (Spirogyra), spiral (Spirogyra), girdle shaped (Ulothrix).

Movements

Chloroplasts change their position due to either cyclists or direction and intensity of illumination. The movement of chloroplasts in response to light is called phototactic movements. Under strong light the chloroplasts come to lie one behind the other with their edges towards the light. This minimizes the light absorption. This is called parastrophe. In moderate light, chloroplast arrange themselves towards the illuminated side of the cells with their flat sides facing the sun called epistrophe. In dark arrangement of chloroplast is known as apostrophe. The movement is due to blue light photoreceptor called phototropin.

Size: The chloroplasts of shade plants are larger than those of sun plants. The chloroplasts of higher plants are of 5-10μm long.

Number: Higher plants have 20-35 chloroplasts per cell, in some cases about 500 or more.

Structure: It is a vesicle bounded by an envelope of two unit membrane and filled with fluid matrix.

Membranes: Each membrane is about 50-70Å thick. The two membranes are separated by narrow fluid filled inter membrane space. The membrane resemble plasma membrane in structure. The outer membrane is smooth and freely permeable and contains protein channels called porin. The inner membrane has selectively permeability and rich in proteins and contains permeases. These regulate the movement of metabolite into and out of chloroplast. Inner mitochondrial membrane is in folded to provide large surface area known as cristae.

Lamellae: Lamellae often take the form of flattened ovoid sac thylakoid which lie closely packed one on another forming grana. A thylakoid encloses a space called as loculus bounded by single membrane. The thylakoids are interconnected by branching tubules termed as frets. Thylakoids occur singly in red algae, in pairs in cryptophytes. Thylakoid membrane contains rounded particles called quantosomes. A quantosome contain 250 molecules of chlorophyll, amount necessary for photosynthesis.

Matrix: It is colorless ground substance called stroma. The chloroplast stroma contains proteins, lipids, small circular, double helical DNA molecule, RNA molecule. Proteins in matrix are enzymes meant for dark reaction of photosynthesis.

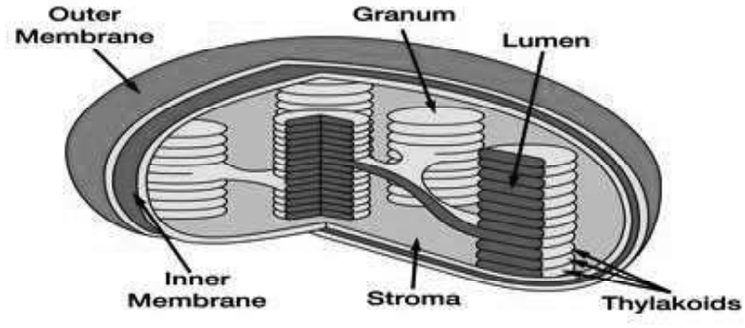

Fig 1.18 *Diagram of a Chloroplast*

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Photosynthesis in Chloroplasts

Photosynthesis takes place in two set of reaction, namely light reaction and dark reaction. These are explained below:

Light Reaction: The chlorophyll molecules trap the radiant energy of sunlight. This energy is used to remove electrons and protons from water to form oxygen. Electrons are transferred through thylakoid membrane to electron acceptor NADP+. The movement of electrons is coupled to transport of protons across membrane from stroma to thylakoid lumen. The protons move down concentration gradient from lumen to stroma which leads to synthesis of ATP from ADP. The process is called photophosphorylation.

Dark Reaction: These reaction occur in the absence of light. Using the energy of ATP and NADPH generated by light, energy poor carbon dioxide is converted into energy rich 6 carbon sugar. Process is called carbon dioxide fixation.

1.3.6 Lysosome

Membrane bound secretion vesicles containing enzymes for intracellular digestion. They are the important products of the secretory pathway in the cells. They are specialized membrane bound secretion vesicles containing enzymes for intracellular digestion. It was reported by the Belgian cytologist and biochemist Christian de Duve in 1955. His findings were based on biochemical studies. In 1956, Novikoff observed lysosomes with the help of electron microscope. It occurs in all animal cells. Some mammalian red blood corpuscles lack lysosomes. They occur in protist, fungi and plants. Lysosomes are small vesicles bounded by single membrane and contain hydrolytic enzymes in form of granules of 5-8 nm. About 60 types of enzymes occur in them. The important enzymes discovered are acid phosphatases, sulphatases, proteases, nucleases, lipases, glycosidases are called acid hydrolases as they function in acidic medium with pH4-5. Acidic conditions are maintained inside by pumping of protons by ATP dependent pumps.

Lysosomes are called suicide bags because of presence of large number of digestive enzymes. In plants and fungi their function is taken over by vacuoles. In animals, they are abundant in macrophages, Kuffer cells etc. They are abundant in white blood corpuscles and secretory cells of pancreas, spleen, liver and kidneys. Lysosomes are evenly distributed in the cytoplasmic matrix. Certain meristematic cells of roots of plants have irregular lysosomes. A lysosome is a tiny sac bounded by a single unit membrane of lipoprotein. It contain dense granular material, which contain hydrolytic enzymes. The common enzymes in lysosomes are proteases, nucleases, glycosidases, lipases, sulphatases and phosphatases which hydolyse proteins, nucleic acids, polysaccharides, lipids, organic sulphate and phosphate respectively. However not all enzymes are found in one lysosome.

There are many kinds of lysosomes containing different set of enzymes. Thus they are heterogenous organelles. They store the hydrolyzing enzymes of the cell. Their membrane prevent the enzyme from escaping into the cytoplasm. The materials needeing hydrolysis must enter the lysosome so their enzyme remain isolated from cytoplasm. In injured and dead cells, the lysosome membrane ruptures, releasing the enzymes that lyse the weakened cells. In intact cells, compounds like cholesterol and cortisone prevent rupturing of lysosome membrane.

Structure: Lysosomes show polymorphism due to various origin and function. They are of four types, i.e., primary, secondary, autophagic and residual bodies. Now terms primary and secondary have become obsolete. They are of three types- Heterophagic lysosomes, autophagic lysosomes and residual bodies.

1. **Heterophagic Lysosome (Digestive Vacuoles):** Lysosomes with extracellular material for digestion. The ingested matter is enclosed in membrane lined phagosome. Phagosome fuses with endosome to produce digestive vacuole. Fusion of lysosome with other membrane bound vesicles is highly selective. Lysosome fuses only with vesicles containing materials to be digested. The membranes of lysosomes and vesicles having material have some sort of recognition system. Lysosome may fail to fuse with phagosome. The internal materials are acted upon by acid hydrolases. The solubilised products of digestion are passed out into cytosol through diffusion.
2. **Autophagic Lysosomes (Autophagosomes):** A cell may digest its own organelles such as mitochondria and ER. This process is called autophagy or autolysis. Primary lysosome fuse together about the unwanted organelles forming large sac known as autophagic vacuole. The enzymes of lysosome digest the organelles thus enclosed. The products of digestion enter the sytoplasm through lysosome membrane and reprocessed into new molecules. Autophagic vacuoles develop in liver cells to digest cell components in a starving animal**.** Self-eating of degenerate intracellular structure. Destruction of various organelle in RBCs occur through autophagy. Autophagy of larger structure is called macrophagy. Autolysis is self-destruction of cell, tissue or organ with the help of lysosome. It occurs in diseased, ageing and dead cells.
3. **Residual Body (Tertiary Lysosomes):** Those lysosome in which only indigestible materials have been left. In secondary lysosome, the enzymes digest the incoming materials. The products of digestion pass through lysosome membrane into matrix for use as a source of nutrition. Indigestible matter, such as bacterial cell walls that are resistant to hydrolases remains in secondary lysosome. A secondary lysosome left with indigestible matter is known as residual body. The residual body meets the cell membrane and residue is released by exocytosis in protozoans. Residual bodies are stored in cells in vertebrates and play role in ageing process. The residual bodies are result of enzymes in lysosomes and cause certain diseases. It is pass outwardly and fuse with plasma membrane to throw the debris into external environement by ephagy. Residual bodies remain inside the cell which leads to pathological disease (storage disease) like hepatitis, Tay sach's disease, polynephritis, hurler's disease (Refer Figures 1.19 and 1.20).

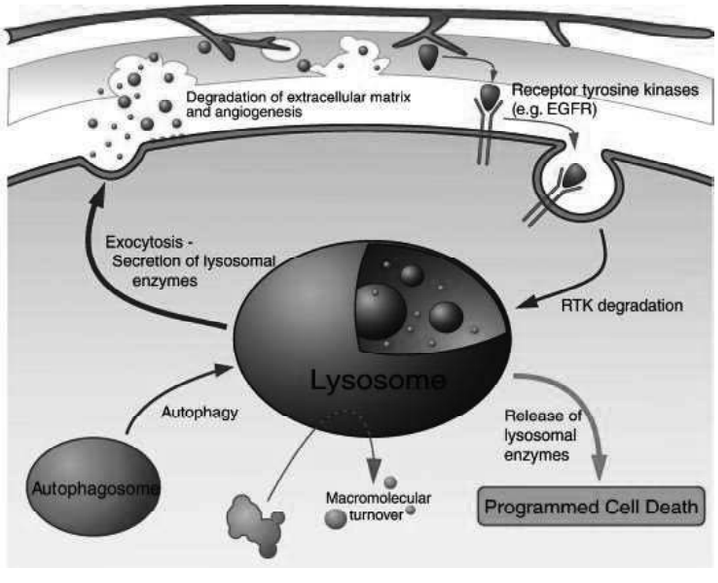

Fig 1.19 *Diagrammatic Representation of a Lysosome*

Lysosomes are considered to be arise from the Golgi complex or smooth endoplasmic reticulum. They originate as membranous vesicles containing enzymes that are stored in Golgi complex and received from the rough endoplasmic reticulum.

Functions

Here below are the functions of lysosomes:

- Lysosomes devour foreign substance, toxic bacteria and other microbes and take part in natural defense of the body.
- Lysosome perform intracellular scavenging by removing old useless organelles.
- Lysosomes provide enzymes required for breaking membrane of eggs.
- The residual bodies do not undergo exocytosis, instead remain inside the cell and cause disease, i.e., polynephritis, hepatitis.
- They cause breakdown of ageing and dead cells.
- In metamorphosis, certain embryonic parts like tail, gills are digested through lysosomes.
- They are essential for cell division by overcoming agents that represses mitotic cycle.
- They remove carcinogens by engulfing them.
- Active hormone thyroxine is formed by hydrolysis of thyroglobulin by lysosomes.

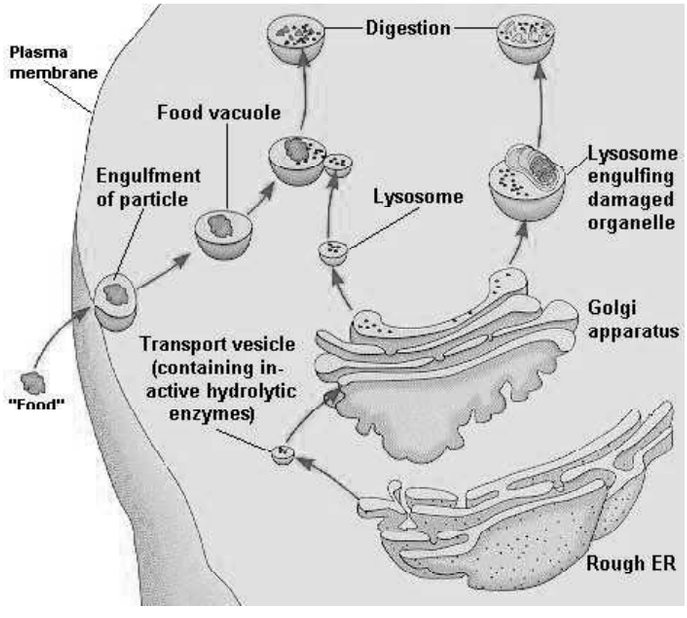

Fig 1.20 *Diagrammatic Representation of Digestion with Lysosomes*

1.3.7 Plasma Membrane

Plasma membranes are subcellular structures, approximately 10nm thick, that form a protective boundary around the cell as well as the cell's organelles. They serve to both impede foreign material from entering the cell, and prevent the cellular contents from leaking out. With the structural makeup of the lipid bilayer conferring membranes unique physical and chemical properties, these structures also contribute to diverse and critical cellular functions.

Membranes are composed of lipids and proteins, balanced in equal proportions by mass. The current views on membrane structure are derived from the Fluid-Mosaic Membrane Model (F-MMM), which depicts them as two-dimensional fluids made up of lipid-bilayers interspersed with proteins. The fluidic nature of membranes is due to the constant

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rotational or lateral motion of both lipids and proteins. While lipids provide the basic structure of membranes, the proteins carry out a vast array of specialized functions, from ion and small molecule transport to the regulation of signalling pathways.

Functions of the Plasma Membrane

Physical Barrier: The plasma membrane surrounds all cells and physically separates the cytoplasm, which is the material that makes up the cell, from the extracellular fluid outside the cell. This protects all the components of the cell from the outside environment and allows separate activities to occur inside and outside the cell.

The plasma membrane provides structural support to the cell. It tethers the cytoskeleton, which is a network of protein filaments inside the cell that hold all the parts of the cell in place. This gives the cell its shape. Certain organisms such as plants and fungi have a cell wall in addition to the membrane. The cell wall is composed of molecules such as cellulose. It provides additional support to the cell, and it is why plant cells do not burst like animal cells do if too much water diffuses into them.

Selective Permeability: Plasma membranes are selectively permeable (or semi-permeable), meaning that only certain molecules can pass through them. Water, oxygen, and carbon dioxide can easily travel through the membrane. Generally, ions, for example sodium, potassium and polar molecules cannot pass through the membrane; they must go through specific channels or pores in the membrane instead of freely diffusing through. This way, the membrane can control the rate at which certain molecules can enter and exit the cell.

Endocytosis and Exocytosis: Endocytosis is when a cell ingests relatively larger contents than the single ions or molecules that pass through channels. Through endocytosis, a cell can take in large quantities of molecules or even whole bacteria from the extracellular fluid. Exocytosis is when the cell releases these materials. The cell membrane plays an important role in both of these processes. The shape of the membrane itself changes to allow molecules to enter or exit the cell. It also forms vacuoles, small bubbles of membrane that can transport many molecules at once, in order to transport materials to different places in the cell.

Cell Signaling: Another important function of the membrane is to facilitate communication and signaling between cells. It does so through the use of various proteins and carbohydrates in the membrane. Proteins on the cell 'mark' that cell so that other cells can identify it. The membrane also has receptors that allow it to carry out certain tasks when molecules such as hormones bind to those receptors.

Plasma Membrane Structure

Phospholipids: The membrane is partially made up of molecules called phospholipids, which spontaneously arrange themselves into a double layer with hydrophilic heads on the outside and hydrophobic tails on the inside. These interactions with water are what allow plasma membranes to form.

Proteins: Proteins are wedged between the lipids that make up the membrane, and these transmembrane proteins allow molecules that couldn't enter the cell otherwise to pass through by forming channels, pores or gates. In this way, the cell controls the flow of these molecules as they enter and exit. Proteins in the cell membrane play a role in many other functions, such as cell signaling, cell recognition, and enzyme activity.

Carbohydrates: Carbohydrates are also found in the plasma membrane; specifically, most carbohydrates in the membrane are part of glycoproteins, which are formed when a carbohydrate attaches to a protein. Glycoproteins play a role in the interactions between cells, including cell adhesion, the process by which cells attach to each other.

Fluid Mosaic Model: Technically, the cell membrane is a liquid. At room temperature, it has about the same consistency as vegetable oil. Lipids, proteins, and carbohydrates in the plasma membrane can diffuse freely throughout the cell membrane; they are essentially floating across its surface. This is known as the fluid mosaic model, which was coined by S.J. Singer and G.L. Nicolson in 1972.

1.3.8 Ribosome

The ribosome is a complex molecule made of ribosomal RNA molecules and proteins that form a factory for protein synthesis in cells. In 1955, George E. Palade discovered ribosomes and described them as small particles in the cytoplasm that preferentially associated with the endoplasmic reticulum membrane. Along with other scientists, Palade discovered that ribosomes performed protein synthesis in cells, and he was awarded the Nobel Prize in 1974 for his work.

Each ribosome has a large component and a small component that together form a single unit composed of several ribosomal RNA molecules and dozens of proteins. The ribosome is responsible for translating encoded messages from messenger RNA molecules to synthesize proteins from amino acids. The ribosome translates each codon, or set of three nucleotides, of the mRNA template and matches it with the appropriate amino acid in a process called translation. The amino acid is provided by a transfer RNA (tRNA) molecule. Each newly translated amino acid is then added to the growing protein chain until the ribosome completes the process of protein synthesis.

Structure

Ribosomes are made of proteins and Ribonucleic Acid (abbreviated as RNA), in almost equal amounts. It comprises of two sections, known as subunits. The tinier subunit is the place the mRNA binds and it decodes, whereas the bigger subunit is the place the amino acids are included.

Both subunits comprise of both ribonucleic acid and protein components and are linked to each other by interactions between the proteins in one subunit and the rRNAs in the other subunit. The ribonucleic acid is obtained from the nucleolus, at the point where ribosomes are arranged in a cell.

The structures of ribosomes include:

- Situated in two areas of the cytoplasm.
- They are seen scattered in the cytoplasm and a few are connected to the endoplasmic reticulum.
- Whenever joined to the ER they are called the rough endoplasmic reticulum.
- The free and the bound ribosomes are very much alike in structure and are associated with protein synthesis.
- Around 37 to 62% of RNA is comprised of RNA and the rest is proteins.
- Prokaryotes have 70S ribosomes respectively subunits comprising the little subunit of 30S and the bigger subunit of 50S. Eukaryotes have 80S ribosomes respectively comprising of little (40S) and substantial (60S) subunits.
- The ribosomes seen in the chloroplasts of mitochondria of eukaryotes are comprised of big and little subunits composed of proteins inside a 70S particle.
- Share a center structure which is very much alike to all ribosomes in spite of changes in its size.

NOTES

- The RNA is arranged in different tertiary structures. The RNA in the bigger ribosomes is into numerous continuous infusions as they create loops out of the center of the structure without disturbing or altering it.
- The contrast between those of eukaryotic and bacteria are utilized to make antibiotics that can crush bacterial disease without damaging human cells.

Ribosomes Size

Ribosomes comprise of two subunits that are suitably composed and function as one to translate the mRNA into a polypeptide chain amid protein synthesis. Due to the fact that they are made from two subunits of differing size, they are a little longer in the hinge than in diameter. They vary in size between prokaryotic cells and eukaryotic cells.

The prokaryotic is comprised of a 30s (Svedberg) subunit and a 50s (Svedberg) subunit meaning 70s for the entire organelle equal to the molecular weight of 2.7×106 Daltons. Prokaryotic ribosomes are about 20 nm (200 Å) in diameter and are made of 35% ribosomal proteins and 65% rRNA.

Notwithstanding, the eukaryotic are amidst 25 and 30 nm (250–300 Å) in diameter. They comprise of a 40s (Svedberg) subunit and a 60s (Svedberg) subunit which means 80s (Svedberg) for the entire organelle which is equal to the molecular weight of 4×106 Daltons.

Location

Ribosomes are organelles located inside the animal, human cell, and plant cells. They are situated in the cytosol, some bound and free-floating to the membrane of the coarse endoplasmic reticulum.

They are utilized in decoding DNA (DeoxyriboNucleic Acid) to proteins and no rRNA is forever bound to the RER, they release or bind as directed by the kind of protein they proceed to combine. In an animal or human cell, there could be up to 10 million ribosomes and numerous ribosomes can be connected to the equivalent mRNA strand, this structure is known as a polysome.

Function

When it comes to the main functions of ribosomes, they assume the role of bringing together amino acids to form particular proteins, which are important for completing the cell's activities.

Protein is required for numerous cell functions, for example directing chemical processes or fixing the damage. Ribosomes can yet be discovered floating inside the cytoplasm or joined to the endoplasmic reticulum.

The other functions include:

- The procedure of creation of proteins, the deoxyribonucleic acid makes mRNA by the step of DNA transcription.
- The hereditary information from the mRNA is converted into proteins amid DNA translation.
- The arrangements of protein assembly amid protein synthesis are indicated in the mRNA.
- The mRNA is arranged in the nucleus and is moved to the cytoplasm for an additional operation of protein synthesis.
- The proteins which are arranged by the ribosomes currently in the cytoplasm are utilized inside the cytoplasm by itself. The proteins created by the bound ribosomes are moved outside the cell.

Taking into consideration their main function in developing proteins, it is clear that a cell ca not function in the absence of ribosomes.

NOTES

'Check Your Progress'

6. How many enzymes are present along with the ER membranes for synthetic activities?
7. What does the SER provide?
8. What is the Golgi apparatus?
9. What are plastids?
10. What are lysosomes called suicide bags?

1.4 SUMMARY

- Progress of science depends not only on the scientists ability to observe and infer but also on the tools and techniques available to them for study.
- More precise and sophisticated tools and techniques enable the scientists to give detailed and accurate information of the object studied.
- Biology was confined to the study of plants and animals or their parts visible to the naked eye till 1665 when an English scientist Robert Hooke discovered cells in a thin slice of cork examined under a primitive microscope assembled by him.
- Compound microscopes is commonly used in the laboratories to view extremely minute organisms and parts and sections of larger organisms.
- Phase contrast is one of the best methods for viewing transparent or translucent specimens without staining the specimens and without a loss of resolution.
- Microdensitometry, or microspectrophotometry, is the measurement of the concentration or mass of a chromophore in microscopically defined regions, and is governed by well-established laws of physics.
- A microdensitometer is an optical instrument used to measure optical densities in the microscopic domain.
- Flow cytometry is a technique used to detect and measure physical and chemical characteristics of a population of cells or particles.
- A flow cytometry analyzer is an instrument that provides quantifiable data from a sample.
- The process of collecting data from samples using the flow cytometer is termed 'acquisition'.
- Autoradiography is a photographic technique used to localize a radioactive substance within a solid specimen; also known as radioautography.
- Immunological technique is the method to locate an antigen to a particular position of the cell of specific antibody for a particular protein to be studied.
- Ion-exchange chromatography has application in separating amino acids and proteins.
- Nuclear Magnetic Resonance Spectroscopy (NMR) is a method for detecting interaction between the nuclei of an atom with the magnetic field of the electromagnetic radiation.

NOTES

- Nucleus is a specialized double membrane bound protoplasmic structure which carries all the genetic information for controlling cell metabolism and transmitting the information to next generation.
- Nucleus is present in all eukaryotic cells, but certain mature cells do not have nucleus. The mammalian red blood cells lose their nuclei at maturity and survive for few months only. The food conducting phloem cells called sieve tubes also lose their nuclei at maturity but remain functional for several years.
- Nucleus is found in peripheral position in plant cell due to development of large central vacuole, also in adipose cells.
- Interphase nucleus is differentiated into 5 parts, namely- nuclear envelope, nucleoplasm, nuclear matrix, chromatin and nucleolus.
- Smooth ER of muscle cells is called sarcoplasmic reticulum. It releases and reabsorbs calcium.
- The rough ER is more sparsely distributed in plant cells. The membrane bears number of gated channels or translocons in area of attached ribosome to pass polypeptide into channel of ER for transport.
- RER offers extensive surface on which protein synthesis can be carried on by ribosomes. The newly formed protein enters the ER membranes, becoming part of membrane structure or pass into the ER lumen.
- The proteins in ER lumen are processed and enclosed in spherical membrane bound vesicle which pinches off from the ER.
- Proteins synthesized by ribosomes enter the channels of RER both as intracellular and extracellular transport.
- Sequestering calcium ions with in cytoplasm of skeletal and cardiac muscle cells. The regulated release of Ca^{2+} from the SER triggers contraction
- The SER has enzymes that bring about detoxification in the liver i.e. converts harmful materials such as pesticides, carcinogens into harmless ones for excretion by the cell.
- The Golgi apparatus also called Golgi complex is system of membranes which takes part in membrane transformation, secretion and production of biochemicals which is noticeable with both light and electron microscope.
- Golgi apparatus varies in size and form in different cell types but has similar organization for any kind of cells. For example, it is well developed in secretory and nerve cells, but small in muscle cells.
- Golgi apparatus brings about membrane transformation, that is converting one type of membrane (i.e., that of ER) into other types (i.e., selectively permeable plasma membrane, differentiated membrane of lysosome).
- Cell organelles of aerobic eukaryotes which take part in oxidative phosphorylation and Kreb cycle of aerobic respiration therefore called as power house of cell.
- Inner membrane is selectively permeable and permeable to only some metabolites. It is rich in double phospholipid called cardiolipin (having four fatty acids) which make membrane impermeable to ions.
- Inner membrane bears minute spaced particles known as elementary particles or oxysomes.
- Synthesis of many amino acids occur in mitochondria. The first formed amino acid are glutamic acid and asparatic.

- Plastids are the semiautonomous organelles having DNA and double membrane envelope which synthesizes various types of organic compounds like fatty acids, amino acids, purines, pyrimidines, etc., found in plant cells and certain protists.
- Proplastids are small spherical, colorless structures which occur in meristematic cells.
- Leucoplasts are colorless, while chromoplast is coloured and occurs in cells exposed to sunlight. Chromoplast with green pigment or chlorophyll is known as chloroplast.

1.5 KEY TERMS

- **Resolving power**: Resolving power is the ability of a microscope to show two closely lying points as two separate points.
- **Magnification:** Magnification is the ratio of the size of the image to that of the object.
- **Simple microscope**: A simple microscope is one which uses a single lens for magnification, such as a magnifying glass.
- **Microdensitometer**: A microdensitometer is an optical instrument used to measure optical densities in the microscopic domain.
- **Plastids:** These are membrane bound organelle found in plant cells, algae and some eukaryotes. They are the sites of manufacture and storage of chemical compounds.
- **Porins:** These are barrel proteins that cross cellular membrane and act as a pore, through which molecules can diffuse.
- **Autolysis:** This is the self-destruction of cell, tissue or organ with the help of lysosome. It occurs in ageing, dead and diseased cells
- **Macrophagy:** It refers to the self-eating of degenerate intracellular structure mainly large sized structures.
- **Oxysomes:** It is the inner membrane of mitochondria that possesses tennis like particles called oxysomes which function as ATP synthetase.
- **Vitellogenesis:** It is a Golgi apparatus that functions as centre around which yolk is deposited in animal oocytes and is called vitellogenesis.
- **Phototactic movement:** It is referred to the movement of chloroplast in response to light.

1.6 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Phase contrast is one of the best methods for viewing transparent or translucent specimens without staining the specimens and without a loss of resolution.
2. Cytochemical methods are used to locate specific chemical constituents within the cells by differentiating a particular part from other parts by colouring them with a specific stain or dye. It is done either by the use of certain dyes or by using the substrates of enzymes, for example, Schiff's reagent used in Feulgen staining, is used to localize the presence of DNA in a cell.
3. By cell fractionation method different organelles of cells such as nucleus, mitochondria, ribosomes, etc. having different particle size and weight are separated by rotating them in a centrifuge at different speeds.

4. Flow cytometry is a technique used to detect and measure physical and chemical characteristics of a population of cells or particles. Uses for flow cytometry include:
   - Cell Counting
   - Cell Sorting
   - Determining Cell Characteristics and Function
   - Detecting Microorganisms
   - Biomarker Detection
   - Protein Engineering Detection
   - Diagnosis of Health Disorders, such as Blood Cancers
5. Immunological technique is the method to locate an antigen to a particular position of the cell of specific antibody for a particular protein to be studied. Immunofluorescence is another slightly modified technique used to study cells under fluorescence microscopy to locate the distribution of the antigen in the cells.
6. As many as 30-40 enzymes are present along with ER membranes for various synthetic activities.
7. The SER provides surface for the synthesis of fatty acids, phospholipids, glycolipids, steroids and visual pigments.
8. The Golgi apparatus is system of membranes which takes part in membrane transformation, secretion and production of biochemical.
9. Plastids are the semiautonomous organelles having DNA and double membrane envelope.
10. Lysosomes are called suicide bags because of presence of large no of digestive enzymes.

1.7 QUESTIONS AND EXERCISES

Short Answer Questions

1. Distinguish between simple and compound microscope.
2. What is resolving power?
3. What is radioactive tracer technique?
4. What is spectroscopy?
5. Briefly discuss the functions of ER.
6. Name and describe the types of Endoplasmic Reticulum.
7. Describe the structure of Golgi apparatus.
8. Describe the ultrastructure of chloroplast.
9. Which organelle is called suicide bag? Describe its origin and function.
10. Write a brief note on quantosomes?

Long Answer Questions

1. Write a note on electron microscope, TEM and SEM.
2. Discuss about some cell biology tools and techniques.
3. Elaborate a note on microdensitometry.

4. Explain what flow cytometry is and also discuss about its principle, procedure and application.
5. Write in detail about the top techniques that are widely used in cell biology.
6. What is a nucleus? What are the functions of a nucleus? Explain in detail with the help of a diagram.
7. Which Endoplasmic Reticulum is connected with detoxification? Discuss.
8. What is Rough Endoplasmic Reticulum? How is it different from Smooth Endoplasmic Reticulum? Explain in detail.
9. From where do lysosomes arise? Give a detailed answer from your learning of the text. Also support your answer with a diagrammatic representation.
10. Write a detailed note on the Golgi apparatus. Also name three types of elements that form the Golgi apparatus.
11. Write a detailed note on photosynthesis in chloroplasts.

1.8 FURTHER READING

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NOTES

UNIT 2 CHROMOSOMAL ABERRATION, POLYPLOIDY AND MUTATION

Structure

2.0 INTRODUCTION

A chromosome abnormality, disorder, anomaly, aberration, or mutation is a missing, extra, or irregular portion of chromosomal DNA. It can be from an atypical number of chromosomes or a structural abnormality in one or more chromosomes. Chromosome mutation was formerly used in a strict sense to mean a change in a chromosomal segment, involving more than one gene. The term 'karyotype' refers to the full set of chromosomes from an individual; this can be compared to a 'normal' karyotype for the species via genetic testing. A chromosome anomaly may be detected or confirmed in this manner.

Polyploidy is the state of a cell or organism having more than two paired (homologous) sets of chromosomes. Most species whose cells have nuclei (eukaryotes) are diploid, meaning they have two sets of chromosomes one set inherited from each parent. However, polyploidy is found in some organisms and is especially common in plants. In addition, polyploidy occurs in some tissues of animals that are otherwise diploid, such as human muscle tissues.

In biology, a mutation is the alteration of the nucleotide sequence of the genome of an organism, virus, or extrachromosomal DNA. Mutations result from errors during DNA replication (Especially during meiosis) or other types of damage to DNA, such as may be caused by exposure to radiation or carcinogens, which then may undergo error-prone repair (especially microhomology - mediated end joining), or cause an error during other forms of repair, or else may cause an error during replication. Mutations may also result from insertion or deletion of segments of DNA due to mobile genetic elements.

In this unit, you will study about chromosomal aberration and their role in evolution, polyploidy and a euploidy and its role in evolution, mutation: types, basis and role in crop improvement in detail.

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2.1 UNIT OBJECTIVES

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

- Understand the chromosomal aberration and their role in evolution
- Explain polyploidy and a euploidy and its role in evolution
- Discuss mutation: types, basis and role in crop improvement

2.2 ROLE OF CHROMOSOMAL ABERRATION IN EVOLUTION

A consequence of chromosomal structural aberration in populations is related to evolutionary change including speciation. Chromosomal aberrations are associated with position effects that may be significant in natural selection. More important for evolution is the genetic isolation that is caused by inversions and translocations. Speciation in the Drosophila group of dipterous insects has been related to chromosome inversions. These structural changes occur in chromosomes of individual flies and are carried homozygous in populations. Populations have developed over a period of time with different chromosome inversions. Each may be isolated because mating of flies from a particular population with those of another population carrying a different inversion result in sterile hybrids. This strengthens the boundaries around a particular population and prevents the exchange of genes between related populations. Speciation in Drosophila has been associated with a series of different inversions that occurred by chance in breeding populations and were recognized in different taxonomic groups. Translocations have been shown to occur in certain plant groups and to cause genetic isolation thus promoting evolutionary stability in populations.

Chromosomal Aberrations

Structural and numerical deviations from the norm that affect many genes at once are called chromosomal aberrations. They are sometimes called chromosomal mutations, but most cytogeneticists prefer to use the term 'mutation'.

Despite the incredible precision of meiosis, chromosomal aberrations do occur, and they are more common than one might think. They are responsible for great economic benefit in agriculture. Unfortunately, they are also responsible for many human genetic malformations. It is estimated that five out of every 1000 humans are born with serious genetic defects attributable to chromosomal anomalies. An even greater number of embryos with chromosomal defects are aborted spontaneously, far more than ever reach term.

Changes in chromosome numbers are called **euploidy**, i.e., when there is the addition or deletion of whole sets of chromosomes and **aneuploidy** is when a single chromosome is added to or subtracted from a diploid set. A set of chromosomes contains one member of each homologous pair as would be present in the nucleus of a gamete. The most common kind of euploidy is **polyploidy**, the carrying of one or more additional sets of chromosomes. Such aberrations are much more common in plants than in animals. Animals are much less tolerant of chromosomal aberrations, because sex determination requires a delicate balance between the numbers of sex chromosomes and autosomes. Many domestic plant species are polyploid (cotton, wheat, apples, oats, tobacco, and others), and perhaps 40% of flowering plants are believed to have originated in this manner. Horticulturists favor polyploids and often try to develop them because they have more intensely colored flowers and more vigorous vegetative growth (Refer Figure 2.1)

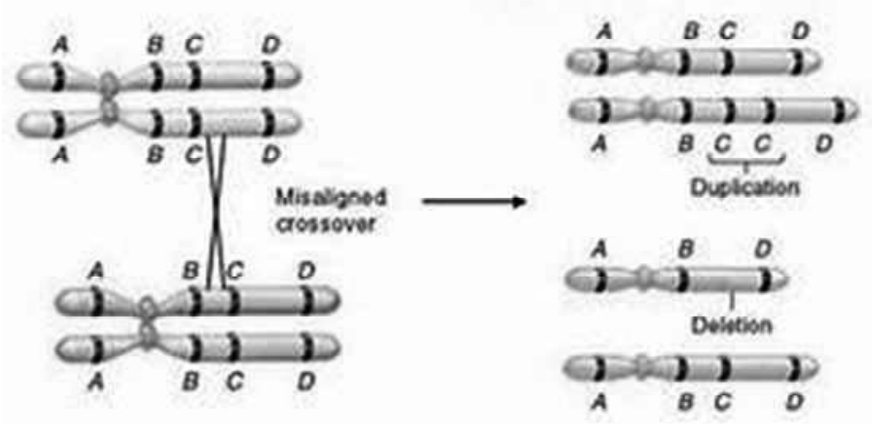


Fig. 2.1 *Chromosomal Aberrations*

Aneuploidy is usually caused by failure of chromosomes to separate during meiosis (nondisjunction). If a pair of chromosomes fails to separate during the first or second meiotic divisions, both members go to one pole and none to the other. This results in one gamete having n – 1 number of chromosomes and another having n + 1 number of chromosomes. If the n – 1 gamete is fertilized by a normal n gamete, the result is a monosomic animal. Survival is rare because the lack of one chromosome gives an uneven balance of genetic instructions. Trisomy, the result of the fusion of a normal n gamete and an n + 1 gamete, is much more common, and several kinds of trisomic conditions are known in humans. Perhaps the most familiar is **trisomy** 21, or **Down syndrome**. As the name indicates, it involves an extra chromosome 21 combined with the chromosome pair 21, and it is caused by nondisjunction of that pair during meiosis. It occurs spontaneously, and there is seldom any family history of the abnormality. However, the risk of its appearance rises dramatically with increasing age of the mother; it occurs 40 times as often in women over 40 years old as among women between the ages of 20 and 30. In cases where maternal age is not a factor, 20% to 25% of trisomy 21 is due to nondisjunction during spermatogenesis; it is paternal in origin and is apparently independent of the father's age.

A syndrome is a group of symptoms associated with a particular disease or abnormality, although every symptom is not necessarily shown by every patient with the condition. An English physician, John Langdon Down, described the syndrome in 1866 that we now know is caused by trisomy 21. Because of Down's belief that the facial features of affected individuals were mongoloid in appearance, the condition has been known as mongolism. The resemblances are superficial, however, and the currently accepted names are trisomy 21 and Down syndrome. Among the numerous characteristics of the condition, the most disabling is severe mental retardation. This, as well as other conditions caused by chromosomal aberrations and several other birth defects, can be diagnosed prenatally by a procedure involving amniocentesis. The physician inserts a hypodermic needle through the abdominal wall of the mother and into the fluids surrounding the fetus (not into the fetus) and withdraws some of the fluid, which contains some fetal cells. The cells are grown in culture, their chromosomes are examined, and other tests done. If a severe birth defect is found, the mother has the option of having an abortion performed. As an extra 'bonus', the sex of the fetus is learned after amniocentesis. How? Alternatively, determination of concentrations of certain substances in the maternal serum can detect about 50% of Down syndrome fetuses, which is less invasive than amniocentesis. Ultrasound scanning is not a reliable method.

Structural aberrations involve whole sets of genes within a chromosome. A portion of a chromosome may be reversed, placing the linear arrangement of genes in reverse order (**inversion**); non-homologous chromosomes may exchange sections (**translocation**); entire blocks of genes may be lost (**deletion**); or an extra section of chromosome may attach to a normal chromosome (**duplication**). These structural changes often produce phenotypic changes. Duplications, although rare, are important for evolution because they supply additional genetic information that may enable new functions.

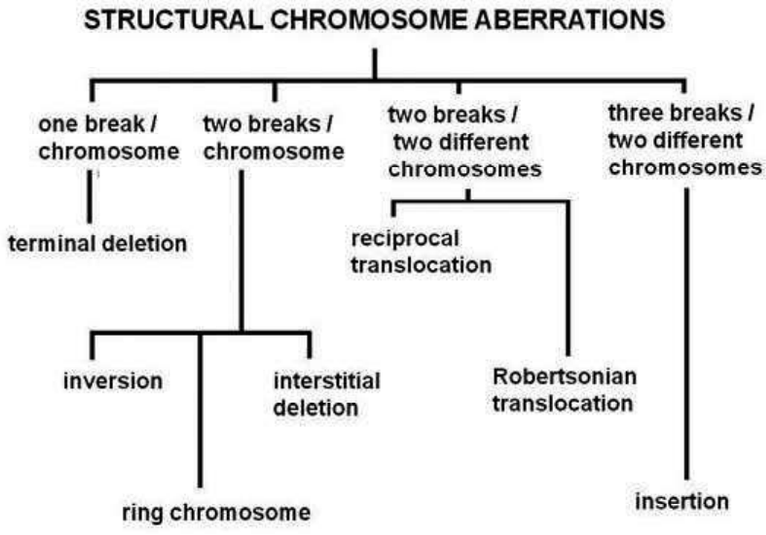


Fig. 2.2 *Structural Chromosome Aberrations*

The following points highlight the three main importance of chromosomal aberrations. The importance are:

- Role in Evolution
- Role in Genetic Analysis
- Role in Plant Breeding

Role in Evolution: The study of the karyotypes of different species has revealed interesting facts about the plant kingdom. It has been demonstrated that individuals in wild populations are to some extent heterogenous cytologically and genetically. In some cases, even if the genes are identical they may be ordered in a different way, owing to alterations of the chromosomal segments. These changes have an important bearing on the evolution of species. Observation of chromosomal organization and of the different karyotypes in the individual, the species, genera and the major systematic groups indicates that chromosomal aberration is involved in the process of evolution. One of the most frequent causes of evolution is a change in the order of genes as a result of chromosomal aberrations. Thus chromosomal aberrations play a vital role in evolution as they generate variation in a natural population

Natural selection maintains the polymorphism for gross chromosomal changes in plants like Oenothera, Datura, Rhoeo, Clarkia, Campanula, etc. Reciprocal translocations along with alternate chromosome segregation ensure the maintenance of heterozygosity and prevent the expression of lethal genes.

Duplication ensure the perpetuation of genes which are present in single dose. Inversions prevent pairing between homologous parts and suppress crossing over.

Role in Genetic Analysis: Chromosomal aberrations have been very helpful in genetic analysis. Translocated strains of maize were used to demonstrate that a cytological exchange

of chromosome parts results into genetic recombination. In different crop species, translocations, duplication and deficiencies have been used in mapping of gene locus.

Role in Plant Breeding: Aberrations result in altered linkage relationships and this has been exploited for breeding experiments

2.2.1 Euploidy and its Role in Evolution

Most diploid sexually reproducing organisms have an alteration between a haploid and a diploid state in the life cycle. The haploid state, mostly confined to germ cells, is characterized by the presence of a single set of **chromosomes (n)**. When two haploid germ cells produced by a male and a female parent unite during fertilization, the zygote formed contains two haploid sets of chromosomes and it becomes **diploid (2n)**. Mitotic divisions of the zygote produce a diploid adult organism of which each cell contains one haploid set of chromosomes from male parent, the other from the female parent. Since both sets of chromosomes are morphologically identical, pairs of identical chromosomes exist in a cell nucleus. The two members of a pair are said to be homologous to each other. When meiosis starts in the germ mother cells, homologous chromosomes attract each other and start pairing. The exactness of pairing depends upon the identical nature of the homologues. In euploidy an organism acquires an additional set of chromosomes over and above the diploid complement. If one additional set is present the condition is known as **triploid (3n)**, if two then tetraploid, addition of three sets is called **pentaploid (5n)**. This is known as a polyploidy series and the individuals are said to show euploidy.

Classification of Euploidy

Depending upon the source of the additional chromosome set, euploids are classified into two types, autopolyploids and allopolyploids

Autopolyploids: Autopolyploids arise when the additional sets originate from the same species. For example, if the haploid set of a species is designated A, the diploid is AA, triploid AAA, tetraploid AAAA.

Autotetraploids can arise through one of the following ways:

- Fertilization of an egg by two or more sperms giving rise to a zygote with three or more sets of chromosomes.
- Normal mitotic division in the diploid zygote in which chromosomes.
- Failure of meiotic division in germ mother cells so that unreduced diploid gametes are formed instead of haploids.

Although autopolyploids have homologous genomes, yet those having odd numbered sets of chromosomes, such as 3n, 5n, 7n, 9n, and so on, show a high degree of sterility. This is because during meiosis, pairing between two homologous chromosomes only results in normal segregation of the haploid set into gametes.

If three homologues are present they may or may not become paired to form a trivalent. Since pairing in any region is restricted to only two homologues at a time, third homologue may fail to pair and remain a univalent, or may pair at some places to form a trivalent.

During anaphasic segregation at Meiosis I, two of these homologues may move into one daughter cell, and the third into the other daughter cell. Since all chromosomes of the haploid set have three homologues each, their random distribution or independent assortment will cause the resulting gametes to have varying numbers of different homologues.

NOTES

In this way a true haploid or a true diploid gamete would be rarely formed. Instead, unbalanced gametes with chromosome numbers ranging between n and 2n would be formed. Such gametes are not viable and triploids consequently are sterile.

It is noteworthy that most popular varieties of seedless watermelons, bananas, Indian carpet grass, and European pears and apples are triploid. These triploid plants have resulted from fertilisation between diploid gametes from tetraploid plants and haploid gametes from diploid plants. Once formed, the triploid plants are healthy and robust and are propagated through asexual cuttings.

Autotetraploids are either fertile or only partially sterile. As there are no univalents and trivalents formed, the four homologues can segregate to form viable diploid gametes, so that tetraploids are often fertile. Groundnut, potatoes and coffee are well known examples of autotetraploid species.

Among cereals, autotetraploid rye is grown in Sweden and Germany. Some of the giant sized plants of Oenothera lamarckiana which de Vries first noticed in Holland and attributed to a mutation, had later turned out to be autotetraploids.

Polyploidy is more common in plants than in animals. More than 50 per cent of angiosperms are known to be polyploids. There are some explanations for this. Plants are mostly hermaphrodites or bisexual organisms in which sex chromosomes do not play a significant role in normal growth and development.

Allopolyploidy: Allopolyploidy is the second type of euploidy where the additional set of chromosomes comes from a different species. For example, suppose a diploid species with two chromosome sets AA crosses naturally or artificially with another species BB. The offspring produced would be AB which is viable but sterile.

This is because during meiosis the chromosomes belonging to the set A do not find homologous partners in chromosomes of B. Due to failure of pairing at anaphase I, the chromosomes move at random towards the two poles. Thus each gamete gets an unbalanced mixture of A and B chromosomes and sterility results.

There is one way of restoring fertility to a sterile hybrid (AB). If during mitotic division in the AB hybrid all the chromosomes are allowed to divide but cell division is inhibited, the result would be a tetraploid nucleus with two sets of A and two sets of B chromosomes (AABB).

It is possible to induce amphidiploidy artificially by treating young buds or seeds with the alkaloid colchicine, a mitotic poison which inhibits spindle formation, consequently cell division. This leads to all the duplicated chromosomes becoming included in a single tetraploid nucleus.

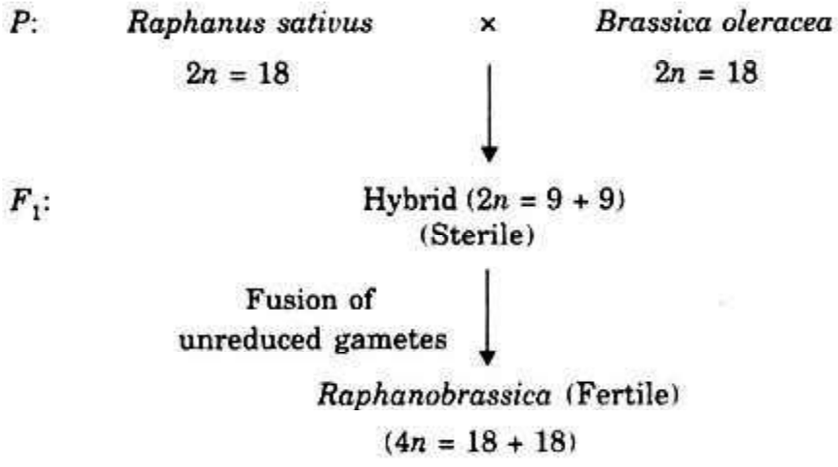
Raphanobrassica is an interesting example of a newly synthesised genus for illustrating allopolyploidy. In 1927 a Russian geneticist Karpechenko made a cross between *Raphanus sativus* (radish) and *Brassica oleracea* (cabbage) with the aim of producing a new plant that would have the roots of radish, and in the aerial portions would bear cabbage. The hybrid that was actually formed had the roots of cabbage and tops of radish plant.

The hybrid produced between radish and cabbage proved useless economically. But it proved very important genetically. Both radish and cabbage plants are diploid with 18 chromosomes.

Thus gametes from each parent had 9 chromosomes, and their union produced the F_1 hybrid with 18 chromosomes. This hybrid was sterile because the 9 chromosomes of radish did not pair with the 9 chromosomes of cabbage. Sometimes however, viable pollen and ovules were produced having all 18 chromosomes.

Fusion of such unreduced gametes produced tetraploid (4n = 36) plants with 18 chromosomes of radish and 18 of cabbage. Pairing took place amongst the radish chromosomes to form 9 pairs; similarly the cabbage chromosomes also formed 9 pairs.

Normal segregation gave rise to viable gametes. The hybrid therefore became fertile and was given the name of a new genus Raphanobrassica. This is a beautiful demonstration of how a new genus can be artificially synthesised through allopolyploidy.



The genus Triticale demonstrates the efforts of man to create a new cereal by crossing wheat and rye. A hexaploid Triticum (2n + 44) is crossed to the diploid Secale (2n = 14).

'Check Your Progress'

1. Define euploidy and aneuploidy.
2. How is aneuploidy caused?
3. Define the term syndrome.
4. What are the three main importance of chromosomal aberrations?
5. What is allopolyploidy? Give its example.

2.3 ROLE OF MUTATIONS IN CROP IMPROVEMENT

Variation in the individuals either induced by environment or due to the hereditary change during genetic recombinations. But the variations or changes which suddenly appear in an individual and can be inherited to the progeny, is called mutation (Dwain).

According to Bateson: Mutation is discontinuous variation.

According to Sinnott DON, Dobzhansky: Mutation is any change in gene or genotype.

According to Hugo de Vries: Mutation is defined as any sudden and drastic heritable change in gene which is not traceable or ascribable to segregation or recombination. He first recognized the sudden changes in Oenothera Lamarkiana and studied the hereditary and came to this condition in 1901.

Mutations may be induced or natural it is brought about by any change in the genetic makeup either in chromosome level or in gene level. It may be structural or compositional or numerical changes which cause the change in character of an individual. A character may be lost or gained.

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Mutation play important role in plant breeding and also displays the key role in evolution of species.

Role of Mutation in Plant Breeding

Plant breeding aims at improving the crop quality but improving the heredity through the cross hybridization technique. In plants mutations can be artificially incused by mutagenic agents and there utilization for production of new superior varieties of species from traditional variety. This process is called mutation breeding.

The history of mutation breeding in India started in 1935 at Bose research institute, Calcutta and established at IARI, New Delhi in 1959.

- **Mutation Breeding in Wheat (Triticum Species):** By the application of Eradication and chemical mutagens mutation being used to introduce in wheat varieties. By this process the resistance variety NP836 was derived from NP 799 at India Agricultural Research Institute (IARI), New Delhi. This is done by application gamma ray on NP 799 variety of wheat. Thus NP 836 is a mutant variety.
- **Mutation Breeding in Rice (*Oryza sativa*):** Mutation breeding in rice is very common in south and south-east Asia. In rice certain chemical mutagens has been used to produce polyploid varieties of rice and hybridised with the diploids producing high yielding and resistant varieties.

The high yielding varieties of rice produced by mutation breeding is P 500.28. This variety is obtained from the T-1145 variety at Bose's institute, Calcutta.

- **Mutation Breeding in Cotton (Gossypium):** Mutation beading achieved evolving improved variety in cotton. A caiety named indore-2 was developed from 'Malwa Upland -4' by X-ray treatment.

Mutations in the variety mesilla cabala by X-ray treatment result increase in 40-50% in fiber production. M.A.2 H-190. Indore-2 L.SS Bury-0394, 320-F and H-14 are mutation improved varieties of cotton.

- **Mutation Breeding in Sugarcane:** Both eradication and chemical mutagens are used to induce maturation in sugarcane. In sugarcane nodal buds are exposed to radiation in field and the mutant buds or tillers are selected in F1 and F2 generations through artificial crosses.

Some popular higher quality sugarcane varieties evolved through mutation breeding are H.M.658, H.M. -661, Co-213, Co-602, Co-612, etc.

- **Mutation Breeding in Potato:** Mutations also introduce in potato crop through mutation breeding. It aims at the production of early harvesting varieties and high yielding variety.

These includes eradication and chemical mutagenic products and through cross breeding. This is done in flowering or by exposing the seed tubers.

Role of Mutation in Evolution

Mutation play a key role in evolution and origin of new species. According to the mutation theory as propounded by Hugo de Vries (1901), a new species arise not by gradual accumulation of small variations, but by the appearance of permanent and sudden change in character which is unpredictable. He explained the process as the mutation.

While working on the plant named Oenothera Lamarkiana de Vries marked sudden heritable changes and put forwarded that:

- New species arises due to mutations.
- Mutation brings the chances of selection.
- Mutation takes place nearly at all directive and may involve any character.

In Oenothera chromosome number is 14 but de Vries observed that in some generations this chromosome number tend to vary and in some it was found 15, 16, 20, 22, 24, 27 ,28, 29 and 30. This results in variation of flower size shape arrangement of buds and size of seeds, etc.

Stebbins (1971) has proposed some basic process of evolution as in terms of neo Darwinism and pointed out that evolution is brought about by gene mutations or chromosomal mutation.

According to Stebbins

- Mutation is gene level causes alterations in the structure and position of gene on chromosome called point mutation.
- This results in the alteration of phenotype of an organism.
- Changes in basic chromosome number either any addition of loss of any set or parts of them cause appearance of disappearance of new characters.
- Once the mutation in gene level or chromosomal level is firmly established in populations, they are subjected to natural selection.

Natural selection is the key process of organic evolution, also called chromosomal mutation, any of a number of structural changes in chromosomes. These changes result from abnormal divisions within the chromosomes, generally accompanied by reunification of the resulting segments in combinations other than those existing in the original chromosomes.

Structural changes may occur within a single chromosome or between homologous and nonhomologous chromosomes. Such changes include the loss (deletion) or gain (duplication) of a segment of the chromosome, or the exchange (translocation) of a segment of the chromosome with another segment. In inversion, a chromosome segment is deleted, turned through 180°C, and reinserted at the same position on the chromosome. Deletion and duplication disrupt the genebalance, thus altering the organism's characters. Inversions change only the sequence of the genes in the chromosome, whereas in translocations the genes of the shifted segment move to another linkage group.

Neither inversions nortranslocations disrupt the gene balance or alter the organism's phenotypic characters.

When meiosis occurs among heterozygotes that contain one normal chromosome and one chromosome with an inversion, itis difficult for these chromosomes to come together, and crossing over between them does not take place or is infrequent.Aneuploid gametes often appear, and consequently heterozygotes are less fertile than homozygotes, in which bothchromosomes of a given pair either have an inversion or are normal. Since heterozygotes have chromosomes in whichtranslocation occurs, heterozygotes produce many aneuploid gametes and are consequently less fertile than homozygotes.

Chromosomal aberrations may arise spontaneously, but they are more likely to occur under the influence of physical andchemical factors. Minor deletions and duplications may result from irregular crossing over. Chromosomal aberrations play animportant role in the evolution of organisms: duplications are the main cause of increases in the number of genes.

Inversionsand translocations may result in genetically isolated homozygous individuals that are more fertile than heterozygotes. In allchromosomal aberrations, the gene position effect is sometimes observed, in which a gene shifted to a new site on a chromosome exerts a different effect on the organism's phenotype.

Chromosomal aberrations have a practical application in that they may be induced in order to change the linkage groups ofgenes to produce economically valuable characters in organisms.

'Check Your Progress'

6. How Hugo de Vries defined mutation?
7. What are the possibilities related to mutation?
8. What is the use of mutation?

2.4 SUMMARY

- A consequence of chromosomal structural aberration in populations is related to evolutionary change including speciation.
- Chromosomal aberrations are associated with position effects that may be significant in natural selection.
- Speciation in the Drosophila group of dipterous insects has been related to chromosome inversions.
- Populations have developed over a period of time with different chromosome inversions.
- Speciation in Drosophila has been associated with a series of different inversions that occurred by chance in breeding populations and were recognized in different taxonomic groups.
- Translocations have been shown to occur in certain plant groups and to cause genetic isolation thus promoting evolutionary stability in populations.
- Structural and numerical deviations from the norm that affect many genes at once are called chromosomal aberrations.
- Changes in chromosome numbers are called euploidy when there is the addition or deletion of whole sets of chromosomes and aneuploidy when a single chromosome is added to or subtracted from a diploid set.
- A set of chromosomes contains one member of each homologous pair as would be present in the nucleus of a gamete.
- The most common kind of euploidy is polyploidy, the carrying of one or more additional sets of chromosomes.
- Animals are much less tolerant of chromosomal aberrations, because sex determination requires a delicate balance between the numbers of sex chromosomes and autosomes.
- Many domestic plant species are polyploid (cotton, wheat, apples, oats, tobacco, and others), and perhaps 40% of flowering plants are believed to have originated in this manner.
- Horticulturists favor polyploids and often try to develop them because they have more intensely colored flowers and more vigorous vegetative growth.

- Aneuploidy is usually caused by failure of chromosomes to separate during meiosis (nondisjunction).
- Trisomy, the result of the fusion of a normal n gamete and an n + 1 gamete, is much more common, and several kinds of trisomic conditions are known in humans.
- A syndrome is a group of symptoms associated with a particular disease or abnormality, although every symptom is not necessarily shown by every patient with the condition.
- Structural aberrations involve whole sets of genes within a chromosome.
- A portion of a chromosome may be reversed, placing the linear arrangement of genes in reverse order (inversion); non-homologous chromosomes may exchange sections (translocation); entire blocks of genes may be lost (deletion); or an extra section of chromosome may attach to a normal chromosome (duplication).
- Natural selection maintains the polymorphism for gross chromosomal changes in plants like Oenothera, Datura, Rhoeo, Clarkia, Campanula, etc.
- Reciprocal translocations along with alternate chromosome segregation ensure the maintenance of heterozygosity and prevent the expression of lethal genes.
- Duplication ensure the perpetuation of genes which are present in single dose.
- Inversions prevent pairing between homologous parts and suppress crossing over.
- Translocated strains of maize were used to demonstrate that a cytological exchange of chromosome parts results into genetic recombination.
- Most diploid sexually reproducing organisms have an alteration between a haploid and a diploid state in the life cycle.
- The haploid state, mostly confined to germ cells, is characterized by the presence of a single set of chromosomes (n).
- Mitotic divisions of the zygote produce a diploid adult organism of which each cell contains one haploid set of chromosomes from male parent, the other from the female parent.
- The exactness of pairing depends upon the identical nature of the homologues.
- In euploidy an organism acquires an additional set of chromosomes over and above the diploid complement.
- Depending upon the source of the additional chromosome set, euploids are classified into two types, autopolyploids and allopolyploids.
- Autopolyploids arise when the additional sets originate from the same species. For example, if the haploid set of a species is designated A, the diploid is AA, triploid AAA, tetraploid AAAA.
- During anaphasic segregation at meiosis I, two of these homologues may move into one daughter cell, and the third into the other daughter cell.
- Polyploidy is more common in plants than in animals. More than 50 per cent of angiosperms are known to be polyploids.
- Allopolyploidy is the second type of euploidy where the additional set of chromosomes comes from a different species
- Mutations may be induced or natural it is brought about by any change in the genetic makeup either in chromosome level or in gene level. It may be structural or compositional or numerical changes which cause the change in character of an individual.

- Mutation play important role in plant breeding and also displays the key role in evolution of species.
- Plant breeding aims at improving the crop quality but improving the heredity through the cross hybridization technique.
- In plants mutations can be artificially incused by mutagenic agents and there utilization for production of new superior varieties of species from traditional variety. This process is called mutation breeding.
- Mutation beading achieved evolving improved variety in cotton. A caiety named indore-2 was developed from 'Malwa Upland -4' by X-ray treatment.
- Natural selection is the key process of organic evolution, also called chromosomal mutation, any of a number of structural changes in chromosomes.
- Structural changes may occur within a single chromosome or between homologous and non-homologous chromosomes.
- Chromosomal aberrations play animportant role in the evolution of organisms: duplications are the main cause of increases in the number of genes. Inversionsand translocations may result in genetically isolated homozygous individuals that are more fertile than heterozygotes.
- Chromosomal aberrations have a practical application in that they may be induced in order to change the linkage groups of genes to produce economically valuable characters in organisms.

2.5 KEY TERMS

- **Allopolyploidy**: Allopolyploidy is the second type of euploidy where the additional set of chromosomes comes from a different specie.
- **Syndrome:** A syndrome is a group of symptoms associated with a particular disease or abnormality.
- **Mutation:** Mutation is defined as any sudden and drastic heritable change in gene which is not traceable or ascribable to segregation or recombination.
- **Euploidy**: Changes in chromosome numbers are called euploidy, i.e., when there is the addition or deletion of whole sets of chromosomes.
- **Aneuploidy:** Aneuploidy is when a single chromosome is added to or subtracted from a diploid set.

2.6 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Changes in chromosome numbers are called euploid**y**, i.e., when there is the addition or deletion of whole sets of chromosomes and aneuploidy is when a single chromosome is added to or subtracted from a diploid set.
2. Aneuploidy is usually caused by failure of chromosomes to separate during meiosis (nondisjunction). If a pair of chromosomes fails to separate during the first or second meiotic divisions, both members go to one pole and none to the other. This results in one gamete having n – 1 number of chromosomes and another having n + 1 number of chromosomes.

3. A syndrome is a group of symptoms associated with a particular disease or abnormality, although every symptom is not necessarily shown by every patient with the condition.
4. The following points highlight the three main importance of chromosomal aberrations:
   - Role in Evolution
   - Role in Genetic Analysis
   - Role in Plant Breeding
5. Allopolyploidy is the second type of euploidy where the additional set of chromosomes comes from a different species. For example, suppose a diploid species with two chromosome sets AA crosses naturally or artificially with another species BB. The offspring produced would be AB which is viable but sterile.
6. According to Hugo de Vries mutation is defined as any sudden and drastic heritable change in gene which is not traceable or ascribable to segregation or recombination.
7. Mutations may be induced or natural it is brought about by any change in the genetic makeup either in chromosome level or in gene level. It may be structural or compositional or numerical changes which cause the change in character of an individual. A character may be lost or gained.
8. Mutation play important role in plant breeding and also displays the key role in evolution of species.

2.7 QUESTIONS AND EXERCISES

Short-Answer Questions

1. Give a short note on chromosomal aberrations. Distinguish between euploidy and aneuploidy.
2. Draw a well-labelled diagram of structural chromosome aberrations.
3. What are the ways in which autotetraploids can arise?
4. What is allopolyploidy?
5. Discuss in brief the role of mutation in plant breeding.

Long-Answer Questions

1. Elaborate a note on chromosomal aberration and their role in evolution.
2. Discuss about the importance of chromosomal aberrations.
3. Explain polyploidy and a euploidy and its role in evolution.
4. Discuss mutation types, basis and role in crop improvement.

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UNIT 3 SEX DETERMINATION AND GENETIC MAPPING

Structure

3.0 INTRODUCTION

A sex-determination system is a biological system that determines the development of sexual characteristics in an organism. Most organisms that create their offspring using sexual reproduction have two sexes. Occasionally, there are hermaphrodites in place of one or both sexes. There are also some species that are only one sex due to parthenogenesis, the act of a female reproducing without fertilization. In many species, sex determination is genetic: males and females have different alleles or even different genes that specify their sexual morphology. In animals this is often accompanied by chromosomal differences, generally through combinations of XY, ZW, XO, ZO chromosomes, or haplodiploidy. The sexual differentiation is generally triggered by a main gene, i.e., a 'sex locus', with a multitude of other genes following in a domino effect.

Genetic mapping is the process of determining the order of and relative distance between genetic markers, i.e., specific sequences or heritable elements that generate a phenotype on a chromosome based on their pattern of inheritance. Genetic mapping in bacteria relies on chromosome transfer between cells.

There are two distinctive types of 'Maps' used in the field of genome mapping: genetic maps and physical maps. While both maps are a collection of genetic markers and gene loci, genetic maps' distances are based on the genetic linkage information, while physical maps use actual physical distances usually measured in number of base pairs. While the physical map could be a more 'accurate' representation of the genome, genetic maps often offer insights into the nature of different regions of the chromosome, for example the genetic distance to physical distance ratio varies greatly at different genomic regions which reflects different recombination rates, and such rate is often indicative of euchromatic (usually gene-rich) vs heterochromatic (usually gene poor) regions of the genome.

In this unit, you will study about sex determination in plants, genetic mapping, genetic recombination in eukaryotes and interaction of genes in detail.

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2.1 UNIT OBJECTIVES

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

- Understand sex determination in plants
- Explain genetic mapping
- Discuss genetic recombination in eukaryotes
- Explain interaction of genes

3.2 SEX DETERMINATION IN PLANTS

Sex determination is a mechanism which brings about differentiation of sex, i.e., male and female amongst organisms. Sex determination is of three types:

- **Progamic:** The sex is determined before fertilization by the type of available egg. It occurs in those cases where females are heterogametic while males are homogametic (ZO-ZZ, ZW-ZZ).
- **Syngamic:** The sex is determined at the time of fertilization by the type of fusing sperm. It is found in those cases where males are heterogametic (XY-XX, XO-XX).
- **Epigamic:** The sex of the offspring is determined after fertilization during development as in case of marine worm.

A number of theories have been given in the past about the mechanism of sex determination, i.e., as follows:

- **Metabolic Differentiation Theory:** According to Riddle (1917), male progeny is produced if metabolism favour increased metabolism, increased water content but less storage of proteins. Female progeny is obtained when anabolism is less, water content is less but stored protein is more.
- **Alternate Dominance Theory:** Castle (1903) believed that reproductive cells are heterozygous in relation to sex. A male progeny is produced if male determinants are abundant while female progeny is got when female determinants become dominant.
- **Heterozygosity Theory:** Correns (1906) thought that the riddle of sex lay in the fact that one parent is homozygous while the other is heterozygous.
- **Quantitative Theory:** Goldschmidt (1911, 1923 and 1927) performed reciprocal crosses between Japanese and European Gypsy Moth. Intersexes also appeared in which one type of sex was present in early stage but later on changed to the other sex without alteration in chromosome complement. The result was explained by presuming the formation of andreas (male enzyme) and glynase (female enzyme) which were produced in greater concentration in the Japanese variety of Gypsy moth. Y chromosome plays little role in determination of sex. Gynandromorphs develop if in 2X female, one X is lost in certain in certain early divisions.
- **Genic Balance Theory:** The theory proposed by Bridges (1921) is based on his work on fruit fly, Drosophila melanogaster. Genic balance theory believes that every individual contains genes for both maleness and femaleness in its genotype. The sex is determined by the preponderance of the genes of one sex over those of the other. According to Bridges, autosomes of Drosophila carry genes for maleness while its X chromosomes possess genes for femaleness. Fertility of male is due to genes present

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on Y chromosomes. Sex of the individual is decided by the ration between X chromosomes to autosomes with 0.5 indicating maleness, less than 0.5 supermale, more than 0.5 but less than 1 forming intersex, 1 producing female and more than 1 developing in super females.

Other Theories: Sex of the offspring is determined by the following ways:

- **Age and Vigour Theory:** It depends upon the sex of the more vigorous parent.
- **Origin of Ovum Theory:** Ovum of right ovary produces male child while that of left forms female child.
- **Timing of Fertilization:** According to Thury, an ovum fertilized immediately after release produces female offspring while the one fertilized later give rise to male offspring.

Organisms of many species are specialized into male and female varieties, each known as a **sex**. Sexual reproduction involves the combining and mixing of genetic traits: specialized cells known as gametes combine to form offspring that inherit traits from each parent. The gametes produced by an organism define its sex: males produce small gametes, for example, spermatozoa, or sperm, in animals; pollen in seed plants while females produce large gametes (ova, or egg cells). Individual organisms which produce both male and female gametes are termed hermaphroditic. Gametes can be identical in form and function (known as isogamy), but, in many cases, an asymmetry has evolved such that two different types of gametes (heterogametes) exist (known as anisogamy).

Physical differences are often associated with the different sexes of an organism; these sexual dimorphisms can reflect the different reproductive pressures the sexes experience. For instance, mate choice and sexual selection can accelerate the evolution of physical differences between the sexes.

Among humans and other mammals, males typically carry an X and a Y chromosome (XY), whereas females typically carry two X chromosomes (XX), which are a part of the XY sex-determination system. Humans may also be intersex. Other animals have different sex-determination systems, such as the ZW system in birds, the X0 system in insects, and various environmental systems, for example in crustaceans. Fungi may also have more complex allelic mating systems, with sexes not accurately described as male, female, or hermaphroditic.

Types of Sex Determination

- **Environmental Determination of Sex:** Nutrition plays an important role in determination of sex in some potentially hermaphrodite animals. Marine mollusk becomes female if reared alone. In the company of a female animal, the young one develops into male. The female animal secretes a chemical in its environment which induces the development of maleness in the young animal. A similar phenomenon occurs in marine worm Bonellia viridis. It has free swimming ciliated larva which develop into either sex, female or male. The female is long with 2.5 cm body and over a metre long proboscis. The male is 0.13 cm. It lives as parasite in the gonoduct of female. If larva settles down at an isolated place, it develops into female worm.

In crocodile and lizards, high temperature induce maleness while low temperature causes development of femaleness. In turtle, males are predominant below 28 degree Celsius, females above 33 degree Celsius.

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- **Non-Allosomic Genetic Sex Determination**
  - **Bacteria:** In bacteria, sex is determined by the presence or absence of fertility factor located on a plasmid. Plasmid is a small additional ring of DNA.
  - **Simple Eukaryotes:** In neurospora, sex determining genes are found on ordinary chromosomes. They form distinct mating types like A and B.
  - **Maize**: It is monoecious flowering plant which bears both male inflorescence and female inflorescence. The two develop under the influence of separate genes.
  - **Drosophila:** Autosomes also bear genes for influencing sex.
- **Allosomic Sex Determination:** In most animals and some plants, sex is determined by specific chromosomes called sex chromosomes, allosomes or idiochromosomes. Henking (1891) discovered a condensed chromatin or X body in the primary spermatocytes of firefly.

The Different Types of Sex Chromosomes

- **XX-XY Type:** In most insects including fruit fly Drosophila and mammals including human beings the females possess two homomorphic sex chromosomes named XX. The males contain two heteromorphic sex chromosomes XY. The Y chromosome is shorter and heterochromatic. Despite differences in morphology, the Y chromosomes pairs with X chromosome during meiosis.

  X linked genes are present on non-homologous parts of X chromosomes. They are known as sex linked genes. Human beings have 22 pairs of autosomes and one pair of sex chromosomes. All the ova formed by female are similar in their chromosome type (22 + X). Females are homogametic.
- **XX-XO Type:** In roundworms and some insects, the females have two sex chromosomes XX, while the males have only one sex chromosome X. There is no second sex chromosome. So males are designated as XO. The females are homogametic because they produce only one type of eggs. The males are heterogametic with half the male gametes carrying X chromosome while the other half being devoid of it. The sex ratio produced in the progeny is 1:1.
- **ZW-ZZ Type:** In birds and some reptiles both the sexes possess two sex chromosomes but unlike human beings the females contain heteromorphic sex chromosomes while the males have homomorphic sex chromosomes. Because of having heteromorphic sex chromosomes, the females are heterogametic and produce two types of eggs (A+Z) and (A+W).
- **ZO-ZZ Type:** This type of sex determination occurs in some butterflies and moths. It is opposite the condition found in cockroaches and grasshoppers. The females have odd sex chromosomes (AA+ZO) while the males have two homomorphic sex chromosomes (AA+ZZ). The females are heterogametic. They produce two types of eggs, male forming with one sex chromosome (A+Z) and female forming without the sex chromosome (A+O). The males are homogametic forming similar types of sperms. The two sexes are obtained in the progeny in 50:50 ratio.
- **Haplodiploidy:** It is a type of chromosomal sex determination in which sex chromosomes don't differentiate. Haplodiploidy occurs in some insects like bees, ants and wasps. Male insects are haploid because they develop parthenogenetically from unfertilized eggs. Parthenogenetic development of males is called **arrhenotoky**.

Sex determination is an important developmental event in the life cycle of all sexually reproducing plants. Recent studies of sex determination in many plant species, from ferns to maize, have been fruitful in identifying the diversity of genetic and epigenetic factors that are involved in determining the sex of the flower or individual. In those species amenable to genetic analysis, significant progress has been made toward identifying mutations that affect sex expression. By studying the interactions among these genes, pictures of how sex-determining signals are perceived to activate or repress male- or female-specific genes are emerging.

'Check Your Progress'

1. What is sex determination?
2. When does sex determination occur in epigamic?
3. How is female progeny obtained?
4. When is male progeny produced?

3.3 GENETIC MAPPING

Gene mapping describes the methods used to identify the locus of a gene and the distances between genes. The essence of all genome mapping is to place a collection of molecular markers onto their respective positions on the genome. Molecular markers come in all forms. Genes can be viewed as one special type of genetic markers in the construction of genome maps, and mapped the same way as any other markers.

Researchers begin a genetic map by collecting samples of blood or tissue from family members that carry a prominent disease or trait and family members that don't. Scientists then isolate DNA from the samples and closely examine it, looking for unique patterns in the DNA of the family members who do carry the disease that the DNA of those who don't carry the disease don't have. These unique molecular patterns in the DNA are referred to as polymorphisms, or markers.

The first steps of building a genetic map is the development of genetic markers and a mapping population. The closer two markers are on the chromosome, the more likely they are to be passed on to the next generation together. Therefore, the 'co-segregation' patterns of all markers can be used to reconstruct their order. With this in mind, the genotypes of each genetic marker are recorded for both parents and each individual in the following generations. The quality of the genetic maps is largely dependent upon these factors: the number of genetic markers on the map and the size of the mapping population. The two factors are interlinked, as a larger mapping population could increase the 'resolution' of the map and prevent the map being 'saturated'.

In gene mapping, any sequence feature that can be faithfully distinguished from the two parents can be used as a genetic marker. Genes, in this regard, are represented by 'traits' that can be faithfully distinguished between two parents. Their linkage with other genetic markers are calculated in the same way as if they are common markers and the actual gene loci are then bracketed in a region between the two nearest neighbouring markers. The entire process is then repeated by looking at more markers which target that region to map the gene neighbourhood to a higher resolution until a specific causative locus can be identified. This process is often referred to as 'positional cloning', and it is used extensively in the study of plant species The great advantage of genetic mapping is that it can identify the relative position of genes based solely on their phenotypic effect.

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Early Genetic Maps

- Alfred Sturtevant created the first genetic map of a chromosome from the fruit fly (*Drosophila melanogaster*) in 1913.
- He determined that genes were arranged on chromosomes in a linear way, like beads on a necklace, and that genes for specific traits are located in particular places.
- He proposed that the frequency of 'crossing over' (recombination) between two genes could help determine their location on a chromosome.
- He realised that genes that were far apart on a chromosome are more likely to be inherited separately simply because there is a larger region over which recombination can occur.
- In the same way, genes that are close to each other on the chromosome are more likely to be inherited together.
- By finding out how often various characteristics are inherited together it is possible to estimate the distance between the genes. A map of where the genes are in relationship to each other on the chromosomes can then be drawn. This is called a linkage map.
- Genes that are on the same chromosome are said to be 'linked' and the distance between these genes is called a 'linkage distance'. The smaller the distance the more likely two genes will be inherited together.
- The same concept of studying how traits are passed on was applied to develop the first human genome map.
- If two (or more) characteristics were seen to be frequently inherited together in a family, for example blonde hair and blue eyes, it suggested that the genes for the two characteristics were close together on a particular chromosome.

Modern Genetic Maps

- With more recent genetic mapping techniques, the position of genes is worked out from finding the exact frequency of genetic recombination that has occurred.
- To produce a genetic map, researchers collect blood or tissue samples from members of a family, some of whom have a certain disease or characteristic.
- The researchers then isolate the DNA from samples taken from each individual and closely examine it to find unique patterns in the DNA of those individuals with the disease/characteristic, that are not present in the DNA of the individuals who do not have the disease/characteristic.
- These are referred to as markers and are extremely valuable for tracking inheritance of characteristics or diseases through several generations of a family.
- One type of DNA marker, called a microsatellite, is found throughout the genome and consists of a specific repeated sequence of bases.
- If a particular gene is close to a DNA marker on the chromosome, it is more likely that the gene and marker will stay together during the recombination process and are therefore more likely to be passed down along the family line (inherited) together.
- In the same way, if a DNA marker and gene are frequently separated by the recombination process it suggests that they are far apart on the chromosome and are less likely to be inherited together.
- The more DNA markers there are on a genetic map the more likely it is that one of them will be located close to the disease or trait-associated gene.

- While genetic maps are good at giving you the bigger picture, they have limited accuracy and therefore need to be supplemented with further information gained from other mapping techniques, such as physical mapping.

Basic Logic of Genetic Mapping

- **Experimental Crosses of Inbred Strains:** Because we can know the original genetic makeup of the inbred strains and can control breeding and other environmental factors, we can study under controlled conditions the correlation of genotype and phenotype. Peak is relatively broad, so a few hundred markers suffice to cover an entire genome.
- **Association Mapping in Humans**: This is, in principle, similar to experimental crosses of inbred strains, but is complicated by the uncontrolled features of population history and inadequate knowledge of the mode of inheritance of the trait. Success of this method depends on association (correlation) between genetic markers and the phenotype of interest. Peak is very narrow, so many, perhaps 500000, genetic markers are required to cover a genome.
- **Recombination (Linkage) Mapping in Humans:** Relatives have similar traits because they have similar genotypes at those loci where there are genes that influences those traits. Identity By Descent (IBD) at a locus t measures genetic relatedness at t. Hence we should expect to find the gene(s) that influence a trait that is shared by related individuals to be found in regions where those individuals are IBD. This involves analysis of covariance (of genotype and phenotype) and generates a signal that is usually weaker, but much wider than for association mapping. Since one utilizes family relationships going back only a few generations, there are fewer difficulties due to population history.
- **Association Mapping with Family Controls:** It has advantages and disadvantages of both association mapping in humans and recombination (linkage) mapping in humans.
- **Admixture Mapping:** It utilizes historical phenotype and genotype differences between populations that for the most part have intermarried only relatively recently, for example, Asians and Europeans. One studies the correlation between phenotypes and the population origin of genetic markers.

With the help of processes like linkage and crossing over following facts were established:

- The genes are present in linear fashion on chromosomes.
- The genes are located in definite positions on chromatids.
- Linkage groups are equal to number of chromosomal pairs in any diploid organism.
- The strength of linkage is inversely proportional to the distance between two linked genes.
- Chances of crossing over increase in distantly placed genes.

The chromosomal maps represent the condensed, graphic representations of relative distance between the genes in a linkage group, expressed in percentage of recombination, located in a single group of chromosomes. Thus, the graphic representation of genes is known as chromosome maps. Strutevant was the first to construct a genetic map based on his idea of recombination frequencies that shows the location of various genes on a chromosome. Recombination frequenct is defined as one map unit or one centimorgan. Map is one dimensional.

NOTES

Morgan (1911) predicted that frequency of crossing over is governed by distance between genes. Thus probability of occurrence of crossing over between two particular genes increase as the distance between them becomes larger. The cross over frequency is directly proportional to distances between genes. A map unit is equal to 1% cross over (recombinants). It represents the linear distance along the chromosome for which a recombination frequency of 1% is observed. Genetic maps of chromosomes are also known as chromosome maps. Here chromosomes are shown by straight lines, proportional to their length with position of genes. These maps represent linkage groups.

Cytological Basis of Crossing Over

Morgan first proposed crossing over to explain the formation of recombinant combination of genes that were shown to be linked by genetic data. He hypothesized that this linkage was the result of location of these genes on the same chromosome. If crossing over occurs, one may expect to observe it cytologically. Cross shaped structure in which two of the four chromatids of homologous chromosomes pair appear to exchange pairing partners are detected in cytological studies of meiosis in many organisms.

Crossing over occurs after chromosome duplication, when each homologous chromosome is represented by two chromatids. Each pair of synapsed homologs is called tetrad, because it consists of four chromatids. Crossing over refers to the exchange of segments of chromosomes whereas recombination implies the formation of new combination of genes. Thus crossing over occurs in completely homozygous organisms, but new combination can be formed only in organism that are heterozygous at two or more loci.

'Check Your Progress'

5. What is gene mapping?
6. What is the need for genome mapping?
7. What is the first steps of building a genetic map?

3.4 GENETIC RECOMBINATION IN EUKARYOTES

Four Major Sources of Genetic Variability from Meiosis and Fertilization are as follows:

- **Genetic Recombination:** It occurs in prophase I (crossing over/chiasmata); synaptonemal complex is a protein framework supporting chromosomes to exchange segments and make new combinations.
- **Random Segregation of Paternal/ Maternal Chromosomes:** Chromosomes in each homologous part randomly segregates to each spindle pole.
- **Alternative Combinations at Meiosis II:** Segregation again of chromosomes during anaphase II; each homologous pair has one recombinant chromatid and one non-recombinant chromatid; could randomly segregate to either spindle end to create new allele combination.
- **Random Fertilization, Male/ Female Gamete Combine:** The fusing of the random male and female gametes increases the genetic diversity by even more. 1/ (2^23) ^2. 23 sex chromosomes each; two gametes.

Mobile Elements: Particular segments of DNA, can move from one area to another; cut and paste DNA and backbones; but do not need homology, (jumping genes) moves within genome of a given cell.

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Transposable Elements (TEs)

Mechanism of movement/ non-homologous recombination, Transposition (Refer Figure 3.1)

- TEs leaves it original location and moves to new location.
- TEs copies itself, sends new copy to the new location.

TEs must have contact with section of DNA they want to integrate into; not free agent (jumping genes= inaccurate).

TEs increase variability; biological mutagens.

Two Major Bacterial TEs

- **Insertion Sequences (IS):** It is simple, small, only carry genes for transposition, transposase catalyzes recombination of bacterial TE into DNA.
- **Transposon**: It is inverted repeat sequence (insertion sequences) surrounding the central regions for 1+ genes.

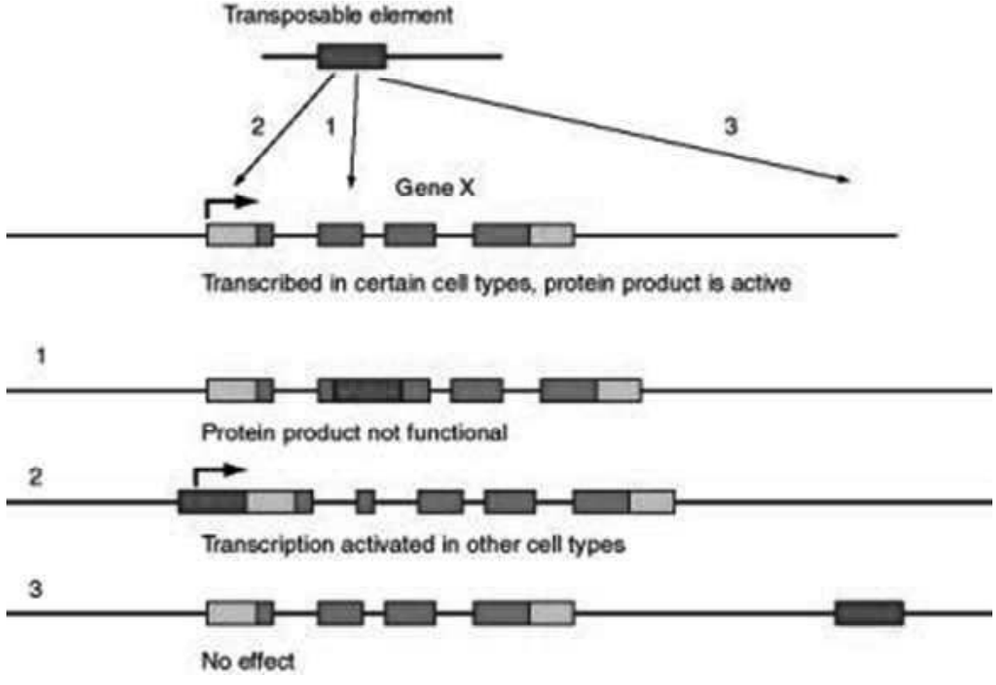

Fig. 3.1 *Transposable Element*

Many Antibiotics Carried as Transposon

- Barbara McClintock, first discovered TEs in eukaryotes.
- Retrotransposons: DNA element; transcribes intermediate RNA copy of TE. Reverse transcriptase, uses RNA to make DNA copy; new DNA copy inserted into new location.

Useful in understanding and experimenting with the genome of organisms:

- Available for many organisms in the literature and at Web sites.
- Maps based on RF are supplemented with maps based on molecular markers, segments of chromosomes with different nucleotide sequences.

Genetic recombination occurs when genetic material is exchanged between two different chromosomes or between different regions within the same chromosome. We can observe

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it in both Eukaryotes (like animals and plants) and Prokaryotes (like archaea and bacteria). Keep in mind that in most cases, in order for an exchange to occur, the sequences containing the swapped regions have to be homologous, or similar, to some degree.

The process occurs naturally and can also be carried out in the lab. Recombination increases the genetic diversity in sexually reproducing organisms and can allow an organism to function in new ways.

Examples of Genetic Recombination

Part of Your Making: Genetic recombination occurs naturally in *meiosis*. Meiosis is the process of cell division that occurs in eukaryotes, such as humans and other mammals, to produce offspring. In this case, it involves crossing-over (Refer Figure 3.2). What happens is that two chromosomes, one from each parent, pair up with each other. Next, a segment from one crosses over, or overlaps, a segment of the other. This allows for the swapping of some of their material, as you can see in the illustration below. What we end up with is a new combination of genes that did not exist before and is not identical to either parent's genetic information. Note that recombination is also observed in mitosis, but it does not occur as often in mitosis as it does in meiosis.

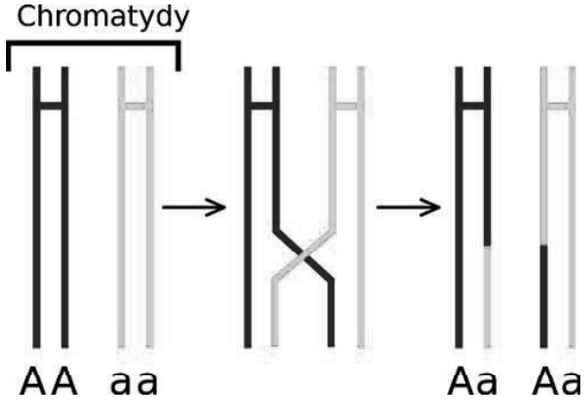

Fig. 3.2 *Crossing Over*

Natural Self-Healing: The cell also can also undergo recombinational repair, for example, if it notices that there is a harmful break in the DNA: the kind of break that occurs in both strands. What we observe is an exchange between the broken DNA and a homologous region of DNA that will fill the gaps. There are also other ways that recombination is used to repair DNA.

Functions of Genetic Recombination: This is a relatively new technology that is allowing scientists to change genes and organisms by manipulating DNA. What makes this so important is the fact that it has improved our understanding of diseases and, consequently, has expanded our ways of fighting them.

As you might expect, DNA segments are joined together in this Technology. For example, a gene can be cut out from a human and introduced into the DNA of a bacterium. The bacterium will then be able to produce human protein that is otherwise only made by humans. The same thing is done in gene therapy. Let us assume a person is born without a particular essential gene, and is suffering from an illness due to the absence of that gene. Scientists can now introduce the missing gene into that person's genome by using a virus that infects humans. First, they join the needed gene with the virus's DNA and then they expose the person to that virus. Since all viruses blend their DNA with their host's DNA, the gene that is added by the scientists ends up being part of the person's genome.

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Types of Genetic Recombination

Scientists have observed the following types of recombination in nature:

- **Homologous (General) Recombination:** As the name implies, this type occurs between DNA molecules of similar sequences. Our cells carry out general recombination during meiosis.
- **Non-Homologous (Illegitimate) Recombination:** Again, the name is self-explanatory. This type occurs between DNA molecules that are not necessarily similar. Often, there will be a degree of similarity between the sequences, but it's not as obvious as it would be in homologous recombinations.
- **Site-Specific Recombination:** This is observed between particular, very short, sequences, usually containing similarities.
- **Mitotic Recombination:** This does not actually happen during mitosis, but during interphase, which is the resting phase between mitotic divisions. The process is similar to that in meiotic recombination, and has its possible advantages, but it is usually harmful and can result in tumors. This type of recombination is increased when cells are exposed to radiation.

Prokaryotic cells can undergo recombination through one of these three process, i.e., as follows:

- **Conjugation:** It is where genes are donated from one organism to another after they have been in contact. At any point, the contact is lost and the genes that were donated to the recipient replace their equivalents in its chromosome. What the offspring ends up having is a mix of traits from different strains of bacteria.
- **Transformation:** This is where the organism acquires new genes by taking up naked DNA from its surroundings. The source of the free DNA is another bacterium that has died, and therefore its DNA was released to the environment.
- **Transduction**: It is gene transfer that is mediated by viruses. Viruses called *bacteriophages* attack bacteria and carry the genes from one bacterium to another.

'Check Your Progress'

8. When does genetic recombination occur?
9. What is insertion sequences?
10. Define transposon.

3.5 INTERACTION OF GENES

The emphasis has been given to the fact that genes studied by Mendel were segregating independently of each other. What is not yet been mentioned is that these genes must have been functioning independently of one another.

If each gene were expressing itself in a separate test tube, it would be reasonable to expect all genes to be functionally independent. But these genes are not in separate test tube, they are located in the same nuclei of the same cells. Thus expression of an allele of one gene will alter the expression of one or more of allele of a second gene. An example of gene interaction was reported by William bateson and his associate R.C Punett. Bateson began to conform and extend Mendels work after its discovery in 1900 and became pioneer

in transmission genetics. Domestic breeds of chickens have different comb shape. Wyandottes have a type of comb called rose whereas brahmas have a pea comb. Leghorns have single combs. The investigators crossed wyandottes and brahmas and all the F_1 chickens had walnut combs, a phenotype not expressed not expressed in either of parent. When F_1 chickens were mated among themselves and large F_2 populations were produced. A dihybrid ratio was recognized but phenotypes were different from those of parents. These two phenotypes were expressed as a result of gene product interaction. Genes R and P were non allelic, but each was dominant over its allele. When R and P were together, as in F_1, the two different products interacted to produce a walnut comb. The two non-allelic genes R and P acted independently in different ways, similar to ways in which codominant allele act.

Allelic Interaction: Multiple Alleles

An allele is a specific form of gene. It may be mutant allele, resulting in an altered phenotype or wild type allele producing active gene product and normal phenotype. Different wild type alleles occur in population. They all produce phenotype within wild type range. Similarly mutant allele of gene exhibit wide range of gene product activity from no activity to wild type level of activity. Alleles may produce a whole series of variable phenotype ranging from an extreme mutant phenotype to wild type phenotype. When more than two different forms of gene exist in a species, they are referred as multiple alleles. For example, Gene controlling eye color in fruit fly. Drosophila have red eyes, but vast array of eye colour have been studied extensively for many decades. Mutant allele of one gene result in flies with eye color ranging from pure white through series of intermediate color upto wild type red when present in homozygous condition, as shown in Figure 3.3.

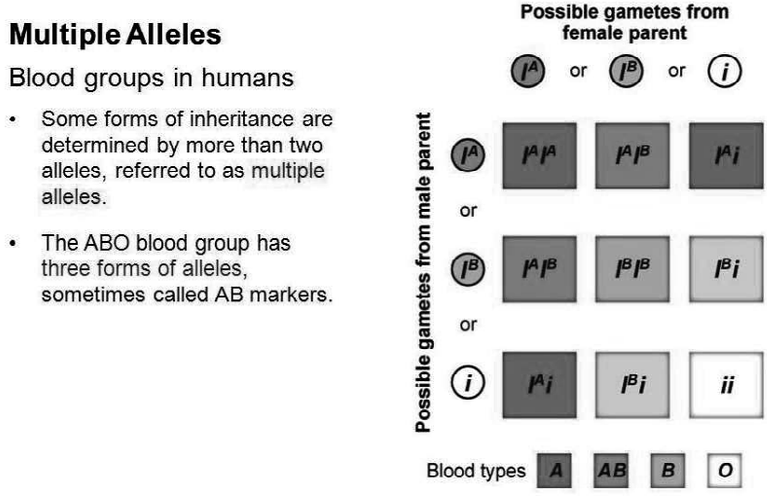

Fig. 3.3 *Multiple Allels*

ABO Blood Type Alleles in Humans

The ABO locus has three common alleles: I^A, I^B and I^o. I^A and I^B are codominant (both A and B antigens on their red blood cells) and I^O is recessive (no antigen) on their red blood cells. The ABO locus controls the type of glycolipids found on the surface of erythrocytes by specifying the type of glycosyl transferase synthesized in the red blood cells. The specific type of glycolipids on the red cell surface provide the antigenic determinants that react with specific antibodies present in blood serum

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Rh Factor Alleles in Human

The Rh factor was discovered in 1940 by K. Landsteiner and A.S Wiener form rabbits immunized with the blood of monkey Macaca rhesus. A test for Rh incompatibility is accomplished by placing a drop of blood on a slide and introducing anti Rh serum. Agglutination of erythrocytes indicates incompatibility whereas even distribution of erythrocytes indicates no reason. Cross matching of the Rh factor as well as of ABO types of donor and receipient blood is used to avoid incompatibility agglutination reaction following transfusion. Blood is exchanged between the mother and the foetus during childbirth. Thus Rh negative mothers are often immunized by blood from Rh positive foetus to which they give birth. Usually no illeffects are associated with exposure of mother to Rh positive antigen during first child birth. Rh positive children carried by same mother may be exposed to antibodies produced by mother against Rh antigen which are carried across placenta in blood serum. Children develop symptoms of haemolytic jaundice and anemia, condition known as erythroblastosis fetalis.

Codominance

When the dominant character is not able to suppress, even incompletely the recessive character and both the characters appear side by side in F1 hybrids, the phenomenon is called co dominance. For example in cattles, if a cattle with black coat is crossed to a cattle with white colour, F1 hybrid possess roan coat. In roan coat, both black and white patches appear separately. So the alleles which are able to express themselves independently when present together are called co dominant alleles (Refer Figure 3.4).

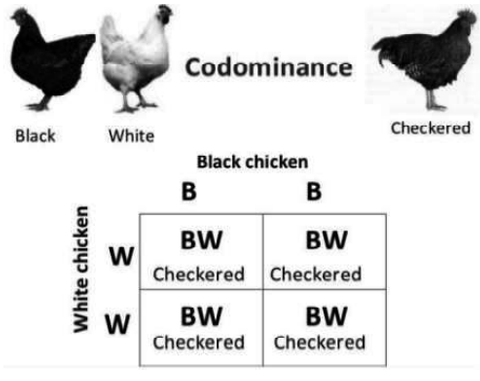

Fig. 3.4 *Condominance*

Incomplete Dominance

The law of dominance is not always correct as there can be found where the complete dominance is absent. In such cases some traits of F_1 phenotype is intermediate between those of parental traits. In incomplete dominance the genes of allelomorphic pair are not expressed as dominant and recessive but express themselves partially when present together in a hybrid. As a result F1 hybrid show characters intermediate to effect of two genes of the parents, for example, *Mirabilis Jalapa* (Refer Figure 3.5).

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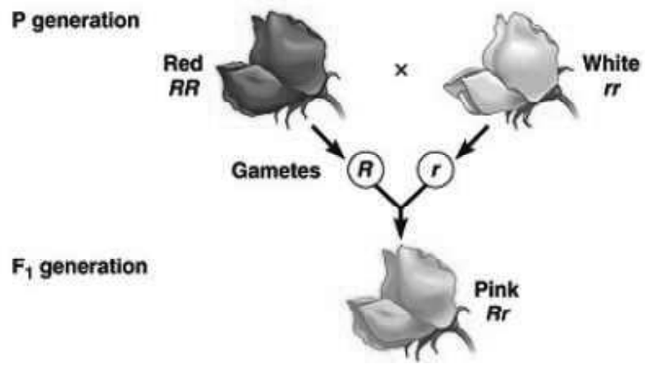

Fig. 3.5 *Incomplete Dominance*

Pleiotropy: It refers to multiple phenotypic effects of a single mutant gene. The traits that are affected have no physiological connection. The most evident expression of a pleiotropic gene is called as major or primary effect, while less evident effects are called as minor or secondary effects. Pleiotropic genes usually change the normal Mendelian ratio. A classic example of pleiotropy is sickle cell anemia. It appears due to presence of pleiotropic gene Hb^s. This leads to formation of haemoglobin –S. This is one of the secondary effects. The gene Hb^s is lethal in homozygous condition but has a slight detectable effect in heterozygous condition. Sickle cell anemia is common in person of African descent and is also found in other parts of the world where malaria is or has been major cause of death (Refer Figure 3.6).

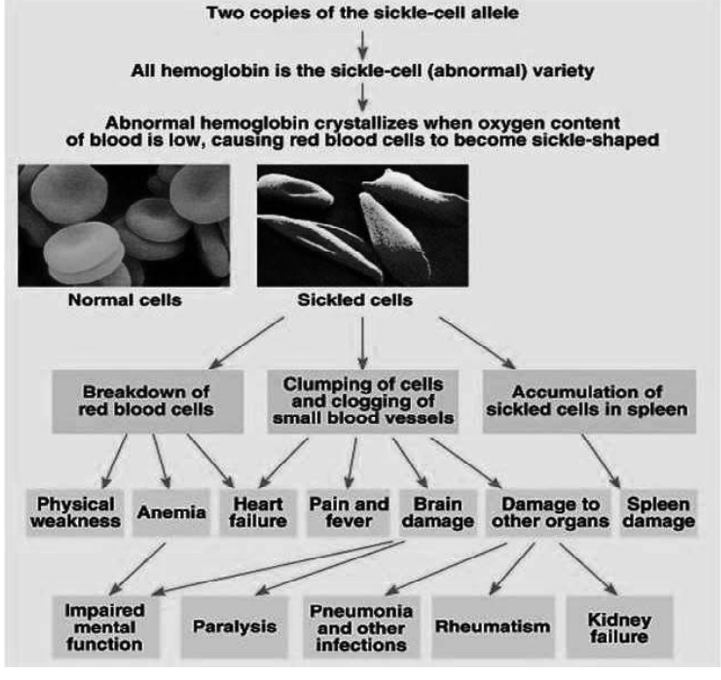

Fig. 3.6 *Sickle Cell Anemia*

Nonallelic Interaction: Epistasis

The functional interaction between different genes occur when an allele or genotype at one locus inhibits the expression of a non-allele or genotype at distinct locus, such interaction is known as epistasis. Any gene that masks the expression of another non allelic gene is epistatic to that gene. Epistasis is the interaction between different genes (non-alleles).Dominance is

the interaction between different alleles of the same gene. Metabolic processes in living organism take place via sequence of enzyme catalyzed reaction. Each step requires the activity of a specific enzyme. Each enzyme is the product of a specific gene. Some enzymes are coded by two or more different genes (Refer Figure 8.7).

Epistasis: It is interaction between 2 nonallelic genes in which one modifies the phenotypic expression of the other. Example: gene for pigment deposition is epistatic to gene for melanin production in mice

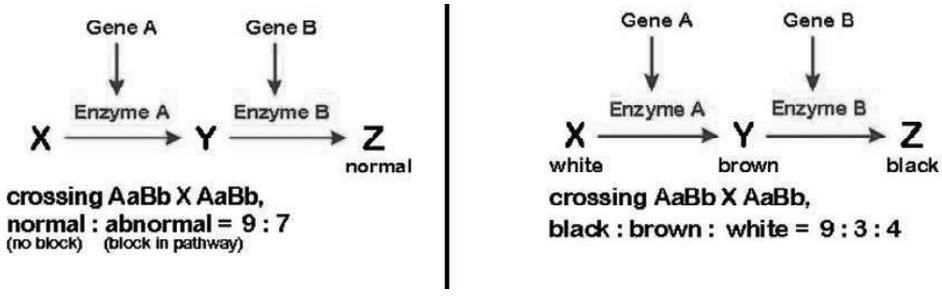

Fig. 3.7 Epistasis

Duplicate Genes: Two or more than two independent genes located on different chromosomes produce the same effect of trait. Such genes are called as duplicate genes (Refer Figure 3.8).

Same effect is given by either of two genes (A) or (B)
Example is Capsella bursa -pastoris (Shepherd's Purse) Fruit shape of two types
a) Triangular (Heart shape) capsule
b) Top shaped (Narrow) capsule

Genetic Expression
Parents AABB X aabb
Triangular Top shaped
P gametes AB ab
F1 (Hybrid) AaBb ---------- triangular

F1 gametes	AB	Ab	aB	ab
AB	AB AB 1	AB Ab 2	AB aB 3	AB ab 4
Ab	Ab AB 5	Ab Ab 6	Ab aB 7	Ab Ab 8
aB	aB AB 9	aB Ab 10	aB aB 11	aB ab 12
ab	ab AB 13	ab Ab 14	ab aB 15	ab ab 16

AB- 1,2,3,4,5, 7,9,10,13=9 (Triangular)
Ab-6,8, 14=3(Triangular)
aB-11,12,15 =3 (Tringular)
ab-16 =1 (top shaped) So the ratio is 15:1
This is example of gene interaction,two genes involved in same pathway.It is based on the idea that some genes may be present more than once in the genome

F2 generation
15 (Triangular): 1 (Top)

Fig. 3.8 Duplicate Genes

Supplementary Genes: There are two independent pairs of genes interacting in such a manner that one dominant factor produces its effect, whether the other is present or not, while the second one produce its effect only in the presence of first (Refer Figure 3.9).

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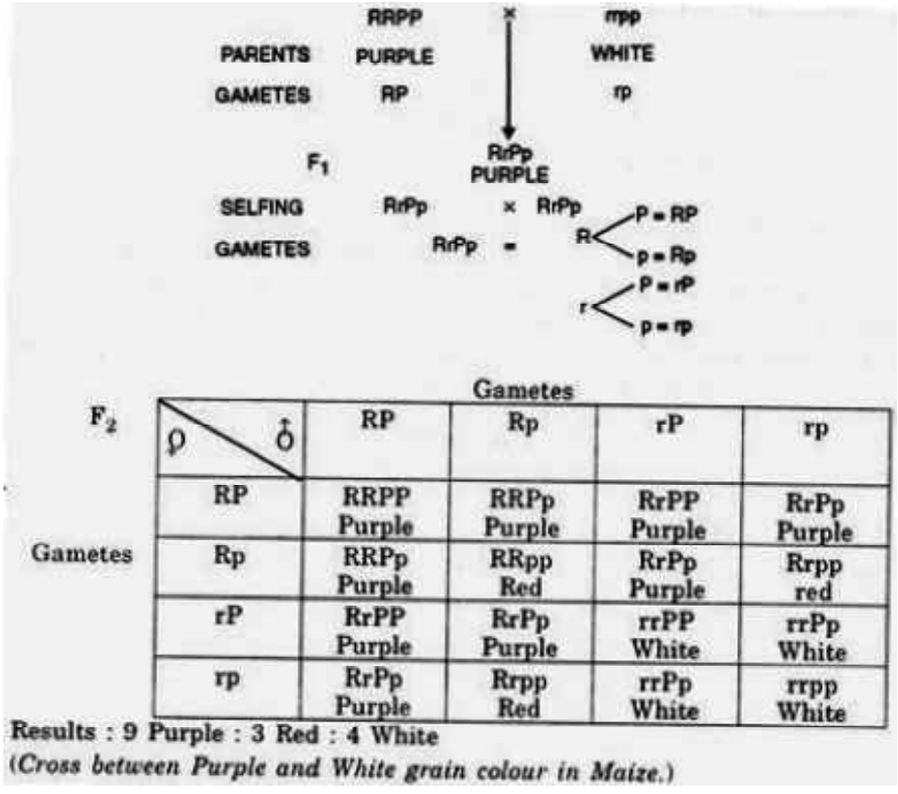

Fig. 3.9 *Supplementary Genes*

Complementary Genes: Two pairs of non allelic genes, which interact to produce only one phenotypic trait, but neither of them if present alone produces the phenotypic trait in the absence of other, for example, in sweet pea as shown below (Refer Figure 3.10).

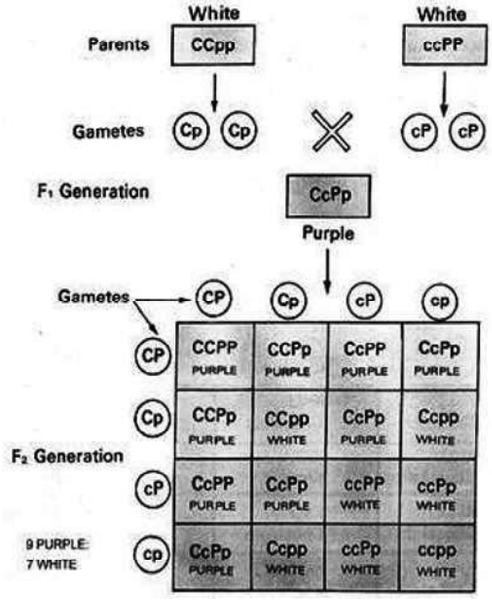

Fig. 3.10 *Complementary Genes*

Polygenic Inheritance: Also called as multiple factor inheritance. They are quantitative traits that are expressed in continous fashion. There are a whole series of traits, many of great economic importance in which the difference are in degree only and are quantitative.

'Check Your Progress'

11. What is an allele?
12. What does allele produce?
13. What happens in incomplete dominance?
14. What is pleiotropy?

3.6 SUMMARY

- Sex determination is a mechanism which brings about differentiation of sex, i.e., male and female amongst organisms.
- In epigamic the sex of the offspring is determined after fertilization during development as in case of marine worm.
- According to Riddle (1917), male progeny is produced if metabolism favour increased metabolism, increased water content but less storage of proteins.
- Female progeny is obtained when anabolism is less, water content is less but stored protein is more.
- A male progeny is produced if male determinants are abundant while female progeny is got when female determinants become dominant.
- Organisms of many species are specialized into male and female varieties, each known as a sex.
- Sexual reproduction involves the combining and mixing of genetic traits: specialized cells known as gametes combine to form offspring that inherit traits from each parent.
- The gametes produced by an organism define its sex: males produce small gametes, for example, spermatozoa, or sperm, in animals; pollen in seed plants while females produce large gametes (ova, or egg cells).
- Individual organisms which produce both male and female gametes are termed hermaphroditic.
- Gametes can be identical in form and function (known as isogamy), but, in many cases, an asymmetry has evolved such that two different types of gametes (heterogametes) exist (known as anisogamy).
- Physical differences are often associated with the different sexes of an organism; these sexual dimorphisms can reflect the different reproductive pressures the sexes experience.
- Among humans and other mammals, males typically carry an X and a Y chromosome (XY), whereas females typically carry two X chromosomes (XX), which are a part of the XY sex-determination system. Humans may also be intersex.
- Other animals have different sex-determination systems, such as the ZW system in birds, the X0 system in insects, and various environmental systems, for example in crustaceans.
- Fungi may also have more complex allelic mating systems, with sexes not accurately described as male, female, or hermaphroditic.

NOTES

- Gene mapping describes the methods used to identify the locus of a gene and the distances between genes.
- The essence of all genome mapping is to place a collection of molecular markers onto their respective positions on the genome.
- Genes can be viewed as one special type of genetic markers in the construction of genome maps, and mapped the same way as any other markers.
- The first steps of building a genetic map is the development of genetic markers and a mapping population.
- The closer two markers are on the chromosome, the more likely they are to be passed on to the next generation together.
- The quality of the genetic maps is largely dependent upon these factors: the number of genetic markers on the map and the size of the mapping population. The two factors are interlinked, as a larger mapping population could increase the 'resolution' of the map and prevent the map being 'saturated'.
- In gene mapping, any sequence feature that can be faithfully distinguished from the two parents can be used as a genetic marker.
- If a particular gene is close to a DNA marker on the chromosome, it is more likely that the gene and marker will stay together during the recombination process and are therefore more likely to be passed down along the family line (inherited) together.
- Admixture mapping utilizes historical phenotype and genotype differences between populations that for the most part have intermarried only relatively recently.
- Homologous (general) recombination occurs between DNA molecules of similar sequences. Our cells carry out general recombination during meiosis.
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NOTES

- Random fertilization, male/ female gamete combine is the fusing of the random male and female gametes increases the genetic diversity by even more. 1/ (2^23) ^2. 23 sex chromosomes each; two gametes.
- An allele is a specific form of gene. It may be mutant allele, resulting in an altered phenotype or wild type allele producing active gene product and normal phenotype.
- Alleles may produce a whole series of variable phenotype ranging from an extreme mutant phenotype to wild type phenotype.
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- A test for Rh incompatibility is accomplished by placing a drop of blood on a slide and introducing anti Rh serum.
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- Cross matching of the Rh factor as well as of ABO types of donor and recipient blood is used to avoid incompatibility agglutination reaction following transfusion.
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- The law of dominance is not always correct as there can be found where the complete dominance is absent. In such cases some traits of F1 phenotype is intermediate between those of parental traits.
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- The most evident expression of a pleiotropic gene is called as major or primary effect, while less evident effects are called as minor or secondary effects.
- A classic example of pleiotropy is sickle cell anemia. It appears due to presence of pleiotropic gene Hbs. This leads to formation of haemoglobin –S.
- Sickle cell anemia is common in person of African descent and is also found in other parts of the world where malaria is or has been major cause of death.
- The functional interaction between different genes occur when an allele or genotype at one locus inhibits the expression of a non-allele or genotype at distinct locus, such interaction is known as epistasis.
- Any gene that masks the expression of another non allelic gene is epistatic to that gene.

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- Epistasis is the interaction between different genes (non-alleles).
- Dominance is the interaction between different alleles of the same gene.
- Metabolic processes in living organism take place via sequence of enzyme catalyzed reaction.
- Epistasis is interaction between 2 non-allelic genes in which one modifies the phenotypic expression of the other
- Duplicate genes refers to two or more than two independent genes located on different chromosomes produce the same effect of trait. Such genes are called as duplicate genes.
- Supplementary genes are two independent pairs of genes interacting in such a manner that one dominant factor produces its effect, whether the other is present or not, while the second one produce its effect only in the presence of first.
- Complementary genes are two pairs of non-allelic genes, which interact to produce only one phenotypic trait, but neither of them if present alone produces the phenotypic trait in the absence of other, for example in sweet Pea.
- Polygenic inheritance also called as multiple factor inheritance. They are quantitative traits that are expressed in continuous fashion.

3.7 KEY TERMS

- **Sex determination:** Sex determination is a mechanism which brings about differentiation of sex, i.e., male and female amongst organisms.
- **Gene mapping:** Gene mapping describes the methods used to identify the locus of a gene and the distances between genes.
- **Insertion Sequences (IS):** Insertion Sequences (IS) is simple, small, only carry genes for transposition, transposase catalyzes recombination of bacterial TE into DNA.
- **Pleiotropy:** Pleiotropy refers to multiple phenotypic effects of a single mutant gene.
- **Codominance:** When the dominant character is not able to suppress, even incompletely the recessive character and both the characters appear side by side in F1 hybrids, the phenomenon is called codominance.
- **Pleiotropy:** It refers to multiple phenotypic effects of a single mutant gene.
- **Epistasis:** Epistasis is the interaction between different genes (non-alleles).
- **Dominance:** Dominance is the interaction between different alleles of the same gene.
- **Duplicate genes:** Duplicate genes refers to two or more than two independent genes located on different chromosomes produce the same effect of trait.
- **Supplementary genes:** Supplementary genes are two independent pairs of genes interacting in such a manner that one dominant factor produces its effect, whether the other is present or not, while the second one produce its effect only in the presence of first.
- **Complementary genes:** Complementary genes are two pairs of non-allelic genes, which interact to produce only one phenotypic trait, but neither of them if present alone produces the phenotypic trait in the absence of other.

NOTES

3.8 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Sex determination is a mechanism which brings about differentiation of sex, i.e., male and female amongst organisms.
2. In epigamic the sex of the offspring is determined after fertilization during development as in case of marine worm.
3. Female progeny is obtained when anabolism is less, water content is less but stored protein is more.
4. A male progeny is produced if male determinants are abundant while female progeny is got when female determinants become dominant.
5. Gene mapping describes the methods used to identify the locus of a gene and the distances between genes.
6. The essence of all genome mapping is to place a collection of molecular markers onto their respective positions on the genome.
7. The first steps of building a genetic map is the development of genetic markers and a mapping population.
8. Genetic recombination occurs in prophase I (crossing over/ chiasmata); synaptonemal complex is a protein framework supporting chromosomes to exchange segments and make new combinations
9. Insertion Sequences (IS) is simple, small, only carry genes for transposition, transposase catalyzes recombination of bacterial TE into DNA.
10. Transposon is inverted repeat sequence (insertion sequences) surrounding the central regions for 1+ genes.
11. An allele is a specific form of gene. It may be mutant allele, resulting in an altered phenotype or wild type allele producing active gene product and normal phenotype.
12. Alleles may produce a whole series of variable phenotype ranging from an extreme mutant phenotype to wild type phenotype.
13. In incomplete dominance the genes of allelomorphic pair are not expressed as dominant and recessive but express themselves partially when present together in a hybrid.
14. Pleiotropy refers to multiple phenotypic effects of a single mutant gene. The traits that are affected have no physiological connection.

3.9 QUESTIONS AND EXERCISES

Short Answer Questions

1. What is quantitative theory?
2. What are non-allosomic genetic sex determination?
3. How many types of sex chromosomes are there?
4. Write a short note on genetic mapping.
5. What is early genetic maps?
6. What is admixture mapping?

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7. What are the types of bacterial transposal elements?
8. What is gene interaction?

Long Answer Questions

1. Elaborate a note on sex determination in plants.
2. What are the types of sex determinations? Describe in detail.
3. Explain what genetic mapping is. Also discuss about early and modern genetic map.
4. What are the basic logics of genetic mapping?
5. Discuss in detail about genetic recombination in eukaryotes.
6. Write a detailed note on interaction of genes. Also cite suitable diagrams.

3.10 FURTHER READING

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UNIT 4 GENETICS: CHROMOSOMES, DNA AND RNA

NOTES

Structure

4.0 INTRODUCTION

In the nucleus of each cell, the DNA molecule is packaged into thread-like structures called chromosomes. Each chromosome is made up of DNA tightly coiled many times around proteins called histones that support its structure. Chromosomes are not visible in the cell's nucleus not even under a microscope when the cell is not dividing. However, the DNA that makes up chromosomes becomes more tightly packed during cell division and is then visible under a microscope. Most of what researchers know about chromosomes was learned by observing chromosomes during cell division.

A gene is the basic physical and functional unit of heredity. Genes are made up of DNA. Genetics is the study of heredity in general and of genes in particular. Genetics forms one of the central pillars of biology and overlaps with many other areas, such as agriculture, medicine, and biotechnology. Genetics arose out of the identification of genes, the fundamental units responsible for heredity. Modern genetics focuses on the chemical substance that genes are made of, called DeoxyriboNucleic Acid, or DNA, and the ways in which it affects the chemical reactions that constitute the living processes within the cell.

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.

In this unit, you will study about Eukaryotic chromosomal organization and special types of chromosomes, DNA and RNA, their structure, function types and replication, transcription and RNA processing in detail.

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4.1 UNIT OBJECTIVES

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

- Understand Eukaryotic chromosomal organization and special types of chromosomes
- Discuss DNA and RNA: Structure, function types and replication
- Explain transcription and RNA processing

4.2 CHROMOSOMES: EUKARYOTIC CHROMOSOMAL ORGANIZATION AND TYPES

Chromosome (Waldeyer, 1888) is a straight or curved rod of hereditary material which is formed through condensation of fibrous chromatin of interphase nucleus. Prophase and metaphase chromosome is made of two strands called chromatids. The two chromatids are attached to each other in the region of centromere. Anaphase chromosome has a single strand. The surface appears smooth but may be irregularly wavy due to presence of a number of small swellings called chromomeres. Each chromosome is differentiated into – pellicle, matrix, chromonemata, primary constriction, secondary constriction, satellite and telomeres.

DNA is the genetic material, and it exists with protein in the form of chromosomes in eukaryotic cells. During most of the life of a cell, chromosomes are in a highly dispersed state called chromatin. During these times, units of inheritance called genes may actively participate in the formation of protein. When a cell is dividing, however, chromosomes exist in a highly folded and condensed state that allows them to be distributed between new cells being produced. The structure of these chromosomes will be described in more detail in the discussion of cell division that follows.

Chromatin consists of DNA and histone proteins. This association of DNA and protein helps with the complex jobs of packing DNA into chromosomes and regulating DNA activity.

There are five different histone proteins. Some of these proteins form a core particle. DNA wraps in a coil around the proteins, a combination called a nucleosome. The fifth histone, sometimes called the linker protein, is not needed to form the nucleosome but may help anchor the DNA to the core and promote the winding of the chain of nucleosomes into a cylinder.

Further folding and the addition of protective proteins result in the formation of chromosomes during mitosis and meiosis.

Not all chromatin is equally active. Some human genes, for example, are active only after adolescence. In other cases, entire chromosomes may not function in particular cells. Inactive portions of chromosomes produce dark banding patterns with certain staining procedures and thus are called heterochromatic regions, whereas active portions of chromosomes are called euchromatic regions.

- **Pellicle:** It is the outer thin but doubtful covering or sheath of the chromosome.
- **Matrix:** It is the ground substance of chromosome which is made up of non-histone proteins, small quantities of RNA and trace of lipids. Both pellicle and matrix are absent in a chromosome. Instead the chromosome has a highly branched fibrous scaffold or core complex of non-histone proteins and RNA which helps in packing of chromenema or chromatin fibre.

- **Chromonemata:** They are coiled and folded DNA histone or chromatin strands which form the bulk of chromosomes. A chromosome possess one or two chromonemata. The strands are coiled, folded and packed in most parts of a chromosome except in the region of constrictions.
- **Centromere or Primary Constriction:** It is narrow very lightly stained area of chromosome where the two chromatids are attached in prophase and metaphase. Primary constriction is called centromere. Centromere has its own small amount of constitutive heterochromatin. It is made up of tandem repeats of satellite sequences of variable lengths. Proteins called centromere proteins are associated with satellite repeats forming heterochromatin not found anywhere else.
- **Secondary Constrictions:** They are narrow areas other than the primary constriction. One type id produced by breaking and fusion of chromosome segments. The other type are metabolically active and function as nucleolar organizers. The nucleolar organizers give rise to nucleoli during interphase. The chromosomes having nucleolar organizer regions are known as nucleolar chromosomes. In human beings 6 chromosomes have nucleolar organizer regions.
- **Satellite:** The area of a chromosome distal to a nucleolar organizer region is called satellite or trabant. It may be small or large. A satellite is knob like in outline and possess heterochromatin. The chromosome bearing a satellite is known as 'sat' chromosome. The word sat is not derived from satellite but from poor staining ability of NOR (sine acid thymonucleinico - without DNA) as its DNA content is low.
- **Telomeres:** The terminal ends of a chromosome is named as telomere. They are highly conserved eukaryotic structures which provide stability to chromosomes. A telomere is a special area of the chromosome which allows the latter to get attached to the nuclear membrane but not to any other chromosome. The special characteristics for telomere is due to presence of repetitive DNA of constitutive heterochromatin (100-1000 repeats). Telomerase is RNA protein enzyme where RNA functions as template for synthesis of DNA repeats of telomere while protein component function as reverse transcriptase.

Functions of Chromosomes

Following are some of the functions of chromosomes:

- Chromosomes contain genes. All the hereditary information is located in the genes.
- Chromosomes control the synthesis of structural proteins and help in the cell division.
- They control cellular differentiation.
- Through directing the synthesis of particular enzymes, chromosomes control cell metabolism.
- Chromosomes can replicate themselves or produce their carbon copies for passage to daughter cells.
- Their haploid or diploid number bring about gametophytic and sporophytic characteristics to the individual.
- Chromosomes form a link between the offspring and the parents.
- Some chromosomes called sex chromosomes determine the sex of the individuals.
- Mutations are produced due to change in gene chemistry.

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Giant Chromosomes

Some cells at certain particular stage of their life cycle contain large nuclei with giant or large sized chromosomes. They are of two types:

- Polytene Chromosomes
- Lampbrush Chromosomes

Polytene Chromosomes: Polytene chromosomes are multistranded somatically paired but repeatedly endoduplicated giant chromosomes. They were reported by E.G Balbiani. In 1881 in certain salivary gland cells of midge chironomus. They are common in salivary glands of larvae of insects and called salivary chromosomes. It is occur in other organs of insects, for example malpighian tubules, fat bodies, foot pads, antipodal cells of embryo sac, endosperm cells and suspensor cells of embryo. Cells having polytene chromosomes are very large in size. DNA content is high. The cells are unable to divide. A polytene chromosome can reach a length of 200-600 micrometre. A polytene chromosome contains 1000 or more times DNA as compared to ordinary somatic chromosomes. Polytene chromosomes are multistranded. They are in permanent prophase stage (Refer Figure 4.1).

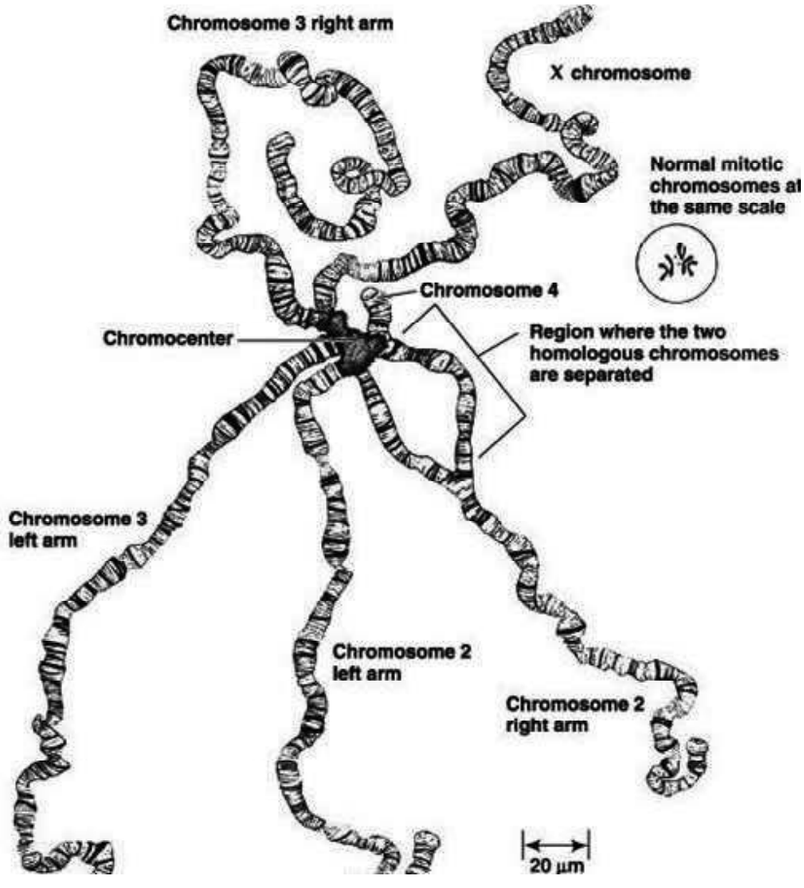

Fig. 4.1 *Polytene Chromosomes*

Depending upon their reaction to basic dyes the polytene chromosomes bear a number of dark bands. They are separated by light areas called interbands. Bands are highly feulgen positive and stain intensely with basic dyes. They are formed by tight packing or folding of chromonemata. In certain developmental stages, the polytene chromosomes bear swellings called chromosome puffs or bulbs. The larger swellings are called **balbiani rings**. In the region of puff, the DNA strands uncoil, protrude out as loops, attract proteinaceous factors and produce a no of copies of m RNA. The mRNA gets temporarily stored in the puff. By

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correlating puffs with different physiological processes, scientist have been able to located genes on polytene chromosomes and prepare chromosome maps.

Lampbrush Chromosomes: They are giant diplotene bivalent chromosomes held together by chiasmata, which possess a large number of multigene lateral loops for large scale synthesis of biochemicals. It occur in yolk rich oocytes of many animals. It may reach a length of 1000 micrometre. A nucleus having lampbrush chromosomes possesses a number of nucleoli. 1600 nucleoli have been recorded in the oocyte of Xenopus (Refer Figure 4.2).

Being in diplotene of prophase I of meiosis lampbrush chromosomes always occur in homologous pairs or bivalents. The two chromosomes of a pair are held together at several points called chiasmata. A lampbrush chromosome has following three parts.

- **Chromosomal Axis:** Each chromosome of the lampbrush pair has an elongated axis which possesses a centromere and two terminal ends or telomeres. Telomeres have small swellings. The axis bears lateral loops on both the sides. The two axial filaments of each chromosome represent the two chromatids.
- **Chromomeres:** The chromosomal axis bears a row of chromomeres of various sizes. The chromomeres represent areas of localized coiling and compaction of the chromosomal axis. They are thicker.
- **Lateral Loops:** Most of the chromomeres give rise to lateral projections or loops. The loops are of various sizes (5 to 1000 micrometre) and configuration. Each loop has a thin end and a thick end. The loop is spread out from the chromosome at its thin end and is wound back into chromomere at the thick end. The lateral loops provide a test tube or lampbrush like appearance to the chromosome pair. Loops are uncoiled and possess replicated parts of DNA or several copies of the same gene.

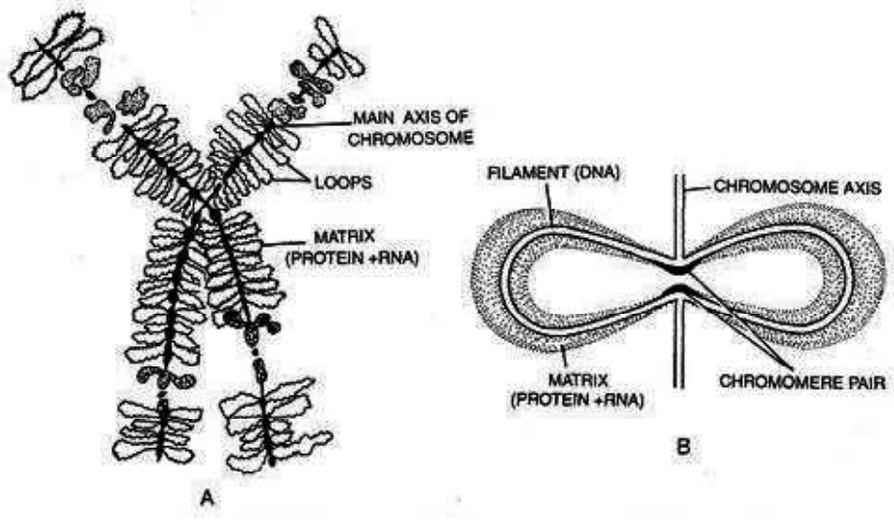

Fig. 4.2 *Lampbrush Chromosomes*

The length of DNA in the nucleus is far greater than the size of the compartment in which it is contained. To fit into this compartment the DNA has to be condensed in some manner. The degree to which DNA is condensed is expressed as its packing ratio.

Packing Ratio: The length of DNA divided by the length into which it is packaged.

For example, the shortest human chromosome contains 4.6×10^7 bp (base pair) of DNA (about 10 times the genome size of *E. coli*). This is equivalent to 14,000 μm of extended DNA. In its most condensed state during mitosis, the chromosome is about 2 μm long. This gives a packing ratio of 7000 (14,000/2).

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To achieve the overall packing ratio, DNA is not packaged directly into final structure of chromatin. Instead, it contains several hierarchies of organization. The first level of packing is achieved by the winding of DNA around a protein core to produce a 'bead-like' structure called a **nucleosome**. This gives a packing ratio of about 6. This structure is invariant in both the euchromatin and heterochromatin of all chromosomes. The second level of packing is the coiling of beads in a helical structure called the 30 nm fiber that is found in both interphase chromatin and mitotic chromosomes. This structure increases the packing ratio to about 40. The final packaging occurs when the fiber is organized in loops, scaffolds and domains that give a final packing ratio of about 1000 in interphase chromosomes and about 10,000 in mitotic chromosomes.

Eukaryotic chromosomes consist of a DNA-protein complex that is organized in a compact manner which permits the large amount of DNA to be stored in the nucleus of the cell. The subunit designation of the chromosome is chromatin. The fundamental unit of chromatin is the nucleosome.

Chromatin: The unit of analysis of the chromosome; chromatin reflects the general structure of the chromosome but is not unique to any particular chromosome

Nucleosome: Simplest packaging structure of DNA that is found in all eukaryotic chromosomes; DNA is wrapped around an octamer of small basic proteins called histones; 146 bp is wrapped around the core and the remaining bases link to the next nucleosome; this structure causes negative supercoiling

The nucleosome consists of about 200 bp wrapped around a histone octamer that contains two copies of histone proteins H2A, H2B, H3 and H4. These are known as the core histones. Histones are basic proteins that have an affinity for DNA and are the most abundant proteins associated with DNA. The amino acid sequence of these four histones is conserved suggesting a similar function for all.

The length of DNA that is associated with the nucleosome unit varies between species. But regardless of the size, two DNA components are involved. **Core DNA** is the DNA that is actually associated with the histone octamer. This value is invariant and is 146 base pairs. The core DNA forms two loops around the octamer, and this permits two regions that are 80 bp apart to be brought into close proximity. Thus, two sequences that are far apart can interact with the same regulatory protein to control gene expression. The DNA that is between each histone octamer is called the **linker DNA** and can vary in length from 8 to 114 base pairs. This variation is species specific, but variation in linker DNA length has also been associated with the developmental stage of the organism or specific regions of the genome.

The next level of organization of the chromatin is the 30 nm fiber. This appears to be a solenoid structure with about 6 nucleosomes per turn. This gives a packing ratio of 40, which means that every 1 μm along the axis contains 40 μm of DNA. The stability of this structure requires the presence of the last member of the histone gene family, histone H1. Because experiments that strip H1 from chromatin maintain the nucleosome, but not the 30 nm structure, it was concluded that H1 is important for the stabilization of the 30 nm structure.

The final level of packaging is characterized by the 700 nm structure seen in the metaphase chromosome. The condensed piece of chromatin has a characteristic scaffolding structure that can be detected in metaphase chromosomes. This appears to be the result of extensive looping of the DNA in the chromosome.

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The last definitions that need to be presented are **euchromatin** and **heterochromatin**. When chromosomes are stained with dyes, they appear to have alternating lightly and darkly stained regions. The lightly-stained regions are euchromatin and contain single-copy, genetically-active DNA. The darkly-stained regions are heterochromatin and contain repetitive sequences that are genetically inactive.

Centromeres and Telomeres

Centromeres and telomeres are two essential features of all eukaryotic chromosomes. Each provide a unique function that is absolutely necessary for the stability of the chromosome. Centromeres are required for the segregation of the centromere during meiosis and mitosis, and telomeres provide terminal stability to the chromosome and ensure its survival.

Centromeres are those condensed regions within the chromosome that are responsible for the accurate segregation of the replicated chromosome during mitosis and meiosis. When chromosomes are stained they typically show a dark-stained region that is the centromere. During mitosis, the centromere that is shared by the sister chromatids must divide so that the chromatids can migrate to opposite poles of the cell. On the other hand, during the first meiotic division the centromere of sister chromatids must remain intact, whereas during meiosis II they must act as they do during mitosis. Therefore the centromere is an important component of chromosome structure and segregation.

Within the centromere region, most species have several locations where spindle fibers attach, and these sites consist of DNA as well as protein. The actual location where the attachment occurs is called the **kinetochore** and is composed of both DNA and protein. The DNA sequence within these regions is called Yeast centromeric DNA (*CEN* DNA). Because *CEN* DNA can be moved from one chromosome to another and still provide the chromosome with the ability to segregate, these sequences must not provide any other function.

Typically CEN DNA is about 120 base pairs long and consists of several sub-domains, CDE-I, CDE-II and CDE-III **(**Centromere DNA Elements). Mutations in the first two sub-domains have no effect upon segregation, but a point mutation in the CDE-III sub-domain com pletely eliminates the ability of the centromere to function during chromosome segregation. Therefore CDE-III must be actively involved in the binding of the spindle fibers to the centromere.

The protein component of the kinetochore is only now being characterized. A complex of three proteins called Cbf-III binds to normal CDE-III regions but cannot bind to a CDE-III region with a point mutation that prevents mitotic segregation. Furthermore, mutants of the genes encoding the Cbf-III proteins also eliminates the ability for chromosomes to segregate during mitosis. Additional analyses of the DNA and protein components of the centromere are necessary to fully understand the mechanics of chromosome segregation.

Telomeres are the region of DNA at the end of the linear eukaryotic chromosome that are required for the replication and stability of the chromosome. McClintock recognized their special features when she noticed, that if two chromosomes were broken in a cell, the end of one could attach to the other and vice versa. What she never observed was the attachment of the broken end to the end of an unbroken chromosome. Thus the ends of broken chromosomes are sticky, whereas the normal end is not sticky, suggesting the ends of chromosomes have unique features. Usually, but not always, the telomeric DNA is heterochromatic and contains direct tandemly repeated sequences. The following table shows the repeat sequences of several species. These are often of the form $(T/A)_x G_y$ where x is between 1 and 4 and y is greater than 1. Table 4.1 illustrates the telomere repeat sequences.

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Table 4. *Telomere Repeat Sequences*

Species	Repeat Sequence
Arabidopsis	TTTAGGG
Human	TTAGGG
Oxytricha	TTTTGGGG
Slime Mold	TAGGG
Tetrahymena	TTGGGG
Trypanosome	TAGGG
Yeast	$(TG)_{1-3}TG_{2-3}$

Notice that the number of TG sequences and the number of cytosines in the yeast sequence varies. At least for yeast, it has been shown that different strains contain different lengths of telomeres and that the length is under genetic control.

The primary difficulty with telomeres is the replication of the lagging strand. Because DNA synthesis requires a RNA template (that provides the free 3'-OH group) to prime DNA replication, and this template is eventually degraded, a short single-stranded region would be left at the end of the chromosome. This region would be susceptible to enzymes that degrade single-stranded DNA. The result would be that the length of the chromosome would be shortened after each division. But this is not seen.

The action of the **telomerase** enzymes ensure that the ends of the lagging strands are replicated correctly. A well-studied system involves the *Tetrahymena* protozoa organism. The telomeres of this organism end in the sequence 5'-TTGGGG-3'. The telomerase adds a series of 5'-TTGGGG-3' repeats to the ends of the lagging strand. A hairpin occurs when unusual base pairs between guanine residues in the repeat form. Next the RNA primer is removed, and the 5' end of the lagging strand can be used for DNA synthesis. Ligation occurs between the finished lagging strand and the hairpin. Finally, the hairpin is removed at the 5'-TTGGGG-3' repeat. Thus the end of the chromosome is faithfully replicated.

Analysis of DNA Sequences in Eukaryotic Genomes

The technique that is used to determine the sequence complexity of any genome involves the **denaturation and renaturation** of DNA. DNA is denatured by heating which melts the H-bonds and renders the DNA single-stranded. If the DNA is rapidly cooled, the DNA remains single-stranded. But if the DNA is allowed to cool slowly, sequences that are complementary will find each other and eventually base pair again. The rate at which the DNA reanneals (another term for renature) is a function of the species from which the DNA was isolated. Below is a curve that is obtained from a simple genome.

The Y-axis is the percent of the DNA that remains single stranded. This is expressed as a ratio of the concentration of single-stranded DNA (*C*) to the total concentration of the starting DNA (*Co*). The X-axis is a log-scale of the product of the initial concentration of DNA (in moles/liter) multiplied by length of time the reaction proceeded (in seconds). The designation for this value is *Cot* and is called the 'Cot' value. The curve itself is called a 'Cot' curve. As can be seen the curve is rather smooth which indicates that reannealing occurs slowing but gradually over a period of time. One particular value that is useful is *Cot½* , the *Cot* value where half of the DNA has reannealed.

NOTES

'Check Your Progress'

1. What are lampbrush chromosomes?
2. Define the term packing ratio.
3. What are secondary constrictions?

4.3 DNA AND RNA: STRUCTURE, FUNCTION TYPES AND REPLICATION

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 pyrimidine 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.

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 photographs 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.

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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). Complementary 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 C = G. Their second big proposal was that the two chains are antiparallel with 5′ → 3′ orientation of one and Y → 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 4.3).

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.

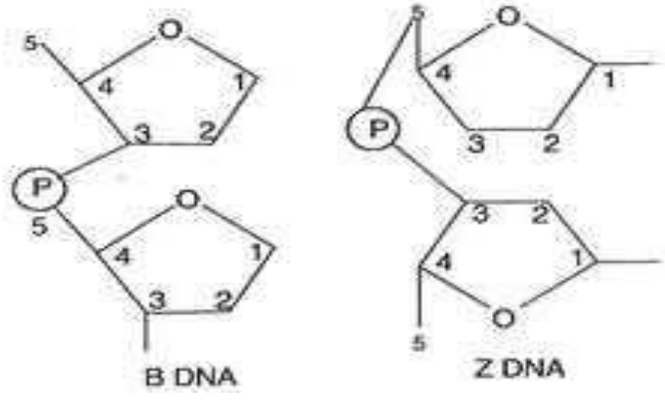

Fig. 4.3 *Orientation of Adjacent Sugar Molecules in B and Z DNA*

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 Mycoplasma. 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 components of DNA. These observations or generalizations are called Chargaff's base equivalence rule.

- 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
- 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
- Sugar deoxyribose and phosphate occur in equimolar proportions.
- A-T base pairs are rarely equal to C—G base pairs.
- The ratio of [A+T] / [G+C] is variable but constant for a species (Refer Table 4.2). It can be used to identify the source of DNA. The ratio is low in primitive organisms and higher in advanced ones.

Table 4.2 *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

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

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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 complementary strands. They are spirally coiled. The two spiral strands of DNA are collectively called DNA duplex (Refer Figure 4.4).

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 (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).

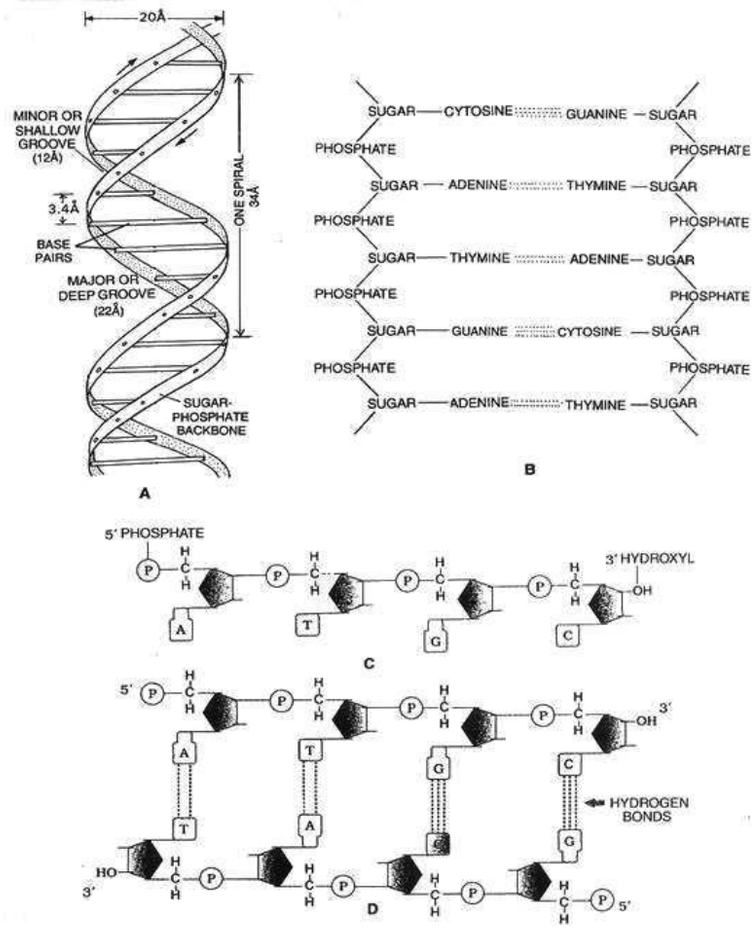

Fig. 4.4 *Structure of DNA*

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Depending upon the type of nitrogen base, DNA has four kinds of deoxyribonucleotides —deoxy adenosine 5- monophosphate (d AMP), 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 5′ of the sugar residue of its own nucleotide and carbon γ of the sugar residue of the next nucleotide by (3 '5') phosphodiester bonds. -H of phosphate and -OH of sugar are eliminated as H_2O during each ester formation.

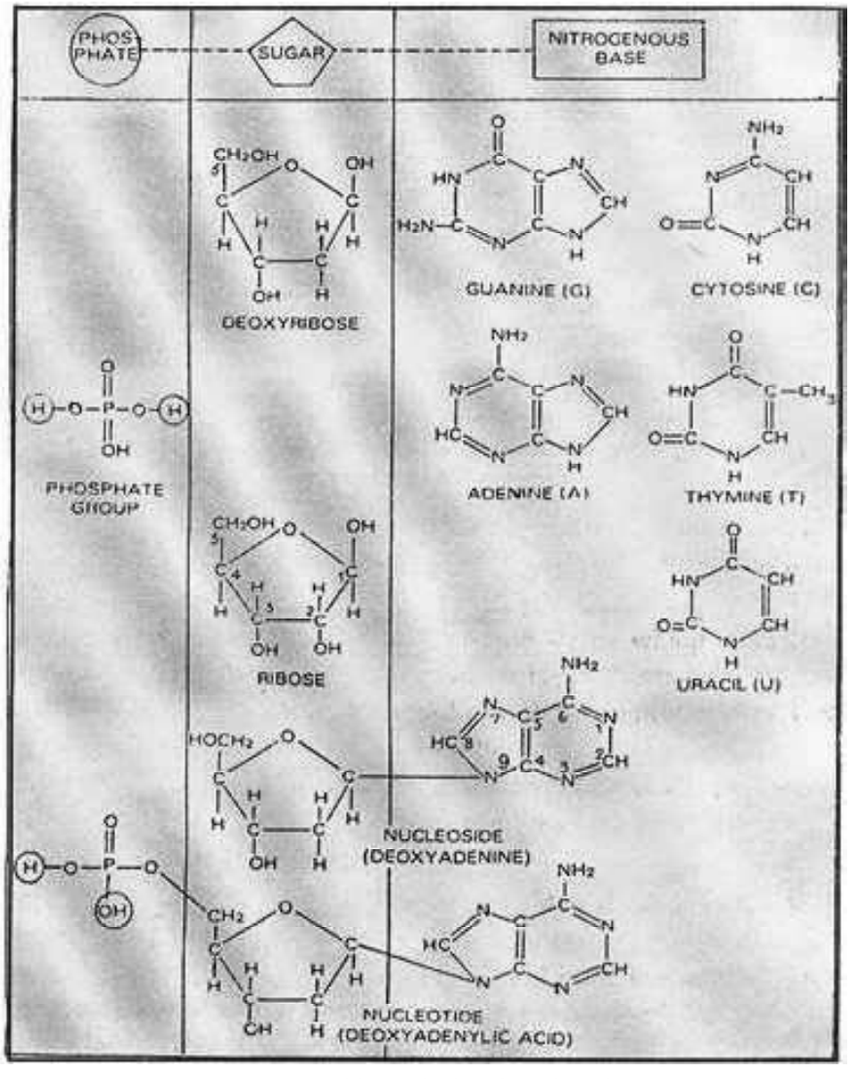

Fig. 4.5 *Building Blocks of Nucleotides*

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 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 $5' \rightarrow Y$ while in the opposite one it is $3' \rightarrow 5'$. 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

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(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 C 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.
- 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 positively 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′ → 5′ (–) strand (template/antistrand) of DNA in 5 → 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

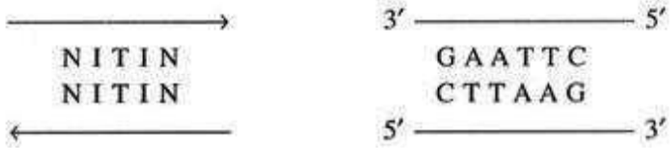
The H-bonds between nitrogen bases of two strands of DNA can break due to high temperature (82-90°C) or low or high pH, so that the two strands separate from each other. It is called denaturation or melting. Since A-T base pair has only 2H bonds, the area rich in A-T base pairs can undergo easy denaturation (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.

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′ → 5′) is GAATTC. It is same in other strand when read in 5′ → 3′ direction. It is similar to palindrome words having same words in both forward and backward direction, e.g., NITIN, MALAYALAM.



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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 interspersed 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 regions 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 polymorphic, 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 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.

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Functions of DNA

- **Genetic Information (Genetic Blue Print):** DNA is the genetic material which carries 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 individual 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).

4.3.1 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.

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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

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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

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 4.6).

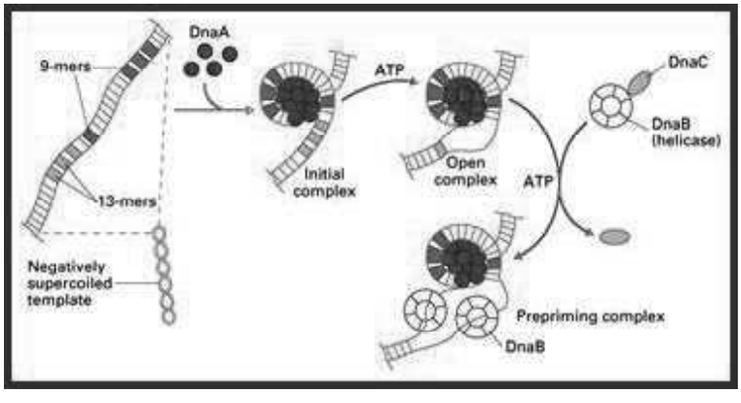

Fig. 4.6 *Initiation - DNA Replication in Prokaryotes*

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

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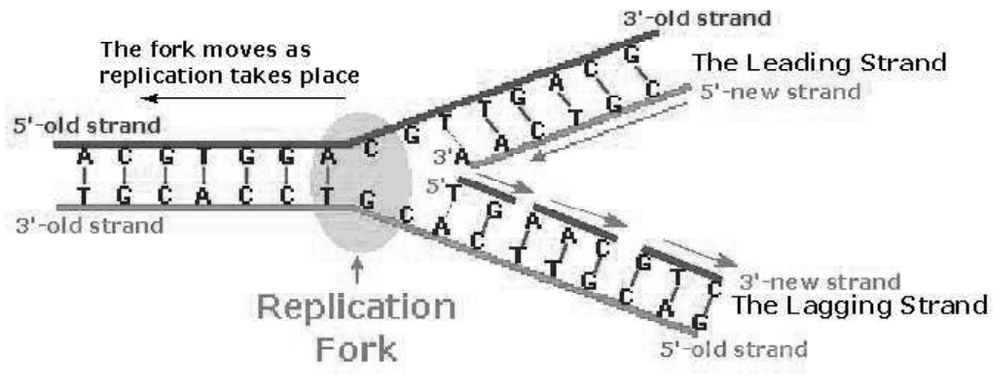

Fig. 4.7 *Elongation - Replication Fork*

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 4.7).

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 4.8).

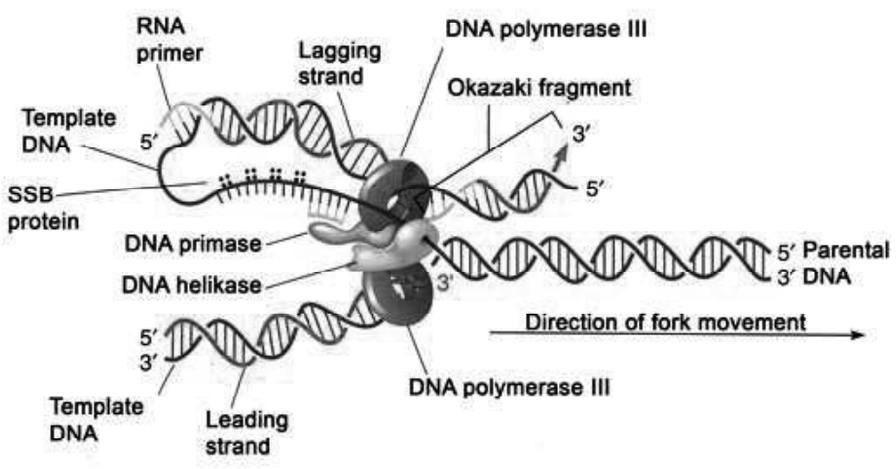

Fig. 4.8 *Mechanism of Lagging Strand Aynthesis*

- 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 by 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 4.9).

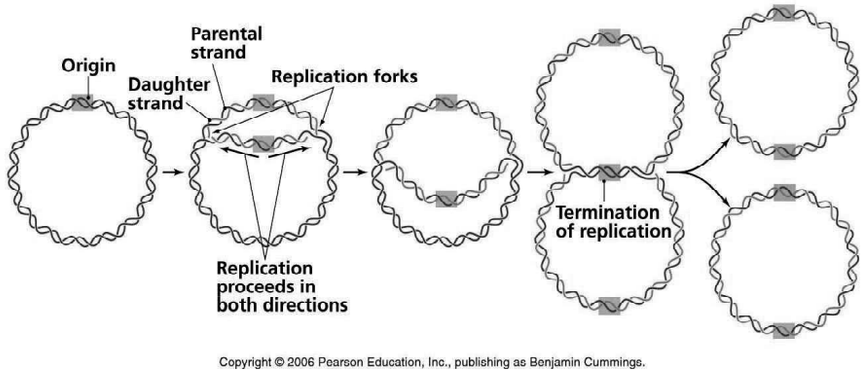

***Fig. 4.9** Termination - DNA Replication in Prokaryotes*

- 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.

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 4.10).

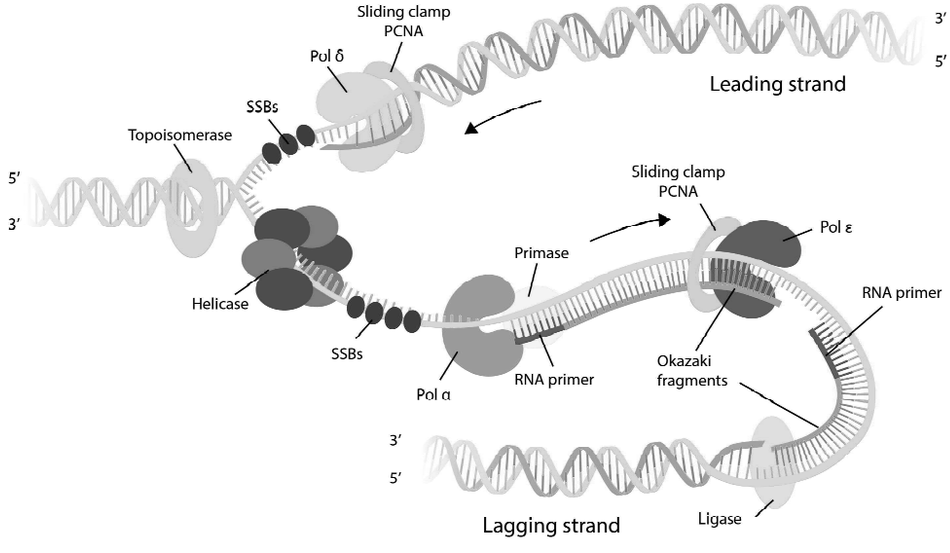

Fig. 4.10 *Initiation Phase - DNA Replication in Eukaryotes*

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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 4.11).

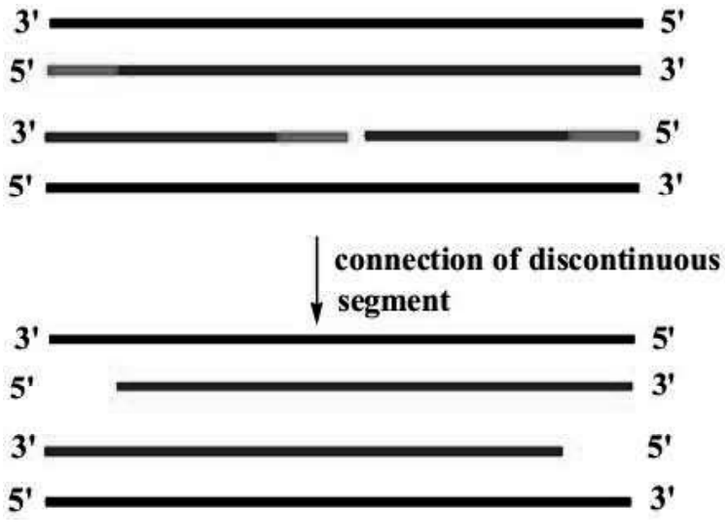

Fig. 4.11 *Elongation Phase - DNA Replication in Eukaryotes*

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 do not 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

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daughter strand and also it synthesizes the same sequences over and over in absence of template strand (Refer Figure 4.12).

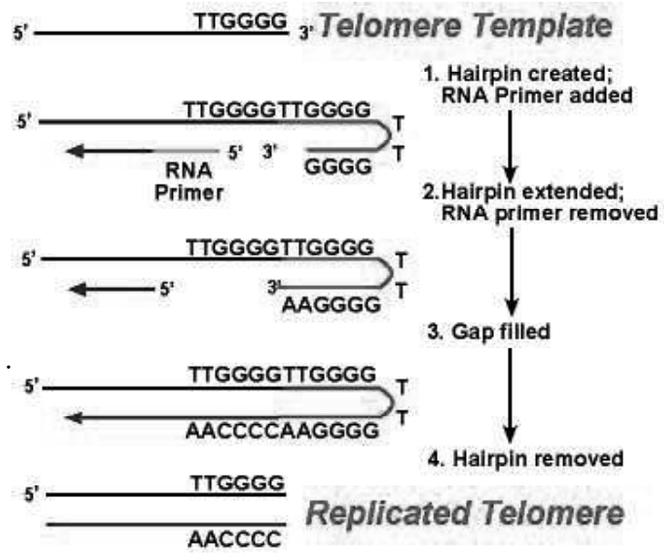

Fig. 4.12 *Telomere Replication - End Replication Problem in Eukaryotic DNA*

- 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 will not be replaced because it is an extra sequence added by copying telomere sequence.
- Finally the integrity of daughter strand is maintained.

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′→ 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′→ 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′→ 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′→ 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′→ 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′→ 5′ direction, in fact Okazaki Fragments are being synthesized in the 3′→ 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 4.13) .

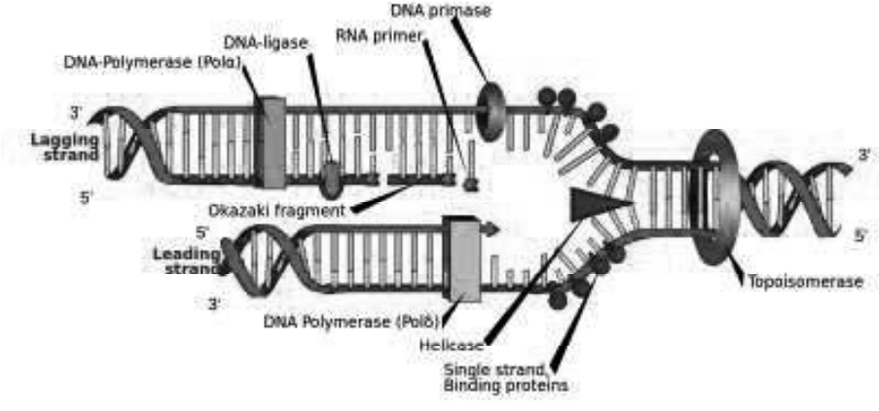

Fig. 4.13 *DNA replication and Consequent Formation of Okazaki Fragments*

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.

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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

- It occurs inside the cytoplasm (Refer Figure 4.14) .
- 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 (Refer Figure 4.15) .

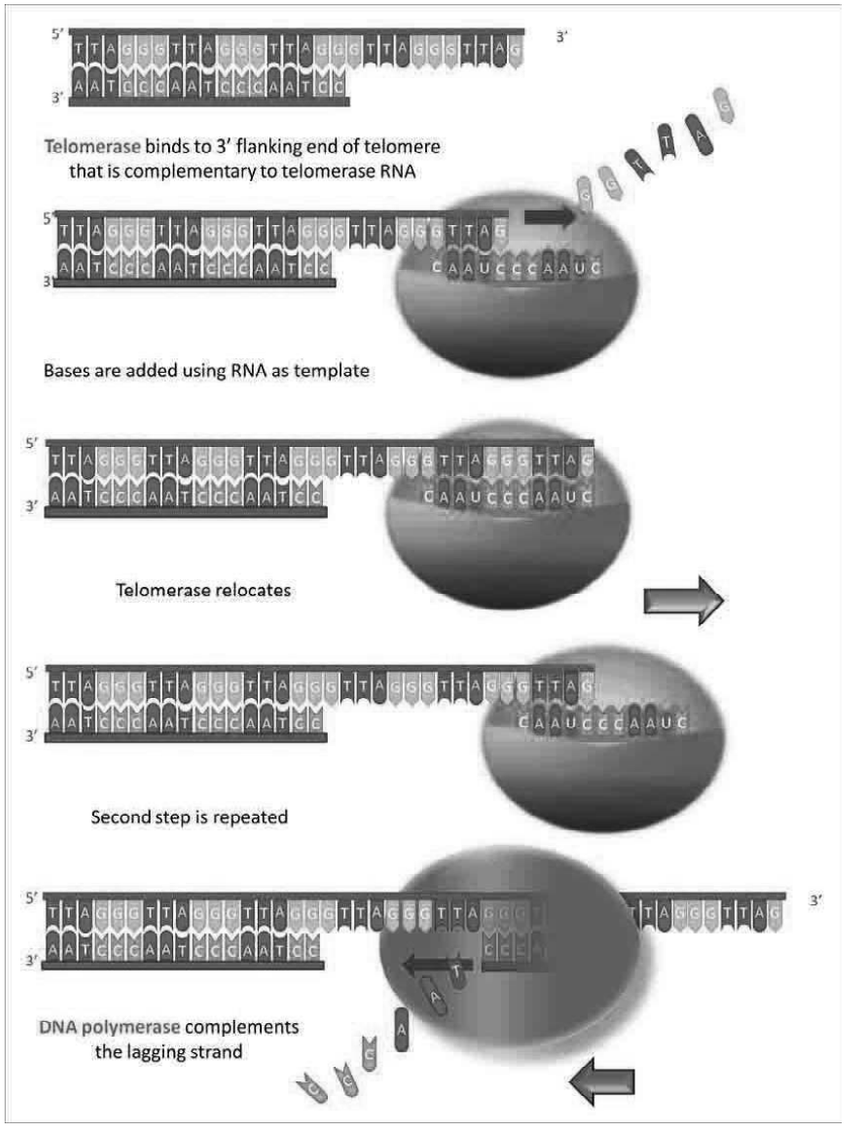

Fig. 4.14 *Prokaryotic DNA Replication*

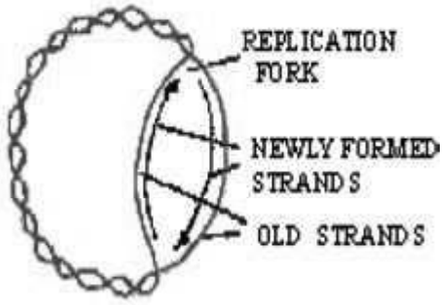

Fig. 4.15 *Replication Fork - Replication*

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

Eukaryotic DNA Replication

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

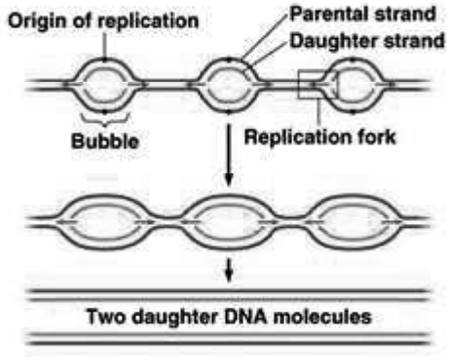

Fig. 4.16 *Eukaryotic DNA Replication*

- 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 (Refer Figure 4.16).

4.3.2 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 4.17).

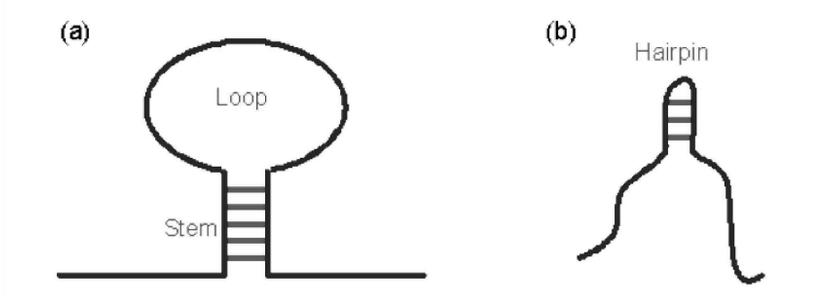

Fig. 4.17 *Secondary Structure of RNA - Stem-Loop and Hairpin.*

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:

- Messenger RNA (mRNA) or template RNA
- Ribosomal RNA (rRNA)
- 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.

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:

- High molecular weight rRNA with molecular weight of over a million, for example 21s-29s RNA
- High molecular weight rRNA with molecular weight below a million, for example 12-8-188 rRNA
- Low molecular weight rRNA, for example 58 rRNA.

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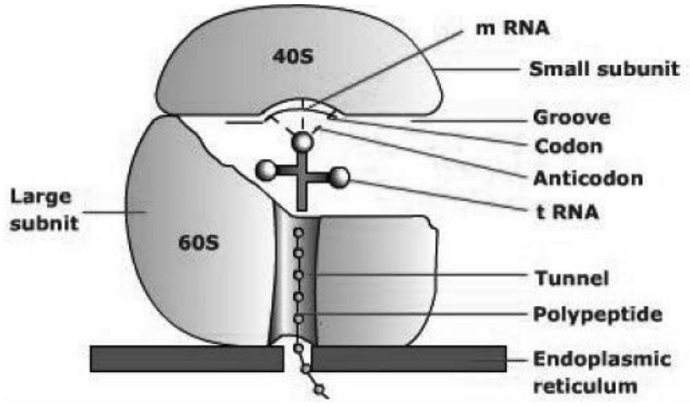

Fig. 4.18 *Ribosomal Subunit and Structure*

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.

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:

- **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.
- **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.
- **The Initiation Codon:** It is AUG in both prokaryotes and eukaryotes.
- **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.

  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 cell and each one serves as the carrier of a specific amino acid to the site of translation. tRNA's are L-shaped molecules.

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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 4.19).

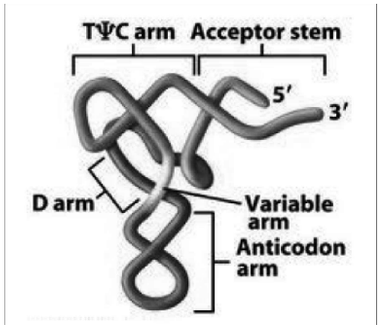

Fig. 4.19 *Double Stranded RNA*

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 4.20)

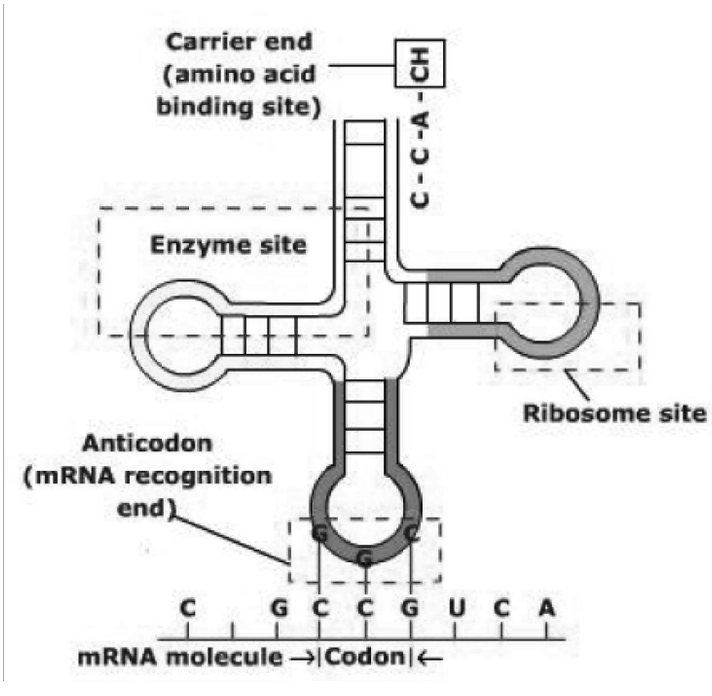

Fig. 4.20 *Cover Leaf Model of tRNA*

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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

- Between bases.
- Between bases and ribose phosphate backbone.
- 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).

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.

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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.

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 4.3 summarizes the key points:

Table 4.3 *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'

4. What is DNA?
5. Give few salient features of B model of DNA of Watson and Crick.
6. Give a differences between Prokaryotic and Eukaryotic DNA replication.
7. How is RNA transcribed from DNA?
8. Name the types of cellular RNA.

4.4 TRANSCRIPTION AND RNA PROCESSING

Transcription is the first step in gene expression, in which information from a gene is used to construct a functional product such as a protein. The goal of transcription is to make a RNA copy of a gene's DNA sequence. For a protein-coding gene, the RNA copy, or transcript, carries the information needed to build a polypeptide (protein or protein subunit). Eukaryotic transcripts need to go through some processing steps before translation into proteins.

A molecule that allows the genetic material to be realized as a protein was first hypothesized by François Jacob and Jacques Monod. Severo Ochoa won a Nobel Prize in Physiology or Medicine in 1959 for developing a process for synthesizing RNA in vitro with polynucleotide phosphorylase, which was useful for cracking the genetic code. 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

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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'.

4.4.1 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 4.21)

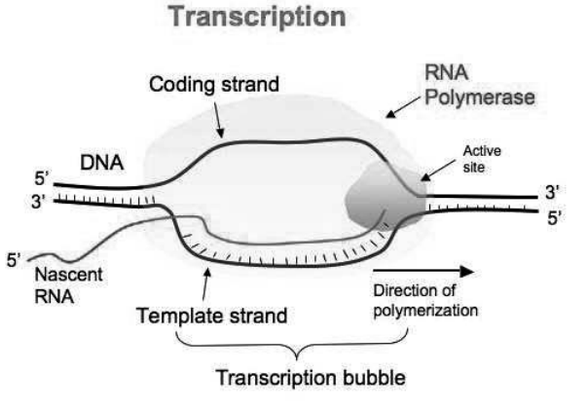


Fig. 4.21 *Transcription*

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.

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.

Steps in Prokaryotic Transcription

NOTES

1. Initiation

- Closed Complex Formation
- Open Complex Formation
- Tertiary Complex Formation

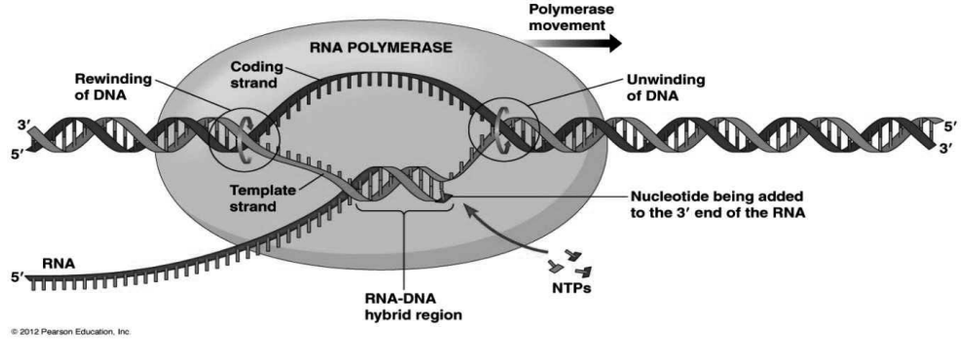
2. Elongation

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 4.22).

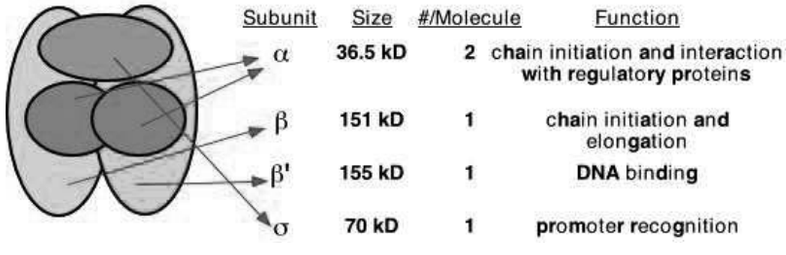


Fig. 4.22 *Holoenzyme*

NOTES

- 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.

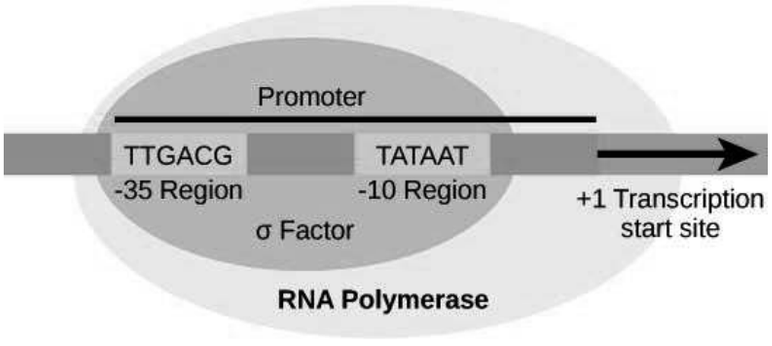


Fig. 4.23 *Promoter Site in E. coli*

σ70 of *E. coli* has Four Region.

- Region1: it includes 1.2 and 1.1 region. Region 1.1 acts as molecular mimic of DNA
- Region2: it recognizes -10 element in promoter. α-helix recognizes -10 element.
- Region 3: it recognizes extended -10 element.
- 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 σ 3.2 acting as mimic of RNA and it lies at middle of RNA exit channel in open complex.

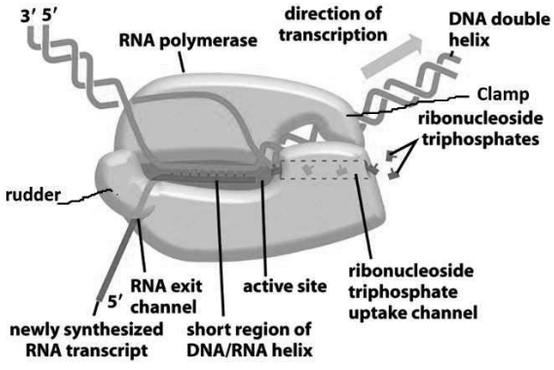


Fig. 4.24 *Tertiary Complex*

- 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 4.24)

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.

3. Termination

There are two mechanism of termination.

(i) Rho Independent:

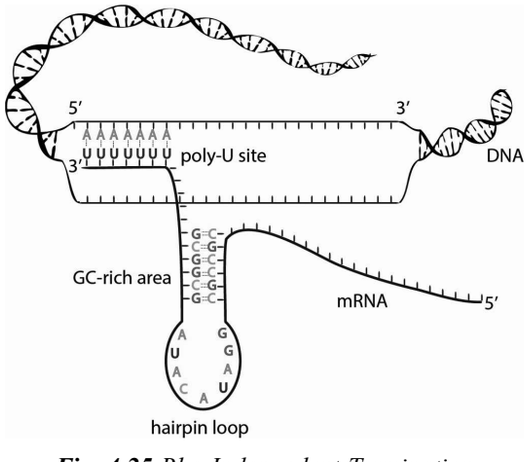


Fig. 4.25 *Rho Independent Termination*

- 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 4.25).

(ii) Rho Dependent

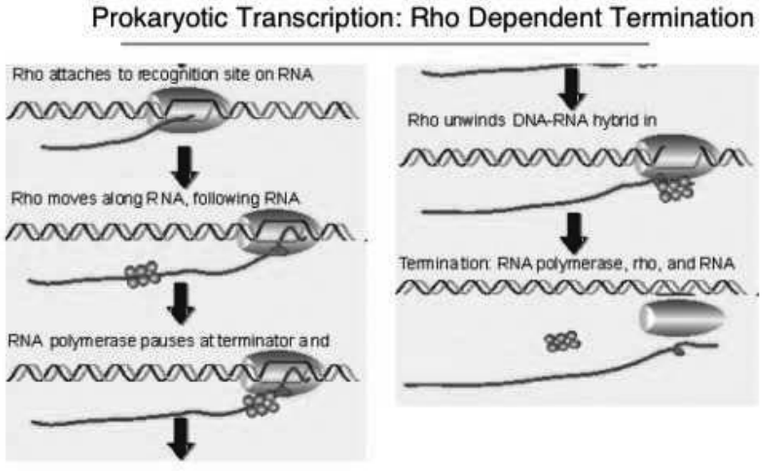


Fig. 4.26 *Rho Dependent Termination*

- 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.

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.

NOTES

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, 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.

NOTES

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.

4.4.2 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.

NOTES

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 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.

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 4.27). 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.

NOTES

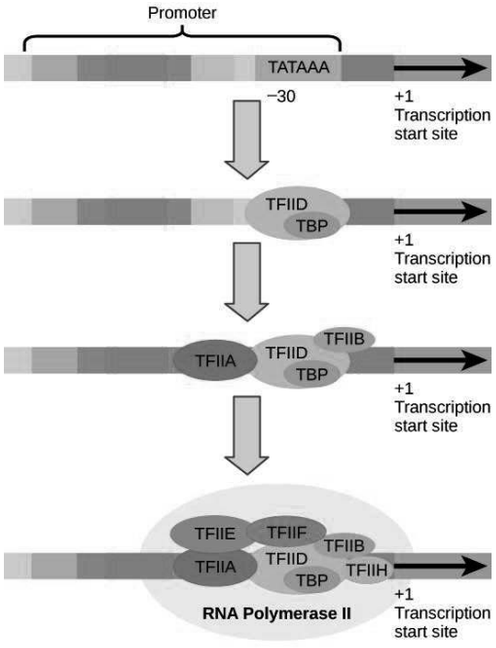


Fig. 4.27 *A Generalized Promoter of a Gene Transcribed by RNA Polymerase II*

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

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:

- **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.

- **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.

  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.

- **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.

- **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.
- **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.
- **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.,

- Pre-Initiation
- Initiation
- Promoter Clearance
- Elongation
- Termination

These are briefly discussed as follows:

- **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).
- **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.

NOTES

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).

- **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.

- **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.

- **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.

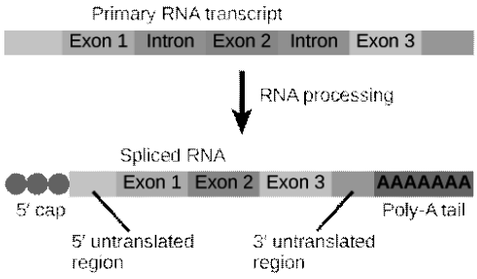


Fig. 4.28 *Eukaryotic mRNA*

Eukaryotic protein genes contain a poly-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'

9. What is transcription?
10. Which enzyme is involved in transcription?
11. Where are RNA polymerase I located?
12. What is the role of tRNAs in translation?

4.5 SUMMARY

- Chromosome is a straight or curved rod of hereditary material which is formed through condensation of fibrous chromatin of interphase nucleus.
- Prophase and metaphase chromosome is made of two strands called chromatids.
- Each chromosome is differentiated into – pellicle, matrix, chromonemata, primary constriction, secondary constriction, satellite and telomeres.
- DNA is the genetic material, and it exists with protein in the form of chromosomes in eukaryotic cells.
- Chromatin consists of DNA and histone proteins. This association of DNA and protein helps with the complex jobs of packing DNA into chromosomes and regulating DNA activity.
- There are five different histone proteins. Some of these proteins form a core particle. DNA wraps in a coil around the proteins, a combination called a nucleosome.

NOTES

- The fifth histone, sometimes called the linker protein, is not needed to form the nucleosome but may help anchor the DNA to the core and promote the winding of the chain of nucleosomes into a cylinder.
- Some cells at certain particular stage of their life cycle contain large nuclei with giant or large sized chromosomes.
- Polytene chromosomes are multistranded somatically paired but repeatedly endoduplicated giant chromosomes.
- In certain developmental stages, the polytene chromosomes bear swellings called chromosome puffs or bulbs. The larger swellings are called balbiani rings.
- Lampbrush Chromosomes are giant diplotene bivalent chromosomes held together by chiasmata, which possess a large number of multigene lateral loops for large scale synthesis of biochemicals.
- Packing Ratio is the length of DNA divided by the length into which it is packaged.
- The first level of packing is achieved by the winding of DNA around a protein core to produce a 'bead-like' structure called a nucleosome.
- Chromatin is the unit of analysis of the chromosome; chromatin reflects the general structure of the chromosome but is not unique to any particular chromosome.
- Nucleosome are simplest packaging structure of DNA that is found in all eukaryotic chromosomes; DNA is wrapped around an octamer of small basic proteins called histones; 146 bp is wrapped around the core and the remaining bases link to the next nucleosome; this structure causes negative supercoiling
- Centromeres and telomeres are two essential features of all eukaryotic chromosomes.
- Each centromeres provide a unique function that is absolutely necessary for the stability of the chromosome.
- Centromeres are required for the segregation of the centromere during meiosis and mitosis, and telomeres provide terminal stability to the chromosome and ensure its survival.
- Centromeres are those condensed regions within the chromosome that are responsible for the accurate segregation of the replicated chromosome during mitosis and meiosis.
- Telomeres are the region of DNA at the end of the linear eukaryotic chromosome that are required for the replication and stability of the chromosome.
- Deoxyribonucleic acid is a molecule composed of two chains that coil around each other to form a double helix carrying the genetic instructions used in the growth, development, functioning, are reproduction of all known living organisms and many viruses.
- The two DNA strands are also known as polynucleotides as they are composed of simpler monomeric units called nucleotides.
- 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.
- Replication of DNA happens before a cell divides to ensure that both cells receive an exact copy of the parent's genetic material.
- DNA replication begins at a specific spot on the DNA molecule called the origin of 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.
- 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.
- DNA replication in eukaryotes occur only in S-phase of cell cycle. However pre-initiation occur in G1 pahse.
- 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.
- 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.
- The process of synthesis of RNA by copying the template strand of DNA is called transcription.
- 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.
- 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.
- 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.
- 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 process of formation of a messenger RNA molecule using a DNA molecule as a template is referred to as transcription.

4.6 KEY TERMS

- **Chromosome:** Chromosome is a straight or curved rod of hereditary material which is formed through condensation of fibrous chromatin of interphase nucleus.
- **Chromatin**: Chromatin is the unit of analysis of the chromosome; chromatin reflects the general structure of the chromosome but is not unique to any particular chromosome.

- **Centromeres**: Centromeres are those condensed regions within the chromosome that are responsible for the accurate segregation of the replicated chromosome during mitosis and meiosis.
- **Telomeres**: Telomeres are the region of DNA at the end of the linear eukaryotic chromosome that are required for the replication and stability of the chromosome.
- **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.
- **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 neighbouring 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.
- **Holoenzyme:** A biochemically active compound formed by the combination of an enzyme with a coenzyme.
- **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.
- **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.

4.7 ANSWERS TO 'CHECK YOUR PROGRESS'

1. Lampbrush chromosomes are giant diplotene bivalent chromosomes held together by chiasmata, which possess a large number of multigene lateral loops for large scale synthesis of biochemicals.
2. Packing ratio is the length of DNA divided by the length into which it is packaged.
3. Secondary constrictions are narrow areas other than the primary constriction. One type id produced by breaking and fusion of chromosome segments. The other type are metabolically active and function as nucleolar organizers. The nucleolar organizers give rise to nucleoli during interphase. The chromosomes having nucleolar organizer

regions are known as nucleolar chromosomes. In human beings 6 chromosomes have nucleolar organizer regions.

4. Deoxyribonucleic Acid is a molecule composed of two chains that coil around each other to form a double helix carrying the genetic instructions used in the growth, development, functioning, are reproduction of all known living organisms and many viruses.
5. 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.
   - Backbone of each polynucleotide chain is made of alternate sugar phosphate groups. The nitrogen bases project inwardly.
6. 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.
7. 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.
8. Three types of cellular RNA have been distinguished as follows:
   - Messenger RNA (mRNA) or template RNA
   - Ribosomal RNA (rRNA)
   - Soluble RNA (sRNA) or transfer RNA (tRNA).
9. The process of synthesis of RNA by copying the template strand of DNA is called transcription.
10. The enzyme involved in transcription is RNA polymerase.
11. RNA polymerase I is located in the nucleolus, a specialized nuclear substructure in which ribosomal RNA (rRNA) is transcribed, processed, and assembled into ribosomes.
12. The tRNAs have a critical role in translation; they serve as the adaptor molecules between the mRNA template and the growing polypeptide chain.

4.8 QUESTIONS AND EXERCISES

Short-Answer Questions

1. What is chromosome?
2. What are telomeres?
3. Give some functions of chromosomes.
4. Write in brief about DNA and its types.

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5. What is repetitive DNA?
6. Give the differences between Prokaryotic and Eukaryotic DNA replication.
7. What are the phases of DNA replication?
8. Describe termination in prokaryotic transcription.
9. Define promoters, enhancers and transcription factors.
10. What are transcription factories in eukaryotes?

Long-Answer Questions

1. Write a detailed note on chromosomes giving its functions, structure and types.
2. Elaborate a note on DNA including its types, function and structure.
3. Write a detailed note on RNA including its types, function and structure.
4. Give a detailed account on mechanism of Prokaryotic and Eukaryotic DNA replication.
5. Explain the DNA replication process in Prokaryotes in detail about its phases.
6. Discuss the mechanism of leading and lagging strands synthesis in DNA replication.
7. Elaborate a note on DNA replication process in Eukaryotes explaining about its phases in detail.
8. Explain about Okazaki fragments in detail.
9. Explain properties of RNA Polymerases I, II and III.
10. Give a detailed note on mechanism of transcription in Eukaryotes.

4.9 FURTHER READING

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UNIT 5 GENE ANALYSIS AND GENETIC CODE

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Structure

5.0 INTRODUCTION

Genetic analysis is the overall process of studying and researching in fields of science that involve genetics and molecular biology. There are a number of applications that are developed from this research, and these are also considered parts of the process. The base system of analysis revolves around general genetics. Basic studies include identification of genes and inherited disorders. This research has been conducted for centuries on both a large-scale physical observation basis and on a more microscopic scale. Genetic analysis can be used generally to describe methods both used in and resulting from the sciences of genetics and molecular biology, or to applications resulting from this research.

Genetic analysis may be done to identify genetic/inherited disorders and also to make a differential diagnosis in certain somatic diseases such as cancer. Genetic analyses of cancer include detection of mutations, fusion genes, and DNA copy number changes.

The genetic code is the set of rules used by living cells to translate information encoded within genetic material (DNA or mRNA sequences) into proteins. Translation is accomplished by the ribosome, which links amino acids in an order specified by messenger RNA (mRNA), using transfer RNA (tRNA) molecules to carry amino acids and to read the mRNA three nucleotides at a time. The genetic code is highly similar among all organisms and can be expressed in a simple table with 64 entries. The code defines how sequences of nucleotide triplets, called codons, specify which amino acid will be added next during protein synthesis. With some exceptions, a three-nucleotide codon in a nucleic acid sequence specifies a single amino acid. The vast majority of genes are encoded with a single scheme. That scheme is often referred to as the canonical or standard genetic code, or simply the genetic code, though variant codes exist. While the 'genetic code' determines a protein's amino acid sequence, other genomic regions determine when and where these proteins are produced according to various 'gene regulatory codes'.

In this unit, you will study about transposable element in Prokaryotes and Eukaryotes, fine structure, analysis of gene, genetic code and positive and negative regulation of genes in detail.

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5.1 UNIT OBJECTIVES

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

- Understand what transposable element in Prokaryotes and eukaryotes are
- Explain the fine structure, analysis of gene
- Discuss genetic code
- Explain positive and negative regulation of genes

5.2 TRANSPOSABLE ELEMENT IN PROKARYOTES AND EUKARYOTES

Transposable elements were discovered by B. McClintock through an analysis of genetic instability in maize. The instability involved chromosome breakage and was found to occur at sites where transposable elements were located. Breakage events were detected by following the loss of certain genetic markers. McClintock used a marker that controlled the deposition of pigmentation in the aleurone, the outermost layer of the endosperm of maize kernels. The endosperm triploid being produced by the union of two maternal nuclei and one paternal nucleus. McClintock marker was an allele of the C locus on the short arm of chromosome 9.

Transposable Elements in Bacteria: Genetic instabilities have been found in bacteria and in many cases have led to the identification of transposable elements. These transposons were the first to be studied at the molecular level and provided important clues about organization and behavior of eukaryotic transposons. The simplest bacterial transposons are the insertion sequences or IS elements. These are less than 1500 nucleotide pairs long and contain only genes involved in promoting and regulating transposition. Two homologous IS elements combine with other genes to form a composite transposon denoted by Tn. The integrating bacteriophage is also considered to be a transposable element because it can insert itself into bacterial chromosome. This phage represents the upper limit of transposon size and contains many genes that are not necessary for the insertion behavior.

IS elements: They are compactly organized. There is a single coding sequence with short, identical or nearly identical sequences at both ends. These terminal sequences are always in inverted orientation with respect to each other, so they are called inverted terminal repeats. Their lengths range from 9 to 40 nucleotide pairs.

When IS elements insert into chromosomes or plasmids, they create a duplication of the DNA sequence at the site of insertion. One copy of the duplication is located on each side of the element. These short (3-12 nucleotide pairs) directly repeated sequences are called target site duplication and thought to arise from staggered breaks in double stranded DNA. IS elements mediate the integration of episomes into bacterial chromosomes. This process involves homologous recombination between IS elements located in the episome and in the chromosome. Composite transposons are created when two IS elements insert near each other. The sequence between them can be transposed by the joint action of the flanking elements.

Medical Significance of Bacterial Transposons: Bacterial transposons are responsible for the transposition of genes controlling resistance to antibiotics from one molecule to another. They are believed to play a role in the rapid evolution of R plasmid. All conjugative R plasmid have at least two components, one segment carrying a set of genes involved in conjugative DNA transfer and a second segment carrying antibiotic and drug resistance gene. The

segment carrying the transfer genes is called resistance transfer factor component, the segment carrying the resistance gene or genes is called **R determinant**. The transmissibility of R plasmids, the transposability of the R determinants and the rapid evolution of compound R plasmids which carry genes for resistance to a whole battery of our most effective antibiotics and drugs are of great concern to medical practitioners. Not only are these plasmids rapidly dispersed within a bacterial species but they are also transmitted across species. For example, *E. coli* R plasmids are known to be transferred to several genera including *Proteus, Salmonella, Haemophilus, Pasteurella and Shigella* all of which include pathogenic species.

Transposable Elements in Eukaryotes

Yeast TY Elements: The yeast carries *Saccharomyces cerevisiae* carries about 35 copies of a transposable element called Ty in its haploid genome. These transposons are about 5900 nucleotide pairs long and are bounded at each end by a DNA segment, which is 340 base pairs long.

The genetic organization of the Ty elements resembles that of eukaryotic retroviruses. These single stranded RNA virus synthesizes DNA from their RNA after entering a cell. The DNA then inserts itself into a site in the genome, creating a target site duplication. The inserted material has the same over all structure as a yeast Ty element and is called provirus.

Maize Transposons: Transposable elements have been found in several plants, maize and snapdragon. The most extensive investigation involves maize, in which several transposons families have been identified.

Ac and Ds Elements: The Ac/ Ds family of maize discovered by McClintock comprises numerous elements scattered throughout the genome. Molecular studies have shown that the functionally autonomous element Ac consists of 4563 nucleotide pairs bounded by an 8 nucleotide pair direct repeat.

Drosophila Transposons: Transposable elements have been discovered in many animals but best information comes from the studies with Drosophila in which as much as 15 % DNA is mobile. Several classes of Drosophila transposons have been identified.

Retrotransposons: The largest group of Drosophila transposons comprises the retrovirus like elements or retrotransposons. These elements are 5000 to 15000 nucleotides pairs long and resemble the integrated forms of retrovirus much like TY elements of yeast. When retrotransposons inserts into a chromosome, it creates a target site duplication with one copy on each side of the transposon. The size of this duplication is characteristic of each retrotransposon family.

The Genetic and Evolutionary Significance of Transposable Elements

Transposable elements also produce chromosomal breakage. This is demonstrated by the behavior of the double Ds elements in maize and by the P elements in Drosophila. Breaks can lead to the loss or rearrangement of chromosomal material. Sometimes transposable elements mediate recombination events between DNA molecules. For example, IS mediated insertion of F plasmids into *E. coli* chromosome. Another is the structural rearrangement of X chromosomes in Drosophila following recombination between homologous transposons that are located in different positions.

Use in Genetic Analysis: The natural ability of transposable element to cause mutations has been harnessed in the laboratory. In several organisms, it is feasible to stimulate the transposition of a particular family of elements, thereby increasing mutation rate. This procedure has an advantage over traditional methods of inducing mutations because a transposable element that has caused mutation by inserting into a gene can serve as a landmark. This

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feature is seen in Drosophila, in which the technique of in-situ hybridization can be used to locate the site of a transposon insertion. In this technique, radioactively labeled transposon sequences are made single stranded and then hybridized to single stranded DNA in the giant chromosomes of the salivary glands. The hybridization reaction takes place on the surface of a microscopic slide, where the chromosomes have been spread by squashing dissected glands. When the hybridization reaction is completed, the location of the radioactive sequences can be determined by autoradiography.

The four transposable genetic elements in prokaryotes are:

- Bacterial Insertion Sequences
- Prokaryotic Transposons
- Insertion-Sequence Elements and Transposons in Plasmids
- Phage mu

Bacterial Insertion Sequences

Insertion Sequences or Insertion-Sequence (IS) Elements: Insertion sequences, or Insertion-Sequence (IS) elements, are now known to be segments of bacterial DNA that can move from one position on a chromosome to a different position on the same chromosome or on a different chromosome. An IS element contains only genes required for mobilizing the element and inserting the element into a chromosome at a new location. Is elements are normal constituents of bacterial chromosome and plasmids.

When IS elements appear in the middle of genes, they interrupt the coding sequence and inactivate the expression of that gene. Owing to their size and in some cases the presence of transcription and translation termination signals, IS elements can also block the expression of other genes in the same operon if those genes are downstream from the promoter of the operon.

IS elements were first found in *E. coli* as a result of their affects on the expression of a set of three genes whose products are needed to metabolize the sugar galactose as a carbon source. Careful investigations showed that the mutant phenotypes resulted from the insertion of an approximately 800 base pairs (bp) DNA segment into a gene. This particular DNA segment is now called Insertion Sequence l (IS 1).

Properties of IS Elements: Is1 is the genetic element capable of moving around the genome. It integrates into the chromosome at locations with which it has no homology, thereby distinguishing it from recombination. This event is an example of transposition event. There are number of IS elements that have been identified in *E. coli*, including IS1, IS2, and IS 10, each present in 0 to 30 copies per genome, and each with a characteristic length and unique nucleotide sequence.

IS 1 is 768 bp long, and is present in 4 to 19 copies on the *E. coli* chromosomes. IS2 is present in 0 to 12 copies on the *E. coli* chromosome and in one copy on the F plasmid, and IS 10 is found in a class of plasmids called R plasmid that can replicate in *E. coli*.

Among prokaryotes, the IS elements are normal cell constituents, that is, they are found in most cells. Altogether, IS elements constitute approx. (Refer Figure 5.1). 0.3% of the cell's genome. All IS elements that have been sequenced, end with perfect or nearly perfect Inverted Terminal repeats (IRs) of between 9 and 41 bp. This means that essentially the same sequence is found at each end of an IS but in opposite orientations.

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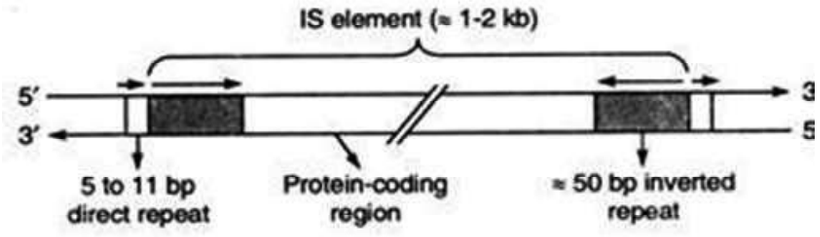

Fig. 5.1 *Structure of Bacterial IS Elements*

IS Transposition: When transposition of an IS element takes place, a copy of the IS element inserts into a new chromosome location while the original IS elements remains in place. That is, transposition requires the precise replication of the original IS element, using the replication enzymes of the host cell. The actual transposition also requires an enzyme encoded by the IS element called transposase.

The IR sequences are essential for the transposition process, that is, those sequences are recognized by transposase to initiate transposition. Is elements insert into the chromosomes at sites with which they have no sequence homology?

Genetic recombination between non-homologous sequences is called illegitimate recombination. The sites into which IS elements insert are called target sites. The process of IS insertion into a chromosome. Firstly, a taggered cut is made in the target site and the IS element is then inserted, becoming joined to the jingle-stranded ends.

The gaps are filled in by DNA polymerase and DNA ligase, producing an integrated IS element with two direct repeats of the target site sequence flanking the IS element. 'Direct' in this case means that the two sequences are repeated in the same orientation. The direct repeats are called target site duplications. The sizes of target site duplication vary with the IS elements, but tend to be small. Integration of some IS elements show preference for certain regions, while others integrate only at particular sequences.

All copies of a given IS element have the same sequence, including that of the inverted terminal repeats. Mutations that affect the inverted terminal repeat sequence of IS elements affect transposition, indicating that the inverted terminal repeat sequences are the key sequences recognized by transposase during a transposition event (Refer Figure 5.2).

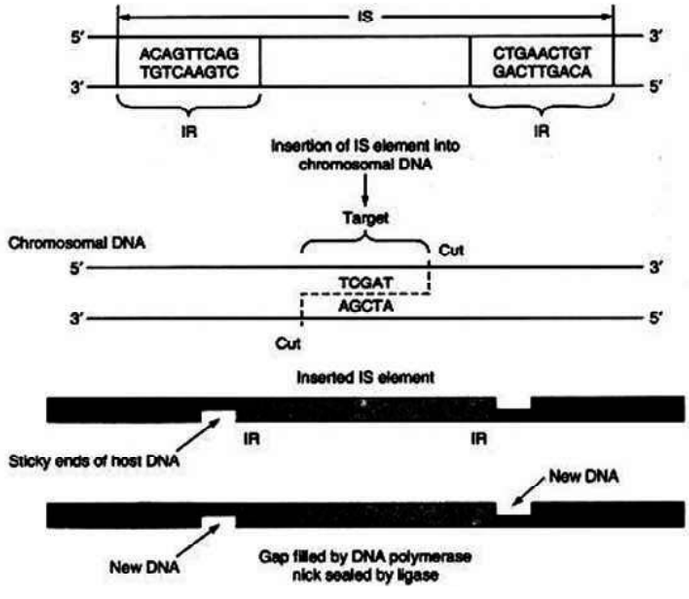

Fig. 5.2 *Schematic Representation of the Integration of an IS Elements into Chromosomal DNA*

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Prokaryotic Transposons

A transposon (Tn) is more complex than an IS elements. A transposon is a mobile DNA segment that, like an IS element, contains genes for the insertion of the DNA segment into the chromosome and for the mobilization of the element to other locations on the chromosome. There are two types of prokaryotic transposons: composite transposons and non-composite transposons.

Composite Transposons: They are complex transposons with a central region containing genes, for example drug resistance genes, flanked on both sides by IS elements (also called IS modules). Composite transposons may be thousands of base pairs long. The IS elements are both of the same types and are called IS-L (for 'left') and IS-R (for 'right'). Depending upon the transposon, IS-L and IS-R may be in the same or inverted orientation relative to each other. Because the ISs themselves have terminal inverted repeats, the composite transposons also have terminal inverted repeats.

The Tn 10 transposon is 9,300 bp long and consists of 6,500 bp of central, nonrepeating DNA containing the tetracycline resistance gene flanked at each end with a 1,400-bp IS element. These IS elements are designated IS10L and IS10R and are arranged in an inverted orientation. Cells containing Tn 10 are resistant to tetracycline resistance gene contained within the central DNA sequence.

Transposition of composite transposon occurs because of the function of the IS elements they contain. One or both IS element supplies the transposase. The inverted repeats of the IS elements at the two ends of the transposon are recognized by transposase to initiate transposition (as with transposition of IS elements).

Transposition of Tn 10 is rare, occurring once in 10 cell generations. This is the case because less than one transposase molecule per cell generation is made by Tn 10 (Refer Figure 5.3). Like IS elements, composite transposons produce target site duplications after transposition.

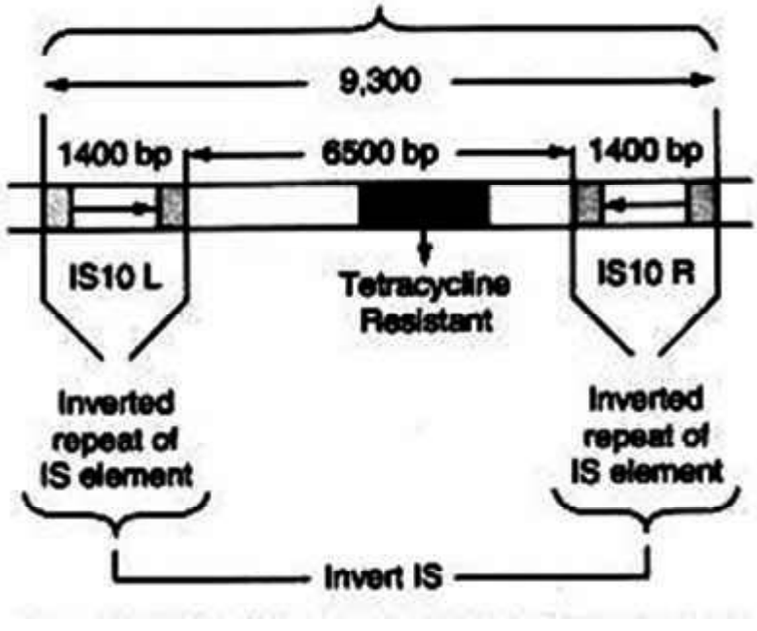

Fig. 5.3 *Detailed Structure of Tn10 Transposon*

Non-Composite Transposons: They like composite transposons, contain genes, such as those for drug resistance. Unlike composite transposons, they do not terminate with IS elements.

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However, they do have the repeated sequences at their ends that are required for transposition. Tn3 is a non-composite transposon.

Tn3 has 38 bp inverted terminal repeats and contains three genes in its central region. One of those genes, bla, i.e., β-lactam-resistant, encodes β-lactamase which breaks down ampicillin and therefore makes cells containing Tn3 resistant to ampicillin. The other two genes, transposase A (tnpA) and Transposase B (tnpB), encode the enzymes transposase and resolvase that are needed for transposition of Tn3. Transposase catalyzes insertion of the Tn into new sites, and resolvase is an enzyme involved in the particular re-combinational events associated with transposition.

Resolvase is not found in all transposons. The genes for transposition are in the central region for non-composite transposons, while they are in the terminal IS elements for composite transposons. Non-composite transposons also cause target site duplications when they move.

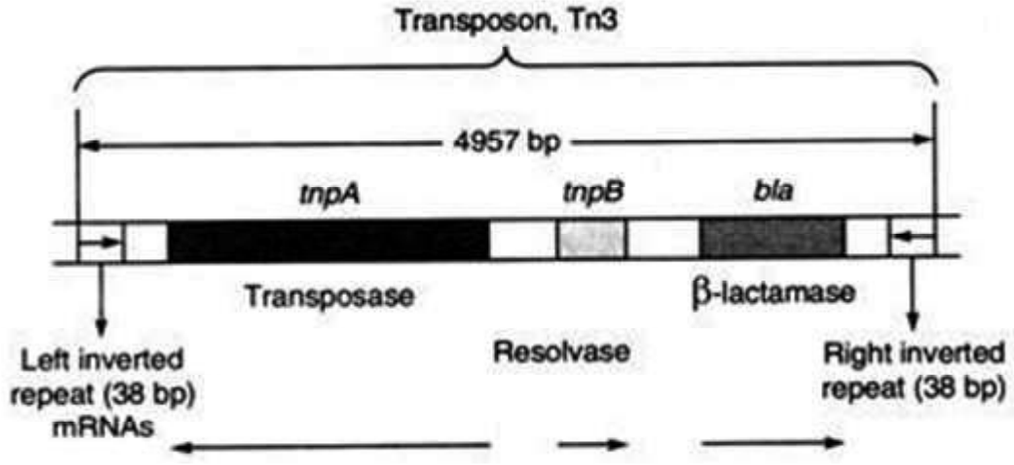

Fig. 5.4 *Detailed Structure of Tn3 Transposon*

Mechanism of Transposition in Prokaryotes: Several different mechanisms of transposition are employed by prokaryotic transposable elements. And, as we shall see later, eukaryotic elements exhibit still additional mechanisms of transposition. In *E. coli*, we can identify replicative and conservative (non-replicative) modes of transposition. In the replicative pathway, a new copy of the transposable element is generated in the transposition event. The results of the transposition are that one copy appears at the new site and one copy remains at the old site. In the conservative pathway, there is no replication. Instead, the element is excised from the chromosome or plasmid and is integrated into the new site

Replicative Transposition: The transposition of Tn3 occurs in two stages. Firstly, the transposase mediates the fusion of two molecules, forming a structure called cointegrate. During this process, the transposon is replicated, and one copy is inserted at each junction in cointegrate. The two Tn3 are oriented in the same direction. In the second stage of transposition, the tnpR-encoded resolvase mediates a site-specific recombination event between the two Tn3 elements. This event occurs at a sequence in Tn3 called res, the resolution site, and generates two molecules, each with a copy of the transposon.

The tnpR gene-product also has another function, namely, to repress the synthesis of both the transposase and resolvase proteins. This repression occurs because the res site is located in between the tnpA and tnpR genes. By binding to this site, the tnpR protein interferes with the synthesis of both gene-products, leaving them in chronic short supply. Consequently, the Tn3 element tends to remain immobile

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Conservative Transposition: Some transposons, such as Tn10, excise from the chromosome and integrate into the target DNA. In these cases, DNA replication of the element does not occur, and the element is lost from the site of the original chromosome. This mechanism is called conservative (non-replicative) transposition or simple insertion. Tn 10, for example transposes by conservative transposition.

Insertion of a transposon into the reading frame of a gene will disrupt it, causing a loss of function of that gene. Insertion into gene's controlling region can cause changes in the level of expression of the gene. Deletion and insertion events also occur as a result of activities of the transposons, and from crossing-over between duplicated transposons in the genome.

IS Elements and Transposons in Plasmids: The transfer of genetic material between conjugating *E. coli* is the result of the function of the fertility factor F. The F factor, a circular double stranded DNA molecule, is one of the example of bacterial plasmid. Plasmids, such as F that are also capable of integrating into the bacterial chromosomes are called episomes. F factor consists of 94,500 bp of DNA that code for a variety of proteins.

The important elements are:

- Transfer gene (tra) required for the conjugation transfer of the DNA.
- Genes that encode proteins required for the plasmid's replication,
- Four IS elements, two copies of IS3, one of IS2, and one of an insertion sequence element called gamma- delta.

It is because the *E. coli* chromosome has copies of these four insertion sequence at various positions that the F factor can integrate into the *E. coli* chromosome at different sites and in different orientations with homologous sequence of the insertion elements.

Another class of plasmids that has medical significance is the R plasmid group, which was discovered in Japan in the 1950s, during the cure for dysentery. The disease is the result of infection by the pathogenic bacterium *Shigella*. *Shigella* was found to be resistant to most of the commonly used antibiotics.

Subsequently, they found that the genes responsible for the drug resistances were carried on R plasmids, which can promote the transfer of genes between bacteria by conjugation, just as the F factor. One segment of an R plasmid that is homologous to a segment in the F factor is the part needed for the conjugal transfer of genes.

That segment and the plasmid-specific genes for DNA replication constitute what is called the RTF (Resistance Transfer Factor) region. The rest of the R plasmid differs from type to type and includes the antibiotic-resistance genes or other types of genes of medical significance, such as resistance to heavy metal ions. The resistance genes in R plasmid are, in fact, transposons, that is each resistance gene is located between flanking, directly repeated segments, such as one of the IS modules. Thus, each transposon with its resistance gene in the R plasmid can be inserted into new location on other plasmids or on the bacterial chromosome, while at the same time leaving behind a copy of itself in the original position.

Phage mu: Phage mu is a normal-appearing phage. We consider it here because, although it is a true virus, it has many features in common with IS elements. The DNA double helix of this phage is 36,000 nucleotides long-much larger than an IS element. However, it does appear to be able to insert itself anywhere in a bacterial or plasmid genome in either orientation. Once inserted, it causes mutation at the locus of insertion-again like an IS element. (The phage was named for this ability: mu stands for 'mutator'.)

Normally, these mutations cannot be reverted, but reversion can be produced by certain kinds of genetic manipulation. When this reversion is produced, the phages that can be

recovered showing no deletion, proving that excision is exact and that the insertion of the phage therefore does not involve any loss of phage material either. Each mature phage particle has on each end a piece of flanking DNA from its previous host. However, this DNA is not inserted anew into the next host. Its function is unclear. Phage mu also has an IR sequence, but neither of the repeated elements is at a terminus.

Mu can also act like a genetic snap fastener, mobilizing any kind of DNA and transposing it anywhere in a genome. For example, it can mobilize another phage, such as λ or the F factor. In such situations, the inserted DNA is flanked by two mu genomes.

'Check Your Progress'

1. What are the four transposable genetic elements in Prokaryotes?
2. What is insertion sequences?
3. How is duplication of the DNA sequence created?

5.3 FINE STRUCTURE, ANALYSIS OF GENE

The gene has been defined as the unit of genetic material controlling the inheritance of one phenotypic characteristic or one trait. The gene is defined as the unit of genetic material coding for one polypeptide. Prior to 1941, the gene was also believed not to be subdivisible by mutation or recombination. The unit of genetic material not subdivisible by mutation or recombination is known to be single nucleotide pair. Many mutable sites separable by recombination exist within each gene. The gene is defined by the cis-trans or complementation test. This test is used to determine whether organisms heterozygous for two mutations have mutant or wild type phenotypes. Cis heterozygote must exhibit the wild type phenotype for the cis-trans test to be informative. If both the cis heterozygote and the transheterozygote containing a given pair of mutations have wild type phenotypes, then the two mutations are in two different genes. If the cis heterozygote has the wild type phenotype and the transheterozygote has the mutant phenotype, then the two mutations are in the same gene. Detailed genetic and cytological analyses of segments of the giant salivary chromosomes in D. melanogaster support the idea that each band of the chromosome contain one gene. If so, Drosophila would have only 5000-6000 genes rather than 1, 00,000 as estimated from the total amount of DNA in the genome and an average of 1000 base pairs per gene. Estimates of the total number of m RNA molecules suggest that Drosophila has about 17,500 genes.

Mendel (1866) employed the term factor for a discrete or particulate unit of inheritance which could be assigned to the expression of a trait and passage from one generation to the next. Sutton (1902) and Boveri (1902) put forward the chromosome theory of inheritance proposing chromosomes to be vehicles of hereditary information and hence Mendelian factors. The term gene was coined by Johanssen (1909) to replace the term of Mendelian factors. According to Johanssen (1909) gene is an elementary unit of inheritance which can be assigned to a particular character. Morgan proved that chromosomes are strings of genes which can undergo recombination and mutation. Belling (1928) equated chromomeres or beads observed in prophasic chromosomes to be equivalent to genes. The classical concept of gene attributes the following characteristics to it.

- It is a unit of hereditary material which occurs as a small indivisible part of a chromosome.
- Gene is a unit of function leading to a physiological activity or phenotypic expression

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- It is a unit of recombination or crossing over
- Gene is a unit of mutation

Gene is neither a unit of recombination nor a unit of mutation. Sturtevant and Morgan found that bar eye locus of Drosophila contains more than one recombination units. Benzer has differentiated three components of a gene- muton, recon and cistron

Muton: It is the smallest segment of genetic material which can undergo change and produce mutation. Muton is a unit of mutation. It can be as small as a single nucleotide.

Recon: It is the smallest segment of genetic material that can get exchanged between two homologous chromosomes through crossing over to produce a recombinant form. A recon may be as small as a single nucleotide because genetic material can rupture between any two adjacent nucleotides.

Cistron: It is a unit of genetic material, DNA that contains encoded information for producing a functional biological product or genetic function. The latter includes the actual synthesis of an RNA or its control. A functional gene is now considered equivalent to a cistron.

Genes and Enzymes

Archibald Garrod (1909) was the first to hint that genes operate through enzymes. He coined the term inborn error of metabolism. Or failure of metabolic machinery of the organisms to perform a particular function due to formation of defective enzymes associated with inheritance of defective genes. Garrod studied alkaptonuria which is a genetic disorder or disease of human beings characterized by brown or black colour of exposed urine. He came to conclusion through pedigree analysis that the disease is caused by the inheritance of recessive gene. Alkapton is produced during metabolism of amino acids, phenylalanine and tyrosine. It is catabolised by an oxidase enzymes to produce carbon dioxide and water. In persons suffering from alkaptonuria the enzyme alkapton oxidase is absent. Homogentisic acid accumulates in the body. The brown black product also accumulates in the body in connective tissue and cartilage. Due to continuous deposition of alkapton in connective tissue, a type of arthritis develop in later years.

One gene one enzyme hypothesis

- Beadle and co-workers found that the red eye colour of Drosophila is controlled by two genes and is caused by the blending of brown and vermillion pigments. A piece of larva destined to form vermillion eye can be made to produce red eye colour if it is placed in the body cavity of larva having red eye because the latter provides its enzyme for brown colour which the transparent lacks.
- In 1944, Beadle and Tatum irradiated Neurospora crassa with X rays and obtained number of nutritional mutants called **auxotrophs**. An auxotroph or nutritional mutant is that mutant which is not able to prepare its own metabolites from the raw materials obtained from outside. Therefore it cannot live in natural environment but can be maintained in culture by providing the required metabolites. The wild type is called **prototroph**. A prototroph or wild type is the normal individual which can synthesize all the complex metabolites required for its growth from raw materials obtained from outside. The prototroph is able to manufacture all the amino acids, purines and pyrimidines from ingredients of minimal medium through enzyme catalysed reactions.

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One Gene One Polypeptide Hypothesis

It has some defects, which are as follows:

- All genes do not produce enzymes or their components. Some of them control other genes. The genes which synthesize enzymes are called structural genes.
- Enzymes are proteinaceous in nature but all proteins are not enzymes.
- Some RNAs also exhibit enzyme activity.
- A protein or enzyme molecule may consist of one or more types of polypeptides.

Yanofsky *et al* (1965) found that the enzyme tryptophan synthetase of bacterium *E. coli* consists of two separate polypeptides, A and B. Polypeptide A is of β type while polypeptide B is of β type. The synthesis of the two polypeptides is controlled by different genes. A change in any of the two genes causes inactivation of tryptophan synthetase through non-synthesis of β and β polypeptide. Inactivation of enzyme stops the synthesis of tryptophan from indole 3-glycerol phosphate and serine. All genes do not code for polypeptides. Some genes code for rRNAs and t RNAs. The genes which code for polypeptides or RNAs are called structural genes. Certain segments of genetic material function as regulatory sequences, for example turning on or off of a structural gene

One Cistron One function

In molecular biology the term gene has been replaced by the term cistron. Only some structural genes or cistrons code for polypeptides through the transcription of m RNAs. There are other structural cistrons or genes which code for tRNAs and r RNAs that aid in the formation of polypeptides. The genetic system also contains a number of regulatory genes or cistrons which control the working of structural genes. A gene is that it is a part of DNA called cistron that has a particular genetic function- it is a unit of heredity.

Types of Genes

A gene is a sequence of DNA that codes for a diffusible product. Genes are of several types i.e., as follows:

- Constitutive Genes (Housekeeping genes): They are those genes which are constantly expressing themselves in a cell because their products are required for the normal cellular activities, for example genes for glycolysis, ATPase
- Non-Constitutive Genes (Luxury genes): The genes are not always expressing themselves in a cell. They are switched on or off according to the requirement of cellular activities, for example gene for nitrate reductase in plants, lactose system in *E. coli.* They are of two types, inducible and repressible:
  - o Inducible Genes: The genes are switched on in response to the presence of a chemical substance or inducer which is required for the functioning of the product of gene activity, for example nitrate for nitrate reductase
  - o Repressible Gene: Those genes which continue to express themselves till a chemical inhibits or represses their acivity.
- Multigenes (Multiple Gene Family): It is a group of similar or nearly similar genes for meeting requirement of time and tissue specific products, for example globin gene family.
- Repeated Genes: The genes occur in multiple copies, for example histone genes, t RNA genes, r RNA genes, actin genes

- Single Copy Genes: The genes are present in single copies. They form 60-70% of the functional genes. Duplicataions, mutations and exon reshuffling between two genes form new genes.
- Structural Genes: Those genes which have encoded information for the synthesis of chemical substances required for cellular machinery. The chemical substances may be a) Polypeptides for the formation of structural proteins (colloidal complex of protoplasm, cell membranes, elastin of ligaments, collagen of tendons or cartilage, actin of muscles, tubulin of microtubules. b) polypeptides for synthesis of enzymes c) Transport proteins like haemoglobin of RBCs, carrier proteins of cell membranes. d) Proteinaceous hormones, for example insulin, growth hormone, parathyroid hormone e) Antibodies, antigens, certain toxins, blood coagulation factors, etc.
- Regulatory Genes: They do not transcribe RNAs for forming chemicals to participate in cellular activities. They are meant for controlling the functions of structural genes. They are promoters, terminators, operators, repressor. Repressor is a protein that is formed through transcription translation route. It does not take part in cellular activity.
- Transposons (Jumping Genes): They are segments of DNA that can jump or move from one place in the genome to another. They were first discovered by McCintock in case of maize when she found that a segment of DNA moved into gene coding for pigmented kernels and produced light coloured kernels. In human beings the most common types of transposons belong to Alu family.
- Processed Genes: They are eukaryotic genes which lack introns. Processed genes have been formed due to reverse transcription. Processed genes are non-functional as they lack promoters.
- Split Genes: (Interrupted Genes): Split genes are those genes which possess extra or non-essential regions interspersed with essential or coding regions. mRNA came in contact with discontinuous stretches of DNA. Loop developed at places of non-contact indicating that they were not represented in m RNA. Segments of a gene which are not represented in its functional RNA are called non-essential parts, spacer DNA, intervening sequences or introns. Parts of a gene which are represented in functional RNA are called essential parts, coding sequences or exons. The terms introns and exons were coined by Gilbert (1985). Split genes are more common in eukaryotes, for example nuclear genes for m RNAs, r RNAs and t RNAs, mitochondrial genes, chloroplast genes, some genes of archaebacteria, eubacteria and viruses. Each gene function as a transcription unit. It produces an RNA copy that exactly corresponds to the whole base sequence of the gene. The original unmodified RNA product of a gene or transcription unit is called primary transcript. It contains portions corresponding to both introns and exons. The phenomenon of removal of introns and joining the exons during processing of RNA is called splicing. The regions of breaking or joining of RNA segments are called splice sites.
- Pseudogenes: They are non-functional genes which possess sequeneces similar to those of functional genes but are unable to express themselves due to defective structure that prevents transcription or translation. The reason for non-functioning can be:
  - o Abolition of signals for initiation of transcription.
  - o Prevention of splicing due to defective exon intron junctions.
  - o Premature termination of translation.
  - o Frame shift mutation that produces a new non-functional product.

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- Transposition are found in almost all genetic systems genes for histones, globins, immunoglobins, histocompatibility antigens, ribosomal proteins, small nuclear RNA (snRNAs), Alu sequences, etc.

Some pseudogenes occur as members of gene family or gene cluster which represents a set of genes descended by duplication and variation of an ancestral gene.

- Overlapping genes: Genes sharing certain common base sequences are called overlapping genes. In $\phi \times 174$ virus, the genome contains 5400 nucleotides (Sanger et al 1977). Sanger's second Nobel prize, 1980). It has ten genes which should have a length of plus 6000 nucleotides. This is possible only if certain genes share some common nucleotides.

Functions of Gene

- Genes are components of genetic material and are thus units of inheritance.
- They control the morphology or phenotype of individuals.
- Genes carry the hereditary information from one generation to the next.
- Replication of genes is essential for cell division.
- They control the structure and metabolism of the body.
- Reshuffling of genes at the time of sexual reproduction produces variation.
- Different linkages are produced due to crossing over.
- Genes undergo mutations and change their expression.
- New genes and new traits develop due to reshuffling of exons and introns.
- Genes change their expression due to position effect and transposons.
- Differentiation or formation of different types of cells, tissues and organs in various parts of the body is controlled by expression of certain genes and non-expression of others.
- Development or production of different stages in the life history is controlled by genes.

Technology is available for constructing recombinant DNA molecules that contain sequences from totally unrelated species and cloning these species in appropriate host cell.

'Check Your Progress'

4. Define gene.
5. What is muton?
6. What is recon?
7. Define the term cistron.

5.4 GENETIC CODE

Genetic code is made up of only four types of nucleotides, the nucleotides of DNA can be positioned in countless ways. A DNA chain of only ten nucleotide length can have 4^{10}. As a single DNA molecule have several thousand nucleotides, a limitless specificity can be incorporated in it.

There is an intimate connection between genes and synthesis of polypeptides or enzymes. Genes or cistrons are made up of nucleotides arranged in a specific manner.

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Polypeptides are formed of amino acids arranged in a specific manner. Likewise polypeptides are formed of amino acids arranged in a specific manner. The arrangement of nitrogen bases seems to be connected with synthesis of polypeptides by influencing the incorporation of amino acids in them. The relationship between the sequence of amino acids in polypeptide and nitrogen base sequence of DNA or m RNA is called genetic code. However DNA contains only four types of nitrogen bases or nucleotides while the number of amino acids is 20. Gamow (1955) said that singlet code can specify only four amino acids, a doublet code only 16 amino acids while triplet code specify all the amino acids. In triplet code three adjacent nitrogen bases constitute a codon that specifies an amino acid in a polypeptide

The different researchers which helped in deciphering the triplet genetic code are:

- Crick *et al* (1961) observed that deletion or addition of one or two base pairs in DNA of T4 bacteriophage disturbed normal DNA functioning. When three base pairs were added or deleted the disturbance was minimum.
- Nirenberg and Mathaei (1961) argued that a singlet code can specify only four amino acids, a doublet code only 16, triplet only 64. As there are 20 amino acids, a triplet code can be operative.
- Nirenberg *et al* (1961) prepared the first synthetic RNA which was polymer of a single nucleotide, uridylic acid. This was carried out with the help of enzyme polynucleotide phosphorylase. They obtained cell free extract of bacterium *Escherichia coli.*
- Khorana synthesized copolymers of nucleotides like UGUGUGUG and observed that they stimulated the formation of polypeptides having alternately similar amino acids as cysteine valine-cysteine. This is possible only if three adjacent nucleotides specify one amino acid
- Ochoa *et al* (1967) synthesized a number of copolymers and assigned a number of triplet codons to specific amino acids.
- Brenner (1965) discovered the first termination codon UAG which was named as amber. Later on two more termination codons were discovered. UAA or ochre and UGA or opal. Termination codons were initially called nonsense codons. Later on it was suggested that they are important codons which function as stop signals for terminating the synthesis of polypeptide chains.
- The triplet codons were confirmed by in-vivo codon assignment through amino acid replacement studies and frame shift mutations.
- The code languages of DNA and m RNA are complementary, for example UUU of m RNA is AAA in DNA, UUC of m RNA is AAG of DNA.

Characteristics

- Triplet Code: Three adjacent nitrogen bases constitute a codon which specifies the placement of one amino acid in a polypeptide.
- Start Signal: Polypeptide synthesis is signaled by two initiation codons- AUG or methionine codon and GUG or valine codon.
- Stop Signal: Polypeptide chain termination is signaled by three termination codons- UAA, UAG and UGA. They do not specify any amino acid and are called non sense codons
- Polarity: This code reads in a fixed direction from 5 to 3 end. By convention, only the code of m RNA is taken in consideration. Code corresponding to m RNA is present on sense, coding or non-template strand of DNA.

- Commaless: The genetic code is commaless and is continuous and is read from a fixed point which is also the beginning of cistron. If a nucleotide is deleted or added, the whole genetic code will read differently. Thus polypeptide having 50 amino acids shall be specified by a linear sequence of 150 nucleotides. If a nucleotide is added or deleted in the middle of this sequence, the first 25 amino acids of polypeptide will be same but next 25 amino acids will be different.
- Non-Overlapping Code: In an overlapping code, a stretch of say 12 nucleotides can code for 11 amino acids while in non-overlapping one it would code for only 4 amino acids. The former condition would naturally be more economical.
- Degeneracy of Code: There are 64 triplet codons and only 20 amino acids, the incorporation of some amino acids must be influenced by more than one codon. Only tryptophan and methionine are specified by single codon. All other amino acids are specified by 2-6 codons. Nine amino acids have two codons each, one amino acid has three codons, and five amino acids have four codons each. Seven codon pairs have similar first two bases but the third base can be any of the two pyrimidines. Six codon pairs have similar first two bases but the third can be any purine. In three codons for IIe the third nitrogen base can be any except G. Only three codons have specific nitrogen base at third place. So total 61 codons are for 20 amino acids and 3 codons – UAA, UAG, UGA are stop signals.
- Universal Code: The genetic code is applicable universally, i.e., a codon specifies the same codon from a virus to a tree or human being.
- Collinearity: Both polypeptide and DNA or m RNA have a linear arrangement of their components. The sequence of triplet nucleotide bases in DNA or m RNA corresponds to the sequence of amino acids in the polypeptides manufactured under the guidance of the former.
- Cistron- Polypeptide Parity- Portion of DNA called cistron specifies the formation of a particular polypeptide. It means that the genetic system should have atleast as many cisterns as the types of polypeptides found in the organisms.

Exceptions

- Different codons: In paramecium and some other ciliates termination codons UAA and UGA code for glutamine
- Overlapping genes: $\phi \times 174$ has 5375 nucleotides that code for 10 proteins which require more than 6000 bases. Three of its genes E, B and K overlap other genes. Nucleotide sequence at the beginning of E gene is contained within gene D.
- Mitochondrial genes: AGG and AGA code for arginine but function as stop signals in human mitochondria. UGA a termination codon corresponds to tryptophan. In Drosophila mitochondria, AGA codes for serine.

Wobble Hypothesis

Degeneracy of the genetic code points towards the fact that the third base of a codon is not very important and that specificity of a codon is determined by the first two bases. It is known fact that the same tRNA can recognize more than one codon differing only at the third position. This pairing is not very stable and is allowed due to wobbling in the base pairing at the third position. Crick (1965, 1966 gave wobble hypothesis). For example anticodon IGC of t RNA can recognize codons GCU, GCC , GCA on mRNA (codes for alanine), IGA anticodon can recognize UCU, UCC, UCA (codes for serine) and IAC can recognize GUU, GUC, GUA (codes for valine).

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Importance: Wobble pairing is a device to overcome the effect of degeneracy of genetic code, mitochondria is able to undertake protein synthesis with small number of tRNAs, It actually speed up protein synthesis. Figure 5.5 shows the structure of gene.

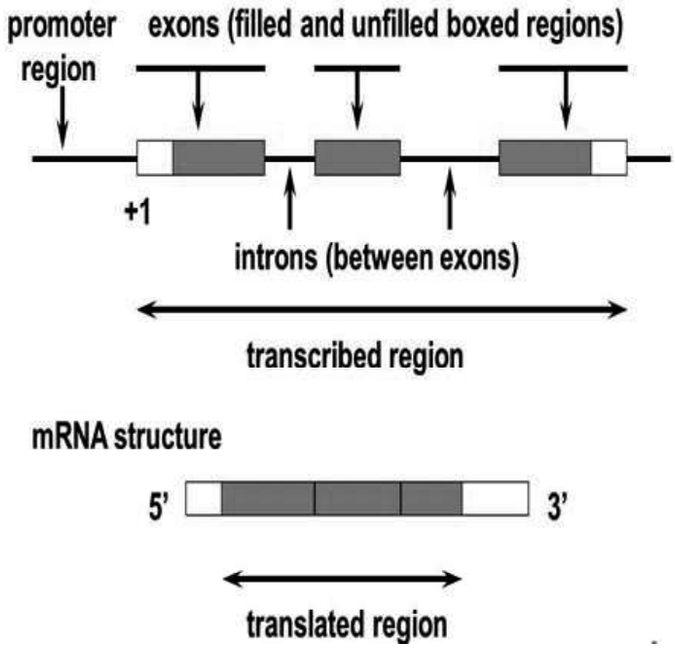

Fig. 5.5 *Gene Structure*

Properties of Gene

Gene has been described by different researchers in various ways. A gene has various structural and functional properties which are briefly described below:

Forms: The alternative form of a gene is known as allele. Generally each gene has two allelic forms. One of these forms in known as wild type and the other as mutant type. Allelic forms are known as dominant and recessive. Some genes have multiple allelic forms, but only two of them are present at a time in a true diploid individual.

Location: Genes are located on the chromosome in a linear fashion like bead on a string. The position which is occupied by a gene on the chromosome is called locus. Studies on linkage, crossing over, sex chromosomes, sex linkage and bacterial transformation and transduction have clearly demonstrated that genes are located on the chromosomes.

Status: Earlier it was believed that genes are the smallest units of inheritance which cannot be divided further. But Benzer demonstrated in 1955 that gene consists of several units of cistron, recon and muton which are the units of function, recombination and mutation within the gene.

Number: Each diploid individual has two copies of each gene and gametic cells have one copy of each gene. Each individual has large number of structural and functional features or characters and each character is controlled by one or more genes.

Thus, each individual has large number of genes. The total number of genes in an individual is always higher than the number of chromosomes. Thus, each chromosome has several genes. The gene number is also fixed per chromosome which may be altered by deletion and duplication.

Sequence: Genes have a specific sequence on the chromosome. The gene sequence is altered by structural chromosomal changes specially translocations and inversions.

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Expression: Genes express in various ways. They may show incomplete dominance, complete dominance, over dominance and lack of dominance. When there is lack of dominance, the expression is intermediate between the two parents. The gene which is expressed is known as dominant gene and which is suppressed is known as recessive gene. The phenotypic expression of genes depends on allelic and non-allelic interactions.

Change in Form: The gene may sometimes change from one allelic form to another. The change in the form of gene is brought out by gene mutation and the changed form of gene is called mutant gene, because generally the change occurs from dominant to recessive form. The reverse change is very rare.

Exchange of Genes: The exchange of genes occurs between non-sister chromatids of homologous chromosomes due to crossing over and between non-homologous chromosomes due to translocation.

Composition: Gene is a macro molecule which is composed of DNA. In most of the organisms, gene is made up of DNA. However, the genetic material in some bacteriophages is RNA.

Duplication: Each gene is duplicated at the time of chromosome duplication or replication. It is believed that chromosome duplication takes place because of gene duplication.

Function: The primary function of each gene is to control the expression of a specific character in an organism. However, sometimes two or more genes are involved in the expression of some characters. The characters which are governed by one or few genes are known as oligogenic traits and those characters which are governed by several genes are referred to as polygenic characters.

In some cases, a single gene has manifold effects, means it controls the expression of more than one character. Such genes are known as pleiotropic genes. Each gene controls the production of one enzyme or one polypeptide chain which in turn governs the expression of specific character.

Segregation: Genes in diploid organisms occur in pairs of alleles. The member of a pair segregates precisely like chromosomes during meiosis. Thus genes show segregation during meiosis.

Interaction: When a character is governed by two or more genes, they sometimes show interaction. In such interaction one gene has masking effect over the other. The masking gene is known as epistatic gene and the gene which is masked or suppressed is called hypostatic gene. Gene interaction leads to modification of normal dihybrid segregation ratio into various other types of ratios.

Linkage: Sometimes two or more genes are inherited together, such genes are referred to as linked genes. Some genes are linked with a particular sex, they are called as sex linked gene.

It is quite clear from the above discussion that there are some similarities or parallel features between chromosomes and genes.

Classification of Genes

Genes can be classified in various ways. The classification of genes is generally done on the basis of:

- Dominance
- Interaction

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- Character controlled
- Effect on survival
- Location
- Movement
- Nucleotide sequence
- Sex linkage
- Operon model
- Role in mutation

Changing Concept of Gene

The concept of gene has been the focal point of study from the beginning of twentieth century to establish the basis of heredity. The gene has been examined from two main angles, i.e., as follows:

- Genetic View
- Biochemical and Molecular View

These aspects are briefly described below:

A Genetic View: The genetic view or perspective of gene is based mainly on the Mendelian inheritance, chromosomal theory of inheritance and linkage studies. Mendel used the term factors for genes and reported that factors were responsible for transmission of characters from parents to their offspring.

Sutton and Boveri (1903) based on the study of mitosis and meiosis in higher plants established parallel behavior of chromosomes and genes. They reported that both chromosomes and genes segregate and exhibit random assortment, which clearly demonstrated that genes are located on chromosomes. The Sutton-Boveri hypothesis is known as chromosome theory of inheritance.

Morgan based on linkage studies in Drosophila reported that genes are located on the chromosome in a linear fashion. Some genes do not assort independently because of linkage between them. He suggested that recombinants are the result of crossing over.

The crossing over increases if the distance between two genes is more. The number of linkage group is the same as the number of chromosomes. The chromosome theory and linkage studies reveal that genes are located on the chromosomes. This view is sometimes called as bead theory.

The important points about the bead theory are given below:

- The gene is viewed as a fundamental unit of structure, indivisible by crossing over. Crossing over occurs between genes but not within a gene.
- The gene is considered as a basic unit of change or mutation. It changes from one allelic form to another, but there are no smaller components within a gene that can change.
- The gene is viewed as a basic unit of function. Parts of a gene, if they exist, cannot function.

The chromosome has been viewed merely as a vector or transporter of genes and exists simply to permit their orderly segregation and to shuffle them in recombination. The bead theory is no more valid for any of the above three points.

Now evidences are available which indicate that:

- A gene is divisible
- Part of a gene can function

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The Gene is Divisible: Earlier it was believed that gene is a basic unit of structure which is indivisible by crossing over. In other words, crossing over occurs between genes but not within a gene. Now, intragenic recombination has been observed in many organisms which indicates that a gene is divisible.

The intragenic recombination has following two main features:

- It occurs with rare frequency so that a very large test cross progeny is required for its detection. Benzer expected to detect a recombination frequency as low as 10^{-6}, the lowest he actually found was 10^{-4}(0.01 x 2 = 0.02%).
- The alleles in which intragenic recombination occurs are separated by small distances within a gene and are functionally related.

Examples of intragenic recombination include bar eye, star asteroid eye and lozenge eye in Drosophila. The bar locus is briefly described below. Lozenge eye and star asteroid have been discussed under pseudo alleles.

Bar Eye in Drosophila: The first case of intragenic recombination was recorded in Drosophila for bar locus which controls size of eye. The bar locus contains more than one unit of function. The dominant bar gene in Drosophila produces slit like eye instead of normal oval eye. Bar phenotype is caused by tandem duplication of 16A region in X chromosome, which results due to unequal crossing over.

The flies with different dose of 16A region have different types of eye as follows:

The homozygous bar eye (B/B) produced both wild and ultra-bar types though at a low frequency which indicated intragenic recombination in the bar locus but the frequency was much higher than that expected due to spontaneous mutations.

Part of a Gene Can Function: It was considered earlier that gene is the basic unit of function and parts of gene, if exist, cannot function. But this concept has been outdated now. Based on studies on rll locus of T4 phage, Benzer (1955) concluded that there are three sub divisions of a gene, viz., recon, muton and cistron.

These are briefly described below:

Recon: Recons are the regions (units) within a gene between which recombination's can occur, but the recombination cannot occur within a recon. There is a minimum recombination distance within a gene which separates recons. The map of a gene is completely linear sequence of recons.

Muton: It is the smallest element within a gene, which can give rise to a mutant phenotype or mutation. This indicates that part of a gene can mutate or change. This disproved the bead theory according to which the entire gene was to mutate or change.

Cistron: It is the largest element within a gene which is the unit of function. This also knocked down the bead theory according to which entire gene was the unit of function. The name cistron has been derived from the test which is performed to know whether two mutants are within the same cistron on in different cistrons. It is called cis-trans test which is described below.

Cis-Trans Test: When two mutations in trans position produce mutant phenotype, they are in the same cistron. Complementation in trans position (appearance of wild type) indicates that the mutant sites are in different cistrons. There is no complementation between mutations within a cistron.

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It is now known that some genes consist of only one cistron; some consist of two or even more. For example, the mutant miniature (m) and dusky (dy) both decrease wing size in Drosophila and map in the same part of X chromosome. But when brought together in dy +/+m heterozygote, the phenotype is normal which indicates that the locus concerned with wing size is composed of at least two cistrons. In the Figure 5.6 below gene structure of Prokaryotes and Eukaryotes is shown.

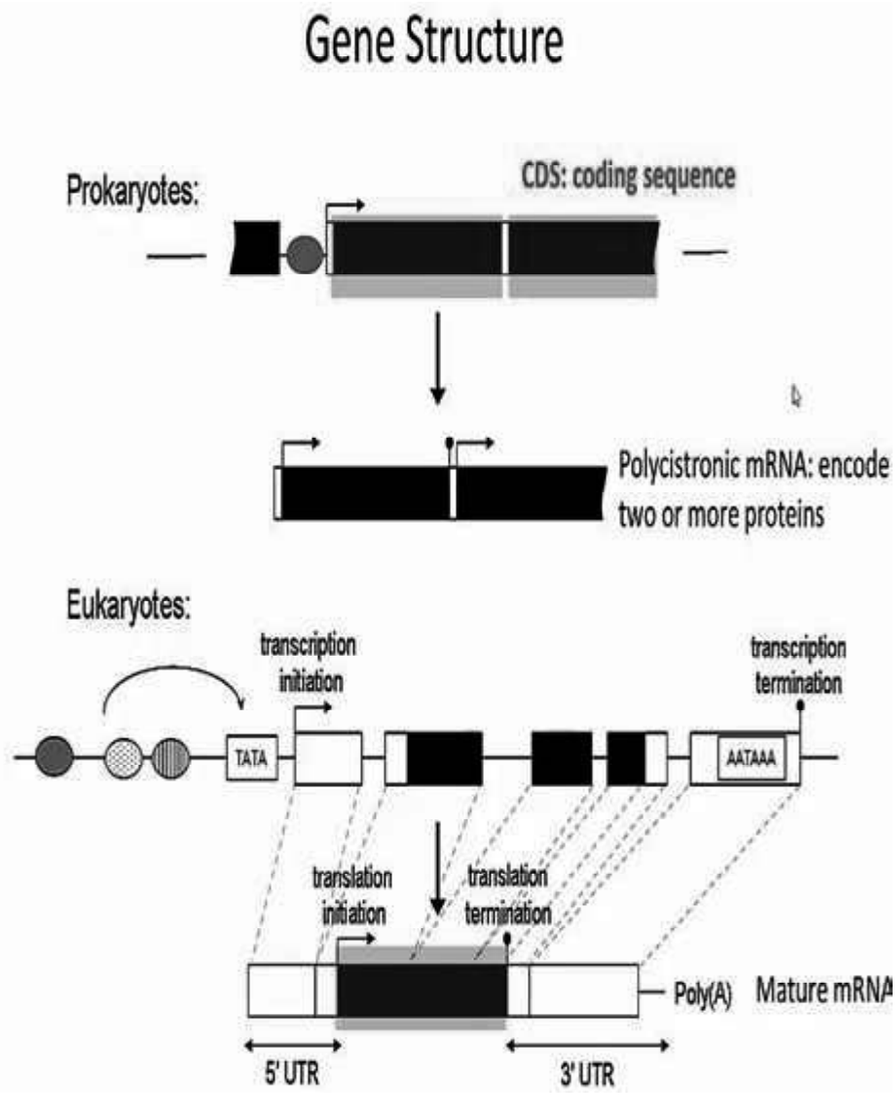

Fig. 5.6 *Gene Structure: Prokaryotes and Eukaryotes*

A Biochemical View: It is now generally believed that a gene is a sequence of nucleotides in DNA which controls a single polypeptide chain. The different mutations of a gene may be due to change in single nucleotide at more than one location in the gene. Crossing over can take place between the altered nucleotides within a gene.

Since the mutant nucleotides are placed so close together, crossing over is expected within very low frequency. When several different genes which affect the same trait are present so close that crossing over is rare between them, the term complex locus is applied to them. Within the nucleotide sequence of DNA, which represents a gene, multiple alleles are due to mutations at different points within the gene.

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Fine Structure of Gene: Benzer, in 1955, divided the gene into recon, muton and cistron which are the units of recombination, mutation and function within a gene. Several units of this type exist in a gene. In other words, each gene consists of several units of function, mutation and recombination. The fine structure of gene deals with mapping of individual gene locus.

This is parallel to the mapping of chromosomes. In chromosome mapping, various genes are assigned on a chromosome, whereas in case of a gene several alleles are assigned to the same locus. The individual gene maps are prepared with the help of intragenic recombination.

Since the frequency of intragenic recombination is extremely low, very large population has to be grown to obtain such rare combination. Prokaryotes are suitable material for growing large population. In Drosophila, 14 alleles of lozenge gene map at four mutational sites which belong to the same locus (Green, 1961). Similarly, for rosy eye in Drosophila, different alleles map at 10 mutational sites of the same locus.

Descriptions about Each Genes: There are some genes which are different from normal genes either in terms of their nucleotide sequences or functions. Some examples of such genes are split gene, jumping gene, overlapping gene and pseudo gene.

A brief description of each of these genes is presented below:

Split Genes: Usually a gene has a continuous sequence of nucleotides. In other words, there is no interruption in the nucleotide sequence of a gene. Such nucleotide sequence codes for a particular single polypeptide chain. However, it was observed that the sequence of nucleotides was not continuous in case of some genes; the sequences of nucleotides were interrupted by intervening sequences.

Such genes with interrupted sequence of nucleotides are referred to as split genes or interrupted genes. Thus, split genes have two types of sequences, viz., normal sequences and interrupted sequences.

Normal Sequence: This represents the sequence of nucleotides which are included in the mRNA which is translated from DNA of split gene. These sequences code for a particular polypeptide chain and are known as exons.

Interrupted Sequence: The intervening or interrupted sequences of split gene are known as introns. These sequences do not code for any peptide chain. Moreover, interrupted sequences are not included into mRNA which is transcribed from DNA of split genes.

The interrupted sequences are removed from the mRNA during processing of the same. In other words, the intervening sequences are discarded in mRNA as they are non-coding sequences. The coding sequences or exons are joined by ligase enzyme.

The first case of split gene was reported for ovalbumin gene of chickens. The ovalbumin gene has been reported to consist of seven intervening sequences. Later on interrupted sequences (split genes) were reported for beta globin genes of mice and rabbits, tRNA genes of yeast and ribosomal genes of Drosophila.

The intervening sequences are determined with the help of R loop technique. This technique consists of hybridization between mRNA and DNA of the same gene under ideal conditions, i.e., at high temperature and high concentration of form amide. The mRNA pairs with single strand of DNA.

The non-coding sequences or intervening sequences of DNA make loop in such pairing. The number of loops indicates the number of interrupted sequences and the size of loop indicates length of the intervening sequence. These loops can be viewed under electron microscope.

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The ovalbumin gene has seven interrupted sequences (introns) and eight coding sequences (exons). The beta globin gene has been reported to have two intervening sequences, one 550 nucleotides long and the other 125 nucleotides long.

The intervening sequences are excised during processing to form mature mRNA molecule. Thus, about half of the ovalbumin gene is discarded during processing. Earlier it was believed that there is co-linearity (correspondence) between the nucleotide sequence and the sequence of amino acids which it specifies.

The discovery of split genes has disproved the concept of co-linearity of genes. Now co-linearity between genes and their products is considered as a chance rather than a rule. Split genes have been reported mostly in eukaryotes.

Jumping Genes: Generally, a gene occupies a specific position on the chromosome called locus. However, in some cases a gene keeps on changing its position within the chromosome and also between the chromosomes of the same genome. Such genes are known as jumping genes or transposons or transposable elements.

The first case of jumping gene was reported by Barbara McClintock in maize as early as in 1950. However, her work did not get recognition for a long time like that of Mendel. Because she was much ahead of time and this was an unusual finding, people did not appreciate it for a long time. This concept was recognized in early seventies and McClintock was awarded Nobel prize for this work in 1983.

Later on transposable elements were reported in the chromosome of *E. coli* and other prokaryotes. In E. coli, some DNA segments were found moving from one location to other location. Such DNA segments are detected by their presence at such a position in the nucleotide sequence, where they were not present earlier. The transposable elements are of two types, viz., insertion sequence and transposons.

Insertion Sequence: There are different types of insertion sequences each with specific properties. Such sequences do not specify for protein and are of very short length. Such sequences have been reported in some bacteria, bacteriophages and plasmids.

Transposons: These are coding sequences which code for one or more proteins. They are usually very long sequences of nucleotides including several thousand base pairs. Transposable elements are considered to be associated with chromosomal changes, such as inversion and deletion.

They are hot spots for such changes and are useful tools for the study of mutagenesis. In eukaryotes, moving DNA segments have been reported in maize, yeast and Drosophila.

Overlapping Genes: Earlier it was believed that a nucleotide sequence codes only for one protein. Recent investigations with prokaryotes especially viruses have proved beyond doubt that some nucleotide sequences (genes) can code for two or even more proteins.

The genes which code for more than one protein are known as overlapping genes. In case of overlapping genes, the complete nucleotide sequence codes for one protein and a part of such nucleotide sequence can code for another protein.

Overlapping genes are found in tumor producing viruses, such as x X 174, SV 40 and G4. In virus xX 174 gene A overlaps gene B. In virus SV 40, the same nucleotide sequence codes for the protein VP 3 and also for the carboxyl-terminal end of the protein VP2. In virus G4, the gene A overlaps gene B and gene E overlaps gene D.

The gene of this virus also contains some portions of nucleotide sequences which are common for gene A and gene C.

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Pseudogenes: There are some DNA sequences, especially in eukaryotes, which are non-functional or defective copies of normal genes. These sequences do not have any function. Such DNA sequences or genes are known as pseudogenes. Pseudogenes have been reported in humans, mouse and Drosophila.

The main features of pseudogenes are given below:

- Pseudogenes are non-functional or defective copies of some normal genes. These genes are found in large numbers.
- These genes being defective cannot be translated.
- These genes do not code for protein synthesis, means they do not have any significance.
- The well-known examples of pseudogenes are alpha and beta globin pseudogenes of mouse.

'Check Your Progress'

8. Give any three characteristics of genetic code.
9. What are the exceptions of genetic code?
10. Where are genes located?
11. What is the function of gene?

5.5 REGULATION OF GENE EXPRESSION: NEGATIVE AND POSITIVE REGULATION

Gene regulation refers to the mechanisms that act to induce or repress the expression of a gene. These include structural and chemical changes to the genetic material, binding of proteins to specific DNA elements to regulate transcription, or mechanisms that modulate translation of mRNA. Regulation of gene expression, or gene regulation, includes a wide range of mechanisms that are used by cells to increase or decrease the production of specific gene products (protein or RNA). 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 colour 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.

In multicellular organisms, gene regulation drives cellular differentiation and morphogenesis in the embryo, leading to the creation of different cell types that possess different gene expression profiles from the same genome sequence. Although this does not explain how gene regulation originated, evolutionary biologists include it as a partial explanation

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of how evolution works at a molecular level, and it is central to the science of evolutionary developmental biology ('evo-devo'). The initiating event leading to a change in gene expression includes activation or deactivation of receptors.

Most of the genes of an organism produce specific proteins (enzymes), which, in turn produce specific phenotypes. The genes whose mRNA transcripts are translated into protein are known as structural genes. Every cell of an organism possesses all the structural genes normally present in the species, but only a small fraction of them are functional in any cell at a given time.

In prokaryotes, cells generally synthesize only those enzymes which they need in a given environment. For example, *E. coli* cells grown in the presence of lactose produce abundant (up to 3000 molecules/cell) β-galactosidase, the enzyme that hydrolyses lactose. However, very little of this enzyme (less than 3 molecules/cell) is produced in the absence of lactose.

In eukaryotes, the cells of different organs produce different proteins needed for their function. Red blood cells contain a high concentration of haemoglobin, while leucocytes (white blood cells) have no haemoglobin at all.

Apparently, there is a precise control on the kinds of proteins or enzymes product in a given tissue or cell at a given time. Such a control on gene activity, i.e., protein production that permits the function of only those genes whose products are required in a given cell at a given time is termed as gene regulation.

Synthesis of enzyme depends mainly on two factors in a degradative process, the synthesis of enzyme depends on the availability of the molecule to be degraded. If the molecule is in more quantity, the enzyme synthesis will be more and vice versa. In a biosynthetic pathway, the synthesis of an enzyme is controlled by the end product. If the end product is more, the enzyme synthesis will be less and vice versa.

There are two types of gene regulation, viz:

- Negative Regulation
- Positive Regulation

Negative Regulation

An inhibitor is present in the cell/system that prevents transcription by inactivating the promoter. This inhibitor is known as repressor. For initiation of transcription, an inducer is required. Inducer acts as antagonist of the repressor. In the negative regulation, absence of product increases the enzyme synthesis and presence of the product decreases the synthesis.

Positive Regulation

An effector molecule (which may be a protein or a molecular complex) activates the promoter for transcription. In a degradative system, either negative or positive mechanism may operate, while in a biosynthetic pathway negative mechanism operates, for example lac operon.

The phenomenon of gene expression can be elaborated further, such as given below:

- Gene expression is the mechanism at the molecular level by which a gene is able to express itself in the phenotype of an organism.
- The mechanism of gene expression involves biochemical genetics. It consists of synthesis of specific RNAs, polypeptides, structural proteins, proteinaceous biochemicals or enzymes which control the structure or functioning of specific traits.

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- Gene regulation is the mechanism of switching off and switching on of the genes depending upon the requirement of the cells and the state of development.
- It is because of this regulation that certain proteins are synthesized in as few as 5-10 molecules while others are formed in more than 100,000 molecules per cell.
- There are two types of gene regulations positive and negative.
- In negative gene regulation the genes continue expressing their effect till their activity is suppressed.
- This type of gene regulation is also called repressible regulation.
- The repression is due to a product of regulatory genes.
- Positive gene regulation is the one in which the genes remain non-expressed unless and until they are induced to do it.
- It is, therefore called inducible regulation.
- Here a product removes d biochemical that keeps the genes in non-expressed state.
- As the genes express their effect through enzymes, their enzymes are also called inducible enzymes and repressible enzymes.

Gene regulation is exerted at four levels:

- Transcriptional level when primary transcript is formed.
- Processing level when splicing and terminal additions are made.
- Transport of mRNA out of nucleus into cytoplasm.
- Translational level.

Important Terms used in Connection with the Regulation of Gene Expression

- **Repressor:** In operon, protein molecules which prevent transcription. The process of inhibition of transcription is called repression.
- **Inducer:** The substance that allows initiation of transcription, for example lactose in lac operon. Such process is known as induction.
- **Co-repression:** A combination of repressor and a metabolite which prevents protein synthesis. Such process is known as co-repression.
- **Inducible enzyme:** An enzyme whose production is enhanced by adding the substrate in the culture medium. Such system is called inducible system.
- **Repressible enzyme:** An enzyme whose production can be inhibited by adding an end product. Such system is known as repressible system.
- **Constitutive enzyme:** An enzyme whose production is constant irrespective of metabolic state of the cell.
- **Negative control:** Inhibition of transcription by repressor through inactivation of promoter, for example in lac operon.
- **Positive control:** Enhancement of transcription by an effector molecule through activation of premotor.
- **Effector:** The molecule that acts as an inducer or co-repressor in the operon model of *E.coli.*

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'Check Your Progress'

12. Define the term gene regulation.
13. What does gene regulation includes?
14. Why is gene regulation important?
15. Give the mechanism of gene expression.
16. How is gene regulation exerted?

5.6 SUMMARY

- Transposable elements were discovered by B. McClintock through an analysis of genetic instability in maize.
- McClintock used a marker that controlled the deposition of pigmentation in the aleurone, the outermost layer of the endosperm of maize kernels.
- Genetic instabilities have been found in bacteria and in many cases have led to the identification of transposable elements.
- IS elements are compactly organized. There is a single coding sequence with short, identical or nearly identical sequences at both ends
- Composite transposons are created when two IS elements insert near each other. The sequence between them can be transposed by the joint action of the flanking elements.
- Bacterial transposons are responsible for the transposition of genes controlling resistance to antibiotics from one molecule to another. They are believed to play a role in the rapid evolution of R plasmid.
- All conjugative R plasmid have at least two components, one segment carrying a set of genes involved in conjugative DNA transfer and a second segment carrying antibiotic and drug resistance gene.
- The segment carrying the transfer genes is called resistance transfer factor component, the segment carrying the resistance gene or genes is called R determinant.
- The Ac/ Ds family of maize discovered by McClintock comprises numerous elements scattered throughout the genome.
- Insertion sequences, or Insertion-Sequence (IS) elements, are now known to be segments of bacterial DNA that can move from one position on a chromosome to a different position on the same chromosome or on a different chromosome.
- The IR sequences are essential for the transposition process, that is, those sequences are recognized by transposase to initiate transposition.
- The sizes of target site duplication vary with the IS elements, but tend to be small. Integration of some IS elements show preference for certain regions, while others integrate only at particular sequences.
- Mutations that affect the inverted terminal repeat sequence of IS elements affect transposition, indicating that the inverted terminal repeat sequences are the key sequences recognized by transposase during a transposition event.

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- Composite transposons are complex transposons with a central region containing genes, for example drug resistance genes, flanked on both sides by IS elements (also called IS modules
- Non-composite transposons like composite transposons, contain genes, such as those for drug resistance. Unlike composite transposons, they do not terminate with IS elements.
- Resolvase is not found in all transposons. The genes for transposition are in the central region for non-composite transposons, while they are in the terminal IS elements for composite transposons.
- Insertion of a transposon into the reading frame of a gene will disrupt it, causing a loss of function of that gene. Insertion into gene's controlling region can cause changes in the level of expression of the gene.
- Deletion and insertion events also occur as a result of activities of the transposons, and from crossing-over between duplicated transposons in the genome.
- The F factor, a circular double stranded DNA molecule, is one of the example of bacterial plasmid. Plasmids, such as F that are also capable of integrating into the bacterial chromosomes are called episomes.
- Phage mu is a normal-appearing phage. We consider it here because, although it is a true virus, it has many features in common with IS elements.
- The gene is defined as the unit of genetic material coding for one polypeptide.
- Cis heterozygote must exhibit the wild type phenotype for the cis-trans test to be informative.
- If both the cis heterozygote and the transheterozygote containing a given pair of mutations have wild type phenotypes, then the two mutations are in two different genes.
- Muton is the smallest segment of genetic material which can undergo change and produce mutation. Muton is a unit of mutation. It can be as small as a single nucleotide.
- Recon is the smallest segment of genetic material that can get exchanged between two homologous chromosomes through crossing over to produce a recombinant form.
- Cistron is a unit of genetic material, DNA that contains encoded information for producing a functional biological product or genetic function.
- Constitutive genes (Housekeeping genes) are those genes which are constantly expressing themselves in a cell because their products are required for the normal cellular activities, for example genes for glycolysis, ATPase.
- Single copy genes are present in single copies. They form 60-70% of the functional genes.
- Genetic code is made up of only four types of nucleotides, the nucleotides of DNA can be positioned in countless ways.
- Genes or cistrons are made up of nucleotides arranged in a specific manner.
- Polypeptides are formed of amino acids arranged in a specific manner.
- The alternative form of a gene is known as allele. Generally each gene has two allelic forms. One of these forms in known as wild type and the other as mutant type.
- Allelic forms are known as dominant and recessive. Some genes have multiple allelic forms, but only two of them are present at a time in a true diploid individual.

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- Genes are located on the chromosome in a linear fashion like bead on a string. The position which is occupied by a gene on the chromosome is called locus.
- Gene is a macro molecule which is composed of DNA. In most of the organisms, gene is made up of DNA. However, the genetic material in some bacteriophages is RNA.
- Genes in diploid organisms occur in pairs of alleles. The member of a pair segregates precisely like chromosomes during meiosis.
- The first case of intragenic recombination was recorded in Drosophila for bar locus which controls size of eye.
- Bar phenotype is caused by tandem duplication of 16A region in X chromosome, which results due to unequal crossing over.
- Recons are the regions (units) within a gene between which recombination's can occur, but the recombination cannot occur within a recon.
- Cis-trans test when two mutations in trans position produce mutant phenotype, they are in the same cistron.
- A gene occupies a specific position on the chromosome called locus.
- Gene regulation refers to the mechanisms that act to induce or repress the expression of a gene.
- The genes whose mRNA transcripts are translated into protein are known as structural genes.
- In prokaryotes, cells generally synthesize only those enzymes which they need in a given environment.
- In eukaryotes, the cells of different organs produce different proteins needed for their function.
- Inducer acts as antagonist of the repressor. In the negative regulation, absence of product increases the enzyme synthesis and presence of the product decreases the synthesis.
- An effector molecule (which may be a protein or a molecular complex) activates the promoter for transcription.
- Gene expression is the mechanism at the molecular level by which a gene is able to express itself in the phenotype of an organism.
- Gene regulation is the mechanism of switching off and switching on of the genes depending upon the requirement of the cells and the state of development.
- A combination of repressor and a metabolite which prevents protein synthesis is known as co-repression.
- An enzyme whose production can be inhibited by adding an end product are known as repressible system.
- Effector is the molecule that acts as an inducer or co-repressor in the operon model of *E.coli.*

5.7 KEY TERMS

- **Illegitimate recombination:** Genetic recombination between non-homologous sequences is called illegitimate recombination.

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- **Target sites:** The sites into which IS elements insert are called target sites.
- **Gene:** The gene is defined as the unit of genetic material coding for one polypeptide.
- **Composite transposons:** Composite transposons are complex transposons with a central region containing genes.
- **Muton:** It is the smallest segment of genetic material which can undergo change and produce mutation.
- **Recon:** It is the smallest segment of genetic material that can get exchanged between two homologous chromosomes through crossing over to produce a recombinant form.
- **Cistron:** It is a unit of genetic material, DNA that contains encoded information for producing a functional biological product or genetic function.
- **Overlapping genes:** Genes sharing certain common base sequences are called overlapping genes.
- **Allele:** The alternative form of a gene is known as allele.
- **Locus:** The position which is occupied by a gene on the chromosome is called locus.

5.8 ANSWERS TO 'CHECK YOUR PROGRESS'

1. The four transposable genetic elements in prokaryotes are:
   - Bacterial Insertion Sequences
   - Prokaryotic Transposons
   - Insertion-Sequence Elements and Transposons in Plasmids
   - Phage mu
2. Insertion sequences, or Insertion-Sequence (IS) elements, are now known to be segments of bacterial DNA that can move from one position on a chromosome to a different position on the same chromosome or on a different chromosome. An IS element contains only genes required for mobilizing the element and inserting the element into a chromosome at a new location. Is elements are normal constituents of bacterial chromosome and plasmids.
3. When IS elements insert into chromosomes or plasmids, they create a duplication of the DNA sequence at the site of insertion. One copy of the duplication is located on each side of the element.
4. The gene is defined as the unit of genetic material coding for one polypeptide.
5. Muton is the smallest segment of genetic material which can undergo change and produce mutation. Muton is a unit of mutation. It can be as small as a single nucleotide.
6. Recon is the smallest segment of genetic material that can get exchanged between two homologous chromosomes through crossing over to produce a recombinant form. A recon may be as small as a single nucleotide because genetic material can rupture between any two adjacent nucleotides.
7. Cistron is a unit of genetic material, DNA that contains encoded information for producing a functional biological product or genetic function. The latter includes the actual synthesis of an RNA or its control. A functional gene is now considered equivalent to a cistron.

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8. Characteristics of genetic code are as follows:
   - Triplet code: Three adjacent nitrogen bases constitute a codon which specifies the placement of one amino acid in a polypeptide.
   - Start signal: Polypeptide synthesis is signaled by two initiation codons- AUG or methionine codon and GUG or valine codon.
   - Stop signal: Polypeptide chain termination is signaled by three termination codons- UAA, UAG and UGA. They do not specify any amino acid and are called non sense codon.
9. Few of the exceptions of genetic code are as follows:
   - Different codons: In paramecium and some other ciliates termination codons UAA and UGA code for glutamine
   - Overlapping genes: Õ× 174 has 5375 nucleotides that code for 10 proteins which require more than 6000 bases. Three of its genes E, B and K overlap other genes. Nucleotide sequence at the beginning of E gene is contained within gene D.
   - Mitochondrial genes: AGG and AGA code for arginine but function as stop signals in human mitochondria. UGA a termination codon corresponds to tryptophan. In Drosophila mitochondria, AGA codes for serine.
10. Genes are located on the chromosome in a linear fashion like bead on a string. The position which is occupied by a gene on the chromosome is called locus. Studies on linkage, crossing over, sex chromosomes, sex linkage and bacterial transformation and transduction have clearly demonstrated that genes are located on the chromosomes.
11. The primary function of each gene is to control the expression of a specific character in an organism. However, sometimes two or more genes are involved in the expression of some characters. The characters which are governed by one or few genes are known as oligogenic traits and those characters which are governed by several genes are referred to as polygenic characters
12. Gene regulation refers to the mechanisms that act to induce or repress the expression of a gene.
13. Regulation of gene expression, or gene regulation, includes a wide range of mechanisms that are used by cells to increase or decrease the production of specific gene products (protein or RNA).
14. 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.
15. The mechanism of gene expression involves biochemical genetics. It consists of synthesis of specific RNAs, polypeptides, structural proteins, proteinaceous biochemicals or enzymes which control the structure or functioning of specific traits.
16. Gene regulation is exerted at four levels:
   - Transcriptional level when primary transcript is formed.
   - Processing level when splicing and terminal additions are made.
   - Transport of mRNA out of nucleus into cytoplasm.
   - Translational level.

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5.9 QUESTIONS AND EXERCISES

Short-Answer Questions

1. What are transposable elements?
2. Give the medical significance of bacterial transposons.
3. Write a short note on bacterial insertion sequences.
4. What is Prokaryotic transposons?
5. Draw a well-labelled diagram of the structure of Tn10 transposon.
6. 'One cistron one function' Explain.
7. Give some functions of genes.
8. What is genetic code?
9. Give the classification of genes.
10. Distinguish between negative and positive gene regulation.

Long-Answer Questions

1. Discuss in detail about transposable element in Prokaryotes and Eukaryotes.
2. Draw a well-labelled diagram of integration of an IS elements into chromosomal DNA.
3. Explain the fine structure of gene.
4. Elaborate a note on analysis of gene.
5. What is genetic code? Explain in detail.
6. Draw a well-labelled diagram of gene structure.
7. Write a detailed note on positive and negative regulation of genes.

5.10 FURTHER READING

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