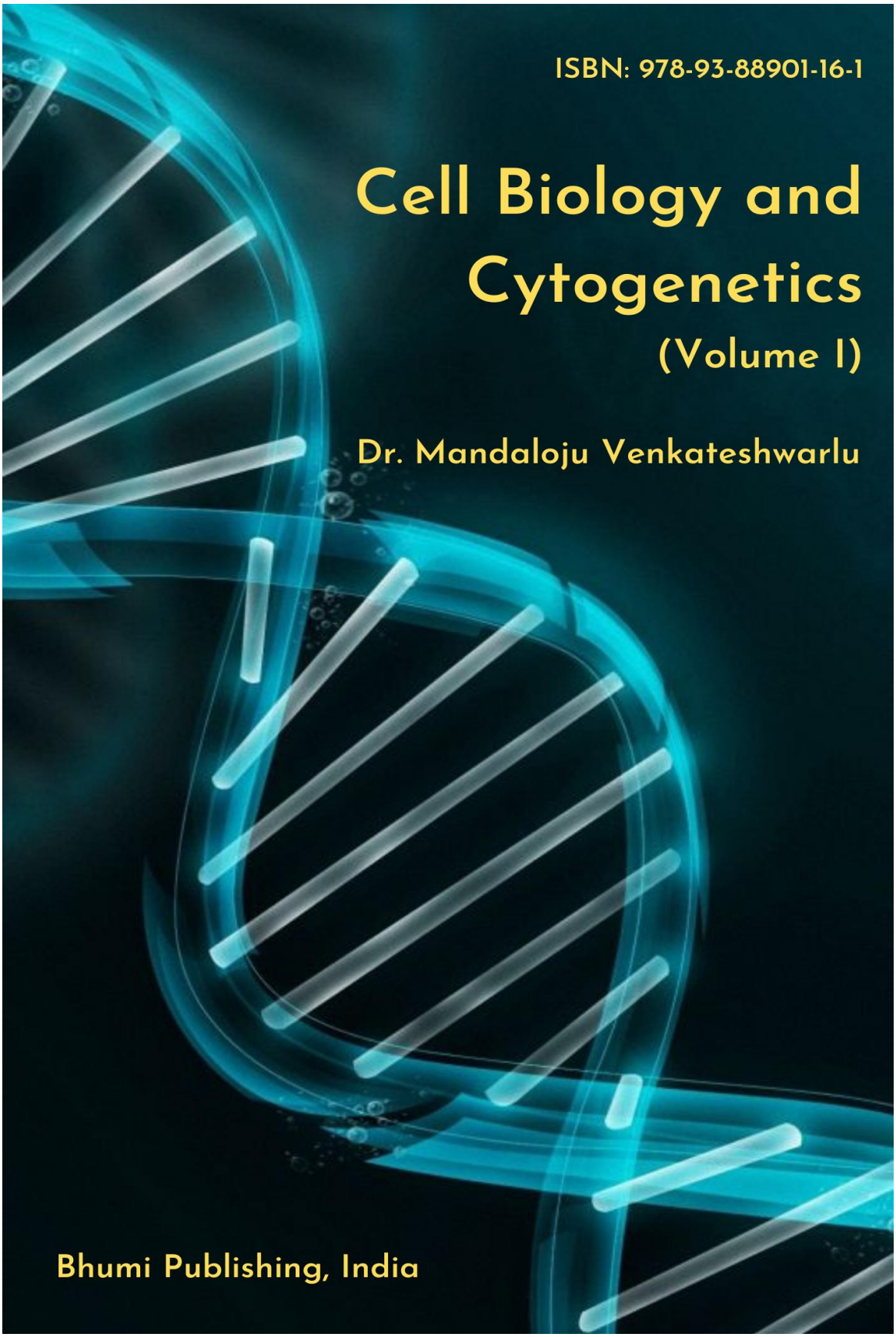
ISBN: 978-93-88901-16-1

Cell Biology and Cytogenetics

(Volume I)

Dr. Mandaloju Venkateshwarlu



Bhumi Publishing, India

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Dr. Mandaloju Venkateshwarlu

M.Sc., Ph.D., (Botany) M.Sc. (Bio-Tech), FSAB, FHAS (L.L.B)

Department of Botany

KAKATIYA UNIVERSITY

WARANGAL – 506 009

Bhumi Publishing

2021

First Edition: 2021

ISBN: 978-93-88901-16-1

Published by:

Bhumi Publishing,

Nigave Khalasa, Kolhapur 416207, Maharashtra, India

Website: www.bhumipublishing.com

E-mail: bhumipublishing@gmail.com

Book Available online at:

https://www.bhumipublishing.com/books/

Preface to the first edition

Cytogenetics, in cell biology, field that deals with chromosomes and their inheritance, particularly as applied to medical genetics. Chromosomes are microscopic structures found in cells, and malformations associated with them lead to numerous genetic diseases. Chromosomal analysis has steadily improved in precision and resolution, and that has led to improvements in the diagnosis of various genetic diseases in all areas of medicine. The study of chromosomes begins with the extraction of intact chromosomes from live cells. Chromosomal analyses often make use of white blood cells (T-lymphocytes), which multiply quickly under cell culture conditions. Chromosomes may also be extracted from skin cells, bone marrow cells, or fetal cells (by amniocentesis or chorionic villus sampling).

The book is written in simple language so that the students can easily grasp the matter. Some important terms has been incorporated, so that the students may search the useful related for competitive examinations. In the recent years included in the syllabus of almost all Indian Universities in various subjects of Biology or Life Sciences as an independent evergreen subject. Exponential growth in many areas of basic fundamentals made it necessary in some cases to write several chapters on the same topic which was covered in a single chapter in the earlier book. Similarly, in the present volume, separate new chapters have been written on topics which in the earlier title either did not figure at all or were each covered very briefly as a part of a chapter. In the present book, for instance in separate chapters have been written on new topics. The students of Biology at the post graduate (P.G.) under graduate (U.G.) levels needed to the recent Global changes and developments.

This book is intended to be an accessible introduction to the cell biology and cytogenetics for junior or senior undergraduate students who have already had an introduction to biological sciences. This engaging and stimulating text focuses on detailed aspects in cell biology.

- **Dr. Mandaloju Venkateshwarlu**

ACKNOWLEDGEMENT

The world is a better place thanks to people who want to develop and lead others. What makes it even better are people who share the gift of their time to mentor future leaders. Thank you to everyone who strives to grow and help others grow. It is the business version of The Lion King *song, "Circle of Life."*

To all the individuals I have had the opportunity to lead, be led by, or watch their leadership from afar, I want to say thank you for being the inspiration and foundation for The Leadership Kakatiya University, Botany Department. *Without the experiences and support from my peers and team at Ultimate Software, this book would not exist. You have given me the opportunity to lead a great group of individuals—to be a leader of great leaders is a blessed place to be. Thank you to friends and colleagues of my Department of Botany, Kakatiya university.*

Several of my students in the laboratory helped me either in writing some of the chapters or in preparing the list of references and appendix given at the end of this volume. There may also be errors and omissions of technical nature, since in a vast and fast expanding subject like Botany Biotechnology; one cannot claim to have known everything, despite his best efforts.

I am thankful to Department Head, BOS, Staff and Research Scholars (Botany) my family members, inspiration and cooperation my wife and children's (M. Hamsini) Teachers, Friends, Students and Well-Wishers (Dursheti Sai Charan, M.B.A., Certified Microsoft Office and Windows Specialist). I hope that this book will be useful to students in Life Sciences.

Having an idea and turning it into a book is as hard as it sounds. The experience is both internally challenging and rewarding. I especially want to thank the individuals that helped make this happen. Complete thanks to Family members and relatives who have supported in making the book.

- **Dr. Mandaloju Venkateshwarlu**

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Chapter 1

Plant Cell

Ultra structure of cell wall

Presence of cell wall is an identifying character of plant cell as it is absent in animal cell. This wall may be absent in some lower plants and cells taking part in the reproduction. For example, cell wall is not found in gametes and zoospores, in lower fungi (slime moulds) as *Synchitrium* which causes wart disease of potato, and *Plasmodiophore*. Bacteria and fungi are placed in plant kingdom because they contain cell wall. Cell wall is not found in *Mycoplasma*.Cell wall is outermost, non living rigid and freely permeable structure.

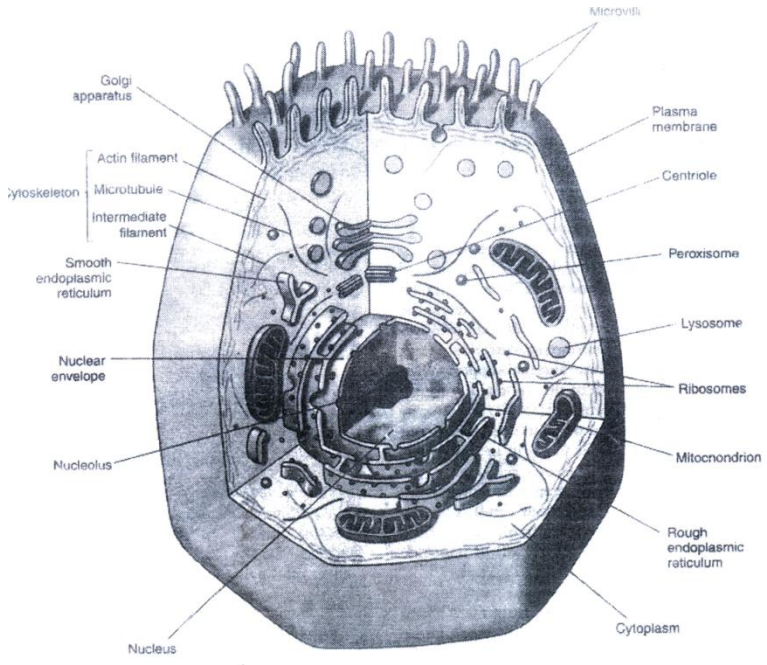


Figure 1.1: Structure of an animal cell. In this generalized diagram of an animal cell, the plasma membrane encases the cell, which contains the cytoskeleton and various cell organelles and interior structures suspended in a semifluid matrix called the cytoplasm. Some kinds of animal cells possess fingerlike projections called microvilli. Other types of eukaryotic cells- for example, many protist cells-may possess flagella, which aid in movement, or cilia, which can have many different functions

During cytokinesis in vesicles of Golgi complex become aligned at the equatorial plate or cell plate or *phragmoplast* (spindle fibre complex). The cell plate forms when Golgi vesicles and endoplasmic reticulum cisternae aggregate in the spindle mid zone area of a dividing cell. The aggregation is organized by the phragmoplast; a complex assembly of microtubules, membranes and the vesicles that form during late anaphase or early telophase. The membranes of the vesicles fuse with each other and with the lateral plasma membrane, to become the new plasma membrane separating the daughter cells. By some physical and chemical changes in cell plate an intercellular amorphous substance functions as cementing material

between two cells. It is made up of *calcium and magnesium pectate.* This layer is called *middle lamella.* Middle lamella is the outermost layer of the cell wall. Plane of cell wall formation is determined by microtubules. The cell formation is always perpendicular to spindle fibres (Fig. 1.4). The characteristic softening of fruit during ripening is mainly due to increase in the solubility of middle lamella.

Primary cell wall

Protoplasm deposit some substance on the middle lamella due to which a soft delicate plastic wall is formed.This is called primary cell wall. Primary wall occurs just below the middle lamella and it appears first in the ontogeny along with the middle lamella. The primary wall consists of cellulose micro-fibrils which are embedded in a highly hydrated amorphous matrix. Approximately 100 long chains of cellulose make up an elementary fibril or micelle. Approximately 20 elementary fibrils constitute a micro fibril. 250 micro-fibrils are interwoven to form a fibril and these fibrils form a network. The matrix consists of two major groups of polysaccharides usually called *hemicellulose* and *pectins* plus a small amount of *structural* proteins (Fig. 1.3).

Cellulose micro-fibrils are relatively stiff structure that contributes to the strength and structural bias of the cell wall. Cell wall is very stable and only breaks down at specific times in development, such as *senescence* and *abscission.* Cellulose has high tensile strength, equivalent that of steel. It is insoluble, chemically stable and relatively imrnune to chemical and enzymatic attack. These properties make cellulose an excellent structural material for building a strong cell wall. Cellulose micro-fibrils are synthesized by large ordered protein complexes called particles rosettes or terminal complexes that are embedded in the plasma membrane.

These structures are believed to contain many units of *cellulose synthase.* In fungi, fibrils are made up of *chitin.* Hemicelluloses are a heterogenous group of polysaccharides and are flexible, in primary cell wall of dicotyledons; the most abundant hemicellulose is *xyloglucan.* In cereal grasses it is β-*D-glucan* and *arbinoxylon.*

Cellulose ⟶ Micelle ⟶ Micro-fibril ⟶ Fibrils ⟶ Network

Like the hemicelluloses, pectins constitute a heterogenous group of polysaccharides, characteristically containing acidic sugars such as *galacturonic acid* and neutral sugars such as *rhamnose, galactose* and *arabinose.* Pectins are the most soluble of the wall polysaccharides, they can be extracted with hot water or with calcium chelators. Pectin forms hydrated gels in which the charged carboxyl groups [COO^-] of neighbouring pectin molecules are ionically linked together via Ca^{++}.

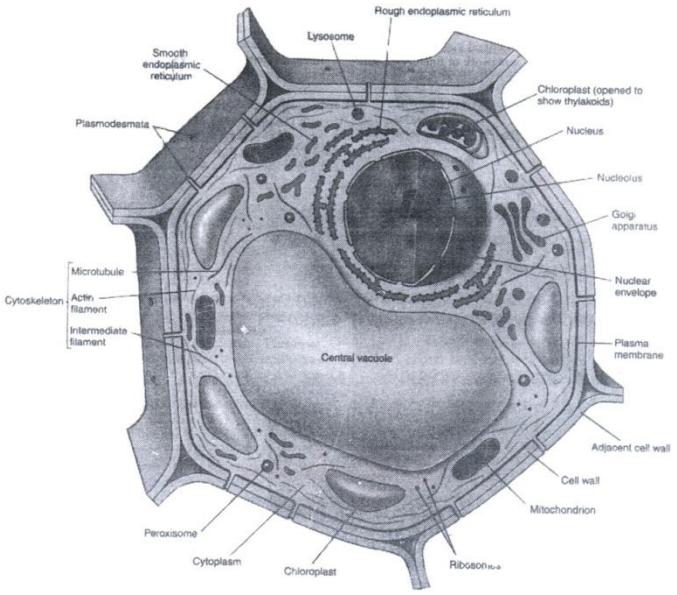


Figure 1.2: Structure of a plant cell. Most mature plant cells contain large central vacuoles, which occupy a major portion of the internal volume of the cell, and organelles called chloroplasts, withinwhichphotosynthesis takes place. The cells of plants, fungi, and some protists have cell walls, although thecomposition of the walls varies among the groups. Plant cells have cytoplasmic connections throughopenings in the cell wall called plasmodesmata

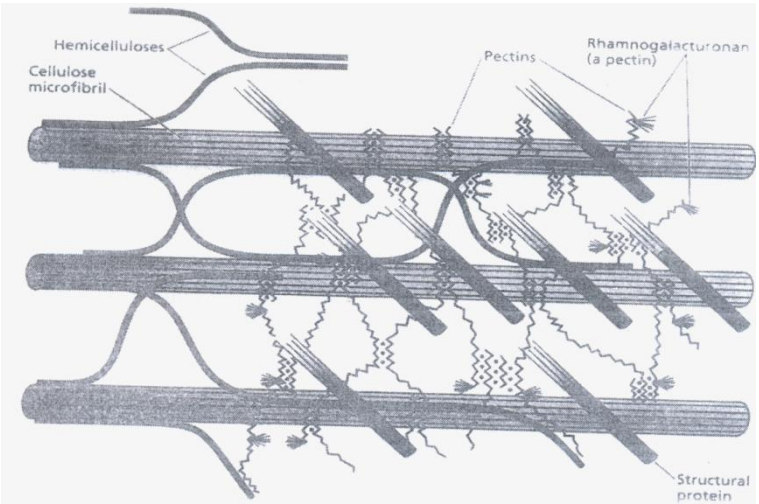


Figure 1.3: Schematic diagram of the major structural components of the primary cell wall and their likely arrangement. Cellulose microfibrils are coated with hemicelluloses (such as xyloglucan), which may also cross-link the microfibrils to one another. Pectin form an interlocking matrix gel, perhaps interacting with structural proteins

Pectins form a gel phase in which the cellulose-hemicellulose network is embedded. They determine the porosity of the cell wall to macro-molecules. The cell wall contains several classes of structural protein. These are hydroxyl proline rich glycoproteins (HRGP), glycine rich protein (GRP) proline-rich protein (PRP) and arabino galactan proteins (AGP) etc. HRGPs are involved in protection against pathogens and desiccation and perhaps in strengthening of walls. HRGPs may also serve as nucleating sites for lignification during the formation of secondary walls. AGPs may function in cell adhesion and in cell signaling during cell differentiation. When Ca^{2+} and Mg^{2+} ions are not present, pectin is a soluble compound. The Ca^{2+} and Mg^{2+}salts of pectin form an amorphous, deformable gel which is able to swell.

The food industries and cooks make use of this property of pectin when preparing jellies and jams. The primary wall also contains much water. In due course of time additional thickenings develop on the inner surface of primary cell wall. These thickenings may be homogenous or localized.

Secondary wall

Secondary walls are often (but not always) impregnated with lignin. Lignin adds significant mechanical strength to cell walls and reduces the susceptibility of walls to attack by pathogens. Lignin also reduces the digestibility of plant material by animals. The incorporation of lignin into the secondary wall gives the cell a woody structure. In lignifications the entire protoplast is used up in the deposition of lignin. Therefore, lignified cells are dead when mature. Thus lignified cells become functional only after they are dead. Lignification is characteristic of woody tissues. Lignification is indication of transition from aquatic habit to terrestrial habit because mechanical support is required in land habit. First, the lignin is deposited in middle lamella and primary wall and later on in secondary wall. Like cellulose, lignin is permeable to water and substances dissolved in it. The cell walls also contain waxes, cutin in and suberin. Cutin in found on the outer wall of epidermal cells of above ground parts of plants. This is formed by some change in fats. This is impermeable to water due to which it greatly reduces water loss due to transpiration.

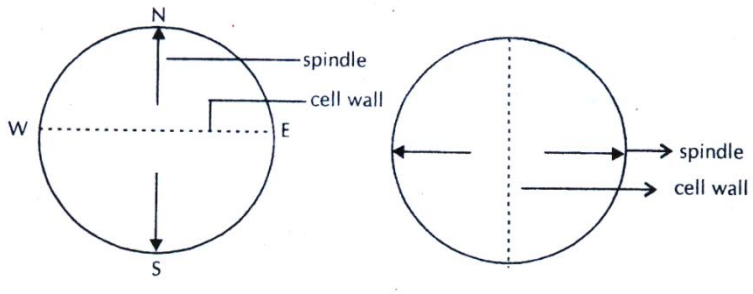


Figure 1.4: This figure shows that cell wall formation is always perpendicular to spindal fibres

The deposition of cutin on cell walls is called *cuticle*. This is specially prominent in *xerophytic plants.*Deposition of suberin in known as suberisation which is chemically fat. Suberisation occurs in the

cells of cork and endodermis. Suberin is also impermeable to water and gases. Suberised cells are dead when mature. Mucilage and gums are found especially in seed coats and cells of aquatic plants. Sometimes mineral crystals are impregnated in cell wall. In grasses these are silica particles. The cell wall of members of family Moraceae, Cucurbitaceae, Acanthaceae and Urticacea contains crystals of calcium oxalate and calcium carbonate.

New substances are deposited on the primary cell wall in two ways (Fig. 1.5 a & b):

(1) Thickening of cell wall may occur due to deposition of permanent wall material as a layer below the first wall. This process is known as aposition.

(2) Sometimes cell wall is thickened due to deposition of permanent wall material in the space between the particles of first wall. This is called intussusception.

(3)

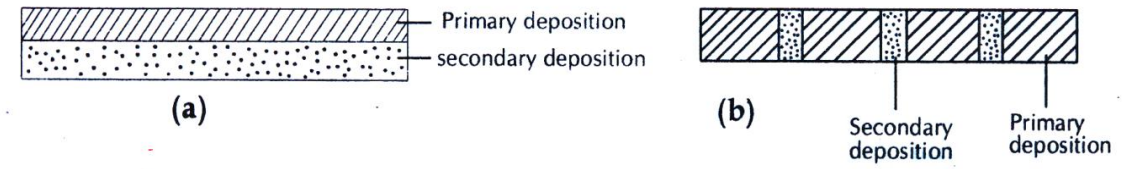


Figure 1.5 (a) Thickening of cell wall by means of apposition (b) Thickening of cell wall by means ofintussusception

Cell wall is not continuous; it has pore through which cytoplasmic bridges known as plasmodesmata, pass and interconnect the protoplast of adjoining cells. This system of living cells is known as symplast. Because all plant cells are separated by cell walls through which mineral nutrients diffuse readily, ions can diffuse across a tissue entirely through the cell wall space without ever entering a living cell. This continuum of cell walls is called the free space or *apoplast* (Fig. 1.6). Coordination of cellular activities requires cell-cell communication. Cells can communicate either symplastically via plasmodesmata or apoplastically through the cell wall.

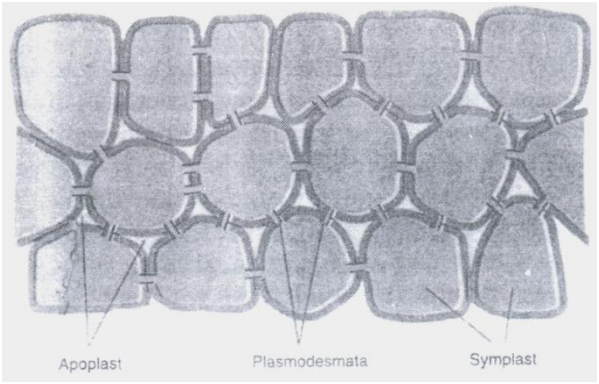


Figure 1.6: Plasmodesmata connect neighbouring cells to form a symplast. The extracellular spaces between the cell walls form the apoplast

Plasmodesmata

These are tubular extensions of the plasma membrane 40 to 50 nm in diameter that traverse thecell wall and connect the cytoplasm of adjacent cells. Intercellular transport of solutes through plasmodesmata is called *symplastic transport.* Each plasmodesma contains a narrow tubule of endoplasmic reticulum called desmotubule.The desmotubule is continuous endoplasmic reticulum of the adjacent cells. Globular proteins are associated with both the desmotubules membrane and the plasma membrane within the pore (Fig. 1.7).

Viruses spread from cell to cell by passing through plasmodesmata after invading the plant. A number of plant viruses, including TMV, cause the synthesis of virus movement proteins which can widen the plasmodesmata to such an extent that a virus particle can slip through it. bBecause sieve tubes lack a nucleus, they are enable to produce mRNA or ribosomes and are therefore unable to synthesize proteins. There is evidence that the functional proteins of the sieve elements are synthesized by companion cells and move into the sieve element through plasmodesmata connecting them. Water can also move through plasmodesmata.

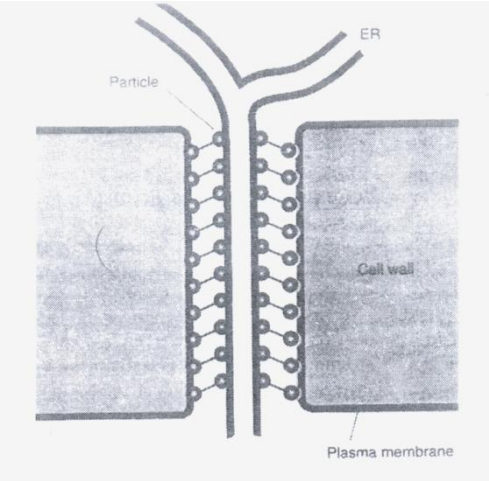


Figure 1.7: Diagram of a plasmodesm

Functions of cell wall

Theplant cell wall is not simply an inert and static exoskeleton. In addition to its role in mechanical restraint, the plant cell wall serves as an *extracellular matrix*, interacts with cell surface proteins, providing positional and developmental information. It contains numerous enzymes and smaller molecules that are biologically active and can modify the physical properties of the wall. The cell wall of prokaryotes, fungi, algae and plants, all serves two primary functions : regulating cell volume and determining cell shape. The cell wall constitutes a kind of exoskeleton that provides protection and mechanical support of plant cells. Fragments of wall, called oligosaccharins, act as hormones. Oligosaccharins, which can result from normal development or pathogen attack, serve a variety of functions including:

(a) Stimulate ethylene synthesis

(b) Induce phytoalexin synthesis

(c) Induce chitinase and other enzymes

(d) Incresase cytoplasmic calcium s and

(e) Cause an "oxidative burst".

It is a major structural barrier to pathogen invasion. This burst produces hydrogen peroxide, superoxide and other active oxygen species that attack the pathogen directly or cause increased cross-links in the wall making the wall harder to penetrate Recognition responses of cell wall are very important for example:

(a) The wall of Roots of legumes is important in the nitrogen-fixing bacteria colonizing the root to form nodules and

(b) pollen-style interactions are mediated by wall chemistry.

In addition to having biological functions, the plant cell wall is important in human economics. As a natural product, the plant cell wall is used commercially in the form of paper, textiles, fibres (cotton, flax, hemp etc), charcoal, lumber and other wood products.

Molicular organization of cell membranes

All cells are enclosed in a membrane that serves as their outer boundary separating the cytoplam from the external environment. Cell membrane is the first living structure of all the cells. They organise complex reaction sequences and are central to both biological energy conservation and cell-to-cell communication. Membranes are flexible, self-sealing and selectively permeable to polar solutes. Membranes of RBCs were first to be studied in detail. Among all animal and plant cells none has been more extensively studied than the mammalian erythrocyte. Because of the red cell's relative simplicity, its membranes are easily separated from other cytoplasmic constituents by Centrifugation following osmotic lysis of cells. The plasma membrane and the membranes of endoplasmic reticulum have many properties in common. In prokaryotes (which lack an endoplasmic reticulum), the plasma membrane plays many of the role of the endoplasmic reticulum of eukaryotic cells.

Structure

At the present time, the most widely accepted model of membrane structure is the fluid mosaic model proposed by S. J. Singer and G. Nicholson. According to this model, the membrane contains a bimolecular lipid layer, the surface of which is interrupted by proteins. The lipid bilayer is effectively a two-dimensional fluid. The bilayer gives cells flexibility and a self-sealing potential being essential when a cell divides. With their ability to break and reseal, two membranes can fuse, as in *exocytosis*, can undergo fission to yield two sealed compartments, as in *endocytosis* or cell division, without creating gross leaks through cellular surfaces.The membrane structure is called a lipid fluid mosaic. Here, laterally

mobile protein molecules, are present in a two-dimensional lipid layer. Such proteins are called intergral proteins. Other proteins are called peripheral proteins because they associate with the periphery of the membrane (Fig.1.8). Peripheral proteins stud the inside and outside of the membrane. The best studied peripheral proteins are located on the internal (cytosolic surface) of the plasma membrane, where they form a fibrillar network that acts as a membrane "skeleton". These proteins provide mechanical support for the membrane and function as an anchor for integral membranes.

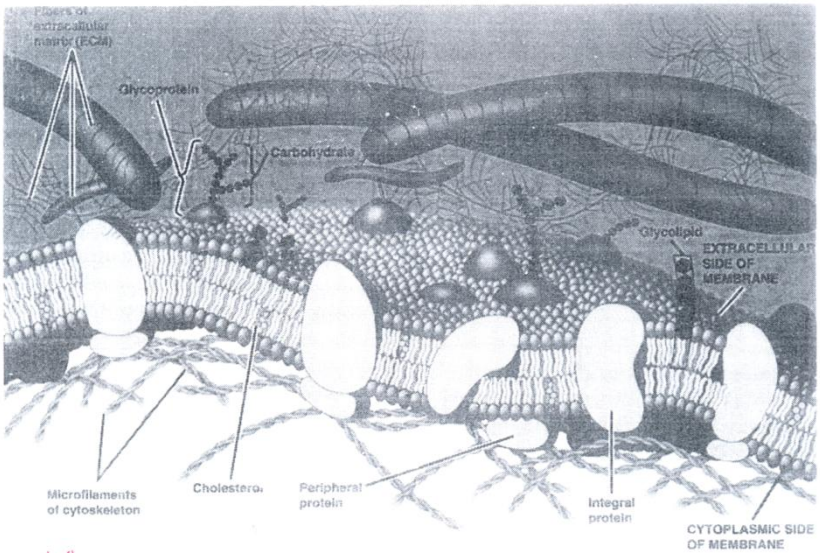


Figure 1.8: The detailed structure of animal cell's plasma membrane (cross section)

Other peripheral proteins on the internal plasma membrane surface function as enzymes, specialised coats or factors that transmit transmembrane signals. Peripheral proteins associated with the external surface of the plasma membrane are typically part of the extracellular matrix. Integral proteins are very firmly associated with the membrane. They contain both hydrophilic and hydrophobic regions. The hydrophillic portions of the protein interact with the polar heads of lipid molecules at each surface of the bimolecular leaflet. Parts of proteins that are buried in the hydrophobic portion of the lipid bilayer are rich in amino-acids with hydrophobic side chains. These side-chains are believed to form hydrophobic bonds with hydrocarbon tails of the membrane phospholipids. Proteins that serve as ion channels are always integral membrane proteins. Integral proteins plays a role in many other cellular processes. They serve as transporters, as receptors for hormones, neurotransmitters and growth factors. They are central to oxidative phosphorylation and photosynthesis, cell-to-cell and antigen-cell recognition in the immune system. Integral proteins are also major players in the membrane fusion that accompanies exocytosis and endocyotsis and the entry of many type of viruses into host cells.

The peripheral proteins and those parts of the integral proteins that occur on the outer membrane surface frequently contain chains of sugars and forms glycoproteins. Some of the lipid at the outer surface is complexed with carbohydrate to form glycolipids. Glycolipids and glycoproteins collectively form glycocalyx and function as "finger print" of the cell. Glycolipids are involved in tissue recognition, as

antigens of ABO blood group. While glycoproteins are involved in cell recognition such as *major histocompatibility complex (MHC).* Glycodipids are always on the outer side of the cell membrane so that their sugars point outwards from the cell into the external aqueous environment. This asymmetry is preserved by the fact that transverse movements of lipids known colloquially as *"flip-flop"* is severely restricted; such movement would involve transferring the polar heads through the central hydrocarbon layer to get to the other side. This is energetically unfavourable and so the asymmetry preserved.

Sterols are also found in biological membranes. It is cholesterol in animal's cells, while in plant cells phytosterols are reported. Cholesterol in membranes acts as a *"fluidity buffer".* It is shorter than the hydrocarbon tails of the polar lipids and inserted between the membrane lipids. It acts as a wedge between the chains near the surface of the membrane and thereby makes the bilayer less permeable to small molecules. It also prevents close packing of the ends of the hydrocarbon tails of the fatty acids and thus lowers the melting point in the centre of the bilayer. Cholesterol "blurs" the melting point of a lipid bilayer. Without cholesterol, the transition from solid to liquid is sharper than when cholesterol is present. Sterols are not found in the membranes of prokaryotic cells (except *Mycoplasma)* and are also absent from the inner membranes of both mitochonodria and chloroplasts, which are believed to be derived evolutionarily from the plasma membranes of prokaryotic cells. However, the plasma membranes of atleast some prokaryotes, including both bacteria and cyanobacteria, contain sterol-like molecules called hopanoids that appear to substitute for sterols in the membrane structure. Nearly all the membrane studied so far appear to contain the same type of lipid molecules. Lipids exhibit a higher degree of mobility in membranes than do proteins. The mobility of lipid and protein molecules in the plasma membrane attests to the membrane fluidity.

C.F. Fox and H.M. McConnell have shown that the degree of fluidity is dependent, on the fatty acid contents of side chains of phospholipids in the membrane. Fatty acid side-chains of membrane phospholipids can be either saturated or unsaturated. The degree of saturation of fatty acid tails is of great importance because the central hydrocarbon core of the lipid bilayer must be fluid rather than solid. Unsaturated hydrocarbon tails reduce the temperature at which a bilayer loses its essential fluidity. Membrane fluidity is essential to allow lateral movement of transmembrane proteins so that they can interact with one another. In addition, such proteins undergo conformational changes needed for ion channels transporters and receptors to function. Without a fluid bilayer such changes might be difficult to accomodate. In membrane phospholipids, one of the fatty acids is typically saturated, the other fatty acid chain usually having one or more *is double bonds.* The double bonds of the unsaturated side chains produce bends (kink) in the hydrocarbon chains, and these give rise to structural deformations that prevent formation of the more rigid crystalline structure. Cells can alter the balance of saturated and unsaturated fatty acids in their membranes as an adjustment to changing temperature or other factors. Membrane proteins have been shown to possess enzymatic activity.

In special situations, such as during hibernation, animals modulate the degree of saturation of cell membrane components to cope with lower body temperature Poikilothermic (cold blooded/ectothermic) animals (all non-vertebrates, fish, amphibia, reptiles), usually have a higher proportion of unsaturated fatty acids than homoiothermic (warm blooded/endothermic), animals (birds and mammals) because body temperature of ectothermic animals becomes lower in cold environments. Lipids rich in unsaturated fatty acids generally remain liquid at temperatures lower (usually 5°C or lower) than those rich in saturated fatty acids. This may be necessary if the lipid is to maintain its function, such as constituent of membranes. To maintain membrane fluidity cells living at low temperatures, have higher proportion of unsaturated fatty acids in their membrane than do cells athigher temperature.

More than 30 enzymes have been detected in isolated plasma membrane. Some ofthem are as follows

1. Adenylate cyclase (AC)
2. Marker enzymes (ME)
3. Alkaline phosphodiesterase; (ME)
4. Leucine amino peptidase-(ME)
5. 5-nucleotidase; (ME)
6. Amino peptidase; (ME)
7. Adenosine triphosphatase; (ME)
8. Acid phosphatase
9. Acetyl c holine esterase
10. Acetyl phosphatase
11. Cellobiase
12. Guanylate cyclase
13. Cholesterol esterase
14. Lactase
15. Maltase
16. Sucrase
17. RNA ase
18. Phospholipase-A
19. UDP glycosidase
20. Na^+/K^+ ATPase

Of all the membrane associated enzymes, Na^+/K^+ ATPase is one of most important because of its role in ion transfer across plasmamembrane. Enzymes disposed in the plasma membrane may be characterised according to the membrane face containing the enzymatic activity. Accordingly *ectoenzymes* are those enzymes whose catalytic activity is associated with theexterior surface of the plasma membrane, the activity of plasma membrane *endoenzymes* is associated with interior of the cell.

(ISBN: 978-93-88901-16-1)

Chapter 2

Nucleus Structure

Nucleus: Ultrastructure

Robert Brown for the first time in 1833 discovered a prominent body within the cell and termed it nucleus. A synonymous term for this organelle is the Greek word karyon. The nucleus can be called as the controlling centre of the cell, since it contains the chromosomes and the genes, which control all activities of an individual cell. The chromosomes which are present within the nucleus are responsible for deciding whether a fertilized egg will develop into a cow, a mango tree or into a man.

Significance of nucleus

Hammerling's experiment

J. Hammerling**,** a German biologist, demonstrated in 1934 that the nucleus determines the characters of the cell and ultimately the characters of the individual. He conducted certain experiments using two species of a green alga, Acetabularia. The two species, namely A. crenulata and A. mediterranea used in this experiment differ in the shape of their caps. While in A. crenulata the cap has loose rays, in A. mediterranea an umbrella-like cap is found.

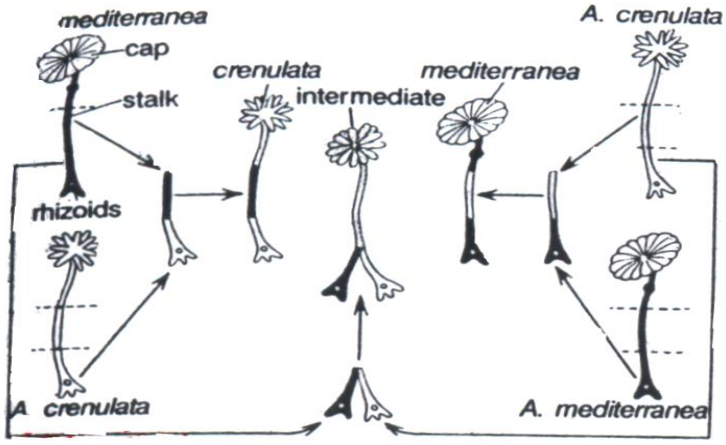


Figure 2.1: Hammerling's experiment in *Acetabularia* showing relative roles of nucleus and cytoplasm

The nucleus in both the species is situated in rhizoid at the bottom of stalk. If cap is cut off, it will develop again and its shape will be that of the original type. However, if after removing the caps, stalk of one species, is grafted on rhizoid (containing the nucleus) of the other species, shape of cap will be determined by nucleus and not by stalk (Fig. 2.1). If the nucleus belongs to A. crenulata, shape of cap will be of the crenulata type and if the nucleus comes from A. mediterranea, cap will be of mediterranea type. When both nuclei are present, shape of cap will be intermediate. This experiment demonstrated clearly that characters of an individual are controlled by nucleus of the cell or cells.

Number, shape and size of nucleus

Generally there is asingle nucleus per cell (mononucleate conditions), but more than one nucleus (polynucleate condition) may be found in certain special cases. There may be many nuclei in a **syncytium,** which is formed due to fusion of cells. A similar multinucleate situation is found in coenocytes commonly found in plants. A coenocyte results by repeated nuclear divisions without cytokinesis. There are variations with respect to shape of nucleus also. It may be spherical, oval to flattened lobe or irregular in shape. In most cases it has a regular outline, but may also have irregular outline. Shape of nucleus also depends on the cell. In spheroid, cuboid or polyhedral cells, nucleus is usually spheroid. In cylindrical, prismatic or fusiform cells, nucleus is ellipsoid. Irregular nuclei are sometimes found in some neutrophylls or leukocytes and branched nuclei are sometimes found in glandular cells. In still other cases like spermatozoa, pyriform or lanceolate nuclei may be found. Size of the nucleus would also vary not only depending upon the type of cell involved but also according to activity of cell. The nucleus will be larger in an active cell, but will be smaller in a resting cell.

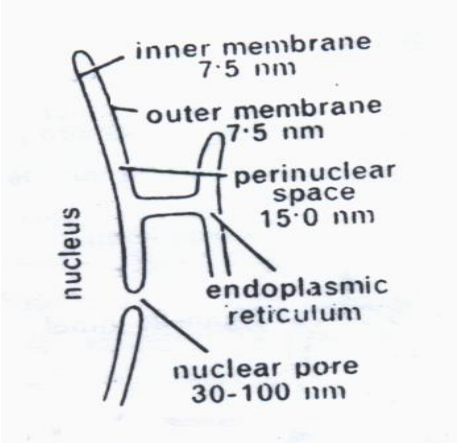


Figure 2.2: Nuclear membrane showing double structure

Nucleus in prokaryotes and eukaryotes

On the basis of presence or absence of well-defined nucleus, living organisms have been classified into two groups by molecular biologists in recent years. These groups are

(i) Prokaryotes, the individuals which do not have a well-organized nucleus and will therefore include viruses, bacteria and blue-green algae; and

(ii) Eukaryotes, which would include the remaining types, which have a well-organized nucleus. The nuclear equivalent of a prokaryotic organism' is known as prokaryon or more commonly as nucleoid rather than a nucleus. The 'prokaryon' or 'nucleoid' does not have a true chromosome, is not enclosed in a nuclear envelope and does not divide by regular mitosis. The nuclei may even be absent in some specialized cells of eukaryotes. For instance mature mammalian red blood cells are also without any nuclei. This is why they are often called as red blood corpuscles rather than cells.

A nucleus may be described as having three important parts, namely, nuclear membrane or nuclear envelope, nucleolus and chromosomes. The fluid, in which nucleolus and chromosomes are present and which is enclosed in nuclear membrane, is called nucleoplasm.

Nuclear envelope

Nuclear boundary of interphase and prophase nuclei is called nuclear membrane or nuclear envelope. It breaks down at the end of prophase and is reformed at the end of the nuclear division. It consists of a double membrane having two unit membranes (Fig. 2.2). The space between two unit membranes varies in width and is known as perinuclear space. Outer membrane is continuous with the endoplasmic reticulum.

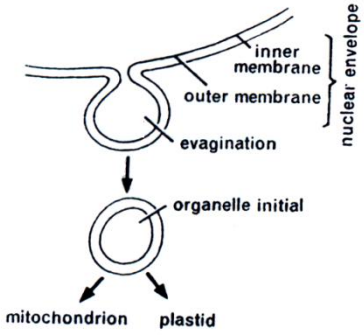


Figure 2.3: **Formation of organelle initial from nuclear envelope**

Each unit membrane is 7,5 nm (1 nm = 10 A) in diameter and perinuclear space may vary from regular 15 nm wide spaces to irregular cavities several hundred times wide. Outline of nuclear envelope is smooth and interrupted by pores which appear circular in surface view. Diameter of these pores varies from 30 nm 100 nm. In sections, it is obvious that at the boundary of these pores, outer and inner unit membranes are joined. These pores provide direct contact between nucleus and cytoplasm and allow import and export of protein and, RNA (particularly export of messenger RNA, which is synthesized in the nucleus and then reaches cytoplasm for protein synthesis). The double membrane also takes part in giving rise to the initials of cell organelles like mitochondria or plastids (Fig. 2.3).

Nuclear pore complex and nucleocytoplasmic traffic

Nuclear pore occupies a central position among the major cellular structures, but still remains one of the least understood structures. However, during the late 1980s and early 1990s, significant progress has been made towards a better understanding of the structure and function of the nuclear pore. During this recent past, new pore proteins have been identified (particularly in yeast), the genes for several of these proteins have been cloned, a number of mutants in these pore proteins have been isolated and detailed mechanism of nucleocytoplasmic traffic has been proposed. Further, the pore has been

reconstituted *in vitro*, a number of 'signal sequences' and one or more 'signal sequence receptors' have been identified and a new 'basket-like structure' has been found attached to the inner side of the nuclear pore.

Structure of nuclear pore complex

The nuclear pore is a large complex structure of 124 million daltons or 30 times the size of a eukaryotic ribosome. The pore is 120nm in diameter and 50nm in thickness. It consists of four separate elements

(i) The scaffold, which includes majority of the pore,

(ii) The central hub or transporter, which carries out active transport (both import and export) of proteins and RNAs,

(iii) Short thick filaments attached to the cytoplasmic side of the pore and

(iv) A newly discovered basket attached to the nucleoplasmic side of the pore. The scaffold is a stack of three closely apposed rings, namely the cytoplasmic ring, the nucleoplasmic ring and a central ring of thick spokes. Each ring has a eightfold symmetry.

The spokes of central ring are attached to the transporter on the inner side, and to the nucleoplasmic and cytoplasmic rings on the outer side. Interspersedbetween the spokes are aqueous channels, 9nm wide, which allow diffusion of proteins and metabolites between the nucleus and the cytoplasm. The transporter is aproteinaceous ring, 36-38nm in diameter and consists of two irises of eight arms each. The two irises are assumed to be stacked atop one another and open sequentially, each like the diaphragm of a camera, to let a nuclear protein or RNA to pass through from the nucleus to the cytoplasm. On the cytoplasmic side of the pore, thick fibres (3.3nm in diameter) extend into the cytoplasm. On the nuclear side, a large basket like structure is found, which consists of eight filaments (each lOOnm long), extending from nucleoplasmic ring of the pore and meeting a smaller ring (60nm in diameter) within the nucleus. This basket may play an important role in RNA export. The detailed structure of nuclear pore complex is shown in Figure 2.4.

Role of nuclear pore in transport

The import and export of proteins and RNAs into and outside the nucleus are facilitated by the presence of signalsequences. Even gold particles 25nm in diameter, if coated with a signal sequence—bearing nuclear protein, are transported into and outside the nucleus. ATP has also been found to be essential for nuclear transport. The transport (this is usually unidirectional) actually involves following two steps:

(i) An ATP-independent, but signal-sequence dependent step involving binding of protein to the pore, and

(ii) An ATP-requiring step, involving translocation through the pore; this step is the only energy requiring step. It has been shown that in the absence of ATP, the protein binds to the pore, but can not be transported to the nucleus.

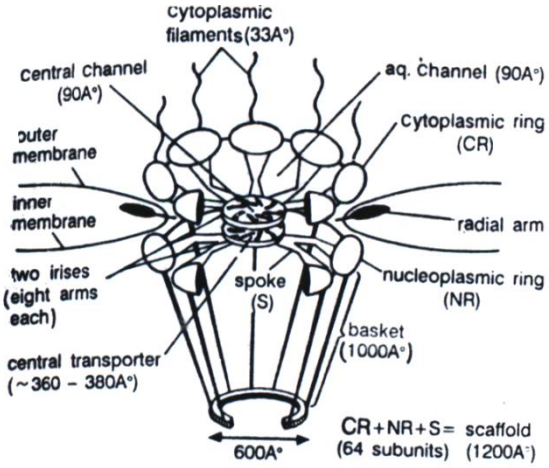


Figure 2.4: Detailed structure of the nuclear pore, showing iris and transporter

(A) Protein import and export

Nuclear proteins,' like other proteins, are synthesized in the cytoplasm, and are later imported into the nucleus. Mostly, these proteins are transported unidirectionally from the cytoplasm to the nucleus and cannot exit the nuclear pore, once they are imported. Such proteins include SV40 T antigen and *Xenopus* nucleoplasmin, which have been studied in some detail. There are few other proteins, which shuttle between nucleus and cytoplasm, either constitutively (irrespective of requirement) or in response to regulatory signals. These include various nuclear proteins like hsc70 and the cAMP dependent protein kinase. It is predicted that these proteins contain an additional signal sequence for protein export. Some of the ribosomal proteins are imported to associate with rRNA, so that the ribosomal subunits are assembled inside the nucleus and then exported through the pore to the cytoplasm, a process which is ATP-dependent. Although several signal sequences for import have been identified, no signal sequences for export are known. These should be discovered only in future.

Signal sequences that have been identified for many nuclear proteins to be imported, have often been compared with SV40 T antigen sequence, Pro-Lys-Lys-Lys-Arg-Lys-Val. In this sequence, mutation of second lysine to threonine greatly reduces import, suggesting the role of this amino acid in import. Many other signal sequences resemble more closely, the first identified signal sequence, i.e. *Xenopus* protein nucleoplasmin, whose gene has also been cloned now. The signal sequence of nucleoplasmin is larger (16 amino acids) than that for T-antigen,and has two independent basic domains separated by 10 spacer amino acids. It is now believed that the bipartite nucleoplasmin signal sequence will be the type most often found in other proteins.

The existence of nuclear signal sequences predicts that there must also exist one or more receptors for these signals. Some such receptors have been isolated (particularly in yeast) and are believed to be located either in the pore or in the cytoplasm. In the latter case, they bind the signal sequence and carry the protein to the pore.

(B) RNA export and import

One of the most vital roles of nuclear pore is the unidirectional export of RNA, including tRNAs, snRNAs (small nuclear RNAs) and processed mRNA. Ribosomal RNA is used in the nucleus for assembly of small subunits, which are later exported, as discussed above. Thus, the rRNA and unprocessed mRNA are not allowed to be exported. It has been shown that for export of processed mRNAs and snRNAs, the 5' monomethyl cap acquired during transcription is essential. Several cap binding proteins have been identified and may help in RNA export. They may work as RNA export receptors or may contain protein export signal, which helps the export, of cap-binding protein-mRNA complex.

Few cellular RNAs are also imported into the nucleus. These include snRNAs (particularly Ul and U2), which are synthesized with monomethyl cap and are first exported to the cytoplasm, to be converted into dimethyl form, which is assembled as snRNPs (small nuclear ribonucleo-proteins). These snRNPs, with the help of a bipartite signal (one for export and other for import), are reimported into the nucleus. T-DNA of Ti plasmid of *Agrobacterium tumefaciens* is an example of the import of DNA into the nucleus.

Nucleolus

In higher organisms, every cell nucleus has a spherical, colloidal body called nucleolus, which is associated with a particular nucleolar organizing chromosome (Fig. 2.5). A special region in this chromosome is known as the nucleolar organizing region to which usually the nucleolus is associated (Fig. 2.6). During meiosis as well as during mitosis, the nucleolus disappears during prophase. It reappears again during telophase and is associated with the same known nucleolar organizing chromosome quite often; more than one nucleus in the same nucleus may also be observed. These several nucleoli may subsequently coalesce and give rise to larger single nucleolus. While chromatin mainly consists of DNA, nucleolus mainly consists of RNA, acidic dyes and basic dyes; phospholipids and alkaline phosphatase are also found. Nucleolar DNA had also been reported in a number of cases. This DNA is believed to represent the nucleolar organizer.

Although various functions have been assigned to nucleolus, presently it is believed that the most important function of nucleolus is synthesis of ribosomal RNA (rRNA). This has been proved by the fact that DNA complementary to rRNA seems to be clustered at the nucleolar organizer.

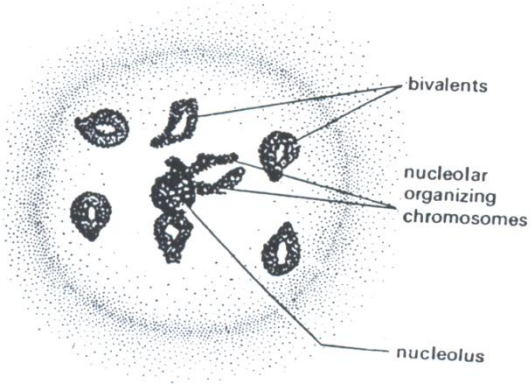


Figure 2.5: A diakinesis cell showing attachment of abivalent wiyh nucleolus

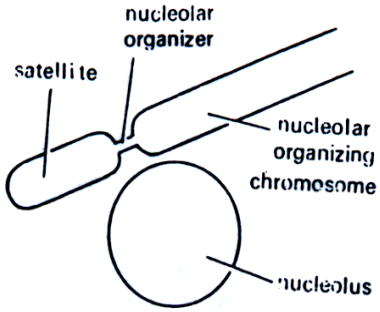


Figure 2.6: A satellited chromosome and an attached nucleolus

The initiation, production and maturation of ribosomes in the nucleolus seem to proceed from centre to the periphery in the following three distinct regions:

(i) Fibrillar centre, where rRNA genes of NOR (nucleolar organizing region) are located; the transcription of rRNA genes (RNA synthesis on DNA template) also takes place in this region,

(ii) Fibrillar component, which surrounds the fibrillar centre and where RNA synthesis progresses. The 80s ribosomal proteins (rps) also bind to the transcripts in this region,

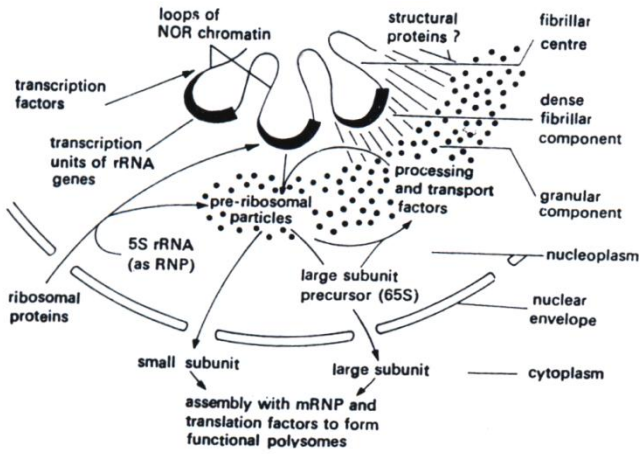


Figure 2.7: Three regions of the nucleolus showing their roles in ribosomal RNA (rRNA) synthesis

(iii) Cortical granular component, which is the outermost region and where processing and maturation of pre-ribosomal particles occurs. These threeregions of the nucleolus and their roles in ribosome formation are shown infigure 2.7.

Nucleic Acids

There are two types of nucleic acids, namely deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Primarily, nucleic acids serve as repositories and transmitters of genetic information.

Brief history

DNA was discovered in 1869 by johann Friedrich Miescher, a Swiss researcher. The demonstration that DNA contained genetic information was first made in 1944, by Avery. Macleod and MacCary.

Functions of nucleic acids

DNA is the chemical basis of heredity and may be regarded as the reserve bank of genetic information. DNA is exclusively responsible for maintaining the identity of different species of organisms over millions of years. Further, every aspect of cellular function is under the control of DNA. The DNA is organized into genes, the fundamental units of genetic information. The genes control the protein synthesis through the mediation of RNA, as shown below

DNA→ **RNA**→ Protein

The interrelationship of these three classes of biomolecules (DNA, RNA and proteins) constitutes the central dogma of molecular biology or more commonly the central dogma of life.

Components of nucleic acids

Nucleic acids are the polymers of nucleotides (polynucleotides) held by 3' and 5' phosphate bridges. In other words, nucleic acids are built up by the monomeric units—nucleotides (It may be recalled that protein is a polymer of amino acids).

Nucleotides

Nucleotides are composed of a *nitrogenous base*, a *pentose sugar* and a *phosphate.* Nucleo tides perform a wide variety of functions in the living cells, besides being the building blocks or monomeric units in the nucleic acid (DNA and RNA) structure. These include their role as structural components of some coenzymes of B-complex vitamins (e.g. FAD, NAD*), in the energy reactions of cells (ATP is the energy currency), and in the control of metabolic reactions.

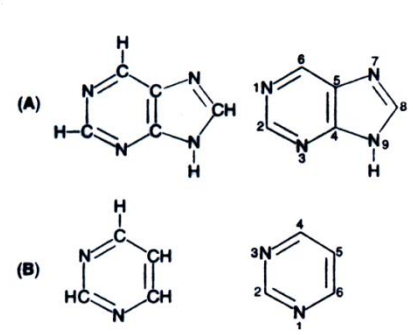


Figure 2.8: General structure of nitrogen bases (A) Purine (B) Pyrimidine (The positions are Numbered according to the international system)

Structure of nucleotides

As already stated, the nucleotide essentially consists of *nucleobase, sugar* and *phosphate.* The term nucleoside refe[r]s to base + sugar. Thus, nucleotide is nucleoside + phosphate.

Purines and pyrimidines

The nitrogenous bases found in nucleotides (and, therefore, nucleic acids) are *aromatic heterocyclic compounds.* The bases are of two types—purines and pyrimidines. Their general structures are depicted in *Fig.2.8.* Purines are numbered in the anticlockwise direction while pyrimidines are numbered in the clockwise direction. And this is an internationally accepted system to represent the structure of bases.

Major bases in nucleic acids

The structures of major purines and pyrimidines found in nucleic acids are shown in *Fig.2.9.* DNA and RNA contain the same purines namely adenine (A) and guanine (G). Further, the pyrimidine cytosine (C) is found in both DNA and RNA. However, the nucleic acids differ with respect to the second pyrimidine base. *DNA contains thymine (T) whereas UNA contains uracil (U).* As is observed in the *Fig.2.9,* thymine and uracil differ in structure by the presence (in T) or absence (in U) of a methyl group.

Tautomeric forms of purines and pyrimidines

The existence of a molecule in a Irefo *(lactam)* and *enol (lactim)* form is known as tautomerism. The heterocyclic rings of purines and pyrimidines with *oxo* functional groups exhibit automerism. The purine—guanine and pyrimidines-cytosine, thymine and uracil exhibit tautomerism. The lactam and lactim forms of cytosine are represented in *Fig.2.10.* At physiological pH, the lactam (keto) tautomeric forms are predominantly present.

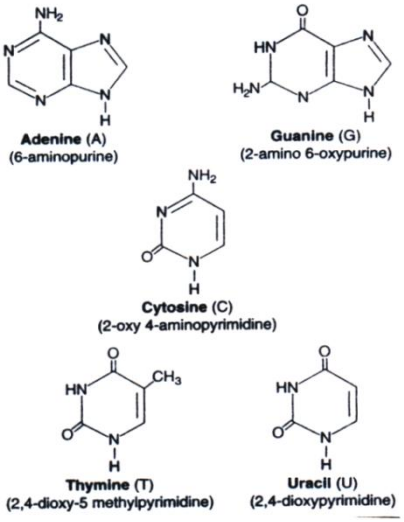


Figure 2.9: Structures of major purines (A, G) and pyrimidines (C, T, U) found in nucleic acids

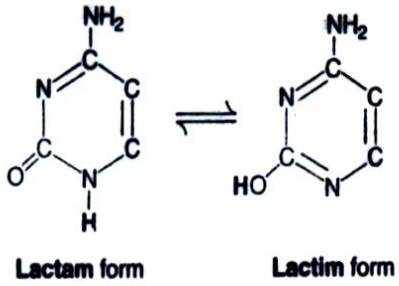


Figure 2.10: Structures of sugars present in nucleic acids (ribose is found in RNA and deoxyribose in DNA; Notethe structural difference at C_2)

Minor bases found in nucleic acids

Besides the bases described above, several minor and unusual bases are often found in DNA and RNA. These include 5-methylcytosine, N^4-acetyl-cytosine, N^6-methyladenine, N^6, N^6-dimethyl-adenine, pseudouracil etc. It is believed that the unusual bases in nucleic acids will help in the recognition of specific enzymes.

Table 2.1 Principal bases, nucleosides and nucleotides

Base	***Ribonucleoside***	***Ribonucleotide***	***Abbreviation***
Adenine (A)	Adenosine	Adenosine 5'-monophosphate or adenylate	AMP
Guanine (G)	Guanosine	Guanosine 5'-monophosphate or guanylale	GMP
Cytosine(C)	Cytidine	Cytidine 5'-monophosphate or cytidylate	CMP
Uracil(U)	Uridine	Uridine 5'-monophosphate or uridylate	UMP

Base	***Deoxyribonucleoside***	***Deoxyribonucleotide***	***Abbrevation***
Adenine(A)	Deoxyadenosine	Deoxyadenosine 5'-monophosphate deoxyor adenylate	dAMP
Guanine (G)	Deoxyguanosine	Deoxyguanosine 5'-monophosphate or deoxyguanylate	dGMP
Cytosine (C)	Deoxycytidine	Deoxycytidine 5'-monophospriate or deoxycylidylale	dCMP
Thymine (T)	Deoxythymidine	Deoxythymidine 5'-monopfi05phate or deoxythymidytate	dTMP

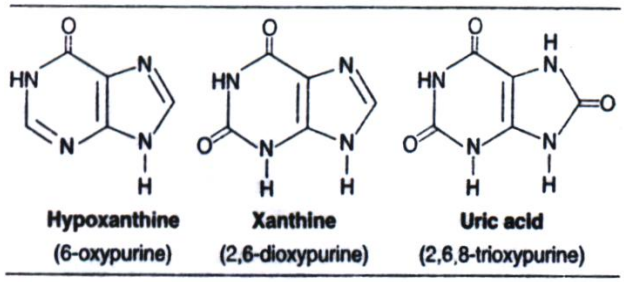


Figure 2.11: Structures of some biologically important purines

Other biologically important bases

Thebases such as hypoxanthine, xanthine and uric acid *(Fig.2.11)* are present in the free state in the cells. The former two are the intermediates in purine synthesis while uric acid is the end product of purine degradation.

Purine bases of plants

Plants contain certain methylated purines which are of pharmacological interest. These include caffeine (of coffee), theophylline (of tea) and theobromine (of cocoa).

Sugars of nucleic acids

The five carbon monosaccharides (pentoses) are found in the nucleic acid structure. *RNA* contains *D-ribose* while *DNA* contains *D-deoxyribcse*. Ribose and deoxyribose differ in structure at C_2. Deoxyribose has one oxygen less at C_2 compared to ribose *(Fig.2.12).*

Nomenclature of nucleotides

The addition of a pentose sugar to base produces a nucleoside. If the sugar is ribose, ribonucleosides are formed. Adenosine, guanosine, cytidine and uridine are the ribonucleosides of A, G, C and U respectively. If the sugar is a deoxyribose, deoxyribo-nucleosides are produced.

The term mononucleotide is used when a single phosphate moiety is added to a nucleoside. Thus adenosine monophosphate (AMP) contains adenine + ribose + phosphate. The principal bases, their respective nucleosides and nucleotides found in the structure of nucleic acids are given in Table 2.1. Note that the prefix 'd' is used to indicate if the sugar is deoxyribose (e.g. dAMP).

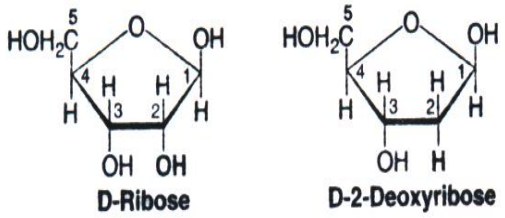


Figure 2.12: Structures of sugars present in nucleic acids (ribose is found in RNA and deoxyribose in DNA; Notethe structural difference at C_2)

The binding of nucleotide components

The atoms in the purine ring are numbered as 1 to 9 and for pyrimidine as 1 to 6 (See Fig.2.8). The carbons of sugars are represented with an associated prime (') for differentiation. Thus the pentose carbons are 1' to 5'. The pentoses are bound to nitrogenous bases by p-N-glycosidic bonds. The N^9 of a purine ring binds with $C_{1(1')}$ of a pentose sugar to form a covalent bond in the purine nucleoside. In case of pyrimidine nucleosides, the glycosidic linkage is between N^1 of a pyrimidine and C'_1 of apentose.

The hydroxyl groups of adenosine are esterified with phosphates to produce 5'- or 3'-rnonophosphates. 5'-Hydroxyl is the most commonly esterified, hence 5' is usually omitted while writing nucleotide names. Thus AMP represents adenosine 5'-monophosphate. However, for adenosine 3'-monophosphate, the abbreviation 3'-AMP is used. The structures of two selected nucleotides namely AMP and TMP are depicted in *Fig.2.13.*

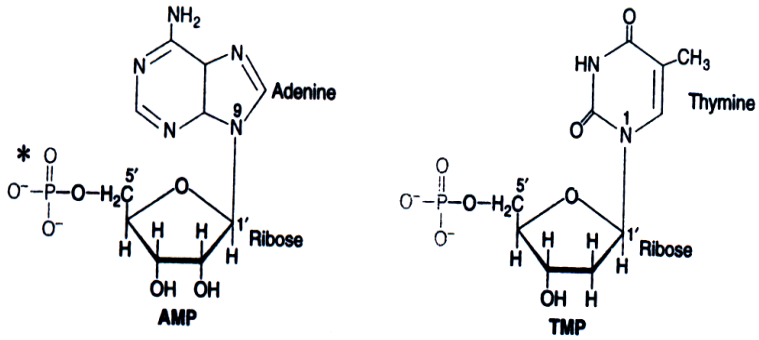


Figure 2.13: The structures of adenosine 5'-monophosphate (AMP) and thymidine 5'-monophosphate (TMP)[Addition of second or third phosphate gives adenosine diphosphate (ADP) and adenosine triphosphate (ATP) respectively]

Nucleoside di- and triphosphates

Nucleoside monophosphates possess only one phosphate moiety (AMP, TMP). The addition of second or third phosphates to the nucleoside results in nucleoside diphosphate (e.g. ADP) or triphosphate (e.g. ATP) respectively. The anionic properties of nucleotides and nucleic acids are due to the negative charges contributed by phosphate groups.

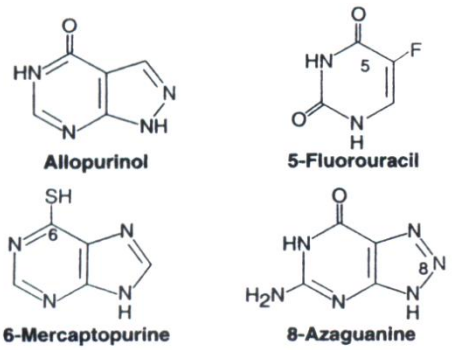


Figure 2.14: Structures of selected purine and pyrlmldine analogs

Purine, pyrimidine and nucleotide analogs

It is possible to alter heterocyclic ring or sugar moiety, and produce synthetic analogs of purines, pyrimidines, nucleosides and nucleotides. Some of the synthetic analogs are highly useful in clinical medicine. The structures of selected purine and pyrimidine analogs are given in *Fig.2.14.*

The pharmacological applications of certain analogs are listed below

1. *Allopurinol* is used in the treatment of hyperuricemia and gout
2. *5-Fluorouracil,* 6-mercaptopurine, 8-aza-guaninc, 3-deoxyuridine, 5- or 6-azauridine, 5- or 6-azacytidine and 5-idouracil are employed in the treatment of cancers. These compounds get incorporated into DNA and block cell proliferation.
3. *Azathioprine* (which gets degraded to 6-mercaptopurine) is used to suppress immunological rejection during transplantation.
4. *Arabinosyladcnint* is used for the treatment of neurological disease, viral encephalitis.
5. Arabinosylcytosine is being used in cancer therapy as it interferes with DNA replication.
6. The drugs employed in the treatment of AIDS namely zidovudine or AZT (3-azido 2',3'-dideoxythymidine) and didanosine (dideoxy-inosine) are sugar modified *synthetic nucleotide analogs.*

Structure of DNA

DNA is a *polymer of deoxyribonucleotides* (or simply deoxynucleotides). It is composed of monomeric units namely deoxyadenylate (dAMP), deoxyguanylate (dGMP), deoxy-cytidylate (dCMP) and deoxythymidylate (dTMP) (It may be noted here that some authors prefer to use TMP for deoxythymidylate, since it is found only in DNA). The details of the nucleotide structure are given above.

Schematic representation of polynucleotides

The monomeric deoxynucleotides in DNA are held together by 3',5'-phosphodiester bridges *(fig.2.15).* DNA (or RNA) structure is often represented in a short-hand form. The horizontal line indicates the carbon chain of sugar with base attached to C_1. Near the middle of the horizontal line is C_3-phosphate linkage while at the other end of the line is C_5- phosphate linkage *(Fig.2.15).*

Chargaff's rule of DNA composition

Erwin Chargaff in late 1940s quantitatively analysed the DNA hydrolysates from different species. He observed that in all the species he studied, DNA had equal numbers of adenine and thymine residues (A = T) and equal numbers of guanine and cytosine residues (C = C). This is known as Chargaff's rule of molar equivalence between the purines and pyrimidines in DNA structure. The significance of Chargaff's rule was not immediately realised. The double helical structure of DNA derives its strength from Chargaff's rule.

Single-stranded DNA, and RNAs which are usually single-stranded, do not obey Chargaff's rule. However, double-stranded RNA which is the genetic material in certain viruses satisfies Chargaff's rule.

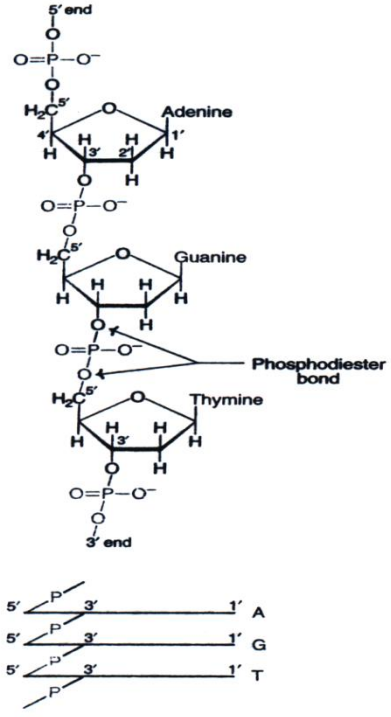


Figure 2.15: Structure of a polydeoxyribonucleotidesegment held by phosphodiester bonds. On the lowerpart is the representation of short hand form ofoligonucleotides

DNA double helix

The double helical structure of DNA was proposed by *lames Watson* and *Francis Crick* in 1953 (Nobel Prize, 1962). The elucidation of DNA structure is considered as a *milestone in* the era of *modern*

biology. The structure of DNA double helix is comparable to a twisted ladder. The salient features of Watson-Crick model of DNA (now known as B-DNA) are described next *(Fig.2.16).*

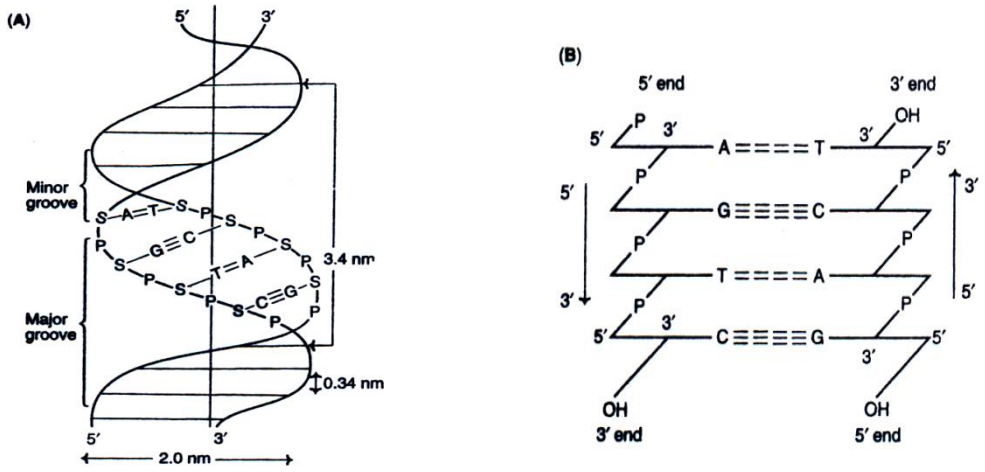


Figure 2.16: (A) Watson-Crick model of DNA helix (B) Complementary base pairing in DNA helix

1. The DNA is a right handed double helix. It consists of *two polydeoxyribonucleotide chains* (strands) twisted around each other on a common axis.
2. The two strands are *antiparallel,* i.e., one strand runs in the 5' to 3' direction while the other in 3' to 5' direction. This is comparable to two parallel adjacent roads carrying traffic in opposite direction.
3. The width (or diameter) of a double helix is 20 A" (2 nm).
4. Each turn (pitch) of the helix is 34 A^0 (3.4 nm) with 10 pairs of nucleotides, each pair placed at a distance of about 3.4 A°.
5. Each strand of DNA has a hydrophilic deoxyribose phosphate backbone (3'-5' phospho-diester bonds) on the outside (periphery) *oi* the molecule while the hydrophobic bases are stacked inside (core).
6. The two polynucleotide chains are not identical but complementary to each other due to base pairing.
7. The two strands are *held together by hydrogen bonds* formed by complementary basepairs *(Fig.2.17).* TheA-Tpairhas2hydrogenbonds while G-C pair has 3 hydrogenbonds.TheGsCisstrongerbyabout50%thanA = T.
8. The hydrogen bonds are formed between a purine and a pyrimidine only. If two purines face each other, they would not fit into the allowable space. And two pyrimidines would be too far to form hydrogen bonds. The only base arrangement possible in DNA structure, from spatial considerations is AT. T-A, G-C and C-G.
9. The complementary base pairing in DNA helix proves *Chargaff's rule.* The content of adenine equals to that of thymine (A = T) and guanine equals to that of cytosine (G = C).

10. The *genetic information resides on* one of the two strands known as *template strand* or sensestrand. The opposite strand is antisense strand. The double helix has (wide) major grooves and (narrow) minor grooves along the phosphodiester backbone. Proteins interact with DNA at these grooves, without disrupting the base pairs and double helix.

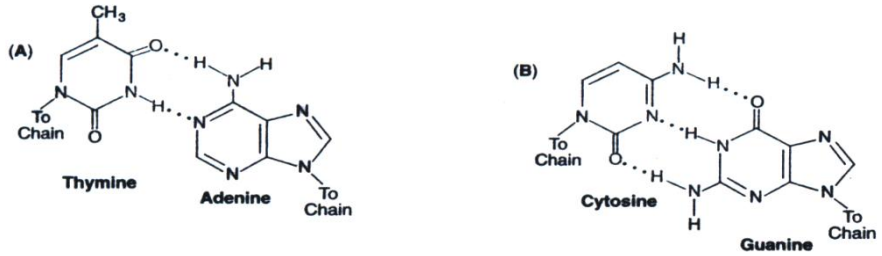


Figure 2.17 Complementary base pairing in DNA

(A) Thymine pairs with adenine by 2 hydrogen bonds

(B) Cytosine pairs with guanine by 3 hydrogen bonds

Conformations of DNA double helix

Variation in the conformation of the nucleotides of DNA is associated with conformational variants of DNA. The double helical structure of DNA exists in at least 6 different forms-A to E and Z. Among these, B, A and Z forms are important *(Table 2.2).* The *B-form of DNA* double helix, described by Watson and Crick (discussed above), is the most predominant form *under physiological conditions. Each* turn of the B-form has 10 base pairs spanning a distance of 3.4 nm.

Table 2.2: Comparison of structural features of different conformations of DNA double helix

Feature	***B-DNA***	***A-DNA***	***Z-DNA***
Helix type	Right handed	Right handed	Left handed
Helical diameter (nm)	2.37	2.55	1.84
Distance per each Complete turn (nm)	3.4	3.2	4.5
Rise per base pair (nm)	0.34	0.29	0.37
Number of base pairs per complete turn	10	11	12
Base pair tilt	$+19^0$	-1.2^0 (variable)	-0.9^0

The Z-form (Z-DNA) is a left-handed helix and contains 12 base pairs per turn. The polynucleotide strands of DNA move in a somewhat 'zig zag' fashion, hence the name Z-DNA. It is believed that transition between different helical forms of DNA plays a significant role in regulating gene expression.The width of the double helix is 2 nm. The A-form is also a right-handed helix. It contains 11 base pairs per turn. There is a tilting of the base pairs by 20° away from the central axis.

Other types of DNA structure

It is now recognized that besides double helical structure, DNA also exists in certain unusual structures. It is believed that such structures are important for molecular recognition of DNA by proteins and enzymes. This is in fact needed for the DNA to discharge its functions in an appropriate manner. Some selected *unusual structures of DNA* are briefly described.

Bent DNA

In general, adenine base containing DNA tracts are rigid and straight. Bent conformation of DNA occurs when A-tracts are replaced by other bases or a collapse of the helix into the minor groove of A-tract. Bending in DNA structure has also been reported due to photochemical damage or mispairing of bases. Certain antitumor drugs (e.g. cisplatin) produce bent structure in DNA. Such changed structure can take up proteins that damage the DNA.

Triple-stranded DNA

Triple-stranded DNA formation may occur due to additional hydrogen bonds between the bases. Thus, a thymine can selectively form two *Hoogsteen hydrogen bonds* to the adenine of A-T pair to form ***T-A-T.*** Likewise, a protonated cytosine can also form two hydrogen bonds with guanine of G-C pairs that results in C-G-C. An outline of Hoogsteen triple helix is depicted in *Fig.2.*18. Triple-helical structure is less stable than double helix. This is due to the fact that the three negatively charged backbone strands in triple helix results in an increased electrostatic repulsion.

Figure 2.18: An outline of Hoogsteen triple helical structure of DNA

Four-stranded DNA

Polynucleotides with very high contents of guanine can form a novel tetrameric structure called *C-quartets.* These structures are planar and are connected by Hoogsteen hydrogen bonds *(Fig2.19A).* Antiparallel four-stranded DNA structures, referred to as *C-tetraplexes* have also been reported *(fig 2.19B).* The ends of eukaryotic chromosomes namely *telomeres* are rich in guanine, and therefore form G-tetraplexes. In recent years, telomeres have become the targets for anticancer chemotherapies. G-

tetraplexes have been implicated in the recombination of immunoglobulin genes, and in dimerization of double-stranded genomic RNA of the human immunodeficiency virus (HIV).

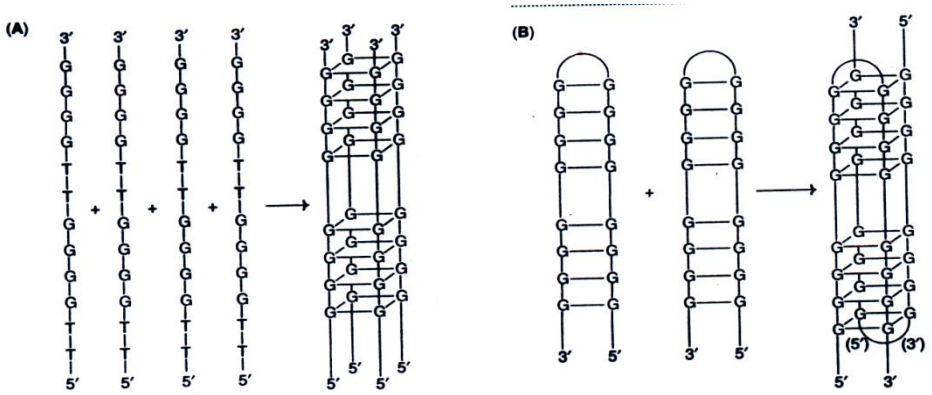


Figure 2.19: Four-stranded DNA structure (A) Parallel G-quartets (B) Antiparallel G-tetraplex

The size of DNA molecule - Uunits of length

DNA molecules are huge in size. On an average, a pair of B-DNA with a thickness of 0.34 nm has a molecular weight of 660 daltons.For the measurement of lengths, DNA double-stranded structure is considered, and expresssed in the form of *base pairs (bp).* A *kilobase pair (kb)* is 10^3 bp, and a *megabase pair (Mb)* is 10^6 bp and a gigabase pair (Gb) is 10^9 bp. The kb, Mb and Gb relations may be summarized as follows:

1 kb = 1000 bp

1 Mb = 1000 kb = 1,000,000 bp

1 Gb = 1000 Mb = 1,000,000,000 bp

It may be noted here that the lengths of RNA molecules (like DNA molecules) cannot be expressed in bp, since most of the RNAs are single-stranded.The length of DNA varies from species to species, and is usually expressed in terms of base pair composition and *contour length.* Contour length represents the total length of the genomic DNA in a cell. Some examples of organisms with bp and contour lengths are listed.

- λphage virus — 4.8×10^4 bp — contour length 16.5 mm.
- *E.coli* — 4.6×10^6 bp — contour length 1.5 mm.
- Diploid human cell (46chromosomes) — 6.0×10^9bp — contour length 2 meters.

It may be noted that the genomic DNA size k usually much larger the size of the cell or nucleus containing it. For instance, in humans, a 2-meter long DNA is packed compactly in a nucleus of about 10um diameter.The genomic DNA may exist in linear or circular forms. Most DNAs in bacteria exist as closed circles. This includes the DNA of bacterial chromosomes and the extra-chromosomal DNA of plasmids. Mitochondria and chloroplasts of eukaryotic cells also contain circular DNA.

Chromosomal DNAs in higher organisms are mostly linear. Individual human chromosomes contain a single DNA molecule with variable sizes compactly packed. Thus the smallest chromosome contains 34 Mb while the largest one has 263 Mb.

Denaturation of DNA strands

The two strands of DNA helix are held together by hydrogen bonds. Disruption of hydrogen bonds (by change in pH or increase in temperature) results in the separation of polynucleotide strands. This phenomenon of *loss of helical structure of DNA* is known as *denaturation {Fig.2.20).* The phosphodiester bonds are not broken by denaturation. Loss of helical structure can be measured by increase in absorbance at 260 nm (in a spectrophotometer).*Melting temperature (Tm)* is defined as the temperature at which half of the helical structure of DNA is lost. Since G-C base pairs are more stable (due to 3 hydrogen bonds) than A-T base pairs (2 hydrogen bonds), the Tm is greater for DNAs with higher G-C content. Thus, the Tm is 65°C for 35% G-C content while it is 70°C for 50% G-C content. Formamide destabilizes hydrogen bonds of base pairs and, therefore, lowers Tm. This chemical compound is effectively used in recombinant DNA experiments.*Renaturalion (or* reannealing) is the process in which the separated complementary DNA strands can form a double helix

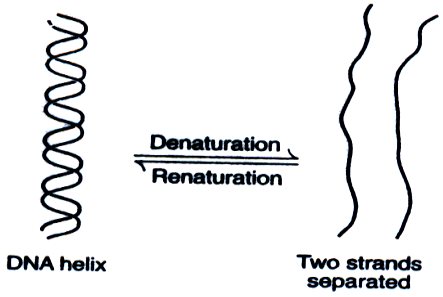


Figure 2.20: Diagrammatic representation of denaturation and renaturation of DNA

Bio chemical concepts

1. DNA is the reserve bank of genetic Information, ultimately responsible for the chemicalbasts of life and heredity.
2. DNA is organized Into genes, the fundamental units of genetic information. Genescontrol protein biosynthesis through the mediation of RNA.
3. Nucleic acids are the polymers of nucleotides. Certain nucleotides serve as B- Complex vitamin coenzymes (FAD, NAD^+, CoA), carriers of high energy intermediates (UDP-glucose, S-adenosylmethionlne) and second messengers of hormonal action (cAMP, cGMP).
4. Uric acid is a purine, and the end product of purine metabolism, that has been implicated in the disorder gout.
5. Certain purinebases from plants such as caffeine (of coffee), theophylline (of tea) and theobromine (of cocoa) are of pharmacological interest.

6. Synthetic analogs of bases (5-fluorouracil, 6-mercaptopurine, 6-azauridlne) are used to inhibit the growth of cancer cells.
7. Certain antitumor drugs (e.g. cisplatin) can produce bent DNA structure and damage it.

Replication of DNA

Introduction

The process by which a DNA molecule produces its identical copies is described as DNA replication. It is a type of self duplication or self reproduction of DNA, where two daughter DNA molecules are formed from a single DNA molecule (parent). The complementary relationship between A and T, and G and C residues ensure that the bases are aligned in the right manner and the sequences of bases in the daughter molecules is exactly the same as in the parent molecule. It results in the production of two double helices identical to the original one and the genetic information content remains unchanged. This type of replication has been described or termed as Semiconservative type.

Mechanisms of replication

But three possible mechanisms of DNA replication have been proposed. They are

1. Dispersive Replication.
2. Conservative Replication.
3. Semi - conservative Replication.

1. Dispersive Replication

In this method the two strands of DNA (parents) break ramdomly and produce seperate fragments. Those pieces replicate and reunite or reassemble to form two new daughter DNA molecules. These new DNA molecules contain a mixture of old and new nucleotides scattered along the chains. The daughter molecules can be described as hybrids. This type of replication is just considered as a possibility. This mechanism is neither accepted nor proved experimentally. (Fig. 2.21)

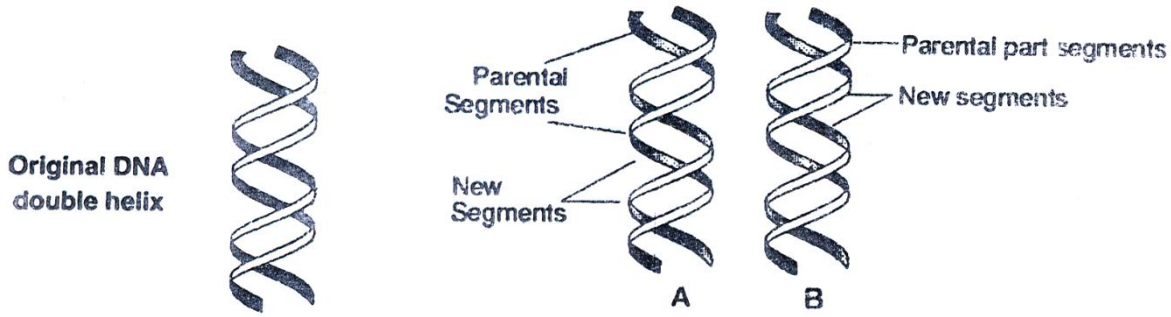


Figure 2.21: Dispersive Replication - A, B with hybrid stands

2. Conservative Replication

In this method two daughter molecules are formed as final products of duplication *o(*which one contains the original two strands of the parental molecule and the other daughter molecules contains two newly synthesized strands. This method, like dispersive type, is also not accepted and also not proved. (Fig.2.22)**.**

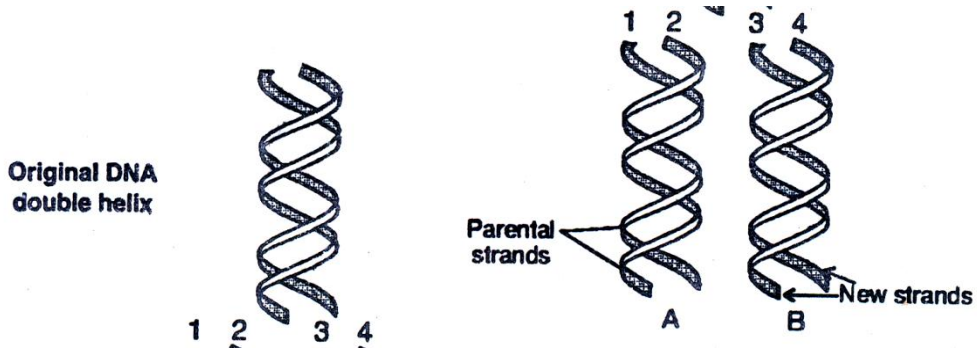


Figure 2.22 Conservative ReplicationA - with parental strands (1 and 2); B - with new strands (3 and 4)

3. Semi conservative Replication

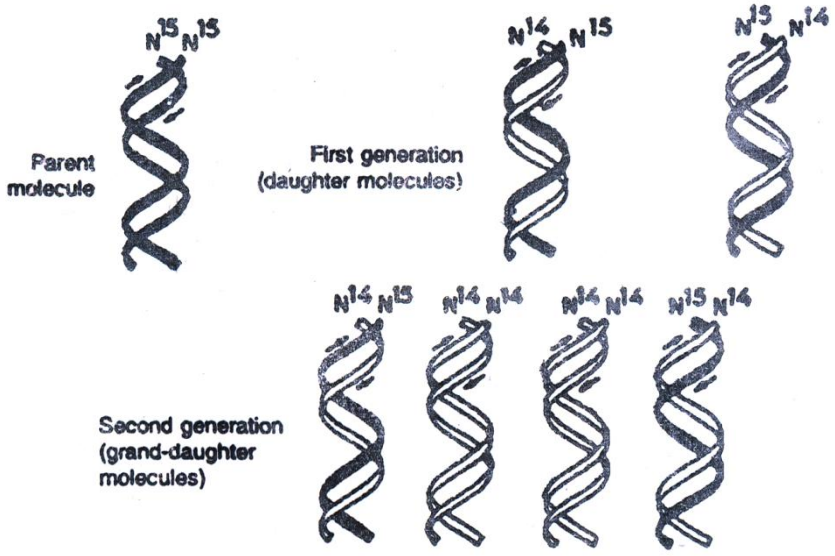


Figure 2.23: Meselson and Stent's experiment to show semiconservative replication of DNA in an environment oflabelednitrogen (^{14}N)

This method of DNA replication was proposed by Watson and Crick. In this model, each strand produces a daughter complementary strand. In this method, the two DNA strands seperate from one another and each strand synthesizes a new strand. The new strands are complementary to the strands from which they are formed. It results in the formation of two daughter DNA molecules. Each daughter

molecule contains a parental strand and a new strand. This method is universally accepted and supported by several experimental evidences.

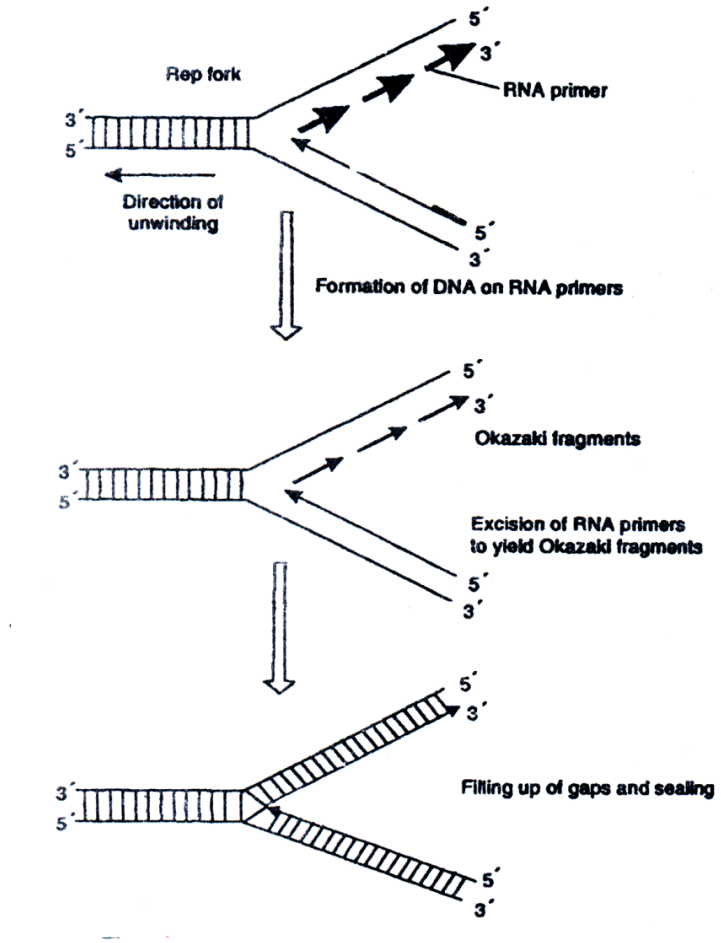


Figure 2.24: Replication of DNA Semi Conservative

Evidences for semiconservative replication

M. Meselson, FW - Stahl, J cairns, JH. Taylor etal conducted experiments differently but provided evidences in favour of semiconservative type of DNA Replication.

Broadly these experiments can be divided into three types -

(a) Meselson - Stahl's Experiments ;

(b) Cairn's Auto radiography experiment;

(c) Taylor's experiment on Viciatfaba roots.

(a) Meselson and Stahl's Experiment

Meselson-and stahl (1958) conducted their experiments on E. Coli by labeling DNA with heavy nitrogen (N^{15}). Theydesignedanexperimenttodetennine the mode of DNA replication.They allowed *E. Coli* cells to grow on cultures containing heavy nitrogen N^{15} for about 14 generations. Then *E. Coli* cells were transferred to a culture median containing normal nitrogen (N^{14}) and allowed to grow there - for one generation (which in E. *Coli* is completed in about 30 mts). If the replication is semiconservative, then

after first generation of replication, one of the two strands would have N^{15} and the other strand would have normal nitrogen (N^{14}). Meselson and stall also studied the density of DNA after first and succeeding generations. The density bands were also analysed thro' ultraviolet absorption spectrum. After first generation they analysed the density bands that were formed and found the density band was exactly midway between the bands formed by N^{15} DNA and N^{14} DNA as expected of a DNA which replicates in a semiconservative fashion.After two generations, it was observed that half the DNA was with intermediate density and half with light bands confirming the Semiconservative made of replication of DNA as predicted and assumed by Meselson - Stahl.

(b) Cairn's Autoradiography Experiment

J. Cairns demonstrated the semiconservative mode of replication of bacterial chromosome by using auto radiography technique.In this technique, tritiated thymidine (H^3 Heavy radioactive use type of hydrogeny) is used to label or tag DNA of £. *Coli* normal thymidine is replaced by H^3 TD). Then *E. Coli*cells are broken to release the intact bacterial chromosome on slides. These slides are covered by photographic emulsion or films and stored in dark. The tritiated thymidine emits particles in dark due to its radioactive decay. These particles expose the films. These films are developed and analysed. If the exposure is light on the auto radiographs, it suggests labelling of one strand of DNA, which indicates semiconservative replication. J. Cairns carried out his auto radiographic experiments on E. *Coli* as out lined above and obtained results as expected, which confirmed the semiconservative mode of DNA replication as established by Meselson - Stahl earlier.

(c) Taylor's experiments

J. H. Taylor etal conducted similar autoradiographic experments like cairns but they selected the root tip cells of Vicia faba as the experimental. The root tips, after the incorporation of tritiated thymidine were transfered to unlabelled medium where in both the chromatids were seen lebelled in the first generation. In the second cycle of duplication one of the chromateds in each chromosome was found to be labelled and the other was normal. Taylor by his experimental results conclusively confirmed the semiconservative mode of DNA replication Taylor tried to establish the semiconservative method thro' the chromosomal replication visualizing a resemblance to each other basically.

Semi conservative method - overview

DNA replication begins with a partial unwinding of the double helix at an area known as 'replication fork'. This unwinding is accomplished by an enzyme known as 'DNA helicase'. This unwound section appears under electron microscope as a *'bubble'*. It is rightly called *"Replication bubble"*.

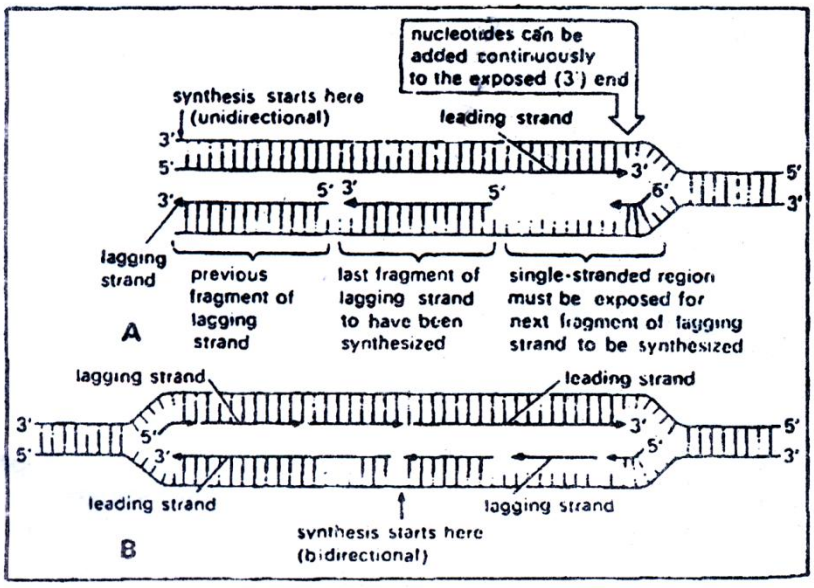


Figure 2.25: Semi discontinuous Types (A) Unidirectional type; (B) Bi directional type

As the two strands seperate, the bases are exposed and the enzyme DNA polymerase moves into a specific position where DNA synthesis will begin. The start point for DNA polymerase is a short segment of RNA known as "RNA primer". This primer acts like a start point or as some sort of 'marker'. This primer is laid down complementary to the DNA template by an enzyme known as "RNA polymerase or primase". The primer helps the DNA polymerase to reach the starting site. The DNA poiymrase after reaching the starting site adds nucleotides one by one in an. exactly complementary manner ie A to T and G to C.

DNA polymerase (being Template dependent) will 'read' the base sequences of bases on the template strand and "synthesize" the complementary strand. The template strand is always read in the 3' to 5' direction. The new DNA strand, as it is complementary, is synthesized always in the 5' to 3' direction DNA polymerase catalyzed the formation of the hydrogen bonds between each striving nucleotide and the nucleotides on the template strand, DNA polymerase also catalyses the reaction between the 5' phosphate on an incoming nucleotide and the free 3' OH on the growing polynucleotide and a phosphodiester bond is formed new DNA (leading) strand can grow only in the 5' to 3' direction. But the strand's growth begins at the 3' end of the template. Only one new strand can begin at 3' end of template DNA. The growth of this strand is continuous. The other strand (Lagging) grows in the opposite direction (being complementary) in a discontinuous fashion. This kind of replication forms a series of short sections of new DNA. These short DNA segments are called 'OKAZAKT fragments (named after their discoverer).

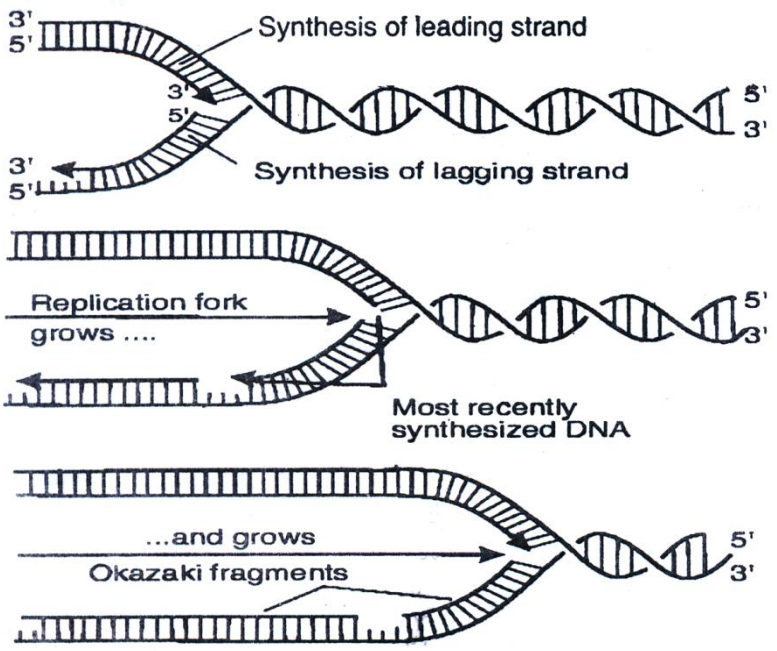


Figure 2.26: Okazaki Fragment

Later these fragments are joined by the action of DNA ligase to produce a continuous strand (Ligation). RNA primers that are joined in the early stages are removed by another type Of DNA polymerase. This enzyme not only removes the primers but also replaces them with deoxynuc3eotides.

Thro' the above process finally two identical new copies of the DNA helix are produced. And in each DNA daughter molecule or Double helix, One strand is the Old Template and the other is newly synthesized. Such replication is described as Semi Conservative since half the DNA (one of the parental strands) remaining in the daughter molecule and preserved in all succeeding generations (Semi Conservation). The above described DNA Replication is described as "Semi discontinuous" with reference to the made of formation of leading strand (continuous formation) and the lagging strand (discontinuous formation). The formation of these above strands may be uni or bidrirectional. Bidirect ional replication has been reported in prokaryotes and several Eucaryotes.

Details of replication in prokaryotes and eukaryotes

The knowledge about the following is essential to under stand the detailed view of DNA Replication in all kinds of organisms. They can be listed as follows

1. DNA Polymerases in Prokaryotes and Eukaryotes
2. Replication Mechanisms in prokaryotes and Eukaryotes.

(1) DNA Polymerases in Prokaryotes and Eukaryotes

(a) ***DNA Polymerases in Prokaryotes***

Three different polymerases namely DNA polymerase I, II, and III are identified in prokaryotes like *E. Coli* DNA polymerase I is used to fill the fragments of the lagging strand. It is also the major enzyme for gap filling during DNA repair.

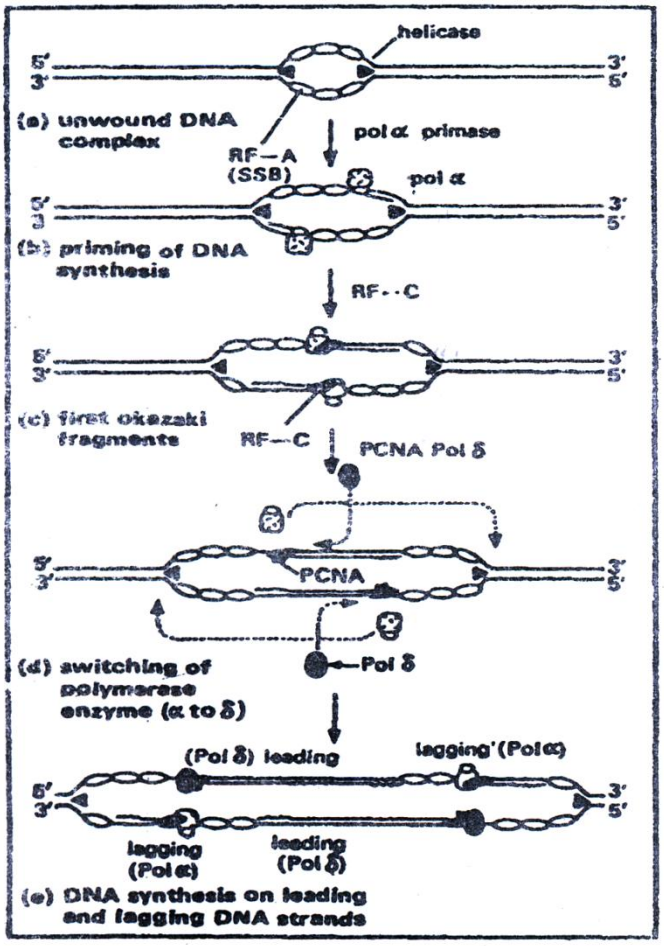


Figure 2.27: Micro level stages In Eukaryotic Relication (DNA pol-a and pol-5 polymers participating) in the replication process

The DNA polymerase II is involved in SOS response to DNA damage and repairs. The DNA replication is mainly carried out by the DNA polymerase III.DNA polymerase I contains two fragments. Of which the larger fragment (Klenow fragment) is mainly involved in removing the RNA primers from leading and lagging strands.The DNA polymerase III consists of several units (muti subunit enzyme) with a molecular weight exceeding 600 KD. The subunits constitute the core part of the polymerase. Some other subunits like P - subunits are also associated with DNA polymerage[7] III. All these subunits together help or promote DNA duplication.

(b) DNA polymerases in Eukaryotes

There are five types of DNA polymerases in Eukaryotic cells. They are depicted as α,β, γ, δ, and ε types.

Table 2.3: Types of DNA polymerases

	Type	Location	Function	Earlier **name**
1	**α** (alpha)	Cytoplasm	Synthesis of lagging strand during DNA Replication	Pol-I
2	**β** (Beta)	Nucleus (only in vertibrates)	DNA repair	
3	**γ** (Gamma)	Mitochondria	Duplication of mt DNA	
4	**δ** (Theta)	Nucleus (in Mammalia)	Synthesis of leading strand during DNA replication	
5	**ε** (Epsilon)	Nucleus (inMammalan cells and in Yeast cells)	DNA repair (proof reading activitiey)	DNA Pol I & II

In Eukaryotes, that both α and β DNA polymerases are involved in the replication of DNA.All the known DNA polymerases, whether pro or Eukaryotic, can extend the pre existing DNA chain in one direction only is 5"to 3'. They cannot initiate chain synthesis de noove.

(2) Replication in Prokaryotes and Eukaryotes

The mechanism of replication though basically similar in fundamental aspects both in prokaryotes and Eukaryotes, they show differences in certain aspects on account of the difference in the organization of the nuclear material and nature of proteins.In both types, replication machinery remains stationary where as DNA undergoing replication moves, (according to the modem concept).

The mechanism of replication of DNA as already explained in the beginning, is almost same and similar in both pro and Eukaryotes in general.But the replication mechanism in Eukaryotes shows several variations to that of the typical type as found in prokaryotes.

(3) Eukaiyotk DNA Replication:

It is complex compared to prokaryotes on account oi following aspects.

(1) Chromosomes contain large amounts of DNA associated with complex proteins like histones.
(2) Replication is related to the cell cycle.
(3) Multiple replications act as replication origin sites (Single Replication in case of Prokaryotes).
(4) Nucleosome complexes are involved in replication.
(5) The mechanism is semi conservative and bidirectional.
(6) There are five types of DNA polymerases as listed already. Two different types of DNA polymerases like α and δ (Alpha and Theta) types help the process of replication.

(7) Okazaki fragments are short having bout 100 to 200 nucleotides ie they have fixed length unlike prokaryotes.

(8) The rate of DNA replication in eukaryotic chromosomes is much slower compared to prokaryotes.

(9) The scheme of DNA replication that involves switching over between DNA poly –α and DNA poly δ is unique to Eukaryotes only.

(10) DNA in Eukaryotes is long and linear where as in prokaryotes and bacteriophages DNA is circular. The circular DNA, show slight variations in the initial stages of Replication unlike in linear DNA type. Generally the mechanism of replication in circular DNA is explained by special models like Cairn's Model (*E. Coli*) and Rolling Circle Model (Bacteriophages).

Organisation of DNA in the cell

As already stated, the double-stranded DNA helix in each chromosome has a length that is thousands times the diameter of the nucleus. For instance, in humans, a 2-meter long DNA is packed in a nucleus of about 10 urn diameter! This is made possible by a compact and marvellous packaging, and organization of DNA inside in cell.

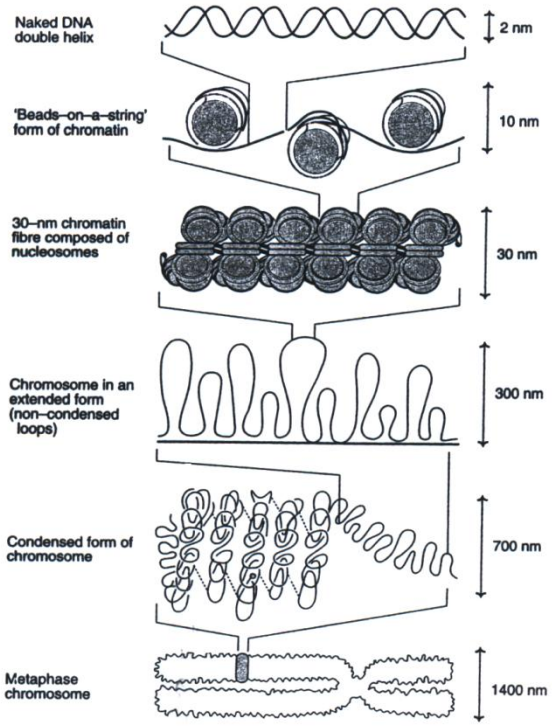


Figure 2.28 Organisation of EukaryoticDNA structure in the form of chromatin and chromosomes

Organization of prokaryotic DNA

In prokaryotic cells, the DNA is organized as a single chromosome in the form of a double-stranded circle. These bacterial chromosomes are packed in the form of nucleoids, by interaction with proteins and certain cations (polyamines).

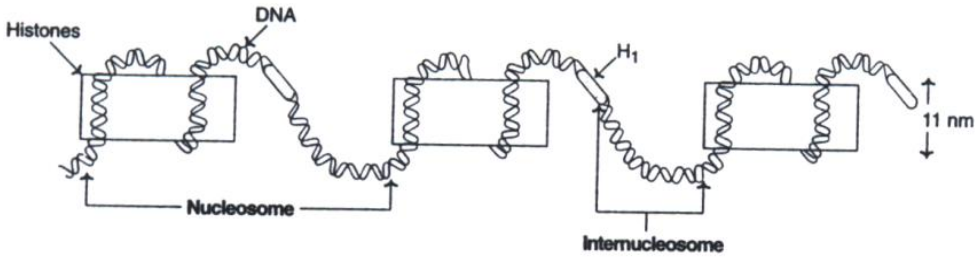


Figure 2.29: Structure of Nucleosomes

Organization of eukaryotic DNA

In the eukaryotic cells, the DNA is associated with various proteins to form *chromatin* which then gets *organized into* compact structures namely *chromosomes (Fig.2.28).*

The DNA double helix is wrapped around the core proteins namely *histones* which are basic in nature. The core is composed *of* two molecules of histones (H2A, H2B, H3 and H4). Each core with two turns of DNA wrapped round it (approximately with 150 bp) is termed as a *nucleosome*, the basic unit of chromatin. Nucleosomes are separated by spacer DNA to which histone H, is attached *(Fig.2.29).* This continuous string of nucleosomes, representing beads-on-a string form of chromatin is termed as 10 nm fiber. The length of the DNA is considerably reduced by the formation of 10 nm fiber. This 10-nm fiber is further coiled to produce 30-nm fiber which has a solenoid structure with six nucleosomes in every turn.

These 30-nm fibers are further organized into loops by anchoring the fiber at A/T-rich regions namely scaffold-associated regions (SARS) to a protein scafold. During the course of mitosis, the loops are further coiled, the chromosomes condense and become visible.

Structure and functions of RNA

RNA is a polymer of ribonucleotides held together by 3', 5'-phosphodiester bridges. Although RNA has certain similarities with DNA structure, they have specific differences

1. **Pentose:** The sugar in RNA is ribose in contrast to deoxyribose in DNA.
2. **Pyrimidine**: RNA contains the pyrimidine uracil in place of thymine (in DNA).
3. **Single strand**: RNA is usually a single-stranded polynucleotide. However, this strand may fold at certain places to give a double-stranded structure, if complementary base pairs are in close proximity.
4. **Chargaff's rule**—not obeyed: Due to the single-stranded nature, there is no specific relation between purine and pyrimidine contents. Thus the guanine content is not equal to cytosine (as is the case in DNA).
5. **Susceptibility to alkali hydrolysis**: Alkali can hydrolyse RNA to 2',3'-cyclic diesters. This is possible due to the presence of a hydroxyl group at 2' position. DNA cannot be subjected to alkali hydrolysis due to lack of this group.
6. **Orcinoi colour reaction**: RNAs can be histologically identified by orcinoi colour reaction due to the presence of ribose.

Types of RNA

The three major types of RNAs with their respective cellular composition are given below

1. Messenger RNA (mRNA) : 5-10%
2. Transfer RNA (tRNA) : 10-20%
3. Ribosomal RNA (rRNA) : 50-80%

Besides the three RNAs referred above, other RNAs are also present in the cells. These include heterogeneous nuclear RNA (hnRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA) and small cytoplasmic RNA (scRNA). The major functions of these RNAs are given in *Table 2.5.*The RNAs are synthesized from DNA, and are primarily involved in the process of protein biosynthesis. The RNAs vary in their structure and function. A brief description on the major RNAs is given.

MessengerRNA (mRNA)

The mRNA is synthesized in the nucleus (in eukaryotes) as *heterogeneous nuclear RNA (hnRNA).* hnRNA, on processing, liberates the functional mRNA which enters the cytoplasm to participate in *protein synthesis.* mRNA has high molecular weight with a short half-life.

The eukaryotic mRNA is capped at the 5'-terminal end by 7-methylguanosine triphosphate. It is believed that this cap helps to prevent the hydrolysis of mRNA by 5'-exo-nucleases. Further, the cap may be also involved in the recognition of mRNA for protein synthesis.

The 3'-terminal end of mRNA contains a polymer of adenylate residues (20-250nucleotides) which is known as *poly (A) tail.* This tail may provide stability to mRNA, besides preventing it from the attack of 3'-exonucleases. mRNA molecules often contai certain modified bases such as 6-methyladenylates in the internal structure.

Table 2.4: Cellular RNAs and their function(s)

Type of RNA	**Abbreviation**	**Functions**
Messenger RNA	mRNA	Transfers genetic information from genes to ribosomes to synthesize proteins
Heterogeneous nuclear RNA	hn RNA	Serves as precursor for mRNA and other RNAs
Ribosomal RNA	tRNA	Transfers amino acid to mRNA for protein bio synthesis
Ribosomal RNA	rRNA	Provides structural frame work for ribosomes
Small nucleor RNA	snRNA	Involved in mRNA processing
Small nucleolar RNA	snoRNA	Plays a key role in the processing of rRNA molecules
Small cytoplasmic RNA	scRNA	Involved in the selection of proteins for export
Transfer-messenger RNA	tmRNA	Mostly present in bacteria. Adds short peptide tags to proteins to facilitate the degradation of incorrectly synthesized proteins.

Transfer RNA (tRNA)

Transfer RNA *(soluble RNA)* molecule contains 71-80 nucleotides (mostly 75) with a molecular weight of about 25,000. There are at least 20 species of (RNAs, corresponding to 20 amino acids present in protein structure. The structure of tRNA (for alanine) was first elucidated by Holley.

The structure of tRNA, depicted in *Fig.5.16,* resembles that of a clover leaf. tRNA contains mainly four arms, each arm with a base paired stem.

1. The acceptor arm: This arm is capped with a sequence CCA (5' to 3') - The amino acid is attached to the acceptor arm.
2. The anticodon arm: This arm, with the three specific nucleotide bases (anticodon), is responsible for the recognition of triplet codon of mRNA. The codon and anticodon are complementary to each other.
3. The D arm: It is so named due to the presence of dihydrouridine.
4. The T ΨC arm: This arm contains a sequence of T, pseudouridine (represented by psi, Ψ) and C.
5. The variable arm : This arm is the most variable in tRNA. Based on this variability, tRNAs are classified into 2 categories :
   (a) Class I tRNAs: The most predominant (about 75%) form with 3-5 base pairs length.
   (b) Class II tRNAs: They contain 13-20 base pair long arm.

Base pairs in tRNA : The structure of tRNA is maintained due to the complementary base pairing in the arms. The four arms with their respective base pairs are given below

The acceptor arm	- 7 bp
The TTC arm	- 5 bp
The anticodon arm	- 5 bp
The D arm	- 4 bp

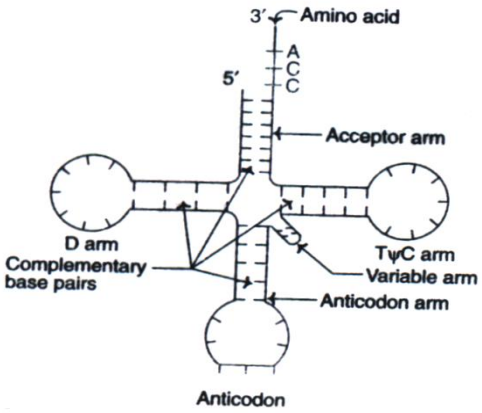


Figure 2.30: Structure of Transfer RNA

Ribosomal RNA (rRNA)

The ribosomes are the factories of protein synthesis. The eukaryotic ribosomes are composed of two major nucleoprotein complexes-60S subunit and 40S subunit. The 60S subunit contains 28S rRNA, 5S rRNA and 5.8S rRNA while the 40S subunit contains 18S rRNA. The function of rRNAs in ribosomes is not clearly known. It is believed that they play a significant role in the binding of mRNA to ribosomes and protein synthesis.

Other RNAs

The various other RNAs and their functions are summarised in *Table 2.4.*

Catalytic rnas—ribozymes

In certain instances, the RNA component of a ribonucleoprotein (RNA in association with protein) is catalytically active. Such RNAs are termed as ribozymes. At least five distinct species of RNA that act as catalysts have been identified. Three are involved in the self processing reactions of RNAs while the other two are regarded as true catalysts (RNase P and rRNA).

Ribonuclease P (RNase P) is a ribozyme containing protein and RNA component. It cleaves tRNA precursors to generate mature tRNA molecules.RNA molecules are known to adapt tertiary structure just like proteins (i.e. enzymes). The specific conformation of RNA may be responsible for its function as biocatalyst. It is believed that *ribozymes* (RNAs) were functioning as *catalysts before the occurrence of protein enzymes,* during the course of evolution.

(ISBN: 978-93-88901-16-1)

Chapter 3

Chromosome Morphology

Chromosomes: Morphology

E. Strasburger in 1875 discovered thread-like structures which appeared during cell division. These thread-like structures were called chromosomes (chroma = colour) due to their affinity for basic dyes. In all types of higher organisms (eukaryota), the well organized nucleus contains definite number of chromosomes of definite size, and shape. The chromosomes cannot clearly seen in the nucleus but can be easily seen during cell division, whether mitosis or meiosis. At leptotene stage of meiotic prophase, chromosomes appear as beaded structures, bead-like nodules being known as chromomeres. Size of chromomeres and interchromomeric regions are not constant, so that every leptotene has its own particular pattern. The DNA is though known to concentrate in the chromomeres, but is believed to be present in the inter-chromomeric regions also. A diploid nucleus has two chromosomes of each type. Two similar chromosomes are known as homologous chromosomes, which come in contact at zygotene and pair lengthwise throughout their length.

Number, size and shape of chromosomes

Chromosome number varies from *2n* = 4 *(n* = 2) in *Haplopappus gracilis* (Compositae) to 2n = >1200 in some pteridophytes. In *Aulacanlha* a radiolarian, a diploid number of approximately 1600 chromosomes is found (consult Swanson, Cytology and Cytogenetics*).* While V normally signifies the gametic or haploid chromosome number, *'2n'* is the somatic or diploid chromosome number in an individual.

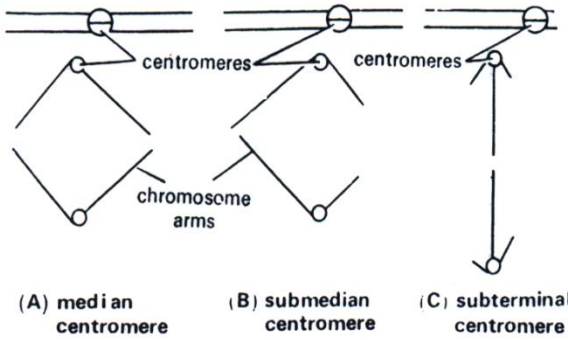


Figure 3.1: Different positions of centromeres controlling shapes of chromosomes at anaphase

In polyploid individuals, however, it becomes necessary to establish an ancestral primitive number, which is represented as *'x'* and is called the base number. For instance, in common wheat *(Triticum aestivum) In* = 42*; n = 21* and*x* = 7, showing that common wheat is a hexaploid (2n= 6*x).*A chromosome is normally measured at mitotic metaphase and may be as short as 0.25|i (u. = .001 mm) in

fungi and birds, or as long as 3Op, in some plants like *Trillium.* As a rule, most metaphase chromosomes fall within a range of 3p. in fruitfly *(Drosophila), 5\x* in man and 8-12µ in maize. Chromosome shape is usually observed at anaphase, when the position of primary constriction or what is also called centromere determines chromosome shape. This constriction or centromere can be terminal, sub-terminal or median in position. The terminal centromere will give rod shaped chromosome, a sub-terminal one would give J-shaped and a median centromere would give a V-shaped chromosome (Fig.3.1).

Morphology of chromosomes

Corresponding to different positions of centromere, chromosomes would be called (i) acrocentric or telocentric, having terminal centromere, (ii) submetacentric having sub-terminal centromere and (iii) metacentric having median centromere. Besides centromere, which produces a primary constriction in chromosomes, secondary constrictions can also be observed in some chromosomes. Such a secondary constriction if present in the distal region of an arm would pinch off a small fragment called trabant or satellite.

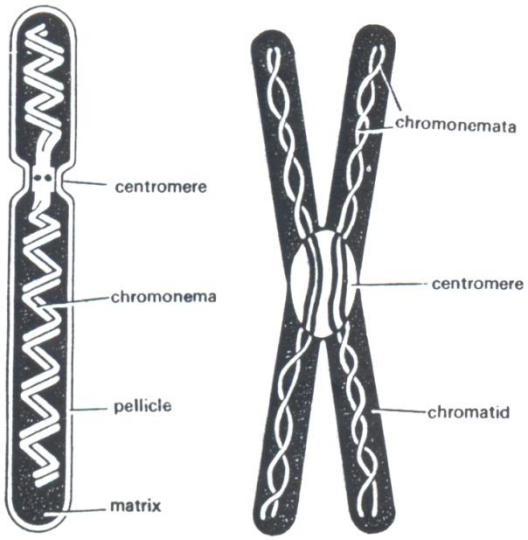


Figure 3.2: Structure of a chromosome and chromatid

The satellite remains attached to rest of the body by a thread of chromatin. Secondary constrictions may be found in other regions also ami are constant in their position, so that these constrictions can be used as useful markers. Secondary constrictions can be distinguished from primary constriction or centromere, because chromosome bends or shows angular deviation only at the position of centromere. Chromosomes having a satellite are marker chromosomes and are called SAT-chromosomes. The chromosome extremities or terminal regions on either side are called telomeres.

If a chromosome breaks, the broken ends can fuse due to lack of telomeres. A chromosome, however, cannot fuse at the telomeric ends, suggesting that a telomere has a polarity which prevents other segments from joining .with it. Telomeres have been studied in great detail at the molecular level

in recent years. Detailed study of chromosome morphology reveals a coiled filament throughout the length of a chromosome. This filament is called chromonema (Vejdovsky, 1912). The chromonemata form the gene-bearing portions of the chromosomes. The chromonemata are embedded in the achromatic substance known as matrix (Fig. 6.9 A). Matrix is enclosed in a sheath or pellicle. Both matrix and sheath are non-genetic materials and appear only at metaphase when the nucleolus disappears. It is believed that nucleolar material and matrix are interchangeable i.e., when matrix disappears, nucleolus appears and vice versa.

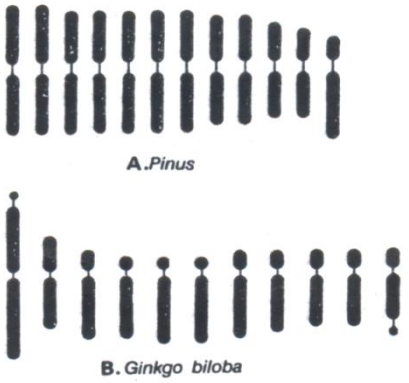


Figure 3.3: (A) A symmetric and (B) an asymmetric karyotype

It would be necessary here to make a distinction between chromonema and chromatid. While a chromatid is a half chromosome, two chromatids being connected at the centromere, the chromonema is a structure which is of a sub-chromatid nature and there can be more than one chromonemata in a chromatid (Fig.3.2 B).

Karyotypes

A group of plants or animals comprising a species is characterised by a set of chromosomes, which have certain constant features. These features include chromosome number, size and shape of individual chromosomes and other attributes listed above. The term karyotype is given to the group of characteristics that identifies a particular chromosome set and is usually represented by a diagram called idiogram, where chromosomes of haploid set of an organism are ordered in a series of decreasing size. The karyotypes of different groups are sometimes compared and similarities in karyotypes are presumed to represent evolutionary relationships.

Karyotype also suggests primitive or advanced feature of an organism. A karyotype showing large differences between smallest and largest chromosome of the set and having fewer metacentric chromosomes, is called asymmetric karyotype, which is considered to be a relatively advanced feature when compared with symmetric karyotypes. A symmetric and an asymmetric karyotype are shown in Fig. 3.3. In 1931 G.A. Levitzky**,** a Russian scientist suggested that in flowering plants there is a predominant trend towards karyotype asymmetry. This trend has been carefully studied in the genus

Crepis of the family compositae. In several cases it was shown that increased karyotype asymmetry was associated with specialized zygomorphic flowers.

Figure 3.4: Chromosomes showing euchromtin and hetero chromatin
(A) Early prophase (B) Late prophase

Euchromatin and Heterochromatin

When chromosomes are stained with stains like acetocarmine or feulgen (basic fuchsin) at prophase, a linear differentiation into regions having dark stain and those having light stain become conspicuous. In 1930's and 1940's Emil Heitz and other cytologists studied this aspect. The darkly stained regions were called heterochromatic and light regions were called dichromatic (Fig.3.4). Heterochromatic regions are constituted into three structures namely chromomeres, chromocentres and knobs. Chromomeres are regular features of all prophase chromosomes, large enough to reveal them, but their number, size; distribution and arrangement are specific for a particular species at a particular stage of development.

Chromocentres are heterochromatic regions of varying size which occur near the centromeres in proximal regions of chromosome arms. At mid-prophase, many chromocentres can be resolved into strings of chromomeres, which are larger than chromomeres found in distal regions. In some dipteran salivary glands, the chromocentres of different chromosomes fuse to form a large chromocentre (Fig. 4.2). The relative distribution of chromocentres is sometimes considered to be of considerable evolutionary value.

Knobs are spherical heterochromatin bodies which may have a diameter equal to the chromosome width but may reach a size having a diameter which is several times the width of the chromosome. Very distinct chromosome knobs can be observed in maize at pachytene stage. Knobs are valuable chromosome markers for distinguishing chromosomes of related species and races.

Constitutive and facultative heterochromatin

Certain regions of chromosomes, particularly those proximal to centromeres are constant, and are called constitutive heterochromaticregions serving as chromosome markers. There are other heterochromatic regions called facultative heterochromaticand represented by whole sex chromosomes

which become heterochromatic only at certain stages. For instance in female humans, one X-chromosome is inactivated or becomes heterochromatic only facultatively. Similarly in plants accessory chromosomes are heterochromatic. In plants also, among dioecious genera like *Melandrium* and *Rumex*, one or both sexchromosomes may undergo partial or complete heterochromatinization.

It is established that DNA replicates in heterochromatic region at a time different than that in euchromatic regions. It is also established that genes in heterochromatic region are inactive, but the earlier belief that no genes are found in heterochromatic regions is not correct because, genes could be located in heterochromatic regions in several cases like *Drosophila* and tomato. The genes in heterochromatic region perhaps become active for a short period. Y chromosome is another example of heterochromatic chromosomes having inactive genes in several dioecious plants and animals.

Single-stranded and multi-stranded hypotheses for chromosomes

When chromosomes are compared in related species which differ widely in DNA content, such differences may be attributed to one of the two causes:

(i) Lateral multiplication of chromonemata leading to multiple strandedness

(or)

(ii) Tandem duplication, where length-wise duplication instead of lateral multiplication is responsible for difference in DNA content. This later condition will retain the single stranded feature of chromosomes.

Although multiple strandedness has been demonstrated in several cases of plants like *Vicia faba* and animals like dipteran salivary gland chromosomes, there are evidences against such a hypothesis to become a generalization. In *Vicia faba* chromosomes, sub-chromonemata were actually observed, while in related V. *saliva* they could not be observed. In other genera also like in *Allium* and *Lolium*, it has been shown that increase in DNA content is mainly brought about by tandem duplication rather than by lateral multiplication leading to multiple stranded features. Therefore, the two hypotheses assuming single stranded and multiple stranded nature of chromosomes are complementary and not exclusive to each other.

Chemical composition of chromosomes

The major chemical components of chromosomes are DNA, RNA, histone proteins and nonhistone proteins. Calcium is also present in addition to these constituents. The relative proportions of different components are given in Table 3.1 for a variety of organisms, which suggest that there is more protein than DNA in chromatin in all cases. DNA As we know, DNA is the most important of chemical components of chromatin, since it plays the central role of controlling heredity. Quantitative measurements of DNA have been made in a large number of cases which are reviewed by H. Rees and R.N. Jones in 1972 (Intern. Rev. Cytol.). The most convenient measurement of DNA is picogram (10^{-12}

g) which is equivalent to 31 cm of double helical DNA It has been found that quantity of DNA varies greatly in cells from different kinds of organisms. The haploid genome of mammals usually contains 1000 times DNA content of bacteria.

Table 3.1: Chemical composition of chromatin (per cent) from various sources

Source	DNA	Histone	Nonhistone	RNA
Pea embryonic axis	39.0	40.0	11.0	10.0
Pea vegetative bud	40.0	52.0	4.0	4.0
Pea growing cotyledon	43.5	34.5	16.0	6.0
Rat liver	37.0	37.0	25.0	1.0
Cow thymus	40.2	46.0	13.5	0.3
Sea urchin blastula	39.2	41.0	19.0	1.0
Sea urchin larva	33.4	29.0	35.0	2.6

Other eukaryotes may similarly have 10 to 100 times the bacterial DNA content. It is interesting to note that a human diploid cell has 174 cm (5-6 picograms) of DNA, so that all cells in a human being may have DNA equal to 2.5 x 10^{10} km (100 g), a length which is equal to 100 times the distance from earth to sun. Similarly a diploid cell of *Trillium* has 37 meters (120 picograms) and that of *Drosophila* salivary glands has 91 meters (293 picograms) of DNA. In comparison of these enormous lengths, the DNA of bacteria measures only 1.1 mm-1.4 mm.

Histones

There are five fractions of histones, which have been differently designated (Table 3.2). HI histone is most easily removed and so is least tightly bound. This may thus be concerned with holding together a chromosome fibre. H3 and H4 are extremely conserved, having same structure in different species and should thus have a common structural role. Histones, isolated from diverse materials showed considerable similarity. It is also assumed that general similarities in histones have been conserved during evolution. This feature alone suggested that these proteins should play a structural role rather than a regulatory role. However, some important chromatin reconstitution and other experiments conducted in 1960s and 1970s established that histones do play a regulatory role. This regulatory role of histones is more of general nature rather than specific and is exercised by repressing the activity of genes.

Non-histones

The non-histone proteins display more but still limited diversity. In a variety of organisms, number of non-histones can vary from 12 to little more than 20. Heterogeneity of these proteins suggested that these proteins are not as conserved in evolution as histones. These non-histone proteins

differ even between different tissues of the same organism suggesting that they regulate the activity of specific genes. Chromatin reconstitution experiments described in 1973 by R.S. Gilmour and J. Paul of Institute for Cancer Research at Glasgow (U.K.), established that specific non-histone proteins switch on specific genes. The results of these experiments were later confirmed in a number of cases.

Table 3.2*:* Old and new standard designations for different kinds of histones isolated from eukaryotic chromosomes

Types of histone	Designation		
	Old		New
	Bonner *et al*,1968	Johns, 1971	Lewin, 1975
1. Lysine rich	I b	f 1	H1
	II b2	f 2a2	H 2A
2. Slightly lysine rich	II b2	f 2b2	H 2B
3. Argine rich	III	f 3	H 3
	IV	f 2al	H 4

Ultrrastructure of chromosomes

In the past, study of chromosome ultrastructure was an important area, where electron microscope had failed to give us a clear picture of organization of DNA. For the study of chromosomes with the help of electron microscope, whole chromosome mounts as well as sections of chromosomes were studied. Such studies had demonstrated that chromosomes have very fine fibrils having a thickness of 2 nm-4 nm (Fig. 3.5).

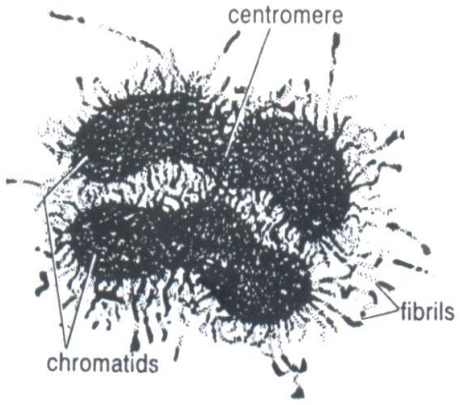


Figure 3.5: Ultra structure of chromosome

Since DNA is 2 nm wide there is possibility that' a single fibril corresponds to a single DNA molecule. However, whether these fine fibrils represent the natural state of DNA or is an artifact was not known. Moreover, the structure corresponding to protein component of the chromosomes was also not known, although the position of proteins was differently shown in different chromosome models.

A meaningful picture of the structure of chromosome could be earlier drawn from available data on ultrastructure of eukaryotic chromosomes. If a single chromatid has a single long DNA molecule, as has been proved, we have no choice but to believe that DNA should be present in a coiled or folded manner. This can also be inferred from the fact that the thickness of a chromosome is usually hundred times that of DNA and the length of DNA found in a chromosome is several thousand times the length of the chromosome. The manner of coiling and folding of DNA was a matter of debate and dozens of important models for this purpose were available. It is not desired here to present these different models, or else to discuss their merits or demerits. A popular model was the folded fibre model (Fig. 3.6) given by E.J. Du Prawin 1965.

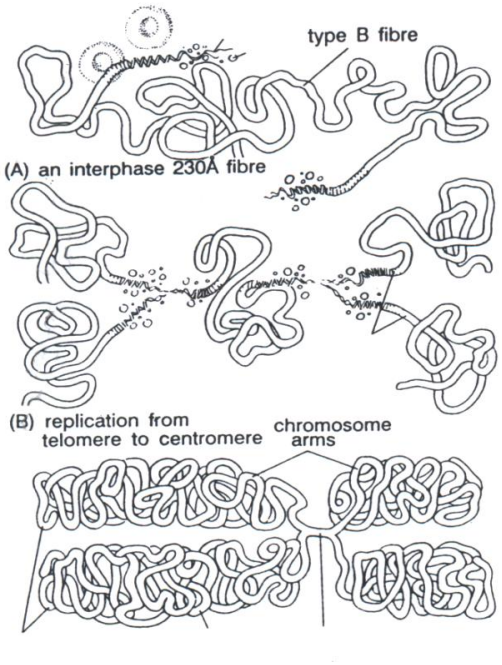


Figure 3.6: Structural organization in a chrommeaccording to folded fibre modle (c) sister fibres folded and held together at centromere unreplicated

The most important and universally accepted model is micleosome model proposed in 1974, this model involving nucleosome concept will be described briefly here.

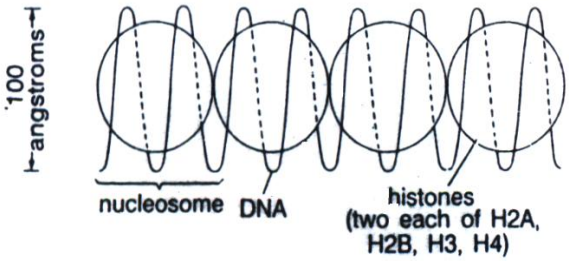


Figure 3.7: Original proposal for nucleosome repeating units, with DNA wound on a series of beads. Actual shape of historic complex or the path of DNA was not known

Structure of chromosomes at molecular level

(a) Nucleosome-subunit of chromatin.

In 1974, R.D. Romberg and J.O. Thomas proposed an attractive mciel for basic chromatin structure involving DNA and histones. They suggested that DNA interacts with a tetramer ($H3_2$-$H4_2$) and two molecules of an oligomer (H2A-H2B), so that a tetramer involving two molecules each of the histones H3 and H4, is associated with two molecules each of the histones H2A and H2B and with 200 base pairs of DNA. This makes a repeating unit (Fig.3.7). One molecule of HI is also associated with each repeating unit. They also proposed that the tetramer makes the core of the unit and oligomers determine the spacing thus giving a flexible structure. This model is supported by biochemical and electron microscopy results. P. Oudet *ex al.* (1975) proposed the term nucleosome for repeating units which were observed as beads on strings under electron microscope.

Nucleosome (12.5 nm in diameter) = 200 base pairs + 2 molecules each of H2A, H2B, H3, H4

(b) Telomere

In recent years the structure of telomeres in a wide variety of organisms has been studied to demonstrate that telomeres are highly conserved elements throughout the eukaryotes, both in structure and function. Telomeric DNA has beer, shown to consist of simple randomly repeated sequences, characterized by clusters of G residues in one strand and C residues in the other. Another feature is a 3' overhang (12-16 nucleotides in length) of the G-rich strand. Some of the telomeric DNA sequences found in eukaryotes are given in Table. 3.3.

Table 3.3*:* **Repeated telomeric DNA sequences in some organisms**

Telomeric DNA repeat	Organisms
1. AGGTT	*Homo sapiens, Physarum, Neurospora, Trypanosoma*
2. GGGGTT	*Tetrahymena. Glaucoma*
3. GGG(G/T)TT	*Paramecium*
4. GGGGTTTT	*Oxyiricha, Srylomychia, Euplotes*
5. AGGG(T/C)TT	*Plasmodium*
6. AGGGTTT	*Arabidopsis* (a higher plant)
7. AGGGTTTT	*Chlamydomonas*
8. (A)$G_{(2_5)}$TTAC	*Schizosaccharomyces pombe* (fission yeast)
9. $G_{(1\text{-}3)}$T	*Saccharomyces cerevisiae* (budding yeast)
10. $G_{(1_8)}$A	*Dictyostelium* (a mold)

The same repeated sequence is found at the ends of all chromosomes in a species (Fig. 3.8) and the same telomere sequence may occur in widely divergent species, such as humans, and some acellular

slime molds (trypanosomes) and fungi like *Neurospora*. At every telomere, as much as 10 kilobases of this repeat sequence may occur. The telomeric DNA is also complexed with non-histone proteins, the complex structure being associated with nuclear lamina, as shown in *Oxyiricha,* a ciliated protozoan. The telomeric DNA is synthesized under the influence of tclomerase, an enzyme which has been shown to be a ribonucleoprotein. Who's RNA component works as a template for synthesis of telomeric DNA repeats.

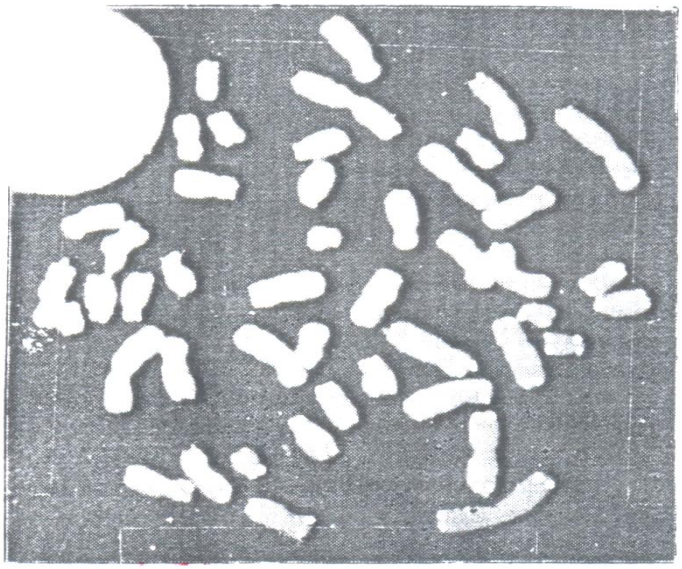


Figure 3.8: A metaphase plate of mitotic chromosomes, hybridized *in situ* with a probe having telomeric DNA repeat sequence. The telomeric sequence can be seen at the ends of all chromosomes.

Function of chromosomes

The function of chromosomes is to carry the genetic information from one cell generation to another. That DNA, which is the only permanent component of chromosome structure, is the sole genetic material. Also, the manner in which DNA stores the genetic information will be dealt elsewhere. The replication of chromosome depends on a very precise replication of DNA.

Organisation of DNA in a chromosome

Organisation of prokaryotic genome

The prokaryotic bacterial cell is much smaller than a eukaryotic cell, ranging from 1-5 nm in diameter. Most eukaryotic cells range in size from 10-100 mm. Bacterial chromosomes are organised into compacted structure, called nucleoids, by interaction of HU and HNS proteins and participation of various cations, polyamines (such as spermine, spermidine etc.), RNA and nonhistone proteins. Several DNA binding proteins have been isolated from E. *coli*, like HU, H, IHF, HLP1, P, H-NS. Many of these proteins resemble the eukaryotic histones. These proteins are some times known as histone- like proteins. HU and H are similar to histones H_2B and H_2A respectively. The most abundant of these are protein HU which binds DNA non specifically by the wrapping of the DNA around the protein.

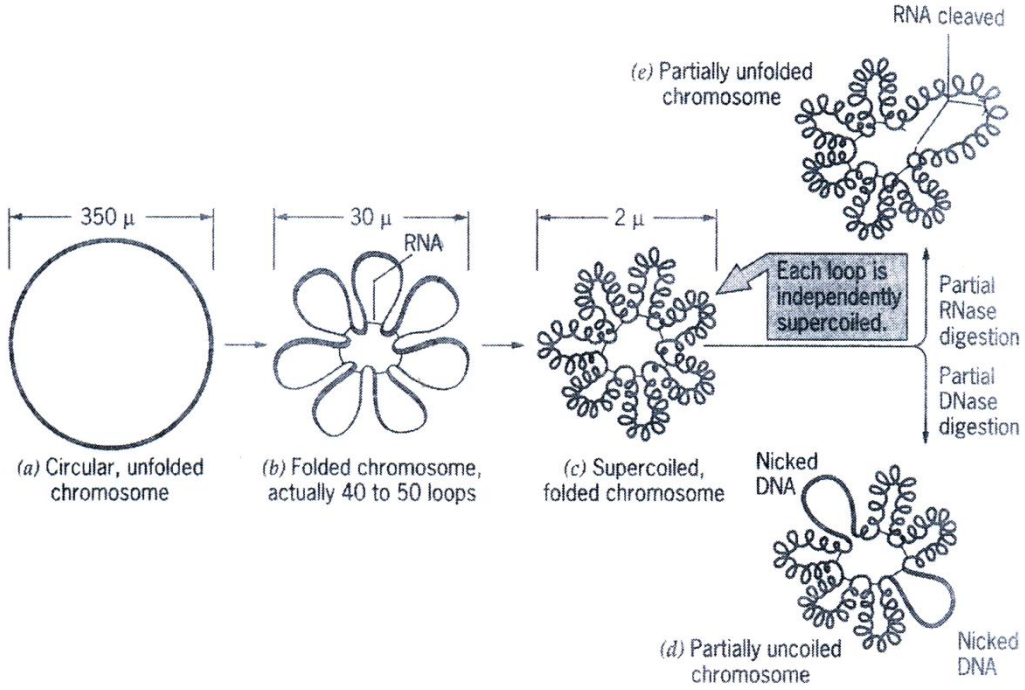


Figure 3.9: Diagram of thestructure of the functional state of the *E.coli* chromosome

A nucleoid is composed of about 80% DNA, 10% protein and 10% RNA and occupies about 25% of the volume of the cell. The HU proteins plays a central role in holding DNA in a tightly compact complex HU has a positive charge that binds to the negative charges present in DNA. The HU proteins bind to DNA and cause the DNA to form tightly packed structures. Thus HU is primarily responsible for the formation of a beaded nucleoid structure seen in prokaryotes. In *E.coli,* the nucleoid consists of single supercoiled DNA molecules organizedinto about 40 loops, each of approximately 10^5 bp of DNA, that merge into a scaffold rich in protein and RNA. In prokaryotic scaffold, the loops are maintained by interaction between DNA and RNA, rather than DNA-protein interactions only, as in eukaryotes.

As a result of formation of nucleoids which have diameters of 2 μm, the *E. coli* genome can easily fit into the cell. The nucleoid is not surrounded by a membrane, nor are there any membrane bounding cytoplasmic structures in prokaryotes.

Organisation of eukaryotic genome

DNA in eukaryotic cells is associated with various proteins to form chromatin. These are dynamic entities whose appearance varies dramatically with the stage of the cell cycle. Just prior to cell division, chromatin becomes organised into compact structures called choromosomes. Metaphase chromosomes are most condensed of normal eukaryotic chromosomes.The eukaryotic chromosomes is tightly packaged. Atleast three levels of organisations are required to package the 10^3 to 10^5 urn of DNA in a eukaryotic chromosome into a metaphase structure a few microns long. The first level of organisation is formation of a "beads-on-a string" structure consisting of DNA associated with a class of

highly basic proteins known as histones. Histones consist of five types of polypeptide of different size and composition, histones HI, H_2A, H_2B, H3 and H4. As a result of their unusually high content of basic amino-acids lysine and arginine (Table 3.4), histones are highly polycationic and interact with the polyanionic phosphate backbone of DNA to produce uncharged nucleoproteins.

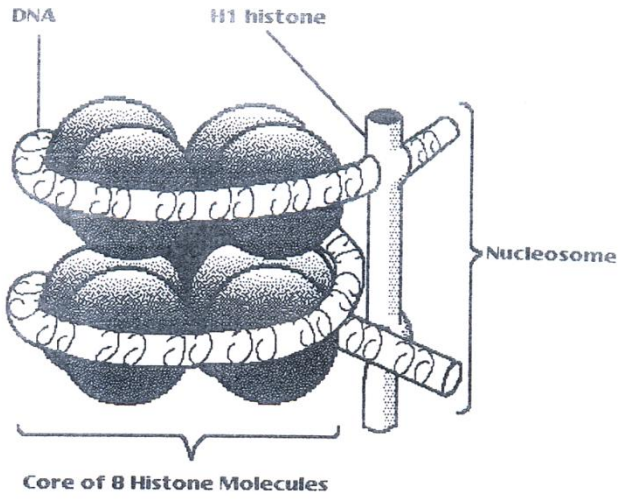


Figure 3.10: Schematic presentation of a nucleosome

Histone proteins have very nearly the same amino acid sequences in all the species from which they have been isolated and characterised. When amino-acid sequences are nearly identical in different species, they are said to have conserved sequences. The most "conserved" histones are H4 and H3 which differ very little even between extremely diverse species e.g., histones H4 from cow, peas, species that diverged 1.26 billion years ago, differ only 2 amino acids. This makes histone H4, the most invariant histone, among the most evolutionarly conserved proteins known. The H_2A and H_2B are less highly conserved but still substantial evolutionary stability, especially within their non basic proteins. Histone HI is quite distinct from the other histones, being large, more basic, and by far the most tissue specific and species specific histones. The erythroid cells of chick embryos contain an HI variants that differs so greatly from other His that, it is named histone H5.In some sperms histones are replaced by another class of small basic proteins called protamines.

Table 3.4: Percentage of Lysine and Arginine" residues in differenthistones

Histones	**No.** of **Residues**	**Lysine (%)**	**Arginines** %
HI	215	29	1
H2A	129	11	9
H2B	125	16	6
H3	135	10	13
H4	102	11	14

The five histone types are presentin molar ratios of approximately HI: $2H_2A$: 2 H,B: 2H3 : 2H4.Histones interact with DNA to form a periodic "beads-on-a string" structure, called a polynucleosome anda nucleosomes is its structural unit. Thus we can say that nucleosome is the basic structural component of eukaryotic chromosomes. Each nucleosome is a disk shaped structure about lOnm (which isactually llnm) in diameter and 5.5 nm (which is actually 6nm) in height that consists of a DNA segment and an octameric histone cluster composed of two molecules each of H,A, H_2B, H3, and H4 histones (Fig 3.10). Each cluster consists of a tetramer consisting of (H3) — $(H4)_2$ with an H_2A — H_2B diamer stacked on each face in the disk.Histones are in contact with the minor groove of DNA and leave the major groove available for the interaction with proteins that regulates gene expression and other DNA functions. A heterogenous group of proteins with species and organ specificity is also present in chromation.

These proteins, grouped together as non histone proteins,consist of several hundred members, most of which are present in trace amounts. Non-histone proteins are not usually positively charged at neutral pH. Many of these are associated with gene expression and regulation of gene expression or duplication of the DNA. For example, DNA polymerase, DNA ligase, single- stranded DNA binding proteins (SSB-Proteins) and RNA polymerase are just a few examples.The Nucleosome core consists of approximately 146 bp of DNA wrapped around the histone octamer complex. The complete nucleosome (as opposed to nuclesome core) contains two full turns of DNA superhelix (a 166 nucleotide pair length of DNA) on the surface of the histone octamer. In polynuclesomes, nucleosomes are joined together by "linker DNA", about -20 to 90 base pair long. This DNA is associated with histone Hi that locks the coiled DNA in place, the resulting complex is called as Chromatosomes. Thus histone HI "Seals off" the nucleosome. Histone HI is not a necessary component in the formation of nucleosomes. The core particle, the linker DNA and histone HI comprise a unit called chromatosome or mononucleosome.

The size of the linker DNA varies from species to species and from one cell type to another. Linkers as short as 8 nucleotide pairs and as long as 114 nucleotide pairs have been reported, although it is usually approximately 55 bp. The first order coiling around the histone proteins reduces the length of the chromosomal DNA about five fold, from about 50 nm of linear DNA to about 10 nm per nucleosome.The first level of chromatin organisation was (i.e nucleosome) pointed out by Roger Kornberg in 1974. Nucleosome core particles migrate over the polynucleosome. DNA sequence. This mobility allows access to the DNA by polymerases and other proteins necessary for transcription and replications. The second order of chromatin organisation involves an additional folding or supercoiling of the 10 nm nucleosome fibre, to produce the 30 nm chromatin fibre characteristic of mitotic and meiotic chromosomes. The basic structural unit of the metaphase chromosome is the 30 nm chromatin fibre. Nucleosomes are visible in 30 nm thick filament.

The 30 nm fibres appear to form by condensation of 10 nm fibres into a Solenoid arrangement involving 6-8 (~6) chromatosomes per solenoid turn. Histone HI is important in the coiling of the 10 nm

filament into the 30nm solenoids (which is actually 34 nm) (Fig.3.11). The exact nature of the second order structure is not fully understood.Finally, in the third level of chromatin organisation, non histone chromosomal proteins form a scaffold that is involved in condensing the 30 nm chromatin fibre into the tightly packed metaphase chromosomes (Fig.3.12). This third level of condensation appears to involve the separation of segments of the giant DNA molecules present in eukaryotic chromosomes into independently supercoiled domains or loops. The radial DNA loops are attached to matrix via AT-rich matrix associated regions (MARs) or Scaffold Attachment Regions (SARs).

Chromosomes

Chromosome is a discrete unit of the genome carrying many genes. They were first observed by Hofmeister (1849) in the pollen grains of *Tradescantia* but the credit of their discovery is generally given to Strasburger (1875). They were named by Waldeyer (1888). Chromosomes are self-replicating bodies and play a pivotal role in heredity, mutation, variation and evolutionary development of the species.Chromosomes are composed of chromatin. Because the chromatin is highly condensed during cell division, the chromosomes are easily seen and described by light microcopy. Each chromatin fibre contains one DNA molecule. The number of chromosomes in the cell nucleus varies considerably among different animals and plant species; however, each species has a specific chromosome number. Sutton believed that chromosomes may be the physical basis for the Mendelian laws of heredity.In plants smallest number of chromosomes occur in *Haplopappus gracilis* (Compositae) where 2n = 4, and largest number of chromosomes are found infern *Ophioglossum* where 2n = 1260. In animals smallest number of chromosomes occur in a nematode *Ascaris megalocephala univalence* where 2n = 2 and largest number of chromosomes occur in *Aulacantha* where 2n = 1600.

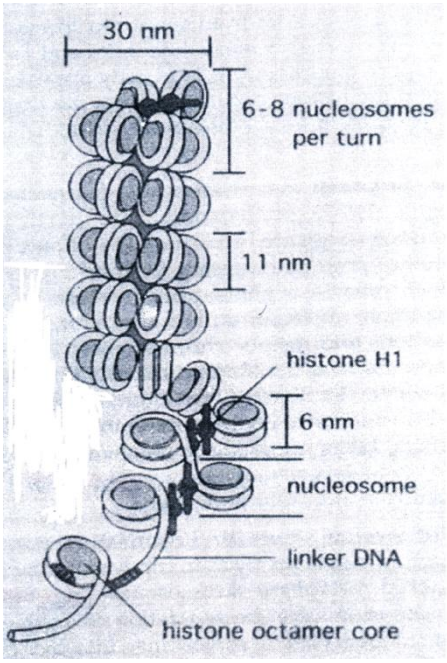


Figure 3.11: Solenoid model of the 30 *nm* chromatin fibre

The plants in general have large-sized chromosomes n comparison to animals. The monocotyteledon plants contain large-sized chromosomes than the dicotyledon plants. In plants smallest chromosome occur in fungi and largest in *Trillium* (Liliaceae).In animals smallest chromosomes are found in birds and the largest chromosomes are giant chromosomes e.g. lampbrush chromosome of certain vertebrate oocytes and polytene chromosome of dipteran insects eg. *Drosophila.*Unrelated organisms may have the same chromosome numbers for example, the potato plant has 48 chromosomes, but so do plum trees and chimpanzees. The size of chromosomes is normally measured at mitotic metaphase and the shape of chromosome is usuallyobserved at anaphase, when the position of primary constriction (centromere) determines chromosome shape. Centromere is not observed in interphase.

In a chromosome the primary constriction or centromere can be terminal subterrninal or median in positions. The terminal centromere will give rod-shaped chromosome, subterrninal, J-shaped and median centromere would give a V-shaped chromosome. In some species there is no defined location of centromere in the chromosome i.e. diffused chromosomes. These chromosomes are called holocentric chromosomes and are found in the members of family Cyperaceae and Juncaceae in plant kingdom.

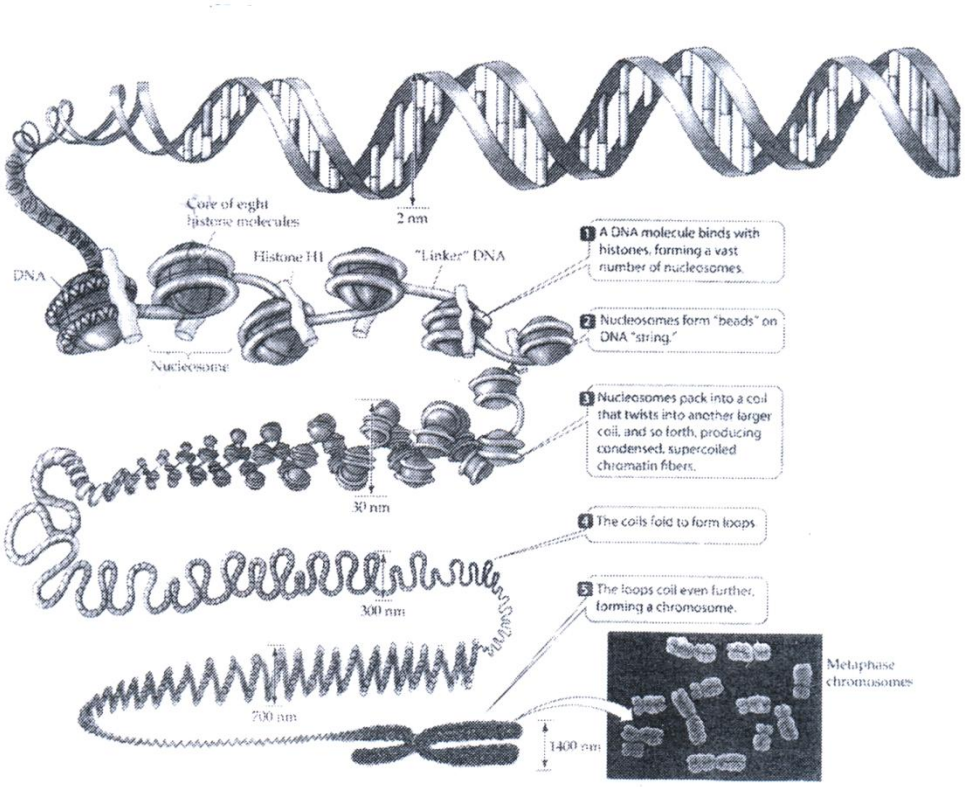


Figure 3.12: DNA Packs into a Mitotic Chromosome : The nucleoscme, formed by DNA and histones, is the essential building block in this highly packed structure

If centromere is absent the chromosome is called acentric. It is usually lost duringcell division because it cannot attach to the microtubules of spindle fibres. The centromere is the site of attachment to the microtubules of spindle and acts as the focus of chromosome movement during the anaphase of cell

division. In addition to primary constriction, some chromosomes have other constriction called secondary constriction. Secondary constrictions are associated with nucleoli and are called nucleolar organiser regions. Secondary constrictions if present in the distal region of an arm would pinch off a small fragment called satellite. A satellite remains attached to the rest of the body by a thread of chromatin called stalk DNA (Fig.3.12).

Stalk DNA synthesizes rRNA, therefore, cells performingprotein synthesis should at least have a pair of SAT-chromosomes (Sine acid thymonucleinico which refers to low DNA % in this region). Throughout the length of the chromosome is found a filamentous thread called *chromonemata*. It is gene bearing part of the chromosome. All along the length of chromosomes, granular structures are found which are called *chromomeres.* Around the chromonemata is semi-fluid matrix, matrix is surrounded by pellicle or sheath. Matrix and pellicle are non-genic part and appear only at metaphase when the nucleolus disappers. Since eukaryotic chromosomes are linear, each has two ends, referred to as telomeres. The telomers prevent the chromosomal ends from acting in a "sticky" fashion. If a chromosome breaks, the broken ends can fuse due to lack of telomeres. Telomeres also prevent the ends of chromosomes from being degraded by exonucleases and allow chromosomal ends to be properly replicated.

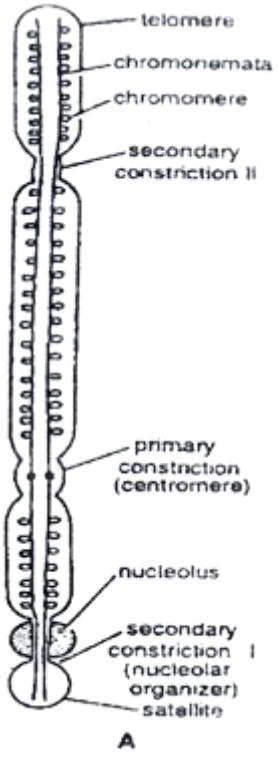


Figure 3.13: Structure of Eucaryotic chromosome

Most telomeres isolated so far are repetitions of sequences of 5-8 bases. In human beings, the telomeric sequence is TTAGGG, in *Trypanosoma.* TTAGGG, in Paramecium GGGGTT, in *Arabidopsis thaliana* TTTAGGG, and is repeated 300 to 500 times at the end of each chromosome in humans. This is

highly conserved sequence (TTAGGG), found in all vertebrates studied as well as in unicellular trypanosomes. Telomeric sequences appear to be added *de novo,* without DNA template assistance by an enzyme called *telomerase,* discovered by Elizabeth Blackburn and her colleagues. Telomerase is a reverse transcriptase (ribonucleo protein). It uses its RNA as a template for adding telomeric repeats to the ends of chromosomes. If telomerase can be deactivated in tumour cells, the cells may stop dividing or die, thereby eliminating the cancer.

Telomerase in generally found only in cells of germline including ESC (embryonic stem cell), unicellular eukaryotes like *Tetrahymena* (unicellular protozoa) and cancer cells. Embryonic stem cells, eukaryotic single cell and cancer cells cannot divide in absence of telomere. In human, 90% of cells divide in absence of telomerase and thus gradually consume the telomeric repeats added earlier in development. The progressive shortening of chromosome ends and eventual loss of genetic information that result has been linked to cell death, and it has been suggested that life span is determined by the number of telomeres with which individual start.

Chapter 4

Special Types of Chromosomes

Special types of chromosomes

The preceding section in this chapter dealing with chromos6mes in eukaryotes was devoted to structure and function of chromosomes as observed in mitotic or meiotic cells in plants and animals. In certain organisms there are special tissues where these chromosomes take up a special structure. Lampbrush chromosomes of the vertebrate oocyte and giant chromosomes of salivary gland cells of dipterans are such special types of chromosomes. Due to special significance of these chromosomes, a relatively detailed account of these two types of chromosomes will be presented.

Lampbrush chromosomes

As indicated earlier, chromosome structure at the same stage of cell division remains constant in the different kinds of cells in the same organism. Chromosomes of a special kind are, however, found in a variety of primary oocyte nuclei in vertebrates (mainly amphibians) as well as in some invertebrates. These chromosomes, known as lampbrush chromosomes, are found during the prolonged diplotene stage of first meiotic division in primary oocytes of amphibians, and in spermatocyte nuclei of *Drosophila.* The lampbrush chromosomes are characterized by a remarkable change in structure. The change in structure includes an enormous increase in length.

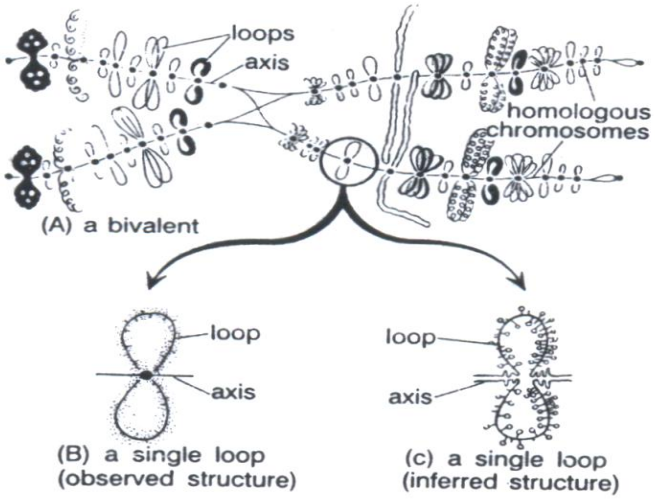


Figure 4.1: Lampbrush chromosomes showing details of structures

These chromosomes maySometimes become even larger than polynemic giant salivary gland chromosomes. The largest chromosome having a length upto 1 mm has been observed in urodele amphibian. The chromosomes seem to have a chromomeric pattern with loops projecting in pairs from majority of chromomeres. One to nine loops may arise from a single chromomere. The size of loops

varies with an average of 9.5u. In inter-chromomeric fibres. These pairs of loops in these chromosomes give them the characteristic lampbrush appearance (Fig. 4.1). Frequently these loops exhibit a thin axis (which probably consists of one DNA double helix) from which fibres project which is covered with a loop matrix consisting of RNA and protein.

The number of pairs of loops gradually increases in meiosis till it reaches maximum in diplotene. As meiosis proceeds further, number of loops gradually decreases and the loops ultimately disappear due to disintegration rather than reabsorption back into the chromomere. H. Ris, however, had thought that the loops were integral parts of chromonemata which are extended in the form of major coils. It is also believed that the loops represent the modified chromosome structures at the loci of active genes. It has been observed that, if the activity of these genes is stopped by actinomycin D (actinomycin D stops synthesis of RNA on DNA template), the.loops will collapse, suggesting that the loops mainly consist of RNA.

Polytene chromosomes

In salivary gland cells of dipteran species, giant chromosomes were observed By E.G. Balbianifor the first time in 1881. The availability of these chromosomes greatly helped the study of cytogenetics in fruitfly *(Drosophila).* These chromosomes may reach a size up to 200 times (or more) the size of corresponding chromosomes at meiosis or in nuclei of ordinary mitotic cells. Another characteristic of these giant chromosomes is that they are somatically paired. Consequently the number of these giant chromosomes in the salivary gland cells always appear to be half that in the normal somatic cells. The giant chromosomes have a distinct pattern of transverse banding which consists of alternate chromatic and achromatic regions. These bands have greatly helped in the mapping of the chromosomes in cytogenetic studies. The bands occasionally form reversible puffs, known as chromosome puffs or Balbiani rings, which are associated with differential gene activation.

The giant chromosomes represent a bundle of fibrils which arise by repeated cycles of endo-reduplication of single chromatids, Endo-reduplication means that the chromatin replicates without cell division, as a result of which the number of chromonemata keeps on increasing. This is why these chromosomes are also popularly known as polytene chromosomes and the condition is described as polyteny. The number of chromonemata (fibrils) per chromosome may reach upto 2000 in extreme cases. Some workers placed this figure as high as 16,000.In *D. melanogaster,* the giant chromosomes are found in the form of five long and one short strands radiating from a single more or less amorphous mass known as chromocentre (Fig.4.2). One long strand corresponds to the X chromosome and the remaining four long strands are the arms of II and III chromosomes. The centromeres of all these chromosomes fuse to form the chromocentre. In the male flies the Y chromosome is also found fused within the chromocentre and is therefore not seen as a separate strand.

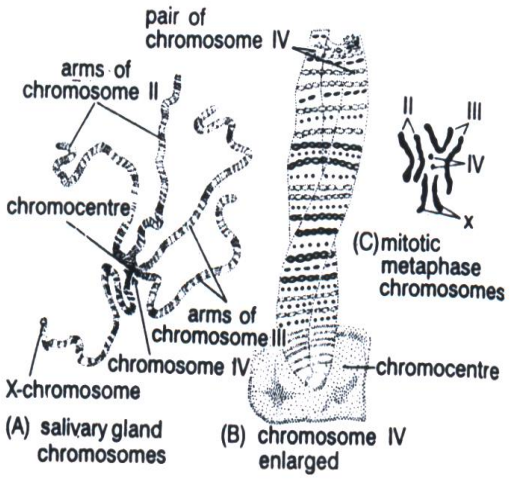


Figure 4.2: Polytene chromosome (A, B) and mitotic chromosomes (C) of Drosophyila melanogaster

How an enormous increase in size of these chromosomes is brought about in salivary glands is not known and various hypotheses are available to explain this issue. The reader should consult Swanson's book, Cytology and Cytogenetics, to geta-relatively detailed account of these hypotheses. It should, however, be emphasized that these giant chromosomes though, comrnonly found in salivary glands, have also been found in malpighian tubules, fat bodies, ovarian nurse cells, gut epithelia and some other tissues.

B - Chromosomes

B-Chromosomes are a particular kind of supernumerary chromosomes, that may or may not be found in an organism as extra chromosomes overfind above the standard diploid or polyploid chromosome complement (see Fig.4.3). The standard complement consists of chromosomes described as A-chromosomes, including sex chromoosomes, if any. The B-chromosomes are found in the naturalpopulations of many plant and aniimal species and are recognized on the basis of their following characteristics:

i. They are dispensable; are not found in all individuals of a species and may not be found in all cells of an individual organism;
ii. They are not homologous with any of the basic A chromosomes;
iii. Their Inheritance is non-Mendelian, sometimes due to non-disjunction during pollen mitosis (as in some plants);
iv. They are usually smaller than A-chromosomes and have their own unique pattern of heterochromatin distribution;
v. In general, they are genetically inert, but may rarely organize nucleoli and carry functional genetic material;
vi. When present in high number, they suppress vigour and fertility;
vii. Their origin and functionare unknown.

Figure 4.3: Amitotic metaphase plate showing the presence of four B chromosomes

B-chromosome in plants have been reported in more than 1000 species distributed over bryophytes (including mosses), ferns, gymnosperms and angiosperms (both monocotyledons anddicotyledons). Mostly, they are found inbut-breeders (cross pollinated species). In the family Gramineae, they are found in 200 species including cereals, and forage grasses belonging to genera *Agrostis, Anthoxanthum, Avena, Bromus, Calamogrostis, Dactylis, Deschampsia, Festuca, Koeleria, Lolium, Pennisetum, Phleum, Secale, Sorghum* and *Zea,* These B-chromosome are often absent in well adapted agronomic strains, and are perhaps eliminated due to selection. The B-chromosomes may sometimes be restricted only to aerial parts (absent in roots e.g. *Aegilops speltoidas, Ae. mutica)* and may also be eliminatedduring meiosis due to their division at anaphase I followed by elimination at anaphase II. B chromosomes may also increase in number due to a drive involving non- disjunction during pollen mitosis, thus leading to the production of plants with two B-chromosomes in the progeny OB x IB.

The most significant effect of B chromosomes is on seedandpollen fertility.Flowering time is generallydelayed by B- chromosomes andseveral characters (plant height, plant weight and tiller number) are adversely affected. A very important effect of B chromosomes relates with their effect on pairing of a chromosomes in

(i) Plants carrying these B-chromosomes and in

(ii) Species hybrids involving a parent carrying B-chromosomes.

In rye, although 0-6 B chromosomes did not influence the chiasmata frequencies, 8 B chromosomes drastically influenced the range of chiasma frequencies among pollen mother cells (PMCs) and among bivalents within PMCs. In maize, the use of various A/B interchange stocks demonstrated that the genetic elements affecting meiotic pairing of A-chromosomes appeared to be widely distributed throughout the length of B-chromosomes. The effect of B-chromosomes in suppressing or promoting meiotic pairing has been shown in a number of intergeneric or interspecific hybrids (wheat x *Aegilops*

sp.; wheat x rye, *Lolium* x *Festuca;* interspecific hybrids within the genus *Lolium*, etc.). For more details, the readers should consult the book B-chromosomes written by Jones and Rees (1982).

Prokaryotic Nucleoids

As indicated earlier, the light microscope could not show any nucleus in the cells of prokaryotes i.e. the bacteria and the blue green algae. These individuals are also designated as the akaryobionta as against the karyobionta having better differentiated nuclei. However, feulgen positive bodies are seen in bacteria and blue-green algae.

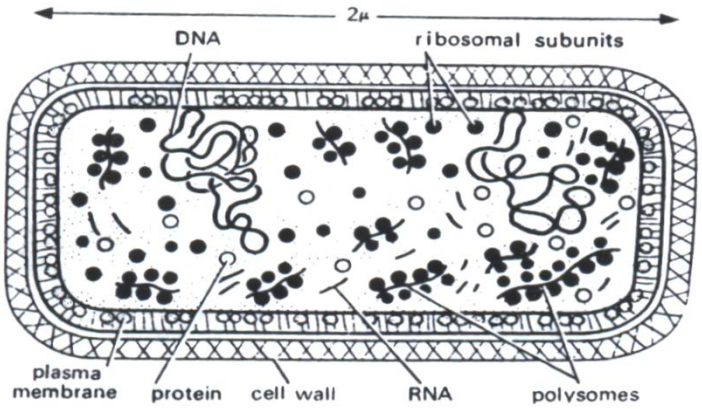


Figure 4.4: A bacterial cell showing details of internal structure

Chapter 5
Cell Division

Cell cycle and itsregulation

The various phases of the growth and reproduction of cell constitute what is called the cell cycle: The four phases of cell cycle are designated as $G_{l,}$ S, G_2 and M (Fig. 5.5). Nuclear DNA is prepared for replication in G. The G_1 phase of the cell cycle is characterised by the synthesis and accumulation of RNA and protein in the cytoplasm. The replication of nuclear DNA occurs in a specific portion of the interphase called the *S phase.* This phase typically lasts for 6-8 hours. Much of the protein synthesis during the S phase is histone synthesis; these histones enter the cell nucleus where they become closely associated with the newly-synthesized DNA. By the end of S phase the cell nucleus contain two full complements of genetic material. The cell then enters the G_2 phase. In G_2 phase cells prepared for mitosis, new proteins are synthesized and the cell approximately doubles in size. The sister chromatids are mirror images of one of the other. In the M phase, the maternal nuclear envelope breaks down, matching chromosomes are pulled to opposite pole of the cell. Each set of daughter chromosome is surrounded by newly formed nuclear envelope and cytokinesis pinches the cell in half, producing two daughter cells.

Some facts about cell cycles

1. The cell cycle may last only an hour with no G_1 phase and with some DNA synthesis occurring during mitosis or soon after mitosis is completed. G1 phase is absent in Amoeba, slime mould and fission yeast.
2. During the M phase, the nuclear content undergo a series of changes and rearrangement. In a human cell, the DNA, which is 10-15 feet long in its dispersed state, condenses to form 46 duplicated chromosomes whose combined length is less than 1/25 of an inch.
3. Bacterial DNA divides without changing its condensation.
4. Cells spend most of their life span in interphase.
5. The most important point in the regulation of cell cycle occurs in the G_1 phase, during which it must decide whether the cell will start a new cell cycle or become arrested in the G_0 state.
6. Centrioles replicate during inter phase, generally in the S-phase.
7. The G_0 phase (quiescent phase) may last hours, days or the life time of the cells.
8. Cells of some tissues (for example liver tissue cells) may exist in non-growing and non-dividing state for a long period of time.
9. In mammals nerve cells do not divide at all after birth. Thus, for a human neuron the interphase period lasts the entire life time of a person.

10. Indeed, the differentiation of certain cells such as cells of muscle and nerve, permanently removes them from the cell cycle.
11. When an cell in G_0 begins to divide again, it reenter the division cycle through the G_1 phase.
12. In human females primary oocytes remain at prophase of meiosis I (diplotene stage) through outchildhood.

Mitosis

Mitosis is a process of nuclear division in which replicated DNA molecules of each chromosome are faithfully partitioned into two nuclei. Mitosis maintains the chromosome number and generates new cells for the growth and maintenance of an organism. Mitosis can take place in either haploid or diploid cells. Although mitosis is a continuous process, for convenience it is usually divided into four major stages (Fig. 5.2).

i) Prophase

Prophase is characterised by the condensation of the chromosome, the disappearance of nucleoli and the nuclear envelope and the formation of the microtubules of the spindle. The chromosomes become distinguishable by light microscopy as a result of their progressive shortening and thickening and eventually are seen to be composed of two sister chromatids held together at the centromere. The sister chromatids are kept together by a complex, called cohesin(Fig.5.1) made up of at least four different proteins.

ii) Metaphase

The chromosomes move to the equator of the cell (process is called as congression). The spindle fibres invade the central area and their microtubules extend between the pole. The chromosomes become attached by the kinetochores. The centromeres of each chromosomesare aligned midway across the spindle on a plane called the equatorial plate. The centromeres are duplicated so that each chromatid becomes an independent chromosome.

iii) Anaphase

The onset of anaphase is characterized by the movement of the chromosomes toward opposite poles of the spindle. The cohesin is broken down and chromatids are separated.

iv) Telophase

The chromosomes reach the poles of the spindle and begin to undergo condensation. Nucleoli reappear, as does a new nuclear envelop enclosing the chromosomes. The spindle breaks down into tubulin subunit. The cell has now reentered the G^ phase of the cell cycle.

The anaphase-promoting complex controls the final stages of mitosis by targeting selected proteins for degradation. Two important proteins targeted for destruction are (1) securin and (2) mitotic cyclin. Thedegradation of securin leads to the release of separin, a protease that triggers the onset of anaphase by cleaving the cohesin proteins that hold sister chromatids together. The degradation of mitoic cyclin leadsto

the loss of MPF activity, which in turn triggers chromosome decondensation and reassembly of thenuclear envelop

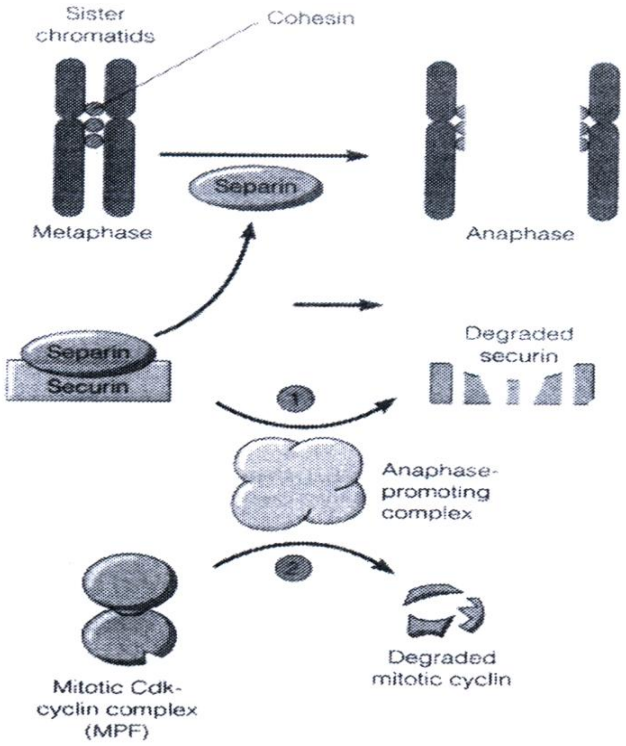


Figure 5.1 Main actions of the Anaphase promoting complex

Cytokinesis

Cytokinesis is the division of cytoplasm. It divides the cell into two halves thereby physically separating the two components of chromosomes. This stage normally follows telophase and leads into the G_1phase of inter-phase. In preparation for cell division, the cell organelles become evenly-distributed towards the two poles of the telophase cells along with the chromosomes. In cell division, all the nuclear and cytoplasmic components are equally-dividedbetween the two daughter cells,*exceptions* are the Golgi apparatus and E.R. It is generally held that Golgi body and E.R. undergo fragmentation during prophase to give rise to large number of *vesicles* that become dipersed throughout the cytoplasm. Fragmentation and dispersal facilitate the partitioning of this membranous network into the two daughter cells.In animal cells the cell surface membrane begins to invaginate during telophase towards the region previously occupied by the spindle equator. Microfilaments in the region are thought to be responsible for drawing in the cell surface membrane to form *a furrow* around the outside surface of the cell. The cell surface membrane in the furrow eventually joins up and completely separates the two cells.

In plant cells, the spindle fibres begin to disappear during telophase everywhere except in the region of the equatorial plane. Here they move outwards in diameter and increase in number to form a barrel shaped region known as phragmoplast-attracts Golgi bodies. Golgi apparatus produces a number of small fluid filled vesicles. The Golgi vesicles are filled with pectin. These vesicles fuse to form a cell

plate which grows across the equatorial plane. The contents of the vesicles contribute to the new middle lamella and cell walls of the daughter cells while their membrane form the new cell surface membranes. Thus, the cell plate is formed by the progressive accumulation of carbohydrate and lipid material that derived from Golgi apparatus. The spreading plate eventually fuses with the parent cell wall and separate the two daughter cells. The new cell walls are called primary cell walls. In certain area the vesicles of the cell plate fail to fuse and cytoplasm of neighbouring daughter cells remain in contact. These cytoplasmic channels are lined by the cell surface membrane and form structures known as plasmodesmata.

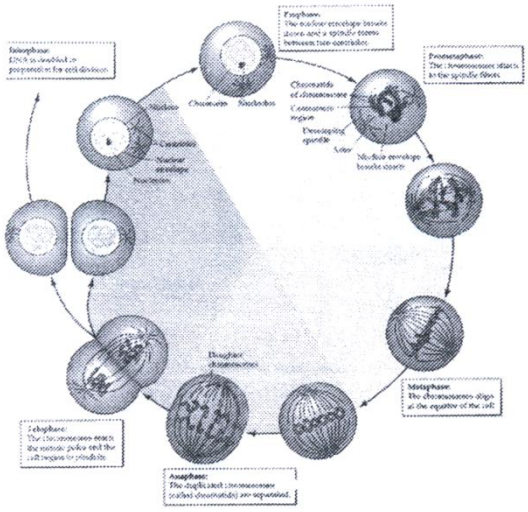


Figure 5.2: Mitosis in animal cells

Mitotic divisions without cytoplasmic divisions occur in many species to produce multinucleated or coenocytic cells. Striated muscles, mycelia of moulds and some phloem cells of plants are examples of multinucleated cells. Plant meristems (e.g. young root tips) are particularly advantageous for the study of cell division, because 10-15% of the cells are in mitosis. Mitosis in which the spindle has centrioles and asters is called astral or amphiastraland is found in animal cells and some lower plants (fungi, moss, fern and some algae but not in red algae). Mitosis in whichcentrioles and asters are absent is called as *anastral* and is found in higher plantsincluding all angiosperms and most gymnosperms.

Kinetochore

Mitotic chromosome of most plant and animal cells has a special region to which some spindle microtubules are attached. This region of the chromosome is called kinetochore - the proteinaceous complex at centromere of each sister chromatid, which eventually is an attachment site for the microtubules of mitotic spindle. The kinetochore is the interface between the visible constriction in the chromosome and the microtubules of spindle (Fig.5.3).

The kinetochore of higher organisms (e.g. mammals) contains proteins and some RNA. Only one microtubule attaches to each centromere during mitosis in a yeast cell (point centromere). Higher eukaryotes have larger centromeric regions that attach more microtubules. These regions are referred to

as regional centromere.At interphase kinetochores appear in the nucleus as subunits that are synthesized in the S phase.

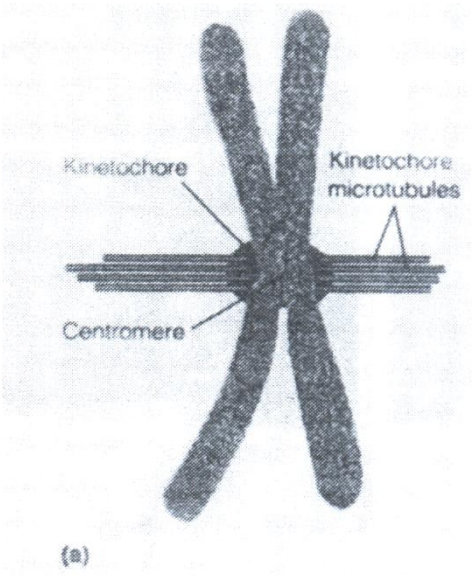


Figure 5.3: Attachment of Chromosomes to the Mitotic Spindle. This figure is a schematic model that summarizes the relationship between the centromere, kinetochores, and kinetochore microtubules of the spindle

The kinetochore may be detected during prophase, before the microtubules become attached to it. The kinetochore is essential for the separation of daughter chromosomes in anaphase. The main function of kinetochores seems to be related to the attachment of chromosomal microtubules. They may also serve as nucleation centres for the polymerization of tubulin. Thus the kinetochore is the site of implantation microtubules in the chromosome. There is usually one kinetochore per chromatid.

Meiosis

Meiosis is a process that includes two sequential nuclear divisions, producing haploid daughter nuclei that contain only one member of each pair of homologous chromosomes, thus reducing the number of chromosomes in half. Meiosis occurs in eukaryotic organisms whose cells contain the diploid number of chromosomes. Meiosis comprises two cell divisions meiosis I and meiosis II (Fig.5.4).

Meiosis I: Comprises prophase I, metaphase I, Anaphase I, Telophase I.

Prophase I: Cytogeneticist have divided the prophase of meiosis I into five stages:

i) Leptotene

(a) The chromosomes become visible as condensation of chromatin begins.

(b) Although DNA duplication has already occurred and they have two chromatids, leptotene chromosomes look single rather double-stranded. These chromosomes show bead-like thickening, the so-called chromomeres.

(c) Leptotene chromosomes have definite polarisation and forms loops whose ends are attached to nuclear envelope at points near centriole. This peculiar arrangement is often called the bouquet.

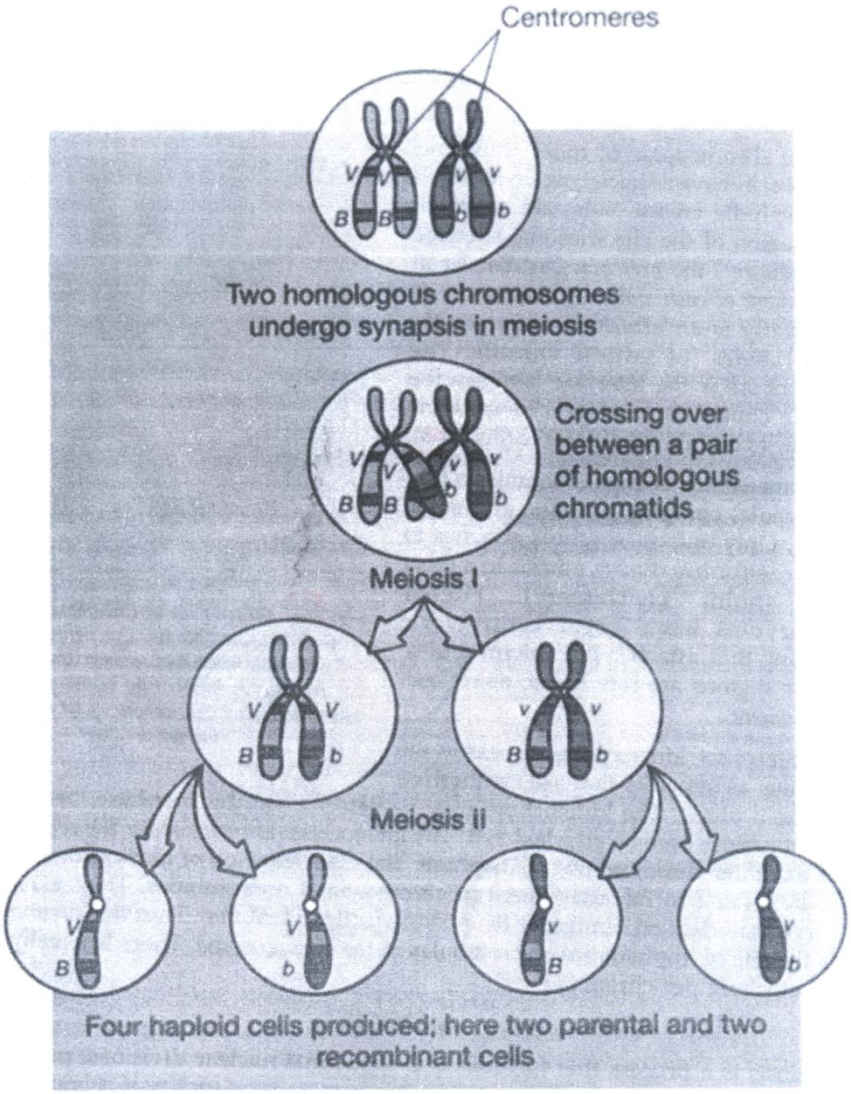


Figure 5.4: Meiosis in Animals

ii) Zygotene

(a) Homologous chromosomes are aligned side by side so that allelic genes are situated adjacent to one another. This phenomenon is called synapsis and each pair is called bivalent one of the pair comes from the male parent and one from the female. Each member of the pair is of the same length, their centromeres are in same position and they usually have the same number of genes arranged in same order.

(b) Pairing is highly specific and involves the formation of a special structure that can be observed under electron microscope and is called synapton-emal complex (SC).

SC is not required for genetic recombination. It functions primarily as a scaffold to allow interacting chromatids to complete their crossover activities.

(iii) Pachytene -

a) Each chromosome is now seen to be composed of two chromatids. Each chromatid has its own kinetochore.

b) The two chromatids of each homologue are callled sister chromatids. During the first meiotic division the kinetochores of the two homologous chromatid behave as a functional unit.

c) During pachytene, two of the chromatids of the homologues exchange segments (i.e. recombine). Here Holiday junctionsare formed.

d) The two chromosomes are seen to be joined at several points along their length. These points are called chiasmata. It can be seen that each chiasma is the site of an exchange between chromatids. As a result, genes from one chromosome may swap genes from the other chromosomes leading to new gene recombination in the resulting chromatids. This is called crossing-over.

The recombination will not be 100% because only two of the four chromatids are involved in crossing-over at each chiasma. As the homologous move apart, they are seen to remain attached to one another at specific points by cross-shaped structure termed chiasmata.

Chiasmata are formed from junction between two of the four chromatids of a bivalent, one from each homologue. Chiasmata are located at sites on the chromosomes where crossing, over between DNA molecules from two chromosomes have previously occurred. These points of attachment provide a striking visual portrayal of the extent of genetic recombination. In humans and other vertebrates every pair of homologues typically contains at least one chiasma and longer chromosomes tend to have two or three of them. The inhibition of excess chiasmata is referred to as crossover interference and is thought to be mediated by synaptonemal complex.

If a chiasma does not occur between a pair of homologous chromosomes, the chromosomes of that bivalent tend to separate from one another after dissolution of SC. This premature separation of homologues often results in non disjunction of homologues and formation of nuclei with abnormal number of chromosomes. Homologous chromosomes may fail to separate from each other during meiosis I or sister chromatids may fail to come apart during meiosis II.When either of these situations occurs gametes are formed that contain an abnormal number of chromosomes- either an extra chromosome or a missing chromosome. Chiasmata are cytological manifestation of genetic recombination (crossing over).

iv) Diplotene

a) The diplotene stage is characterized by the separation of the paired homologous chromosomes except at points where chiasmata are formed.

b) The initimately paired chromosome repels each other and begins to separate.

Bivalent assume particular shape depending upon the number of chiamata -

(i) Bivalent having single chiasma appear as open crosses

(ii) Two chiasmata produce ring shape

(iii) Three or more chiasmata, loops at right angles to each other

Diplotene is a long-lasting period. In the fifth month of prenatal life, for example, human oocytes have reached the stage of diplotene and remain in it until many years later, when ovulation occurs. Unlike the production of sperm in males which only begins at puberty, the production of eggs in females begins before birth. During development of foetus many oogonia are produced. These undergo mitosis and form primary oocytewhich remain at prophase of meiosis I throughout childhood. Primary oocytes are enclosed by a single layer of cells granulosa cells and forms structures known as primordial follicles. About two million of these follicles exist in females just before birth but about 450 ever develop secondary oocyte which are released from ovary during the menstrual cycle. During a woman's fertile years one primordial follicle per month develops into a mature follicle, known as a graffian follicle. This is in response to a hormone FSH. Within each developing follicle, a primary oocyte starts to develop into an egg.

v) Diakinesis

a) During this stage chromosome condensation is completed.

b) The number of chiasmata diminishes.

c) By the end of diakinesis, in general the homologues are held together only at their end, a process that has been termed terminalization.

Metaphase I

The chromosomes that are bivalents become arranged at the equator. The kinetochores of homologous chromosomes attach to spindle fibres arising from opposite poles of the cell. The two sister chromatids behave as a functional unit.

Anaphase I

Homologous chromosomes of each tetrad separate from each other and move to opposite poles of the spindle. The meiotic division is called as reductional divisionbecause it reduces the number of chromosomes to half the diploid number in each daughter cells. For every tetrad there is now one chromosome in the form of a chromatid pair known as a *dyad* or monovalent at each pole of cell.The orientation of the materal and paternal chromosomes of each bivalent on the metaphase I plate in random, the maternal member of a particular bivalent has an equal likelihood of facing either pole, consequently, when chromosomes separate during anaphase I, each pole receives a random assortment of maternal and paternal chromosomes. Thus, anaphase I is the cytological event that corresponds to Mendel's law of independent assortment.

Telophase I-

The separated homologues aggregate at their respective poles so that two nuclear areas are distinguishable. In most organisms, a new nuclear envelope is formed and most consendation of chromosomes occurs. Spindles and spindle fibres usually disappear.

Interkinesis (Interphase II)

It is the period between the end of telophase I and the onset of prophase II. At the interphase between the two meiotic division there is no replication of chromosomes. No further DNA replication occurs. These are now haploid in number although each one consist of two chromatids. This stage is present only in animal cells and varies in length.

Meiotic division II

Since each dyad consist of two sister chromatids, a second mitosis like division is required to reduce each chromosome to a single chromatid (Fig.5.4).

i) **Prophase II** - This stage is absent if interphase II is absent. The nucleoli and nuclear envelope disperse and chromatids shorten and thicken. At the endof prophase II, new spindle fibres appear.

ii) **Metaphase II** - The paired chromatids migrate to the centre of the spindle and attached there to the spindles microtubules.

iii) **Anaphase II** - In anaphase II, sister chromatids separate from one another and are drawn to opposite poles of the spindle. Sister chromatids do not separate in anaphase I. The centromeres divide at anaphase II.

iv) **Telophase II** - The spindle fibres disappear, nuclear envelopes reform around each nucleus which possess half the number of chromosomes of the original parental cell.

Significance of Meiosis

1. Ifmeiosis did not occur fusion of gametes would result in a doubling of the chromosomes for each successive sexually reproduced generation.
2. In addition to reducing the chromosome number as required by sexual reproduction, meiosis increases the genetic variability in a population of organisms from one generation to the next.
3. Independent assortment allows maternal and paternal chromosomes to become shuffled during formation of the gametes and genetic recombina tion allows maternal and paternal alleles on a given chromosmes to become shuffled as well.

By mixing maternal and paternal alleles between homologous chromosomes meiosis generates organisms with novel genotypes and phenotypes on which natural selection can act. Homologous recombination does not change the linear array of genes but it can determine which alleles become linked together on asingle chromosome i.e. genetic recombination which is the primary source of variation creates new alleles not new genes because genes on the homologous chromosomes are same only they have different allelic configuration. In cases in which no crossing-over occurs (in male Drosophila which shows complete linkage) the tetrads tend to fall apart and segregate randomly. Thus crossing over not only increase genetic diversity but also ensures the proper separation of homologous chromosomes.

When two genes are close to one another the probability of crossing-over is less than when they are far apart. The genes present in the chromosomes are found in pairs called alleles. In each homologous chromosome, the gene for each trait occurs at a particular point called a locus. Whereas the law of segregation applies to the behavior of a single pair of genes, the law of independent assortment describe the simultaneous behavior of two or more pairs of genes located in different pairs of chromosomes. Genes that lie in separate chromosomes are independently distributed during meiosis.

Cohesin and Securin

During anaphase, initially, an inhibitory protein called securin binds an enzyme called separin that can break down cohesin; the complex holding the chromatids together. At the correct moment, the cyclosome (APC = anaphase promoting complex) *ubiquitinates* the inhibitor, causing it to break down by proteasomes (refer molecular basis sssof cell division). This liberates the sister chromatids from each other.

Molecular mechanism of cell division

The key enzymes that control the transitions between the different states of the cell cycle and the entry of non-dividing cells into the cell-cycle are the cyclin dependent protein kinases(CDKs). Protein kinases are enzymes that phosphorylates proteins using ATP. The mechanism regulating the progression of cell through their division cycle is highly conserved in evolution.

Most of the details centre on the enzyme cdc2 kinase,which has changed little in its structure, and none in its functions, during a billion years of evolution. This enzyme is one part of a protein complex called the maturation promoting factor (MPF).Once activated, MPF plays a role in most of the events of the cell cycle, such as

(1) Condensation of chromosome
(2) Dissolution/disintegration of nuclear lamina and nuclear envelope.
(3) Organisation of spindle apparatus
(4) Re-organisation of actin filaments to achieve cytokinesis.
(5) Segregation of the chromosomes into daughter cells during mitosis.
(6) M phase kinase appears to phosphorylate HI histone, which is very good substrate for the kinase. Phosphorylation of HI histone is connected with chromosome condensation during mitosis.

Evidence indicates that events in the nucleus do not control the cell cycle. Instead an autonomous oscillator - a set of chemical reactions in the cytoplasm-controlscell division with the regularity of a clock. The basic mechanism of this control involves cyclin proteins activating and inactivating MPF. MPF is a dimer with protein kinase activity. The two subunits are p^{34} (catalytic unit) and p^{45} (regulatory subunit i.e. cyclin) (Fig. 5.5).

The catalytic subunit (also called cdc2) carries out phosphorylation of the target proteins. During interphase, p34 is present in an inactive form, this inactive form associates with cyclins to form inactive

dimer molecules. The inactive form of M phase kinase becomes active in resporise to specific phosphorylation and dephosphorylation events. Activation of the dimer occurs during G_2. The dimer is inactivated by the destruction of cyclins that is its regulatory subunit.In the inactive form of M phase kinase, two amino acids (Threonine -14 and Tyrosine-15) located in the ATP binding site of$p^{34}$are phosphorylated, these amino acids are dephosphorylated in order to activate the M phase kinase. In addition, a phosphorylation event occurs at threonine-161 of p34, this phosphate group is essential for M phase kinase activity. Most likely threonine-161 is phosphorylated during G_2 and is dephosphorylated at the end of mitosis.

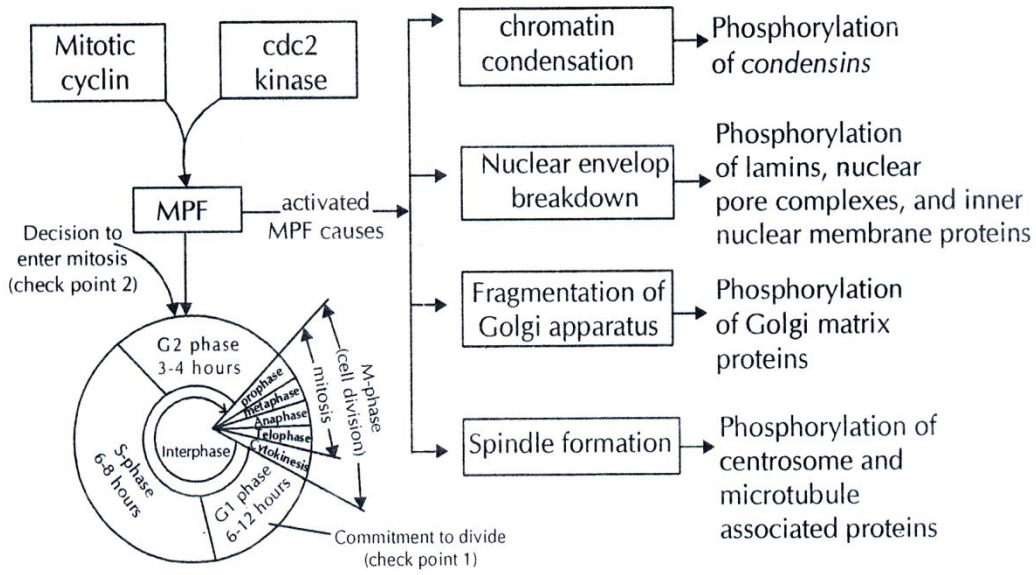


Figure 5.5: Cell cycle. This figure shoes different phases of cell cycle and possible roles of mitosos promoting Factor

Cell cycle check points

Cells are considered to check their mass, state of DNA replication and DNA damage, etc at specific points in the cell cycle. They are allowed to go to pass such a point only if all the necessary conditions are satisfied, otherwise the cell cycle is stopped at this point; these points are called check point (Fig.5.6). There are three check points in the cell cycles of eukaryotes.

(1) G_1check point

(2) G_2 check point

(3) spindle assembly check point

In yeast, G_1check point is called START while in animal cells G_1 check point is called the RESTRICTION POINT. Cells that have successfully passed through the restriction point are committed to S phase, whereas those that have not passed this point can remain in G_1indefinitely, in the resting state called G_0. This point is regulated by a protein kinase which is similar to M phase kinase. G_1phase kinase

is G_1 cyclin dependent protein kinase.At the G_2 check point, located at the boundary between G_2 and M phase, proper completion of DNA synthesis is required before the cell can initiate mitosis.

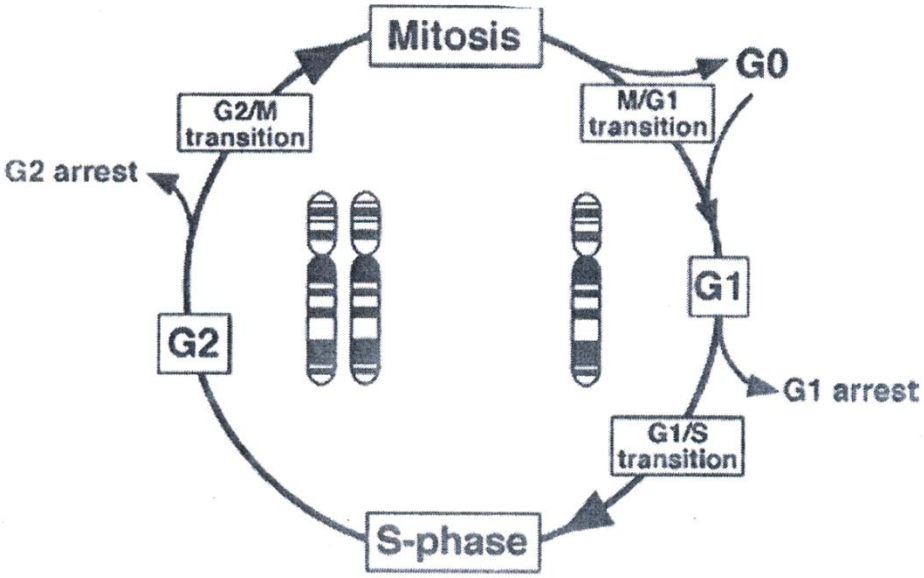


Figure 5.6: Cell cycle check points

In certain cell types, the cell cycle can be indefinitely arrested at this point if cell division is not necessary; under such conditions the cells enter a resting state analogous to G_0. The third cell cycle check point, the spindle assembly check point, is at the junction between metaphase and anaphase.If the two chromatids that make up each chromosome are not properly attached to opposite spindle poles, the cell cycle is temporarily arrested at this point. In the absence of such control mechanism, there would be no guarantee that each of the newly-forming daughter cells would receive a complete set of chromosomes.

If this check point is passed, the cell cycle is allowed to proceed to anaphase in which the chromatids separate due to the activation of an enzyme (a protein) which destroys the *cohesin* proteins holding the chromatid together.G_1check point is the major decision point in animal cells so that a cell going passed the restriction point will complete M phase as well. At this point, a cell evaluates its internal and external environments, and accordingly decides if it is ready to enter S phase. If commitment is made to enter S phase, DNA replication begins and will not stop before it is completed.

In cultured animal cells, this is the major control point so that a cell entering S phase, will normally enter division phase as well, this is probably typical of most diploid cells whether *in vitro* or *in vivo.* The G_2 check point is more important in the mitotic division of fertilized frog eggs and in the yeast *Schizosaccharomyces pombe* (fission yeast because it reproduces by dividing evenly in two, rather than by budding). The G_1check point is more important in the budding yeast *(Saccharomyces cerevisiae).*

Machinery which regulate cell division.

The four control mechanisms modulate the activity of specific cdks

1. Regulation of cdks by phosphorylations

2. Controlled degradation of cyclin
3. Regulated synthesis of cdks and cyclins
4. Inhibition of cdks by specific protein inhibitor that, in turn, control whether a cell will divide, differentiate, become permanentaly quiescent, or begin a new cycle of division after a period of quiescence. Here I am going to discuss (2) in detail:

Controlled degradation of cyclin

Proteolysis of mitotic cyclins, which leads to a decrease in MPF activity, is required for the completion of mitosis. Once mitosis has been initiated, cyclins that have served their purpose by this point in the cell cycle, breaks down with the help of a protein complex called the anaphase promoting complex(APC), also called the cyclosome. Highly specific and precisely timed proteolytic breakdown of mitotic cyclin regulates cdks activity throughout the cell cycle.

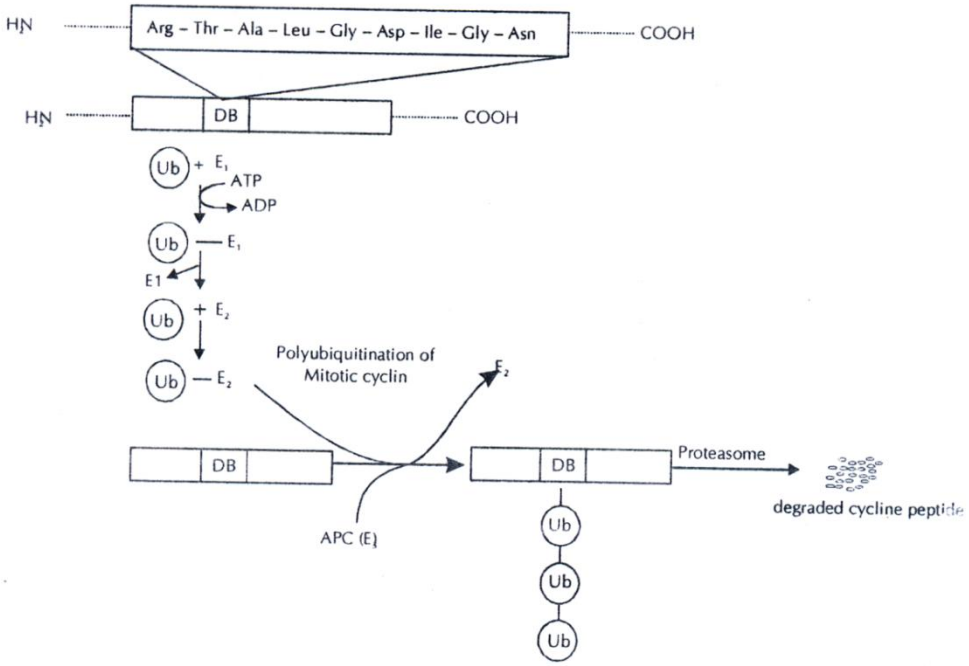


Figure 5.7: Ubiquitin mediated protein degradation

The cyclin contain near their amino terminus a nine residue sequence [Arg-Thr-Ala-Leu-Gly-Asp-Ile-Gly-Asn], the destruction box that is recognised by ubiquitinating enzymes which target for degradations. A protein that recognises this sequence, called DBRP (destruction box recognising protein); initiates the process of cyclin degradation by bringing together the cyclin and other protein ubiquitin. The multi-subunit APC directs specific ubiquitin-conjugating enzymes to polyubiquitinate mitotic cyclins, marking the proteins for rapid degradation by proteasomes (Fig.5.7).

Chapter 6

Mendel's Laws

Mendelism: Law's of Inheritance

Mendel chose garden pea as plant material for his experiments, since it had the following advantages:

1. well defined characters,
2. Bisexual flowers,
3. Predominantly self-fertilization,
4. Easy hybridization.

Besides these features, garden pea, being self-fertilized, had pure lines due to natural self-fertilization for a number of years.

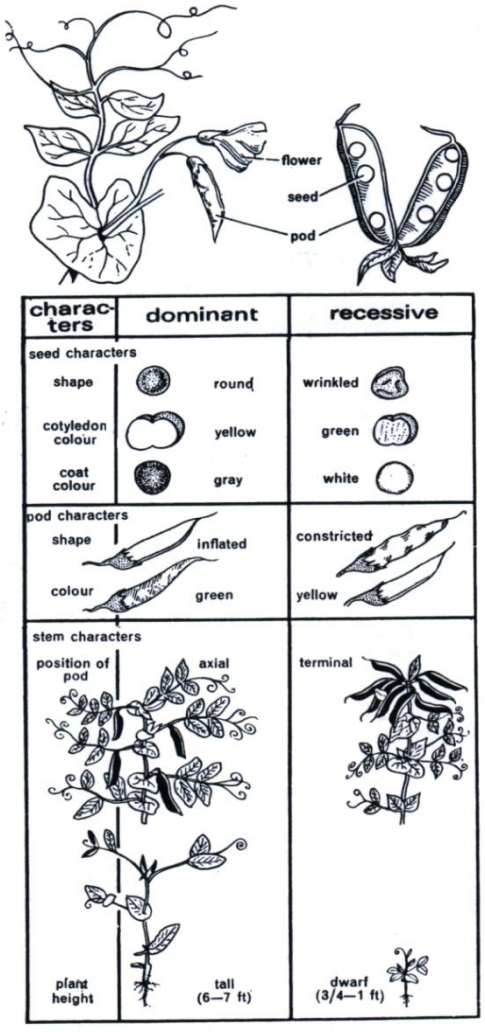


Figure 6.1: Seven pairs of characters used by Gregor Mendel

Therefore, any variety used was pure for the characters it carried.Although hybridization experiments were conducted by earlier workers also as discussed in the previous section, but they

considered the individual as a whole complex of characters. Mendel's success was mainly based on the fact that he considered a single character at one time. Seven pairs of contrasting characters were chosen for the study. These characters are shown in Figure 6.1.

Crossing technique

Since garden pea is self-fertilizing, the anthers have to be removed before maturity. This operation of removal of anthers is called emasculation**.** The stigma is protected against any foreign pollen, with emasculated flower. At the time of pollination,the pollen should be mature and stigma should be receptive.

For each of the seven pairs of characters shown in Figure 6.1, plants with one alternative trait were used as female and those with the other alternative as male. Reciprocal crosses were also made. The population obtained as a result of crossing plants exhibiting contrasting characters is called the first filial generation or F_1 (filial means progeny). The progeny of Fj plants obtained due to self-fertilization represents the second filial generation or F_2. Similarly F_3, F_4, etc. can also be obtained.

Results of Mendel's experiments

Mendel presented the data and conclusions derived from his experiments in a paper entitled "Experiments in Plant Hybridization"which was read before the Brunn Natural History Society in 1865 and was published in the proceedings of the society in 1866.

Table 6.1: Actual dataobtained by Mendel in F_2 segregating progenies in pea

Character studied*	Chromosome location (known now)	Domonant	Recessive	Total	Ratio
1.Seed form (R, r)	7	5474 (*round*)	1850 (wrinkled; rugosus)	7324	2.96 : 1
2. Cotyledon colour (l, i)	1	6022 (*yellow*)	2001 (green)	8023	3.01 : 1
3. Seed coat colour (A, a) or flower colour (violate vs white)	1	705 (*grey*)	224 (white)	929	3.15 : 1
4.Pod form (V, v)	4	882 (*inflated*)	299 (constricted)	1181	2.95 : 1
5.Pod colour (Gp, gp)	5	428 (*green*)	152 (yellow)	580	2.82 : 1
6.Flower position (Fa, fa)	4	651 (*axial*)	207 (terminal)	858	3.14 ; 1
7. Stem length (Le, le)	4	787 (*tall*)	277 (dwarf)	1064	2.84 : 1
Total		**14,949**	**5010**	**19,959**	**2.98 : 1**

*Gene symbols in parentheses are those used currently

This paper was though prepared as a lecture rather than as a research publication, contained Mendel's hypothesis concerning the mechanism of inheritance.Mendel's results can be explained using a specific example. When tall plants were crossed with dwarf plants, all plants in the F[generation were tall. The plants used in the initial cross are referred to as P, and P_2 or parents. When theF_1plants were self-fertilized, both tall and dwarf plants were obtained in the F_2 generation. The tall and dwarf plants were obtained in a ratio close to 3 : 1. Similar patterns were obtained for other six pairs of characters also.

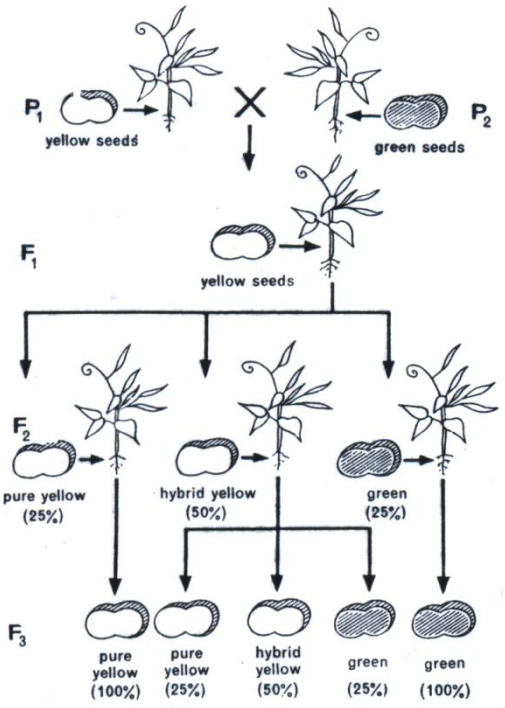


Figure 6.2: Inherittance of stem length showing designation used to represent different genotypes in the parents F_1, F_2, F_3 generations

The pattern of inheritance observed in all the seven cases can be summarized as follows:

1. For any character the F, individuals derived from crosses, between two different varieties having alternative characters, showed only one of the traits and never the other. This feature was expressed as dominance of one trait over the other. The trait which appeared in the F, generation was called dominantand the other which did not appear in the F, population was called recessive.
2. It did not matter which parent variety provided the pollen and which provided the eggs; the results were always the same. In other words, the reciprocal crosses gave the same results.

   The dominant-recessive relationships of the seven pairs of characters are shown in Figure 6.1.

   The results obtained by Mendel with respect to seven pairs of characters are listed in Table 6.1.

The results presented in Table 6.1 indicate that a definite 3 : 1 ratio was obtained in each case in the F_2 generation. The determining agent responsible for each trait was called a 'factor'. Since the recessive trait

was not seen in the F, generation but reappeared in F_2, Mendel could predict the results to be expected in F_3. This will be illustrated with the help of an example.

When plants with yellow seeds were crossed with plants having green seeds, only yellow seeds were obtained in the F_1generation. This showed dominance of yellow seed colour over green seed colour. When F, seeds were sown and seeds were obtained in F_2 generation, these were obtained in a ratio of 3 yellow and 1 green (6022 yellow, 2001 green). Mendel predicted that out of the F_2 yellow seeds sown, one-third should give rise to only yellow seeds in the next generation, while the remaining two-third should again give rise to yellow and green seeds in 3 : 1 ratio. The plants raised from green F_2 seeds were expected to produce only green seeds (Fig. 6.2).The results expected by Mendel as outlined in Figure 6.2 were actually obtained in the F-, generation for five of the seven pairs of characters studied.

Symbols and Terminology

Symbols

English alphabets were used by Mendel to represent the factors. A capital letter like *'A'* signified dominani member and lower case like *'a'* signified the recessive member. Since large numbers of genes are now available, one or more alphabets are chosen to indicate the characters for which they are used. The alphabet is chosen on the basis of the mutant character (mutant character is one which deviates from the wild type; wild type character is one which is most common). For instance, since garden pea is normally tall, the dwarf (affecting stem length) which is considered to be mutant, can be represented by letter *'le'* (lower case in italics is used to indicate recessive nature). The tall will then automatically receive the designation *'Le'* (capital italics showing dominance). It is not necessary that the alphabet used, should always represent the recessive character, since in exceptional cases, a mutant may be dominant and the wild type a recessive (e.g. barred eye, a mutant character in *Drosophila* is dominant and is represented as *'B'* and the normal wild type eye which is recessive is represented as *'b').*

Table 6.2: Summary of two systems of assigning symbols to genes

System for symbols	Recessive mutant, *a*		Domonant mutant, *A*	
	Symbol for wild type allele	**Symbel for mutant allele**	**Symbel for wild type allele**	**Symbel for mutant allele**
Mendelian	A	a	a	A
Newer system	a+ (or $+^{a}$ or +)	a (or a^{-})	A+ (or $+^{A}$ or +)	A (or A^{-})

Each individual receives two doses for each character, one from the father and the other from the mother. Therefore, in the above example pure dwarf will be represented as *'le le'* and pure tall *'Le Le'*. Two doses of a factor present will separate using meiosis before fertilization and each gamete will have a single dose. Therefore, gametes from tall plants can be designated as *'Le'* and those from dwarf plants as *'le'*. The F_1 will then be the product of these two and should be designated as *'Le le'* (Fig. 6.3). This *'Le le'* will be tall because *'Le'* dominates over *'le' (le* = length).

Another method for the use of symbols is to signify the wild type by a sign ' + ' and the mutant type by a capital or a small letter depending upon whether the mutant is dominant or recessive. In the above case, for instance, tall will be represented as +/+, the dwarf as *le/le* and F, as *+/le.* This method has an advantage of using the same sign for every wild character. A ' + ' sign will mean different things at different places depending upon the context in which it is used. An improvement over the above method is to modify the ' + ' sign lo indicate the character for which it is meant. For instance in the above case *'+*^{ie}or *'le*$^{+}$*'* may be used for tall and *'le'* for the dwarf. The two methods of assigning symbols lo genes are summarized in Table 6.2.Although mainly the first method, where only letters are used, will be followed in this book, the students should be familiar with the use of ' + ' sign in genetics. Following these symbols, the cross of tall and dwarf and the F_1, F_2 and F_3 progenies can be represented as shown in Figure 6.3.

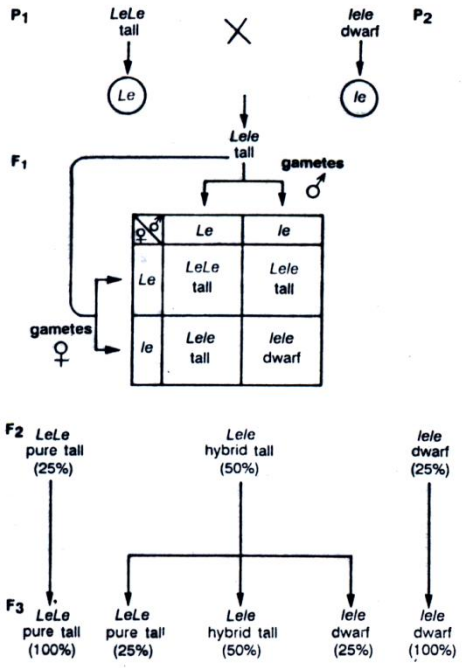


Figure 6.3: Inheritance of stem length showing designations used to represent different genotypes in the parents, F_1, F_2 and F_3 generations

Terminology

The following terms are commonly used in genetics and should be understood:

Gene

In modern sense an inherited factor that determines a biological character of an organism is called a gene. This is a functional unit of hereditary material. In the past, gene was believed to be unit of structure also, but recent knowledge has shown that it is no longer a unit of structure.

Allelomorphs or alleles (homozygous and heterozygous)

Alleles, the abbreviated form of the term 'allelomorphs' (meaning one form or the other) indicates alternative forms of the same gene. For instance, in the above example *'Le'* and *'le' are* two allelomorphs of the gene for plant height. In pure tall or pure dwarf plants, same allele is duplicated *(Le Le* or *le le),* while in hybrid tall, both the alleles will be present *(Lele).* An individual having only one allele or in other words two identical alleles, is known as homozygous *(LeLe* or *lele).* Similarly, an individual, having two different alleles will be called heterozygous or hybrid *(Lele).* In the present usage the term gene and the allele are interchangeable, but while gene can be used for any factor, allele is used with reference to another allele. For instance, while *'Le'* and *'le'* are alleles to each other, they can not be allelic to any other gene.

In certain situations, the terms allele and gene are used interchangeably, and may thus, become confusing. For example, 'dominant gene' and 'dominant allele', both refer to the same thing. This is due to the fact that *alleles* of a gene are considered to be the genes themselves functionally. Monohybrid, dihybrid and trihybrid. Inthe examples discussed earlier in this chapter, single characters each controlled by a single pair of genes or alleles were considered. Such crosses are known as monohybrid crosses and the F_2ratio of 3 : 1 is known as the monohybrid ratio. Similarly crosses can be considered when two or three pairs of genes or alleles arc involved. Such crosses will be called dihybrid and trihybrid crosses and the respective ratios as dihybrid and trihybrid ratios.

Reciprocal crosses

A set of two reciprocal crosses means that the same two parents are used in two experiments in such a way that if in one experiment, 'A' is used as the female parent and 'B' is used as the male parent, in the other experiment 'A' will be used as the male parent and 'B' as the female parent. This was earlier discussed in this chapter.

Backcross and testcross

The F, individuals obtained in a cross are usually selfed to get the F_2 progeny. They can also be crossed with one of the other two parents from which they were derived. Such a cross of F, individual with either of the two parents is known as a backcross. In such backcrosses, when F, is backcrossed to the parent with dominant phenotype, no recessive individuals are obtained in the progeny. On the other hand, when it is crossed with recessive parent, both phenotypes appear in the progeny. While both these crosses

are backcrosses only the cross with the recessive parent is known as a testcross. It is called a testcross, because it is used to test whether an individual is homozygous (pure) or heterozygous (hybrid).

This can be illustrated with the help of the example of tall *(LeLe)* and dwarf *(lele)* plants. In the F, generation only tall plants *(Lele)* appear. These plants can be backcrossed with either of the two parents as shown in Figure 6.4.

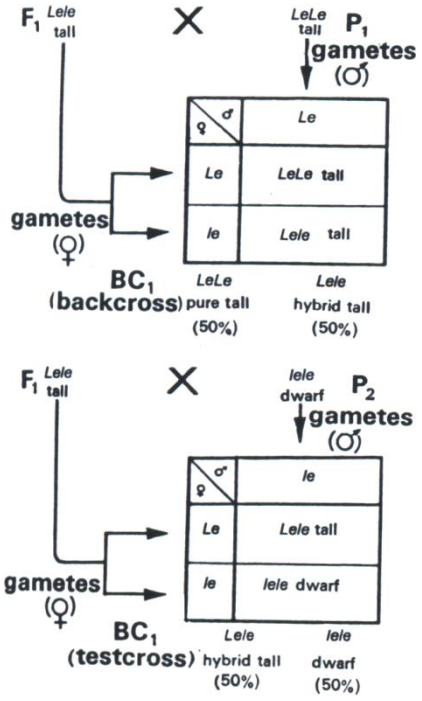


Figure 6.4: Inheritance of plant length in a back cross (A), and test cross (B)

Principle of Segregation

(Law of Purity of Gametes or Mendel's First Law)

Mendel's principle of segregation is inherent in the results of Mendel earlier described in this chapter. It is obvious that though in F, the dominant phenotype appears, the recessive phenotype is not lost but reappears in F_2. This suggested that there is no blending of Mendelian factors in F_1, but that they stay together and only one is expressed. At the time of the formation of gametes, these two factors obviously separate or segregate, otherwise recessive type will not appear in F_2. The gametes which are formed are always pure for a particular character. A gamete may carry either the dominant or the recessive factor but not both as we find in F, individual. This is why it is called either as 'principle of segregation' or as 'law of purity ofgametes'. This concept of segregation is often called Mendel's First principle. While a 3 : 1 ratio in F_2 generation of a monohybrid cross suggested that segregation of alleles does take place, the testcross outlined above confirmed it.

Principle of Independent Assortment (Mendel's second law)

As earlier pointed out, one of the secrets of Mendel's success was that he considered only one character at one time and, unlike earlier workers, did not study the individual as a whole. Such an approach soon enabled him to discover the principle of segregation. From this simplest situation, Mendel tried to analyze cases where two pairs of characters were simultaneously studied. He tried to find out whether one pair of characters is completely independent of another pair or else if there was any deviation.

As we know, crosses where two pairs are involved are known as dihybrid crosses.The classical example of a dihybrid cross is the use of two pairs of characters namely the seed shape and seed colour. The plants with yellow and round seeds (pure) were crossed wrth those having green and wrinkled seeds (pure). The F, seeds were yellow and round. When these F, seeds were grown into plants, F_2 seeds were obtained which showed all the four possible combinations i.e. (i) yellow and round seeds, (ii) yellow and wrinkled seeds, (iii) green and round seeds, and (iv) green and wrinkled seeds. These four kinds of seeds were obtained in 9 : 3 : 3 : 1 ratio (Fig. 6.5).

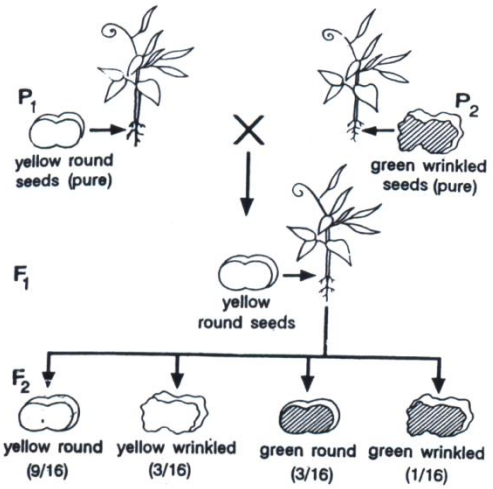


Figure 6.5: Independent assortment of seed shape and seed colour

The above results would be expected if the two pairs of characters are believed to behave independent of each other. In the ratio 9 (yellow round) : 3 (yellow wrinkled) : 3 (green round) : 1 (green wrinkled), we can analyze them for single characters, separately as in Tables 6.3, 6.4 and 6.5. In this analysis it is obvious that, when one character is fixed, the other character exhibits a clear 3 : 1 ratio. Therefore, it can be concluded that the two characters under consideration are assorting (giving rise to different combinations) in an independent manner. This principle is known as 'principle of independent assortment'**.** The independent assortment holds good for two or more than two pairs of characters. The following discussion will be devoted to the theoretical derivation of dihybrid and trihybrid ratios on the basis of independent assortment.

Table 6.3: Relative proportions of four combinations in a dibybrld cross as derived from monohybrld crosses

	Cross	Phenotype	Probability in F_2
1	Monohybrid cross	yellow seeds	3/4
	(seed colour)	Green seeds	1/4
2	Monohybrid cross	Round seeds	3/4
	(seed shape)	Wrinkled seeds	1/4
3	Dihybrid cross	Yellow and round seeds	3/4X3/4=9/16
	(seed colour	Yellow and wrinkled seeds	3/4X1/4=3/16
	& Seed shape)	Green and round seeds	1/4X3/4=3/16
		Green and wrinlled seeda	1/4X1/4=1/16

Table 6.4: Segregation for seed colour among different classes of seed shape

	Population analysed	Phenotype	Proportion	Ratio
1	Whole F_2	Yellow	12/16	3 : 1
		Green	4/16	
2	Among round seeds	Yellow	9/12	3 : 1
		Green	3/12	
3	Among wrinkled seeds	Yellow	3/4	3 : 1
		Green	1/4	

Table 6.5: Segregation for seed shape among different classes of seed colour

	Population analysed	Phenotype	Proportion	Ratio
1	Whole F_2	Round	12/16	3 : 1
		Wrinkled	4/16	
2	Among yellow seeds	Round	9/12	3 : 1
		Wrinkled	3/12	
3	Among green seeds	Round	3/4	3 : 1
		Wrinkled	1/4	

Dihybrid cross

A dihybrid cross is represented in Figure 6.6. This method of working out the F_2 progeny using different kinds of male and female gametes is known as checkerboard. From the checkerboard the different phenotypes and the genotypes can be analysed. Table 6.6 shows a summary of the genotypic and phenotypic .ratios in F_2 progeny.The results of a dihybrid cross in F_2 generation can also be obtained

by the 'Forked line method'shown in Figure 6.7 and Figure 6.8. The forked line method makes use of the consideration of single character at a time.

Dihybrid testcross

In the F_2 progenies, only one of the four phenotypes i.e. green and wrinkled seeds will be homozygous for both characters. The other three phenotypes can be homozygous or heterozygous for one or both the characters. Testcross is used to test whether an individual is heterozygous or homozygous. For instance, if an individual is heterozygous for both characters (as in F_1) and is crossed with double recessive, a phenotypic ratio of 1 : 1 : 1 : 1 will be obtained. Diagrammatic representation in Figure 6.9 will explain it.

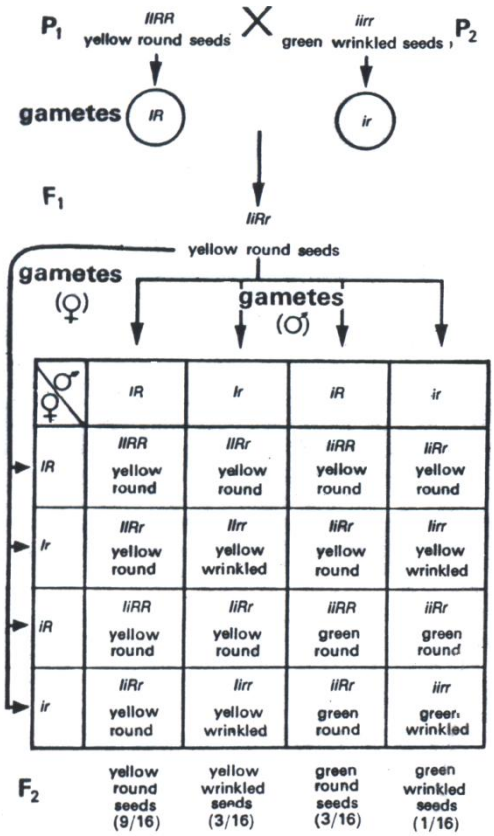


Figure 6.6: Checkerboard showing 16 combinations obtained in cross involving characters for seed shape and seed colour

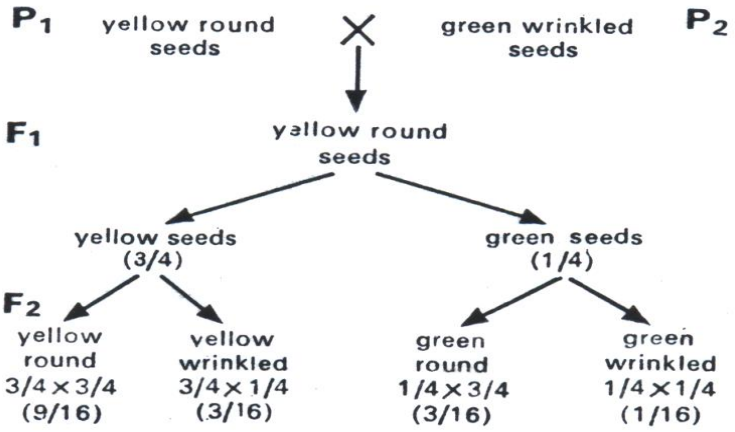


Figure 6.7: Derivation of the phenotypes in a dihybrid cross using forked line method

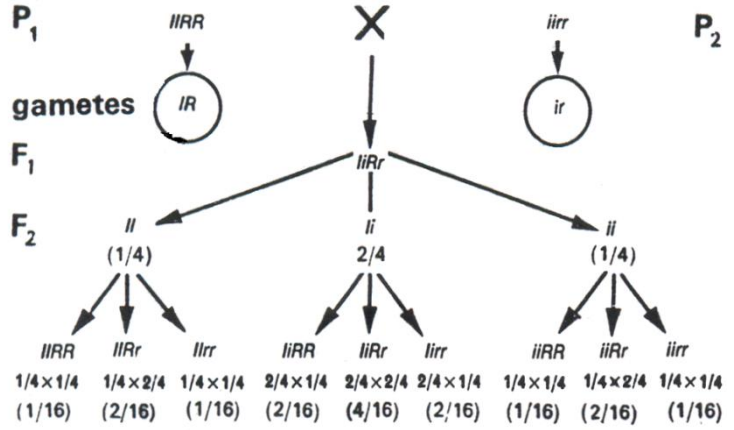


Figure 6.8: Derivation of the genotypes in a dihybrid cross using forked line method

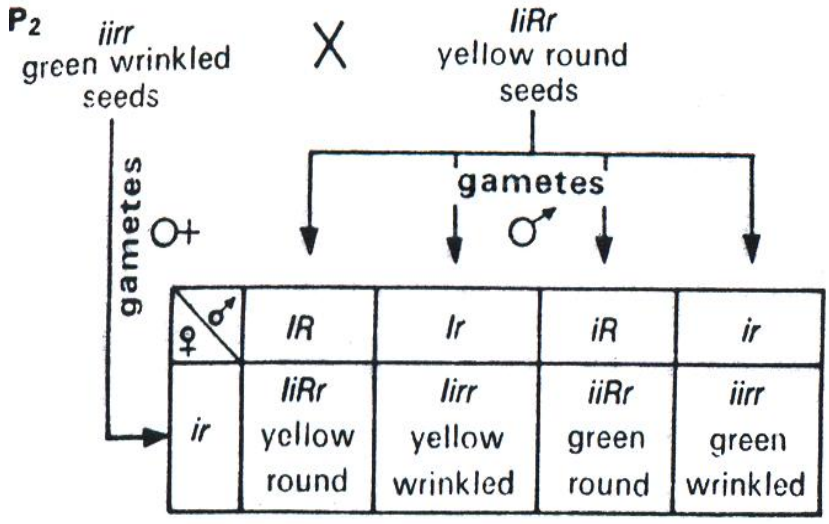


Figure 6.9: Diagrammatic representation of a dihybrid test cross involving seed shape and seed colour

Table 6.6: Summary of the genotypes and phenotypes obtained in F_2 (556 seeds) of the cross shown in Figure 6.6

Genotype	Genotypic ratio	Phenotype	Phenotypic ratio
II RR	1		
II Rr	2		
Ii RR	2	yellow and round (315)	9
Ii Rr	4		
II rr	1	yellow and wrinkled (101)	3
Ii rr	2		
Ii RR	1	green and round (108)	3
Ii Rr	2		
Ii rr	1	green and wrinkled (32)	1
Total	16	556	16

Tribybrid cross

The following three pairs of characters may be considered in a trihybrid cross,

(i) Tall plant vs. dwarf plant,

(ii) Yellow seed vs. green seed,

(iii) Round seed vs. wrinkled seed.

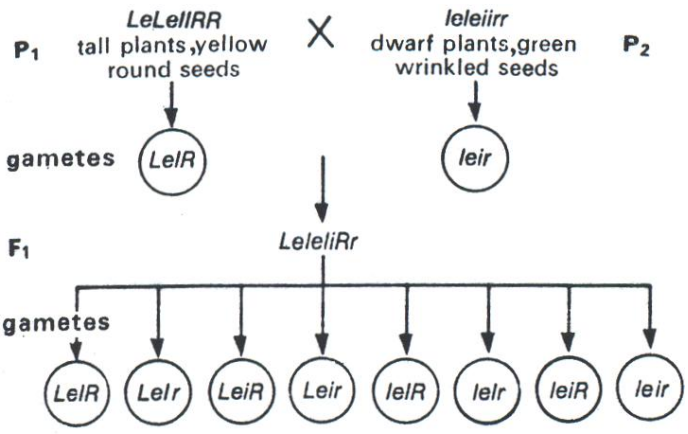


Figure 6.10: A trihybrid cross showing the production of eight kinds of gametes by F_1

The parents *(LeLelIRR* and *leleiirr)* will give rise to the F, hybrids *(LeleliRr)* which are heterozygous for three genes. Eight different types of gametes will be available in equal proportions both on the male side as well as on the female side (Fig. 6.10). A checkerboard technique will involve the drawing of 64 combinations. Therefore, for the study of three or more genes, checkerboard is rather difficult and forked line method is utilized. The diagrammatic representation in Figure 6.11 will show the results of a trihybrid cross.

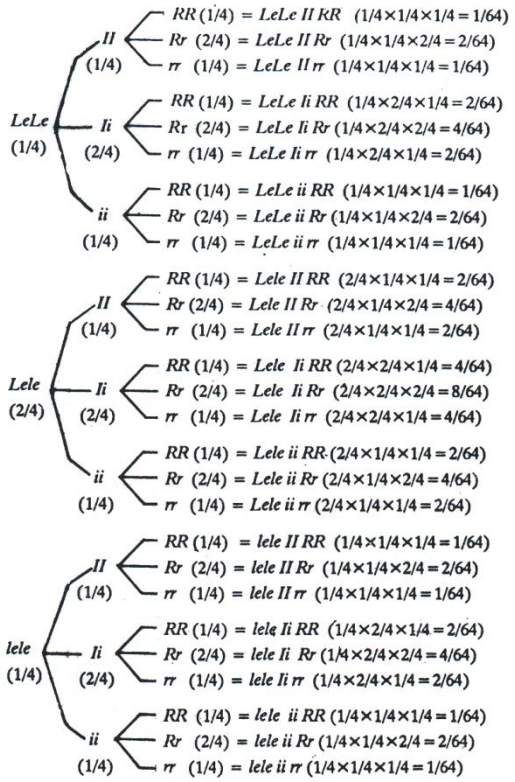


Figure 6.11: The results of a trihybrid cross as derived by forked line method

A summary giving the genotypes, phenotypes and their ratios obtained in a trihybrid cross is presented in Table 6.7.The trihybrid testcross will involve crossing of a trihybrid *(LeleliRr)* with triple recessive *(leleiirr).* The eight different phenotypes described in the previous section will be obtained in 11 : 1:1:1:1:1:1 ratio as shown in Figure 6.12.

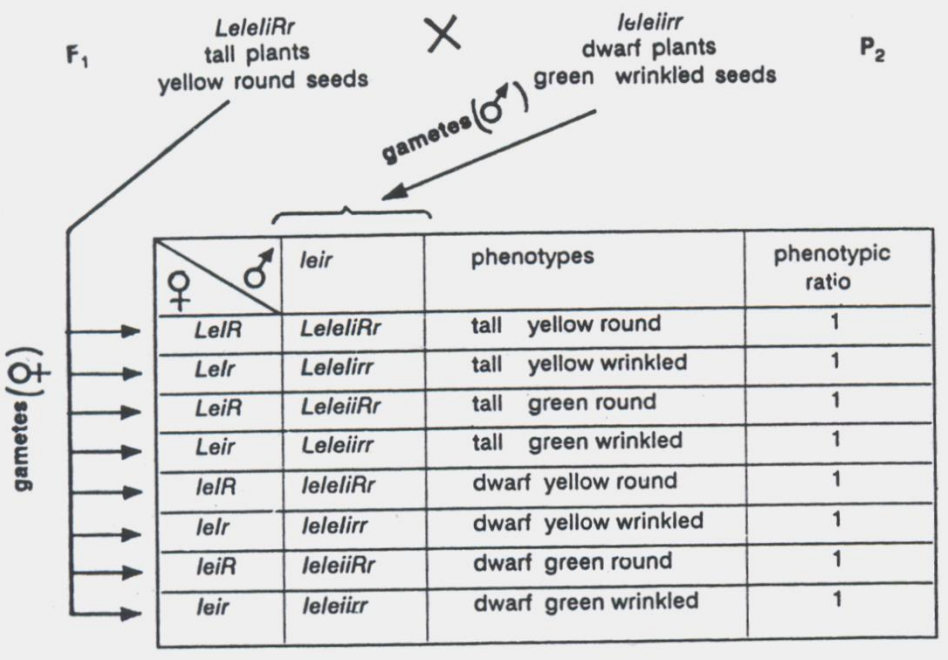
Mendel's Results, Chromosome Theory and Linkage (Present Status)

Mendelian factors (later called 'genes') are located on chromosomes and that genes located on same chromosome, unless. Separated by long distances, will not exhibit independent assortment'.(in other words these will be linked). In view of this we can re-examine the seven pairs of genes used by Mendel and analyze, whether or not they exhibited independent assortment in all possible 21 combinations, when arranged in pairs. This is necessary, because often a parallelism is drawn between seven pairs of characters used by Mendel and the seven pairs of chromosomes found in pea.

Table 6.7 Genotypic and phenotypic ratios in a trihybrid cross (discussed in the text)

Genotype	Genotypic ratio	Phenotype	Phenotypic ratio
LeLeIIRR	1		
LeLeIIRr	2		
LeLeIiRR	2		
LeLeIiRr	4	tall, yellow, round	27
LeleIIRR	2		
LeleIIRr	4		
LeleIiRR	4		
LeleIiRr	8		
LeLeIIrr	1		
LeLeIirr	2	tall, yellow, wrinkled	9
LeleIIrr	2		
LeleIirr	4		
LeLeiiRR	1		
LeLeiiRr	2	tall, green, round	9
LeleiiRR	2		
LeleiiRr	4		
leleIIrr	1		
leleIIRr	2	dwarf, yellow, round	9
leleIiRR	2		
leleIiRr	4		
LeLeiirr	1	tall, green, wrinkled	3
Leleiirr	2		
leleIIrr	1	dwarf, yellow, wrinkled	3
leleIirr	2		
leleiiRR	1	dwarf, green, round	3
leleiiRr	2		
leleiirr	1	dwarf, green, wrinkled	1

This is also used as an argument, albeit erroneously, for independent assortment observed by Mendel, by saying that had he taken more than seven characters, he would have had problems due to linkage. In this connection, we can examine the relationship between different characters with their standard gene symbols and the chromosomes on which each is located (Table 6.7).



♀ \ ♂	*leir*	phenotypes	phenotypic ratio
LelR	*LeleliRr*	tall yellow round	1
Lelr	*Lelelirr*	tall yellow wrinkled	1
LeiR	*LeleiiRr*	tall green round	1
Leir	*Leleiirr*	tall green wrinkled	1
lelR	*leleliRr*	dwarf yellow round	1
lelr	*lelelirr*	dwarf yellow wrinkled	1
leiR	*leleiiRr*	dwarf green round	1
leir	*leleiirr*	dwarf green wrinkled	1

Figure 6.12: A checkboard showing a trihyvrid test cross

In his original paper, Mendel gave results of only two experiments showing independent assortment. One of them involved two characters *(R-r, l-i*) and the other involved three characters *(R-r, I-i, A-a),* such that they represented demonstration of independent assortment among only three of the seven characters in three possible combinations (out of a total of 21 combinations possible among seven characters). For other character combinations, experiments were conducted, but no results are available in Mendel's original paper. However, he claimed that independent assortment was available in all other combinations also.

Extensive linkage studies later conducted by H. Lamprecht (1961), however, demonstrated that seven genes used by Mendel belonged to only four linkage groups. It was also shown that the combinations *i-a, v-fa* and *fa-le* despite each belonging to same linkage group are not linked (due to separation by long distances) and exhibit independent assortment. However, a solitary combination, *v-le* showed linkage (13% recombination) suggesting lack of independent assortment. Therefore, it is obvious that either Mendel did not conduct an experiment for independent assortment between v and*le* or else overlooked the lack of independent assortment in this combination.

Molecular Basis of Mendel's Wrinkled Seed Character

In the year 1990, a group of scientists, working at John Innes Institute at Norwich (U.K.), cloned the pea gene *r (rugosus),* which determines whether the seed is round or wrinkled. It was shown that an isoform of 'starch branching enzyme' (SBE I)is present in round *(RR* or *Rr)* seeds, but absent in wrinkled *(rr)* seeds. By carefully planned experiments it was shown that there was 100% co-segregation of the absence of SBE I with r locus, suggesting that the gene for SBE I is located at r locus. It was also shown"

that in *rr*lines, SBE I gene is interrupted by a small DNA sequence (0.8 kilobases) called 'transposon-like insertion', similar to *Ac/Ds* system in maize. Interruption of SBE I gene by insertion of a foreign sequence, gives an aberrant SBE I enzyme leading to metabolic disturbances in the biosynthesis of starch, lipid and protein. This results in an increase of free sugars due to failure of starch formation. This probably leads to higher osmotic pressure and hence higher water content and larger cell volume earlier observed in *rr*seeds. The seeds lose large proportion of their volume on maturation leading to shrinkage in volume. Since testa does not shrink with the cotyledons, seeds become wrinkled.

In most cases of the study of Mendelian traits, we seldom know the molecular basis, so that the students do not ask the question: how does a gene control the phenotype? With the availability of recent techniques of molecular biology and recombinant DNA, many traits are now being examined for the molecular basis of their development. This approach is receiving support from the recent emergence of 'reverse genetics' (from DNA to phenotype) as opposed, to forward or classical genetics (from phenotype to DNA).

Genetic interactions

Introduction

After the rediscovery of Mendel's work in 1900 by De Vries, Correns and Tshermak, the laws of segregation and independent assortment were confirmed. However in certain instances they appeared to be deviating. This has led to the formation of opinion that Mendel's laws could not be applied universally and there are certain exceptions in some specific instances.Mendel was successful in formulating the laws of heredity because he was lucky enough to choose simple non linked traits. All the seven pairs of contrasting characters he chose in the garden pea, *Pisum sativam* were non linked. Genes for seed colour and flower :olour are now known to be present on the same chromosome. But inkage is not detected in their inheritance since they are far apart Tom each other. They assort independently as Mendel discovered. One pair of characters namely plant height and pod shape, then responsible for these traits show linkage in peas, lonspicuously Mendel did not report the results of the dihybrid cross) pair.

From the Mendelian laws of inheritance, it is noted each trait s determined by a single pair of genes or factors. However Later experiments have proved that many genes may effect and single character. It might also be possible that several pairs of genes 'ocated at different places of same chromosome or different chromosomes interact to produce the same effect of a trait. For example the flower color of sweet pea, feather color of fowls, capsule shapes of shepherds purse etc. The coat colour of mammals *(mice)* is the result of the combined action of several genes, as well She eye colour of Drosophila.

When several non homologous genes interact to produce a phenotypic character, it does not mean that all these have acted together to express that trait. One pair may modify the trait, another

pair may reverse, yet another pair may inhibit and so on. The expression of these genes may sometimes be effected by the 5 environment also. This is known as the Gene Interaction or Factor Interaction.This phenomenon is a deviation from Mendel'sfundamental laws and states that characters are due to the f interaction of various pairs of genes but not a single pair as such. | This is responsible for the alternation of classical Mendelian monoand dihybrid ratios. As noted above the interaction is possible ; between more than two genes also, but it is too complicated; so I interactions involving two genes only are discussed here.

Unfortunately the majority of traits cannot be analyzed in a simple Mendelian fashion. The linkage is also another important factor for the deviation of Mendelian monohybrid classical phenotypic ratio of 3 : 1 or dihybrid ratio of 9 : 3 : 3 : 1 or the test cross ratios of 1:1 and 1:1:1:1.

In this chapter we shall discuss about such phenomena wlvch obscure the genetic nature of many interesting traits in complex organisms. Had Mendel encountered them in his studies they might have thrown him completely off the track. These important modifications of Mendelian mono and dihybrid ratios are explained below on the basis of interaction of genes.

Modification of monohybrid ratios:

Incomplete dominance

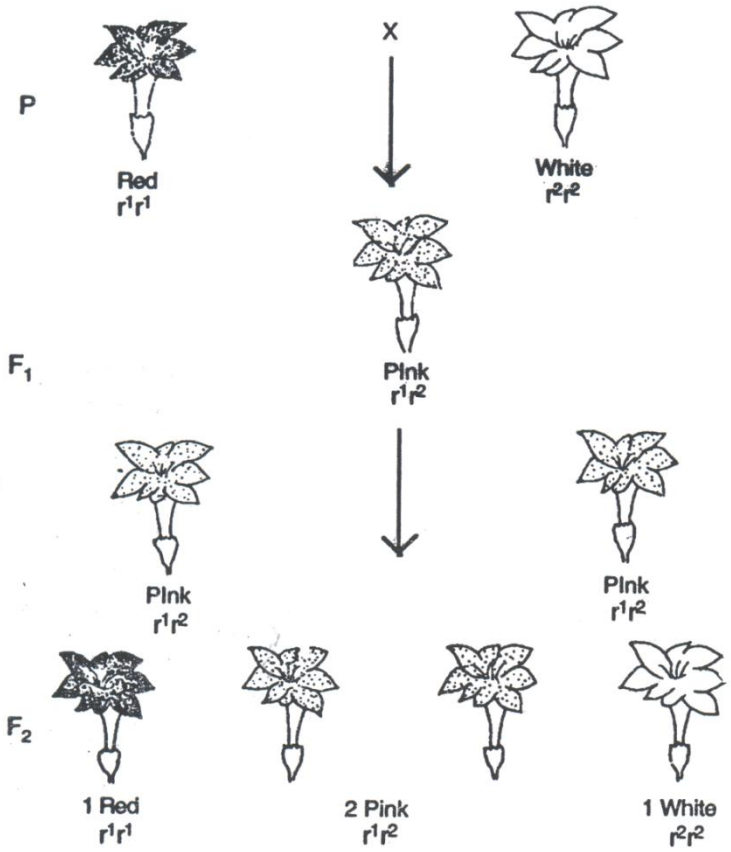


Figure 6.13: A cross in *Mirabilis jalapa* for incomplete dominance

So far we know about the examples of complete dominance or recessiveness, in which phenotypes are indistinguishable from homozygous and heterozygous dominant individuals. A number of dominant alleles in plants and animals do not follow this pattern.In four O' clock plant, Mirabilis jalapa, if a red flowered plant is crossed with a white floivered plant, both are homozygous, the F_1 heterozygotes would bear pink flowers. When these Fj plants are crossed, they produce 1 red coloured, 2 pink coloured and 1 white coloured progeny in F_2 (6.13).

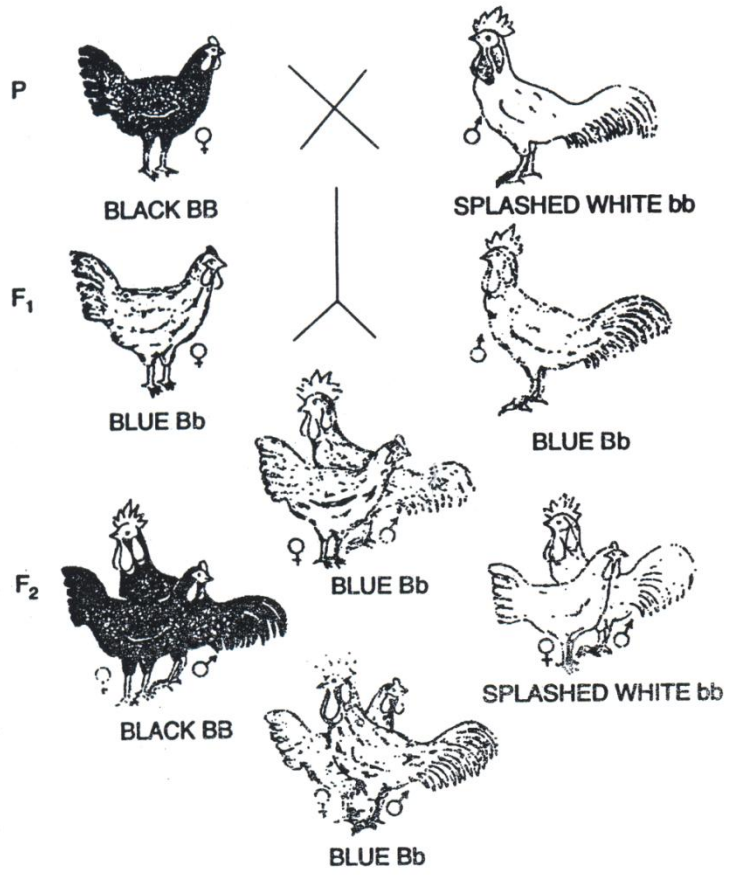


Figure 6.14: Cross between black and splashed white andalusian fowls showing the incomeplete dominance

Similarly if the black andalusian fowl is crossed with splashed white andalusian fowl, Fj fowls are blue in colour and in $F_{2/}$ 1 black, 2 blue and 1 splashed white fowls are obtained. (Fig 6.14).Here the red colour of *Mirabilis jalapa* and black colour of o Andalusian fowls are incompletely dominant. If the genes are not -completely dominant the phenotypic and genotypic ratios would remain the same, i.e., 1:2:1.A similar situation occurs in humans regarding the type of hair. Curly hair is incompletely dominant over straight height. which is a homozygous recessive trait. Heterozygotes in this case will carry wavy hair.

Codominance:

In the inheritance of A B O system of blood groups, the type of antigen present on the RBC of a person will decide his blood group. If he carries 'A' antigen he is 'A' group person and if there is 'B' antigen on the RBC, he is 'B' group person. If a person carries both these antigens he is AB group man. The genotype of 'A' group person is I^AI^A or I^Ai, that of 'B' group person is I^BI^B or I^Bi. I^AI^B person will have AB group of blood.The expression of these alleles is known as codominance in which heterozygotes for two codominant alleles express both the traits determined by the alleles. So the I^AI^A and I^BI^B are codominant alleles.

Modifications of 9 :3 : 3 :1 ratios

Combs in Fowls

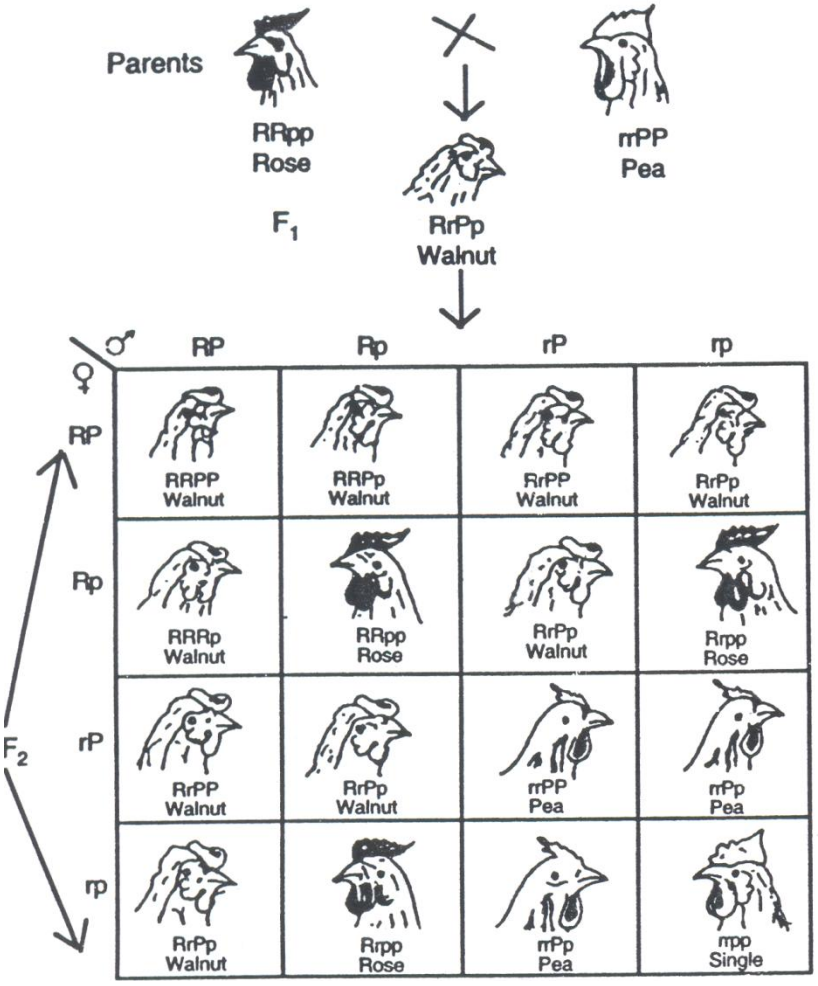


Figure 6.15: Cross of Fowls Showing a ratio of 9 walnut: 3 rose: 3 Pea: 1 single combs

While studying the combs of fowls bateson and punnet noticed a very interesting phenomenon. The Wyandotte breed carries a papillate comb known as *'rose'* comb, while Brahmas breed possesses a high and narrow comb known as *'pea'* comb. Leghorns and such other breeds of similar origin posses a comb known as *"single"*.Both rose and pea are dominant to single variety. If any one of them is crossed

with single a ratio of 3:1 is obtained (3 rose or pea : 1 single). When rose fowl was crossed with pea fowl, an entirely new type namely walnut comb appeared in the Fj progeny. Walnut comb is a characteristic of Malaysian breed.

Explanation

Inbreeding of F, progeny yielded 9 walnut, 3 rose, 3 pea and 1 single varieties in the F_2 generation, i.e., a dihybrid ratio. These results indicate that the interaction of.two dominant genes namely R and P will result in the formation of Walnut comb. It is a novel phenotype appearing in the Fj progeny and 9/16 of F_2 progeny. R and P genes individually yielded Rose and Pea forms in 3/16 each of F_2 progeny. These two genes in recessive condition (r and p) resulted in single comb. When a single was mated with another single the progeny were single indicating the presence of two recessive genes in the single. The single is also a novel phenotype found only in 1/16 of the F_2 progeny. R and P are two independently inherited genes and their interaction in dominant and recessive forms produces walnut and single combs respectively. (Fig 6.15)

Epistasis:

In a heterozygote the dominant allele for a particular trait obscures the effect of the recessive trait. Sometimes two different genes which are not alleles, and present on different chromosomes, both influencing the same part or trait of the organism, the expression of one gene conceals or hides the expression of the other gene. The gene which is suppressing is known as epistatic gene or inhibitor gene and the suppressed gene is called hypostatic gene. This phenomenon of masking is known as *epistasis*

Example (I) 13 : 3 ratio:

The white plumage of white leghorn fowls is dominant over the coloured plumage such as black, barred and other colour patterns. The white plumage of white wyandotte or white plymouthrock is recessive over the coloured varieties. Infact the white leghorns are genetically coloured birds. But their colour is not expressed because of the presence of an epistatic or inhibitor gene. So the gene for colour is hypostatic in white leghorns. Whereas the white wyandottes and plymouthrocks carry these genes in recessive condition for their whiteness.

Explanation

We can name the gene for colour as C and epistatic gene as I. So the white leghorns are I I C C and white wyandottes or plymouthrocks are i i c c genotypically. When these are crossed Fi generation produces white birds; since the inhibitor gene I do not allow the dominant gene C to have its colour expressed. In F_2 also all genotypes with T will be white. When it is in recessive form the effect of gene C surfaces and bird becomes coloured. The genotype i i c c also develops white plumage because colour

gene is in recessive form. Ultimately total number of white birds is 12+1 = 13 and colored birds are 3. ie., 13:3 ratio (Fig 6.16)

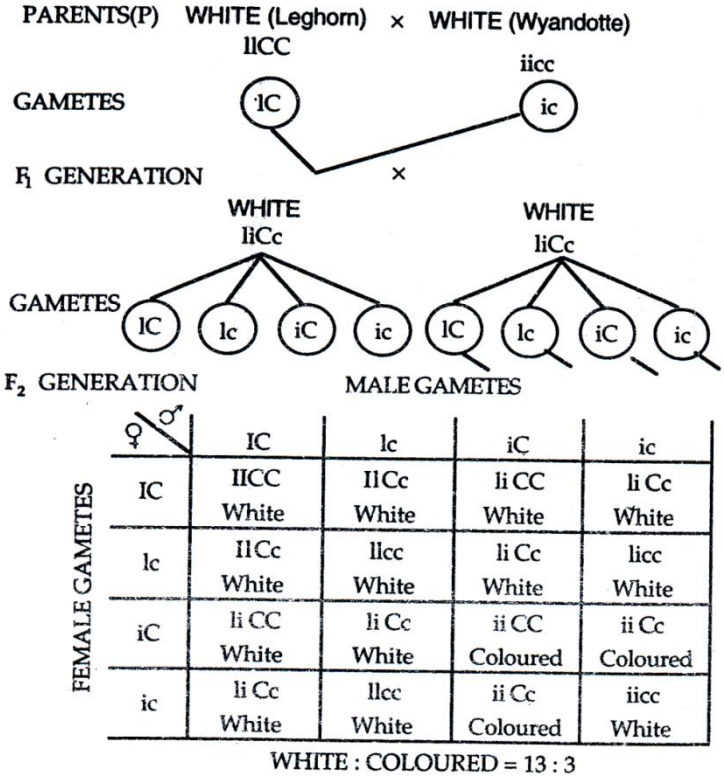


Figure 6.16: Inheritance of epistatic and hypostatic genes F_2 generationshows 13: 3 ratio

Example (II) 12 : 3 :1 ratio

In summer squashes the common fruit colours are white, yellow and green. White when crossed with yellow or green proved to be dominant. Yellow when crossed with green also proved to be dominant. But the yellow gene cannot express itself in the presence of white. So white gene is epistatic over the yellow gene. This fact is clearly revealed by conducting a cross between a white and green summer squashes (WWYY x wwyy)

Explanation:

In this cross the genotypes with W gene bear white fruit, those with Y gene bear yellow fruit. As long as W gene is there Y gene does not express itself and remains hypostatic. When both these genes are recessive the fruit remains in green colour, (ww yy). Thus white, yellow and green squashes will be in 12: 3:1 ratio (Fig 6.17).

Complimentary genes

The complimentary genes are those, which are different but produce similar phenotypic expression, being present in two different individuals. When they come together, an altogether new phenotypic expression is produced in the progeny, because of their complimentary interaction. This is

proved by Bateson and Punnet in a cross they conducted between two *Lathyrus odoratus* plants (sweat pea) bearing white flowers.

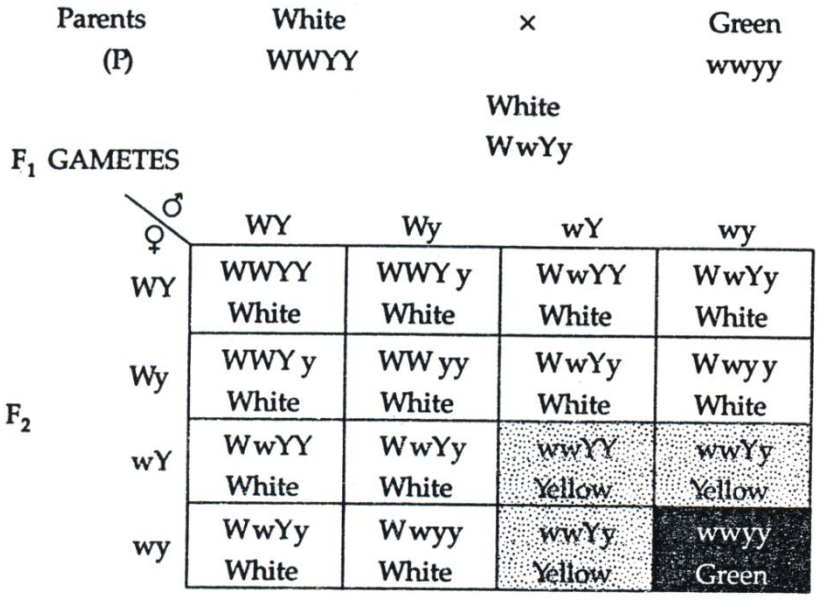


Figure 6.17: Cross between white and green summer squashes, 12 : 3 :1 ratio of white, yellow and green colours

Example

The wild *Lathyrus odoratus* plant possesses purple flowers with red petals. Bateson and Punnet observed that the purple was dominant over white variety. The white colour breeds true. When they crossed two pure white varieties, the *Vi*progeny expressed purple coloured flowers, to their utter surprise, which was similar to the wild variety. When F, hybrids were self pollinated purple and white flowered plants were produced in 9 : 7 ratio in F_2. The white variety on self pollination proved to be breeding true, whereas the purple flowered plants gave rise to progeny of different types. Some gave the ratio of 3 purple : 1 white, while others 9 purple : 7 white and few bred true.

Explanation:

Here the gene for white flowers of one parent is labelled as C and the gene for the same trait of other parent as P. When both these dominant genes are present the progeny exhibited purple flowers. When either of them is present there is no colour and phenotype is white. In this case the terms 3 : 3 : 1 of the classical ratio of 9 : 3 : 3 : 1 got merged resulting in 9 purple and 7 white flowered plants.

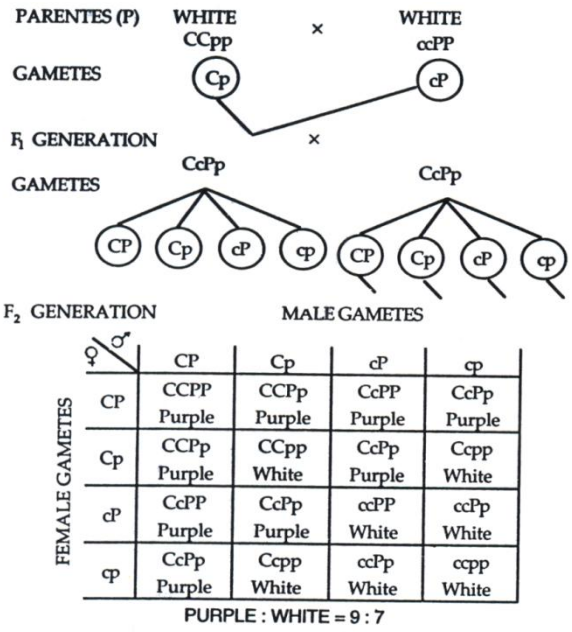


Figure 6.18: Inheritance of complimentary genes showing 9 : 7 ratio in the F_2generation

Physiological Proof:

It is proved physiologically also that one plant with white flowers produces the required substrate and the other plant produces the enzyme which converts this substrate into the purple anthocyanin. The C and P genes owe their responsibility separately when they are seperate. Once they come together these two genes compliment each other *in Vitro.* Theoutcome is the production of the purple anthocyanin. The recessive genotype c c p p also bears white flowers since neither substrate nor enzyme is produced in it. These different pairs of homozygous recessive genes prevented the production of anthocyanin in the two types of parent plants.

Supplementary genes (9 : 3 : 4 ratio):

There are some instances where the interaction of two different genes is such that the dominant gene will produce its effect, irrespective of the presence or absence of the other gene. Whereas the second can only produce its effect in the presence of the first gene. This is known as the supplementary gene interaction.

Example

The inheritance of coat colour in mice, guinea pigs and such other rodents is an example of supplementary gene interaction. The primitive Agouti pattern had given rise to number of othervarieties such as Albino, black etc. The Albino carries a completely white coat with pink or blood red eyes. It not only breeds true but also recessive to any other coat colour. The agouti is a kind of black -yellow mixture. If yellow disappears from this it becomes black. Each hair of this mouse is mostly black except yellow

coloured band at the tip. Hairs on the underside are normally light yellow with black or grey at the base. Black is recessive to agouti and breeds true.

If a cross is conducted between black and Albino. The Fj offspring appears in agouti pattern. The inbreeding of F_x agouties results in the production of 9 agouties : 3 blacks : 4 albinos (Fig 6.19)in F_2.

Explanation

Black color is controlled by the gene, C, it is absent in Albinos. Albino is due to the gene, 'A'. It is absent in blacks, but it is responsible for banding of the hair with yellow color. In albinos even though 'A' gene is present, no color develops in the absence of C. When both these genes come together (CA) the agouti pattern results. Since it is the recollection of the primitive wild character, this phenomenon is also known as the "Reversion" or "Atavism".When the gene 'A' in CA genotype (Agouti) mutates into 'a', black colour is expressed. If 'C mutates into "c", phenotype becomes albino. If these recessive genes 'a' and 'c' reversed their mutation to 'A' and 'C again the primitive agouti pattern reappears, hence the name reversion or Atavism. The last two terms of the classical ratip 9:3:3:1 will combine resulting in the formation of 9:3:4 ratio.

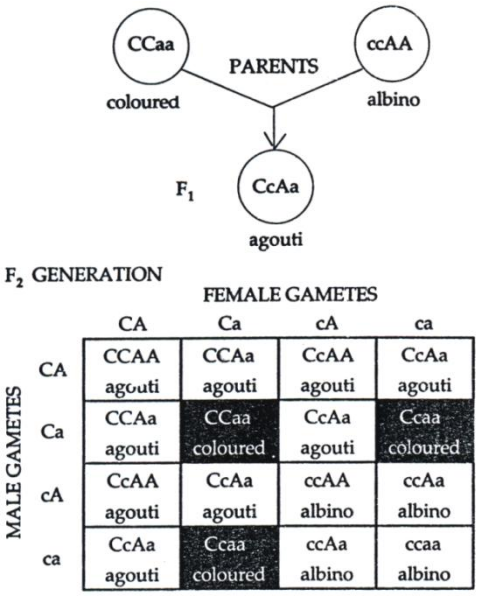


Figure 6.19: Inheritance of supplementary genes showing 9:3:4 ratio in the F_2 generation

Chapter 7

Linkages

Linkage

Mendel studied the inheritance of seven characters in peas, each controlled by a single pair of alleles. He was fortunate, for the garden pea has the seven pairs of chromosomes, and each of the seven pairs of characters was associated with a different pair of chromosomes. Therefore, the genes that were carried by different chromosomes assorted independently and produced 9:3:3:1 dihybrid F_2 ratio and 1:1:1;1 test cross ratio.However, in later years, it was found that the number of genes present in an individual are much more than the number of chromosome pairs possessed by that individual . For example, in *Drosophila*, many hundreds of genes have been studied, yet there are only four pairs of chromosomes. Therefore, it was concluded that, each chromosome carries a large number of genes.

The genes of the same chromosome do not show independent assortment, but are inherited together. They are now said to obey the phenomenon of linkage. Linkage is defined as the tendency of two or more genes to remain together in the original combination in the same chromosome during the process of inheritance for a number of generations. The theory of linkage was proposed by T.H MORGAN in 1910.All those genes which are located on the same chromosome constitute a linkage group. The total number of linkage groups in anorganism is equal to the number of chromosomal pairs. For example, there are 4 linkage groups in *Drosophila*, 23 in man and 7 in sweet pea.

Discovery of linkage

The first exception to MENDEL'S law of Independent Assostment was reported by BATESON and PUNNET (1906) in pea plant. They formulated the hypothesis of coupling and repulsion to explain the unexpected F_2 results of dihybrid cross.

Coupling

Bateson and punnet (1906) crossed a pea plant with blue flowers and long pollen (BBLL) with another one having red flowers and round pollen (bbll) . From previous study they found that the gene for blue colour (B) is dominant over red (b) and long pollen (L) is dominant over round pollen (l). So all the F_1offspring snowed blue flowers and long pollen (BbLl).According to the law of Independent Assostment, the F_1 hybrid was expected to produce four types of gametes (BL,Bl,bL,bl) in equal number. Further, when the $F_l$hybrid was test crossed with the recessive parent (Red, round - bbll), the expected offspring was 1:1:1:1 ratio.But in this case, Bateson and Punnet observed a test cross ratio of 7:1:1:7 insted of normal 1:1:1:1 ratio.

The above results indicate that, the parental phenotypes blue long and red round) are in greater proportion, than the non parental phenotypes (blue round and red long). This shows that the gametes with genes BL and bl are more common. This tendency of some pair of characters (BL and bl) to unite

together and reappear hand to hand in the next generation was termed Gametic Coupling byBateson and Punnet.

	BBLL			**bbll**
Parents	Blue, Round	X		Red, Long
	BBLL			BBLL
F_1 Hybrid		Blue, long		
		BbLl		
Test Cross	BbLl	X		bbll
	Blue long			Red, Round
Gametes	BL Bl bL Bl			bl
	BbLl	Bbll	bbLl	Bbll
	Blue Long	Blue Round	Red Long	Red Round
F_2 Ratio : 1:7:7:1	1	7	7	1

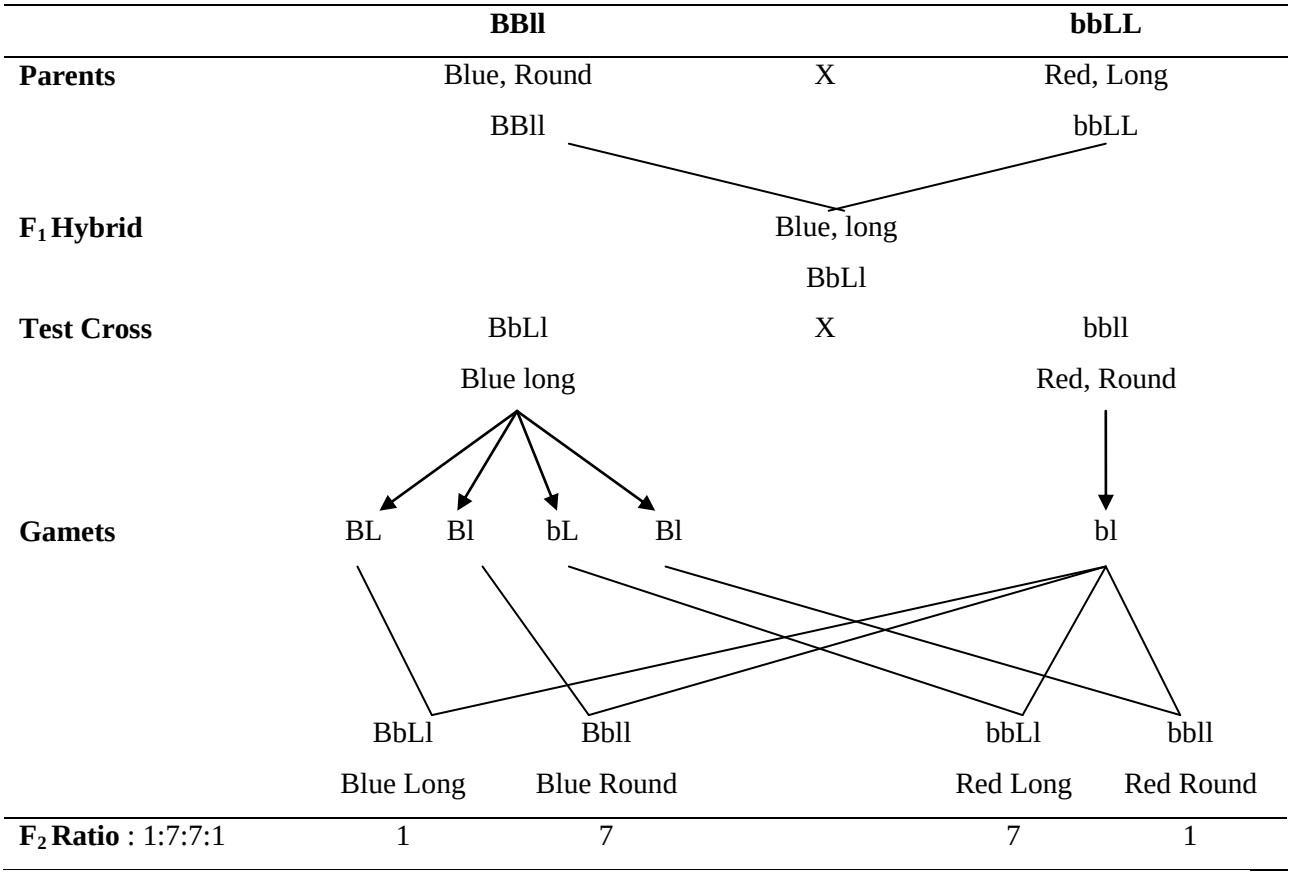
Repulsion

Later, Bateson and Punnet made another cross which involved the same characters but in a different combination. A plant bearing blue flowers and round pollen (BB11) was crossed with another pea plant having red flowers and long pollen (bbLL). The F_1 hybrids 'ere blue and long (BbLl). When they were test crossed with recessive parent (bbll), the F_2 progeney appeared in 1 : 7 : 7:1 ratio, in stead of normal 1:1:1:1 ratio

The above results show that the two dominant alleles (B and L) or recessive alleles (b and l) repelled each other because they came from different parents. The gametes with genotypes BI and bL were formed in more number. Hence the blue round and red long plants were produced in more numbers. This peculiarity was called Repulsion by them.Bateson and Punnet could not give the exact reasons for coupling and repulsion. It was MORGAN in 1910, who discovered that coupling and repulsion are essentially the two aspects of the same phenomenon, LINKAGE

Morgan (1910) while performing experiments with *Drosophila melanogaster*,found that genes located on the same chromosome are tied together arts pass together from generation to generation. While the genes located on different chromosomes assorted freely during gamete formation. Therefore he

proposed that two genes are found in coupling phase or in repulsion phase, because these are present on the same chromosome (coupling) or on two different homologous chromosomes (repulsion). Such genes are then called linked genes and the phenomenon is called linkage.



Chromosome theory of linkage

Morgan and castle have formutaled the Chromosome theory of linkage. It states that:

1. Genes located in the same chromosome are inherited together and show linkage
2. The linked genes are arranged in a linear fashion in the chromosome
3. The degree of linkage is determined by the distance between the two genes. Linkage strength is inversely proportional to the distance between the two genes. Closely related genes show strong linkgae, while genes widely located show weak linkage.

Arrangement of linked genes

Based upon the arrangement of genes in the chromosome, the linkage can be classified into two types.

(1) **CIS arrangement:**

The dominant genes (A and B) are located in one member of the chromosome pair; and the two recessive genes (a and b) are located in the other chromosome . This type of arrangement (AB/ab), with

two dominants on same chromosome is called Cis arrangementThe heterozygote's with such arrangement are know as Cis heterozygote's. In such cases the genes are said to be in coupling phase.

(2) **TRANS arrangement:**

In this type, one daminant gene and one recessive gene are located in one chromosome. The heterozygotes with such arrangement (Ab/aB) are known as trans heterozygotes. In such case, the genes show repulsion.

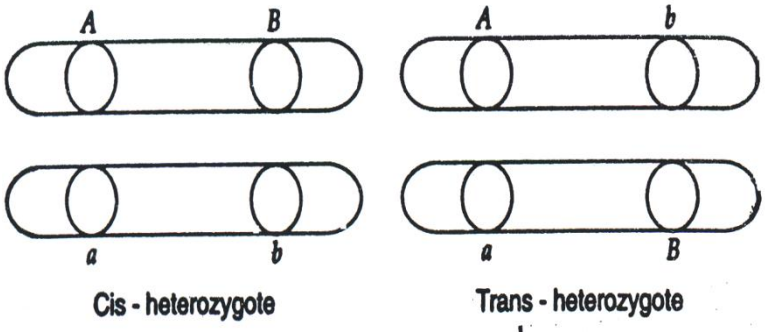


Figure 7.1: CIS arrangement and TRANS arrangement

Kinds of linkage

There are two types of linkage. They are complete linkage and incomplete linkage, depending upon the presence or absence of non - parental combination of linked genes.

Complete linkage:

It is the phenomenon in which two or more genes or characters are inherited together for a number of generations. In this, genes are closely associated and tend to inherit together. As a result, the young ones receive only the parental characters. This phenomenon is very rare. According to bridge, all the genes of male Drosophila remain completely linked.

Example: **Linkage in Drosophila**

In *Drosophila* grey colour is dominant (B) over black colour (b) and long wing (V) is dominant over vestigial wings (v). When a fly with grey body and long wings (BBW) is crossed with another fly having black body and vestigial wings (bbw), the F_1 hybrid show grey body and long wings (BbVv) . When F_1 hybrid is test crossed with the double recessive female parent, the F_2 generation consisted of 50% grey, long flies and 50% black, vestigial flies, like the parents.

Parents:	BBVV	X	bbvv	→	BbVv
Test cross:	BbVv	X	bbvv		
F_2 generation	1 BbVv:		1 bbvv		
	Grey, long		Black vestigial		

Incomplete linkage:

In majority of the cases, the homologous non - sister chromatids exchange segments with one another during meiotic prophase by process of crossing over. This leads to the formation of new combinations among the young ones. This phenomenon is called incomplete linkage.Incomplete linkage is very common and has been observed in maize, Sor£/wm, cotton, rice, tomato, female *Drosophila,* poultry and man.

***Example*: Incomplete linkage in Maize**

Hutchinson (1922) crossed a variety of Maize having seeds that were coloured and normally filled with another having colourless and shrunken seeds. The gene 'C for colour is dominant over V for colourless condition. Similarly gene 'S' for full endosperm is dominant over V for shrunken endosperm. The resulted Fj plants produced coloured and full seeds, having the genotype CS/cs.When One of the dihybrid female is test crossed with a double recessive male plant (cc/ss) four types of seeds are produced in the following proportion.

	BBll			**bbLL**
Parents	Coloured, full CS/CS		X	Colourless, shrunken cs/cs
F1			CS/cs Coloured,Full	
Test cross	Fl coloured, full CS/cs		X	colourless, shrunken cs/cs
Test cross results	Coloured, full CS/cs	Coloured, shrunken Cs/cs	Colourless, full cS/cs	Colourless, shrunken cs/cs
	48%	2%	2%	48%

In the above example, the parental combinations were more frequent than the new combinations. This clearly indicates thatthese characters are linked together (coupling phase) and their genes are located in the same chromosome.

Factors affecting strength of linkage

(1) Closely related genes show strong linkage while genes widely located show weak linkage.

(2) With increasing age the chances of crossing - over are reduced . Therefore, strength of linkage increases.

(3) Increase in temperatrue decresases the strength of linkage.

(4) Exposure to X- rays reduces the strength of linkage.

Significance of linkage:

The phenomenon of linkage has great significace for the living organisms, because it reduces the possibility of variations in gametes unless crossing - over occurs.

Crossing over

` The chromosome theory of linkage proposed by morgan states that genes located in the same chromosome are in a linear fashion.These linked genes inherit generation after generation, when the chromosomes remain intact during inheritance. Linkage, however, is seldom complete. Some times, the linked genes are seperated by reciprocal exchange of genes between chromosomes of homologous pairs. Morgan and Castle (1912) were the first to introduce the term Crossing- over for this exchange of genetic material. The chromatids, in which crossing over has occured, will have new combinations of genes (recombination) which are quite different from those of the parents

Crossing over may be defined as the exchange of chromosomal parts between non-sister chromatids of a pair of homologous chromosomes". R ecombination in Drosophila: A cross between a grey bodied, vestigial winged (BBvv) Drosophila and black bodied, long winged (bbVV) Drosophila produced F_2 hybrids, all them having grey body and long wings (BbVv). When a female fly of this F_1 generation is back crossed to the double recessive males (bbw), four kinds of individuals appeared in the following proportion.

(i)	Grey, Vestigial	:	41.5%	Non-crossovers
(ii)	Black, Long	:	41.5%	83%
(iii)	Black, Vestigial	:	8.5%	Crossovers
(iv)	Grey, Long	:	8.5%	17%

The two new combinations appeared in the F2 generation because crossing over occured between two chromosomes, during the formation of gametes.

Stage at which crossing - over occurs

The Crossing over is an actual exchange of segments between two homologous chromosomes. It means, it must occur when the homologous chromosomes are together in the prophase of meiosis. But prophase of meiosis is a lengthy and complex process. So it is important to know exactly at which stage of meiosis, crossing over occurs. This has been answered by the tetrad analysis in moulds and attached X-chromosome studies in *Drosophila*.During the zygotene stage, the homologous chromosomes become paired. The paring is very precise and is brought about by the mutual attraction between parts of chromosomes. The paired structure thus formed is known as a *diad* (or) *bivalent.* This process is called synapsis.

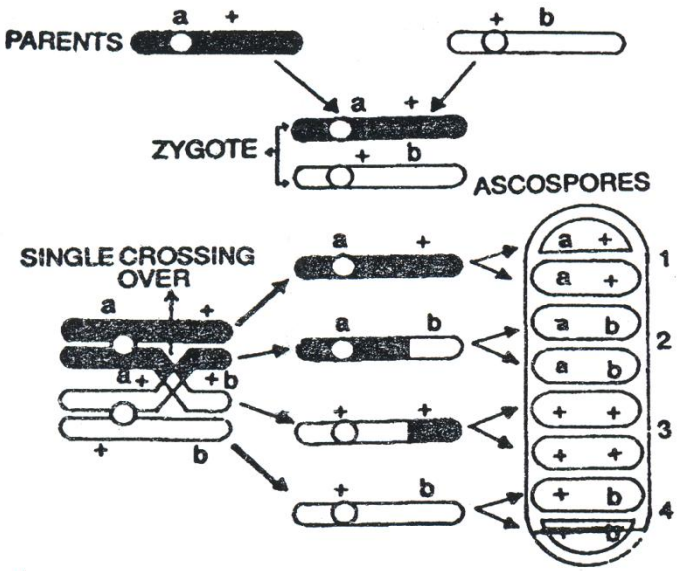


Figure 7.2: Single crossing over in mould showing that crossing-over occurs in tetrad stage

In pachytene stage, each chromosome of a bivalent, splits longitudinally to form two sister chromatids. Thus, the bivalent now consists of four chromatids and is know as the *tetrad* .It is at this stage an exchange of chromatid segments between non-sister chromatids of each tetrad takes place. This is known as crossing over.

Evidence:

Excellent photographs by David stadler of *Neurospora crassa* (red bread mould) demonstrated conclusively that crossing - over takes place in the four - strand stage. If the crossing over occured, in the two strand stage (bivalent) the distribution of ascospores in the ascus would always be 4 and 4. But, the 2-2-2-2 distribution of 4 types of ascospores in an ascus, clearly indicates that crossing over occured at the four strand stage.

Mechanism of crossing over

The crossing - over occurs in the homologous chromosomes during the four - stranded (or) tetrad stage. During pachytene stage, the non - sister chromatids of homologous chromosomes twist over each other. These points of contact are known as chiasmata. At each chaisma, the chromatids break, by the activity of an enzyme called endonuclease. The broken segment of one chromatid is fused with the other chromatid and vice versa. This is brought about by another enzyme ligase. This exchange of chromatid segments between non - sister chromatids is called crossing - over. According to the recent findings, a little amount of DNA sysnthesis (about 0.3% of total genome) takes place during the crossing - over that repairs the broken chromosomes.

After the completion of crossing - over, the non sister chromatids start repelling each other, because the force of attraction between them decreases. The chromatids seperate progressively from the centromere towards the chiasma.The chiasmata also start moving in zipper - like fashion towards theends

of the tetrad. This movement of chiasmata is known as terminalization. Simultaneously the chromatids get shortened and in Diakinesis the homologous chromosomes become seperated except at their ends.

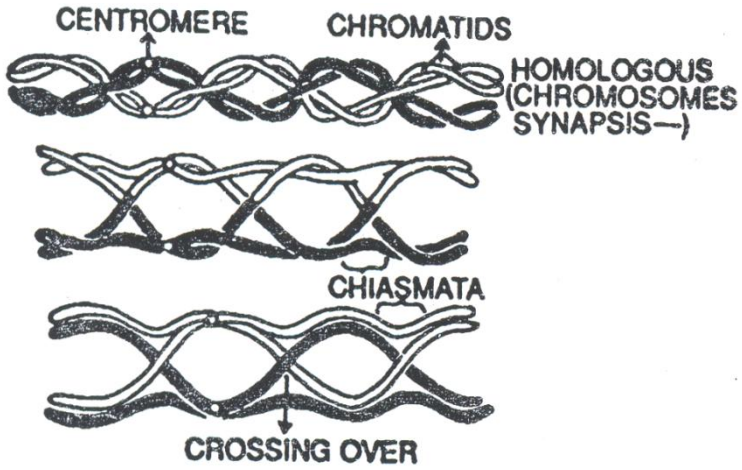


Figure 7.3: Diagram showing mechanism of crossing-over at tetrad stage

Salient features of crossing - over

The essential features of crossing - over are as follows:-

(1) Crossing - over is the exchange of chromatid segments between non - sister chromatids of the homologous pair of chromosomes.

(2) The two chromatids of the same chromosome (sister chromatids) never exchange parts between them.

(3) The number of chiasmata per set of chromosomes depends upon the length of the chromosomes. The longer the length, the greater is the number of chiasmata.

(4) When the genes are located apart from one another, the chance for crossing - over is more.

Theories explaining the mechanism of crossing -over

1. **Janssen's Hypothesis:**

This was putforth by Janssen in 1909. According to this hypothesis, prior to crossing over, the homologous maternal and paternal chromosomes come in pair. In pachytene stage each bivalent becomes doubled to form a tetrad. Of these four chromatids, only two cross each other and the other two do not. In the diplotene stage, the non - sister chromatids over lap with one another and form, chiasma or point of contact. In the chiasma, the chromatids break and they rejoin with the mutual exchange of segments. This theory believes that true chiasmasta are the direct result of crossing over, and there would be one to one relatioship between the chiasmata and crossing over. This theory is also called chiasmata type theory or one plane theory.

2. The Torsion Hypothesis (Darlington, 1935, 37):

According to this theory, the two homologues are rationally coiled about each other, to satisfy a two - by two associations. When new chromatids are formed in each tetrad, the paired eqilibrium and longitudinal cohesion will be upset. Now the attraction between homologues is replaced by a repulsion which forces them apart. When the chromosomes start seperating their rational coil unravels in onedirection, and the sister chromatids unravel in opposite direction, ecause of this a torsion is developed which leads to the breakage f weak non - sister chromatids.

The torsion hypothesis is not much supported hy many ytologists. The principal objection to this theory is that the hromosomes are duplicated before synapsis.

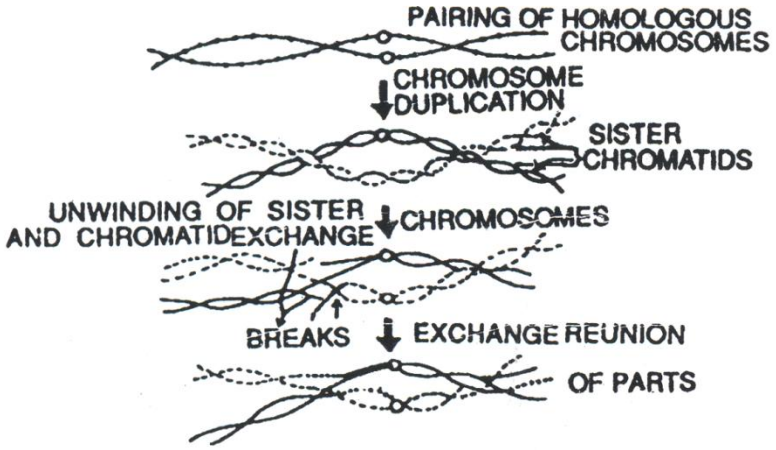


Figure 7.4: Diagramatic representation of Darlington's strain theory ofcrossing-over

3. Breakage and exchange Theory:

Stern and Hottan (1969) have discovered the presencce of two enzymes - endonuclease and ligase. The enzyme endonuclease causes a break in the non - sister chromatids of homologous chromosomes at corresponding points and the enzyme ligase governs the reunion of broken non sister chromatids.

However there exists a lot of controversy about the manner and time of break and exchange.

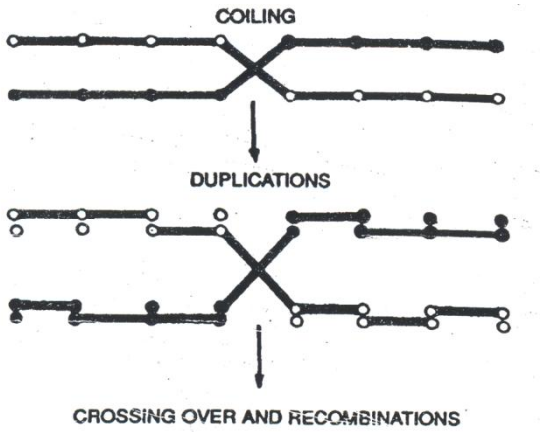


Figure 7.5: Mechanism of genetic recombination as proposed by Belling

a. ***The Contact First Theory*:** This theory was proposed by Serebrovsky. According to him, the non - sister chromatids of homologous chromosomes first touch and cross each other. Then breakage occurs at the points of contact of the chromatids. The broken segments rejoin to form new combinations.

b. ***The Breakage First Theory:*** This was proposed by Muller. According to this theory, the chromosomes first break into two or more segments. The broken segments of non sister chromatids rejoin with an exchange forming chiasma.

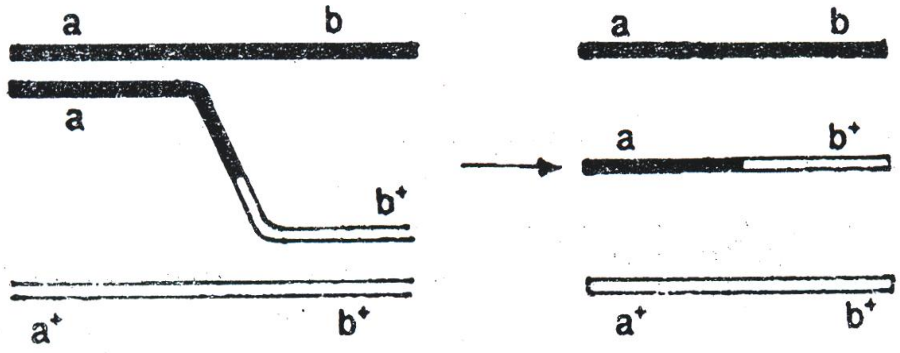


Figure 7.6: Mechanism of genetic recombination based on copy choice mechanism

c. ***The Copy Choice Theory****:* This was proposed by Belling in 1931. According to this theory, crossing over and recombination occur during the synthesis of new chromatids. After pairing of homologous chromosomes, the parent chromatid of each chromosomebegins to replicate. For this the parental chromatids act as templates. It is assumed that a newly synthesized daughter chromatid is derived due to copying of one chromosome upto acertain distance and then switching over to the other chromosome ^for copying the remaining distance. The new chromatids, thus carry information from both parental chromatids.This theory was later abandoned, because it assumed a conservative rather than a semi - conservative replication of DNA duplexes.

Cytological detection of crossing - over

The first cytological demonstration of genetic crossing over was given by Stern (working with *Drosophila)* and H.B. Creighton and Mc Clintock (working with Maize) in 1931.

Stern's Experiment:

Each somatic cell of *Drosophila* contains four pairs of chromosomes in the nucleus. Out of the four pairs, three pairs are autosomes and the remaining one pair is allosomes. The female *Drosophila* carries XX chromosomes and the male *Drosophila* carries XY chromosomes.For his experiment, Stern

managed to obtain a wild type female *Drosophila.* This female has two X chromosomes which are morphologically different from each other.

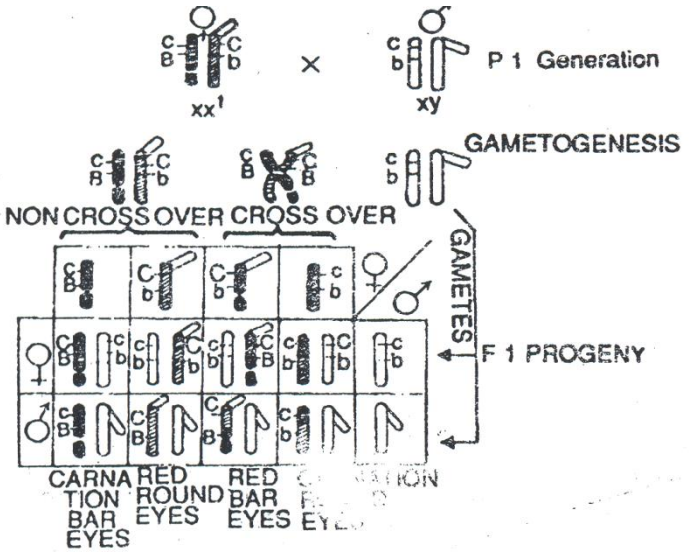


Figure 7.7: Cytological demonstration of Crossing over in *Drosophila*

In this *Drosophila,* one of the X - chromosome is rod shaped and was broken into two pieces. One piece behaved as an independent X chromosome and contained the genes considered by Stern in his experiment. The other smaller piece was attached to a member of fourth pair of chromosomes. The other X - chromosome had a piece of Y - chromosome attached to it. So it had L - shaped appearance. Both the chromosomes can be distinguished by microscopic examination. STERN used these chromosomes as markers for demonstrating crossing - over.The two X chromosomes were also genetically different. The broken X contained a recessive gene carnation (e) for dark ruby eye colour and a dominant gene Bar (B) for narrow band - like shape of eyes. The L - shaped X chromosome contained a dominant allele (C) for red eyes and a recessive allele (b) for normal round eyes.

Stern crossed this female, heterozygous for red and bar eyes (cB/Cb) with a double recessive male having carnation, round eyes (cb/cb). He studied only the female offspring of the F_1 generation. The F_1 female flies appeared in four phenotypic classes. The chromosomes of these females were studied cytologically. The X -chromosome coming from the male parent was found to carry both the recessive genes in all the daughter females. The other X -chromosome received from the mother was found to differ in all the four types of females.

1. Carnation, bar females (cB/cb) - with broken X and without any fragment of Y.
2. Red, round females (Cb/cb) - with unbroken X and with the attached Y fragment.
3. Carnation, round (cb/cb) - with unbroken X and without an attached Y fragment.
4. Red, Bar (CB/cb) - the broken X and with attached Y fragment.

Thus the flies in which crossing - over was observed phenotypically also showed microscopic evidence of exchanges between homologous chromosomes. The physical (or) cytological basis of crossing over was thus established.

Kinds of crossing – over

Depending upon the numberofchiasmata formed, crossing -over may be of the following kinds:

1. **Single crossing – over:** In this type, only one chiasma is formed all along the length of a chromosome. Only one chromatid of each chromosome is involved in crossing - over. It produces two non -cross over gametes and two cross over gametes.
2. **Double crossing - over:** In this type, two chiasmata are formed along the entire length of chromosomes. Both the chiasmata may be between the same chromatids or between different chromatids. Thus two or three or all the four chromatids may be involved in the process of double cross overs. This is of less frequent occurence.
3. **Multiple crossingover**: In this type, crossing over occurs at three, four or more points between any two given points in the chromosome pair. Multiple crossing - over does not occur frequently due to interference in crossing - over.

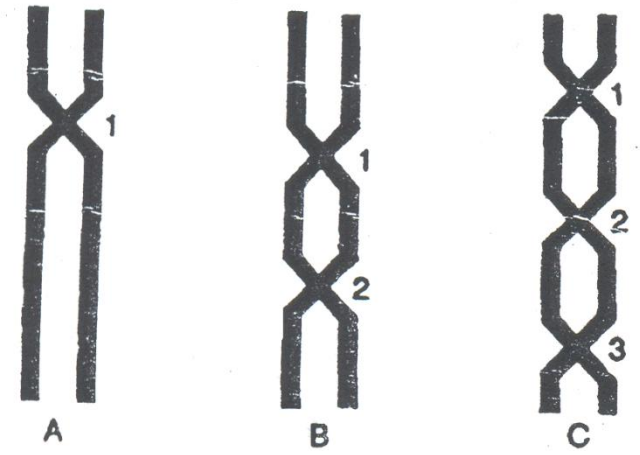


Figure 7.8: Single, Double and Multiple Cross overs

Frequency (percentage) of crossing – over

The frequency with which crossing - over occurs between two genes is expressed in terms of percentage of crossing - over. Normally, the percentage of crossing - over is directly proportional to the distance between the two genes. If the distance between the two genes is more, the chances of crossing over and chiasmata formation are more and vice versa. Thus the percentage of crossing -over between two genes indicate the distance between the two genes.This fact is of great signficance, in working out the relative distances between the genes in a chromosome. Knowing the relative distances, the exact locations of the genes in the chromosomes can be determined. This helps in the construction of chromosomal maps.

Factors controlling frequency of crossing -over

Primarily, the frequency of crossing - over is dependent upon the distance between linked genes. But a number of physiological, enviromental and genetic factors also influence it. These are:-

(1) High and low temperatures increase the frequency of crossing -over (Plogh).
(2) X -rays increase the frequency of crossing - over (Muller).
(3) The frequency of crossing over decreases with increasing age in female *Drosophila* (Bridges).
(4) Gene, mutations may affect the frequency of crossing over. Some mutations are known to decrease the frequency.
(5) Chiasma formation at one point discourages chiasmata formation in the vicinity due to strain on the chromatids. This phenomenon is called *interference*.
(6) Inversions of chromosome segements suppress the crossing -over.
(7) Crossing - over is iess frequent near the centromeres and the tips of chromosomes.

Significance of crossing - over

Crossing - over is a universal phenomenon and has a great significance in generics.

1. Crossing - over provides a direct evidence for the linear arrangement of genes in the chromosomes
2. Frequency of crossing - over is very useful in the construction of chromosome maps. The relative distance between the genes is expressed in terms of percentage of recombination among the genes in a likage group.
3. As a result of crossing - over, new gene combinations are produced. These variations play important role in the process ofevolution.

Construction of genetic maps

The discovery of the phenomenon of linkage and crossing over by T.H Morgan has established the following facts:

1. The number of genes usually exceeds the number of chromosomes in different species; so many genes will be located in the same chromosome.
2. The genes are arranged in a linear order in a chromosome.
3. All the genes present in a chromosome inherit together and form one linkage group.
4. The number of linkage groups corresponds to the number of chromosome pairs, for e.g. 4 in *Drosophila.*
5. The linked genes are occasionally seperated from the members *of* their linkage group by crossing – over
6. The frequency of recombination (or) crossing over between genes depends upon the distance between two genes. Closely placed genes will have less chances of crossing - over.
7. Each gene has a specific order and location in a linkage group of chromosome.

Based upon the above observations sturtevant (1913), astudent of Morgan developed the idea that the percentage of crossing-over could be used as a tool to determine the relative positions of genes in chromosomes. Working on this concept, he emerged with the first chromosome map of *Drosophila.*This map showed the positions of five genes on the X chromosomes of Drosophilia . These five genes were for yellow body (y), white eyes (w), Vermilion eyes (v), miniature wings (m) and rudimentary wings(r). This graphic representation of the genes in a chromosome in now known as chromosome map (or) generic map. The units used in the mapping are called map units or morgan units.

Definition:

The graphic representation of relative distances between linked genes of a chromosome is called linkage (or) genetic map.

Construction of chromosome maps

The entire process of chromosome mapping includes the following steps:-

A. **Determination of linkage groups**

Before starting the genetic mapping of a chromosome of a species, we have to know the exact number of chromosomes present in that species. Then we have to determine the total number of genes of that species by making hybridization experinents between wild, and mutant strains. Linkage groups of the species have to be worked out by studying the percent cross over.

B. **Determination of map distance**

After knowing the total number of genes in each linkage group, the relative distances between each linked gene have to be calculated. This distance is calculated according to the percentage of crossing-over, because, cross over frequency is direciiy proportional to distance between the genes. For example, if the percentage of crossing over is 10%, the map distance between two linked genes is l0 units.

C. **Determination of gene order**

After determining the relative distances between the genes of a linkage qroup, it becomes easy to place genes in their proper linear order. For example if there are three genes A,B, C present in the same chromosome (i.e. these are linked), then these three genes may be presnt in any one of the following orders. These are A-B-C, A-C-B or B-A-C. It is obvious that in one case B is present in the middle, while in others C and A are present in the middle. Therefore, in finding out the linear order, we have to find out the gene which is present in the centre.

D **Interference and coincidence**

In majority of higher organisms, the frequency of double cross over is always less than the excepted value. This is because of interference. The tendency of one Cross-over to reduce the probability

of another cross-over in the vicinity is called interference. This phenomenon was discovered by Muller in 1911. The degree of interference may vary in different regions. It is greatest over a distance and decreases as the distance increases i.e., interference is inversely proportional to the distance between the points of crossing - over.

The strength of interference is usually expressed in terms of a co-efficient of coincidence. It is expressed as a 'ratio between actual number of double cross overs to the expected number of such double cross oilers'. That is:

$$\text{Coincidence} = \frac{\text{Actual number of double cross-overs}}{\text{Expected number of double cross-overs}}$$

When the interference is complete (1%), no double cross overs will be observed and coincidence becomes zero. On the other hand, when interference decreases (becomes lenss thanl) coincidence increases. Coincidence values ordinarily vary between 0 and 1.

Two point test cross

The percentage of crossing over between two linked genes is calculated by test cross in which an F_1dihybrid is crossed with a double recessive parent. Such test cross is called two point test cross because it incolves crossing over at two points.The distance between two genes can be calculated by taking the example of Maize.

When a Maize plant with seeds having colour and full endosperm (CS/CS) is crossed with another plant having recesseve alleles for colourless and shrunken seeds (cs/cs), all the Fj hybrids produced coloured and full seeds (CS/cs). When Fi female hybrids are test crossed with double recessive parent (cs/cs) four types of seeds are produced. These are

Coloured full (CS/cs) - 4032 + Coloured shrunken (Cs/cs) – 149 = 96.4% of the total

Coloured shrunken (Cs/cs) – 149 + Colourless full (cS/cs) – 152 = 3.6% of the total

Total.... 8368

From the test cross data it becomes clear that the genes C and S were located in the same chromosome and are linked together. Only 3.6% individuals were obtained due to crossing over between the genes C and S. Since the percentage of crossing over indicates the relative distance between the linked genes, we can determine the distance between the genes C and S to be 3.6 centimorgans (or) map unis. Thus we may draw a genetic map of that part of chromosome in which is these two genes are located, as

C I ——————— I S

3-6 units

Since double cross over usally do not occur between genes less than 5 centimorans apart, three point test cross are used for genes which are further apart.

Three point test cross

A three point test cross (or) trihybrid test cross gives us the information regarding the relative distances between three linked genes. It also shows us the linear order in which these genes are present in the chromosome.

The preparation of a linkage map can be exemplified by usingan example from maize involving three endosperm characters.

Dominant		**Recessive**
Coloured aleurone (C)	—	Colourless aluerone(c)
F'dl endosperm (S)	—	Shrunken endosperm (s)
Starch endosperm (W)	—	Waxy endosperm (w)

The cross made by Hutchinson (1922) is shown below:

Parents: CsW/CsW x cSw/cSw

Coloured, shrunken, nonwaxy — colour less, full, waxy

Garnets: CsW — cSw

↓

F_1 Hybrid: CsW/cSw

Coloured, full, nonwaxy

Test cross: CsW/cSw x csw/csw

Coloured, full, nonwaxy — colourless, shrunken, waxy

	(♀) gametes	(♂) gametes csw	Progeny
No crossing over	(1) CsW	CsW/csw coloured-shrunken-nonwaxy	2538
	(2) cSw	cSw/csw colourless-full-waxy	2708 — 5,246
Single cross over (C-s)	(3) CSw	CSw/csw coloured-full-waxy	116
	(4) csW	csW/csw colourless-shrunken-nonwaxy	113 — 229
Single cross over (s-w)	(5) Csw	Csw/csw coloured-shrunken-waxy	601
	(6) cSW	cSW/csw colourless-full-nonwaxy	626 — 1,227
Double cross oversbetween (Cs)and(s-W)	(7) CSW	CSW/csw coloured-full-nonwaxy	4
	(8) csw	csw/csw colourless-shrunken-waxy	2 — 6
			Total = 6,708

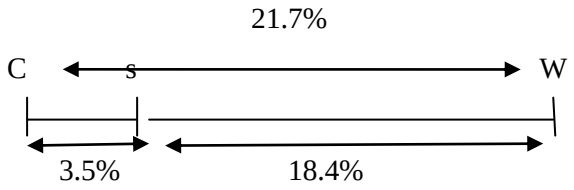
In P_1 parent, C, s, W genes are present together in the same chromosome . Therefore, the progeny showing their seperation is recorded as recombination between them. The frequency of recombination is expressed in percentage of the total number of individuals examined.

Therefore, recombination of C - s = $\frac{229+6}{6708} X\ 100 = 3.5\%$

Recombination of s - W =$\frac{1227+6}{6708}$x 100 =18.4 %

Recombination of C - W =$\frac{229+1227}{6708}$ x 100 = 21.7%

In this case, recombination value of C -W (21.7%) is close to recombination value of (C - s) + (s - W) = 3-5 + 184 = 21-9%. This shows that V is present between C and W. On the basis of the above values, the linkage map of genes C, s and W can be shown as



Chapter 8

Mutations

Mutations:

Mutations in a broad sense include all those heritable changes, which alter the phenotype of an individual. Hugo de Vries used the term 'mutation' to describe phenotypic changes which were heritable. He is, therefore, credited to have differentiated between heritable and environmental variations. However, the term mutation is now used in a rather strict sense to cover only those changes wthich alter the chemical structure of the gene at the molecular level. These are commonly called gene mutations or point mutations. In practice, sometimes it is rather difficult to distinguish between gene mutations and structural changes in chromosomes, because certain structural changes may have same phenotypic effects as gene mutations. Small deficiencies cannot be discovered by cytological observations. Although in Drosophila small deficiencies can also be detected in giant salivary gland chromosomes, in other organisms the only test for a deficiency is that it will not revert back to wild type character. However, gene mutations would be able to give reverse mutations.

The distinction between point mutations and chromosomal aberrations is thus rather superficial. If chromosomes are not studied under the microscope, in certain cases we may not be in ' a position to say with certainty whether a particularphenotypic character is due to point mutation or due to a structural change. Many mutations, described by de Vries in *Oenothera lamarckiana,* are now known to be due to certain numerical and structural changes in chromosomes. These are sometimes described as chromosomal mutations.For instance, 'gigas' mutant in *O. lamarckiana* was later found to be due to polyploidy. The structural and numerical changes in chromosomes have been discussed in Chapters 19 and 20 respectively, and this chapter will be devoted to point (gene) mutations only.

Brief History

The earliest record of point mutations dates back to 1791, when Seth Wrightnoticed a lamb with unusually short legs in his flock of sheep. Wright thought that it would be worthwhile having a whole flock of these short legged sheep, which could not get over the low stone fence and damage the crop in the adjacent fields. In the successive generations, this trait was transferred and a line was developed where all sheep had short legs. This character resulted from a recessive mutation and the short legged individuals were homozygous recessive. Once this mutation occurred in a particular cell, this will be carried in all the cells descending from this parent cell. This point mutation was discoveredat a time when the science of genetics did not even have its birth. The short legged breed of sheep was known as Anconbreed.

The scientific study of mutations started in 1910, when T.H. Morgan started his work on fruitfly, *Drosophila melanogaster,* and reported white eyed male individuals among red eyed male

individuals. Later it was found that the gene for this character is located on sex chromosome (X-chromosome) and expresses itself in a male individual (male individuals have one X-chromosome and one Y-chromosome; the female has two X-chromosomes). When these rare white eyed males were crossed to their sister red eyed females, white eyed females could also be obtained in some cases proving that the females involved were heterozygous (Fig. 8.1).

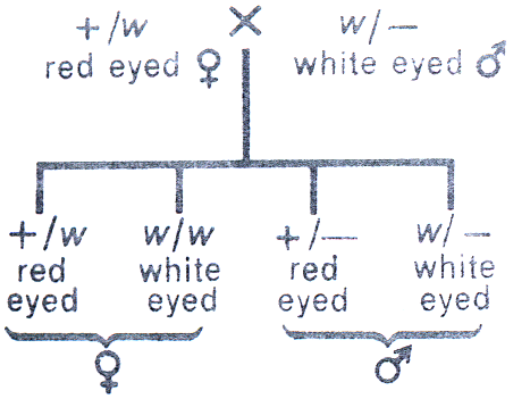


Figure 8.1: Appearance of a white eyed male fly in a cross red eyed female X white eyed male

After the discovery of white eyed mutant, a thorough search for mutants was made by Morgan and his co-workers in *Drosophila* and about 500 different mutations were observed by geneticists all over the world. This search of mutations in *Drosophila* was accompanied with mutation work in other organisms also e.g. maize, snapdragon, rodents, pea, fowl, man, etc. However, in the last 40 years, increasing interest has been observed for mutations in microorganisms, like *Neurospora,* bacteria *(Escherichia coli)* and bacteriophages, since these materials have been found to be very suitable for mutation work.

Range of Mutations

Although changes due to single gene mutations may involve sudden and large changes (like that of Anconbreed of sheep), they can also be so small that one may not be able to notice them. Therefore, earlier concept that, mutations could involve sudden and big changes only, was not correct. Mutations could also be observed which do not involve any change in morphology but only modifynutritional requirements as in *Neurospora.* Similarly, mutations could be observed in pathogens, as a result of which a pathogen could lose or acquire virulence against a particular host.

Stages at which Mutations Occur

Mutations can occur at any stage during the development. Depending upon this stage following situations will be met:

(1) If mutation occurs in a germinal cell, before differentiation of gametes, it would influence several gametes and will thus influence all the individuals derived from these affected gametes.

(2) If mutation occurs in a gamete or a zygote, a single individual will carry the mutation.

(3) If mutation occurs in a cell after the zygote has undergone one or more divisions, only a part of the body will show the mutant character. Such mutations will be called somatic mutations, in contrast to germinal mutationslisted in (1) and (2) above.

Types of Mutations

Various classifications of mutations are known, each based on a definite criterion. A classification based on the method of detection of mutations includes the following main types:

(i) **Morphological mutations**

Involve alterations in external form including colour, shape, size, etc. Examples include albino ascospores in *Neurospora,* kernel colour in corn, curly wings in *Drosophila* and dwarfism in pea.

(ii) **Lethal mutations**

Involve genotypic changes leading to death of an individual. These are perhaps the easiest to score for a study of mutation frequencies (see later for lethal mutations in *Drosophila;* some albino mutations resulting from chlorophyll deficiency are also lethal),

(iii) **Biochemical mutations**

These are identified by a deficiency, so that the defect can be overcome by supplying the nutrient or any other chemical compound, for which the mutant is deficient. Such mutations have been studied mainly in prokaryotes like bacteria and fungi, but sometimes also in eukaryotes like *Drosophila* and humans.

(iv) **Resistant mutations**

These are identified by their ability to grow in the presence of an antibiotic (e.g. streptomycin, ampicillin, cycloheximide) or a pathogen, to which wild type is susceptible. These are very easy to score and therefore have been extensively studied.

(v) **Conditional mutations**

These are those which allow the mutant phenotype (includinglethality) to be expressed only under certaincondition (e.g. high temperature) called restrictive condition. Under other or normal condition permissive condition, the mutant expresses normal phenotype. These mutants, iflethal or semi-lethal can be multiplied underpermissive conditions and shifted to restrictive conditions for specific study. They have been extensively used for study of cell cycle or for a study of DNA replication.

Major emphasis in this chapter will be on morphological and lethal mutations, which are also described as macromutations (identified in individuals) in contrast to micromutations (identified only on the basis of a population, e.g. mutation for yield in a crop).

Spontaneous versus Induced Mutations

Mutations are rare events in nature and are then described as spontaneous mutations. Due to their occurrence, sometimes, it is difficult to identify and score them. This difficulty has been overcome by two methods:

(i) **Selective systems** have been designed, which facilitate the selection of mutants against normal wild type, as in case of biochemical, resistance and conditional mutations, where under certain conditions only mutants will grow permitting selection of one mutant among even a million individuals. Most of the selective systems are used in microorganisms and are discussed in the next chapter,

(ii) **Induced mutations** are used, when selective systems are not available and therefore, frequency of mutations needs to be increased artificially, to allow convenient identification and scoring of mutations.

Mutation Rates and Frequencies

A distinction between rates and frequencies of mutations has often been made. Whenever, such a distinction is made, mutation ratesare described (at a locus or per genome) as probability of a mutation event at a locus (or mutation events at all loci in a genome) over a specific unit of time measured either as an organism generation or as a cell generation or as a cell division (not ashoursor days). It is not easy to monitor and estimate celldivisions or cell generations. Therefore, more often we measure mutation frequencies,which are measured as number of mutants in a population of cells or individuals. The cell population can be gametes or spores. In Figure 8.2, the mutation rateis 1/7 per cell division, but mutation frequencyis 2/8 = 1/4 per cell.

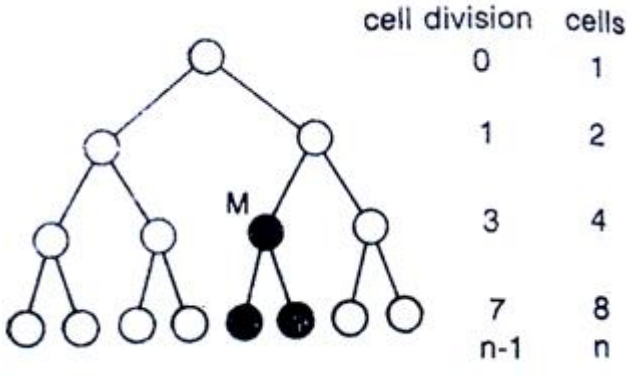


Figure 8.2: A simple cell pedigree showing a mutation at M

Mutation rates at individual loci

Mutation rates, per gene replication or per cell division could be measured in organisms like bacteriophages, bacteria or unicellular algae like *Chlamydomonas,* where from the number of mutant

cells and normal cells, an estimation of number of cell divisions can be made. Some examples of these mutation rates are given in Table 8.1.

Table 8.1: Mutation rates (spontaneous) in some organisms

	Organism	Mutation	Rate (per 10^6 cell divisions or DNA replications)
1	Bacteriophage (T2)	Lysis inhibition, *r*→*r*+	.01
		Host range, *h*+→*h*	.003
2	*E. coli*	Lactose fermentation	
		lac→*lac*+	.20
		Histidine requirement	
		his→*his*+	.04
		his→*his*-	2.00
3	*Chlamidomonas reinhardii*	Streptomycin sensitivity *str-s*→*str-r*	1.00
4	Bone marrow tissue Culture cells (human)	Normalazaguanine resistance	700

Chromosomal aberrations

Structural Changes in Chromosomes

Variations in the structure and number of chromosomes have been observed in natural populations and could also be produced artificially in a variety of organisms. These variations have been extensively studied and can be due to either structural changes or numerical changes. This chapter will be devoted to structural changes in chromosomes, and the next chapter will deal with numerical changes.

Structural changescan be of following types:

(i) Deficiency which involves loss of a part of chromosome,

(ii) Duplication, which involves addition of a part of chromosome,

(iii) Inversion, which involves a reverse order of the genes in a part of chromosome, and

(iv) Translocation, which involves exchange of segments between non-homologous chromosomes.

These structural changes are diagrammatically represented in Figure 8.3, where two non-homologous chromosomes from the complete set are shown. Structural abnormalities may be found in both the homologous chromosomes of a pair, or in only oneof them. When both homologous chromosomes are involved, these are called structural homozygotes e.g., deficiency homozygote, duplication homozygote, etc.

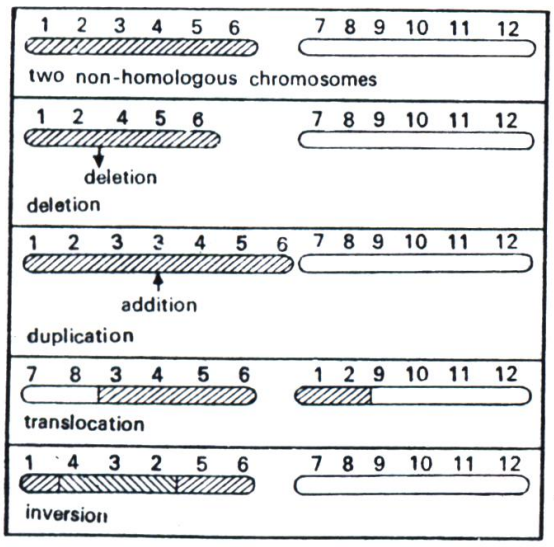


Figure 8.3: Different kinds of changes in chromosome structure

If only one chromosome is involved, this will be called a structural heterozygote. The constitutions of a translocation heterozygote and that of a translocation homozygote are shown in Figure 8.4. In this chapter, a brief and elementary account of different structural changes is presented. For a more detailed account of this subject, reader are encouraged to consult author's advanced book 'Cytogenetics'.

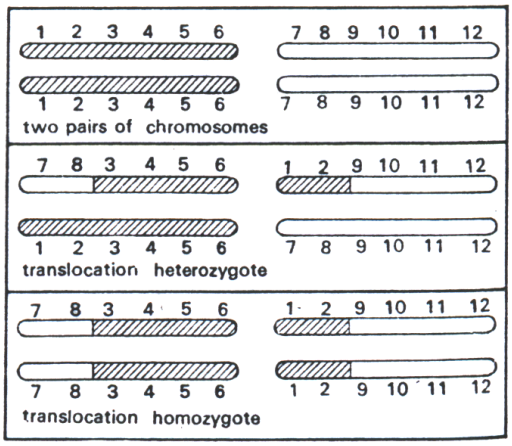


Figure 8.4: Chromosome constitution of a translocation heterozygote and a translocation homozygote

Deficiencies

Deficiency is due to loss of a part of a chromosome. Smaller deficiencies, present in heterozygous condition (only on one of the two homologous chromosomes), can be tolerated by an organism. Such individuals at meiosis will form a loop in a bivalent that can be observed at pachytene stage (Fig. 8.5).

Loops can also be observed in salivary gland chromosomes of *Drosophila* which are found in a permanent state of pairing, so that even small deficiencies could be detected in these chromosomes (Fig

8.6). Deficiencies have an effect on inheritance also. In presence of a deficiency, a recessive allele will behave like a dominant allele (pseudodominance).

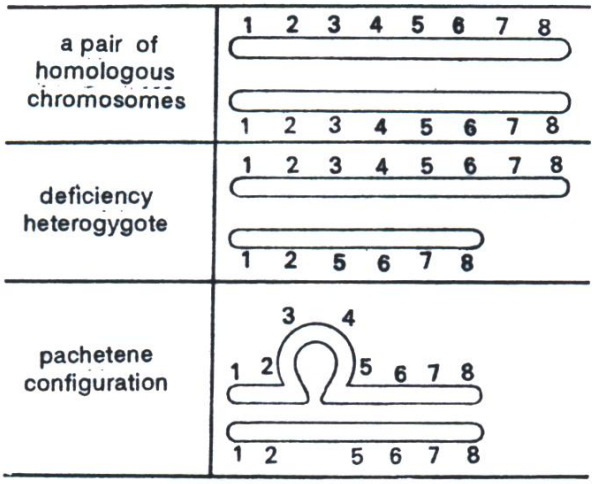


Figure 8.5: Chromosome pairing in a deficiency heterizygote

This principle of pseudodominance exhibited by deficiency heterozygotes has been utilized for location of genes on specific chromosomes in *Drosophila*, maize and other organisms. L.J. Stadler, who was a pioneer in radiation work in plants, devised a method where a homozygous recessive stock was pollinated by irradiated pollen from dominant stock, so that if irradiation induced a deletion, recessive allele will express due to pseudodominance.

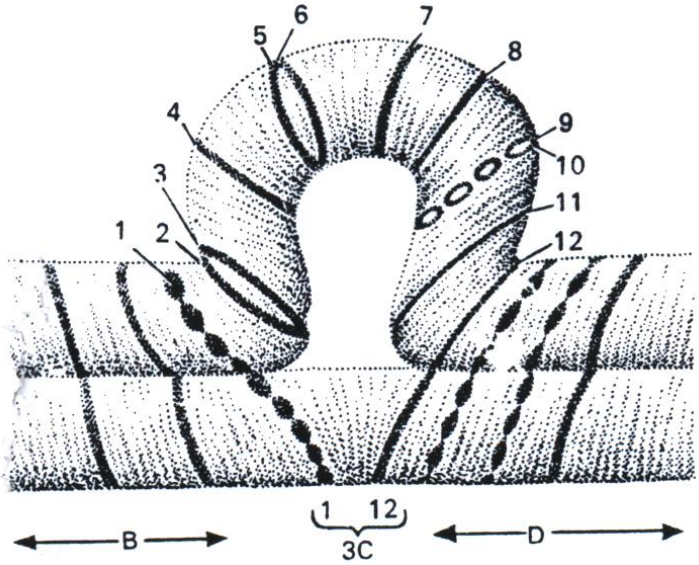


Figure 8.6: Diagrammatic representation of pairing of salivary gland X-chromosomes in a deficiency heterozygote in a o. *Drosophila,* showing a loop. Note that the bands 3C2 to 3C11 are missing in one chromosome

As shown in Figure 8.7, if homozygote *abc*is pollinated by *ABC,* heterozygous F_1*(ABC/abc)* will be produced expressing only dominant characters. If pollen with dominant alleles *ABC* is irradiated, a

deletion may be induced leading to expression of pseudodominance by one or more recessive alleles. If meiosis at pachytene is examined in such a deficiency heterozygote, presence of loop will indicate location of gene. Several genes were located on different chromosomes of maize and tomato, utilizing deficiencies. In *Drosophila* also deficiencies were recorded particularly on X-chromosomes in regions of genes *w* (white eye), fa (facet eye) and v (vermilion coloured eye).Deficiencies have also been recorded in waltzing mice in region of gene v inducing nervous abnormality. In human beings, a deficiency was discovered, which was associated with cat like-cryso that the child carrying this deficiency had a cat like cry and also had microcephally (small head and low mental faculty). This deficiency was found in a segment of chromosome 5.

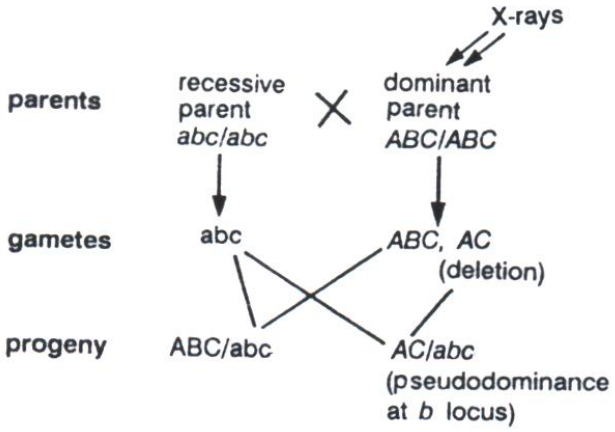


Figure 8.7: Detection of deletion due to psuedodominance

Duplications

Duplications are obtained due to addition of a part of a chromosome. If duplication is present only on one of the two homologous chromosomes, at meiosis (pachytene), cytological observations characteristic of deficiency will be obtained in duplication also (Fig 8.8). Duplication of a chromosome segment, may be brought about by addition at any of the following positions:

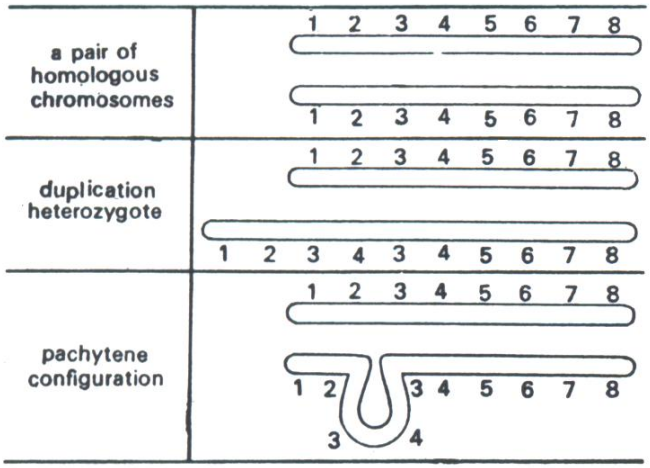


Figure 8.8: Chromosome pairing in a duplication heterozygote

(i) In adjacent region (Fig 8.9a),

(ii) At a displaced position of the same arm (Fig 8.9b),

(iii) On the different arm of the same chromosome (Fig 8.9c) or

(iv) On a different chromosome (Fig 8.9d). Sometimes the duplication may be found as a reverse repeat (Fig 8.9e).

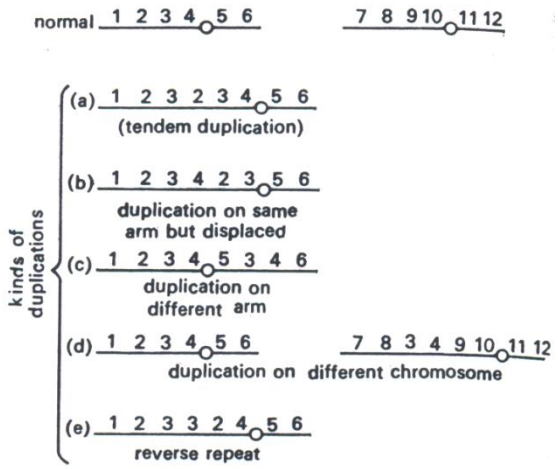


Figure 8.9: Different kinds of duplications in chromosomes

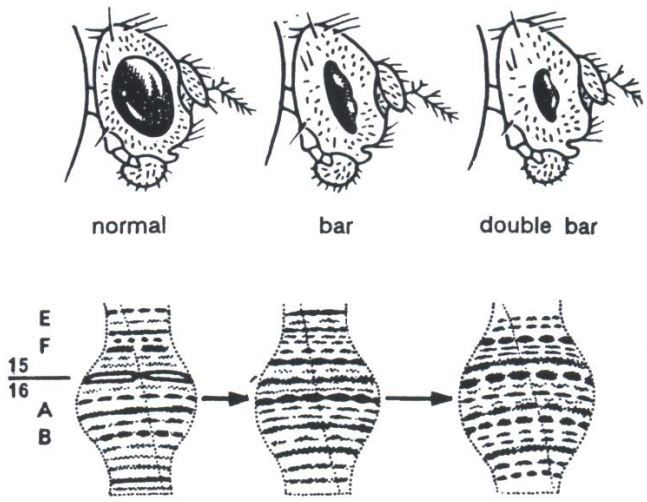


Figure 8.10: A normal eye, bar eye and a double bar eye with corresponding chromosome segments (salivary gland chromosomes) showing duplications

One of the classical examples of duplication in *Drosophila* is Bar eye (Fig 8.10). Bar eye is a character, where eyes are narrower as compared to normal eye shape. This phenotypic character is due to duplication for a part of a chromosome. By the study of giant salivary gland chromosomes, it could be demonstrated that 'Bar' character was due to a duplication in region 16A of X-chromosome. Barred eyes will have slightly different phenotype in heterozygous and homozygous individuals (Fig 8.11). Barred individuals *(16A 16A)* gave rise to ultrabar *(16A 16A J6A)* and normal wild type *(16A)* due to unequal crossing over (Fig 8.12).

Some other duplications known in *Drosophila* lead to following phenotypic effects (i) A reverserepeat in chromosome 4 causes *eyeless* dominant *(Ey);*

(ii) A tandem duplication in chromosome 3 causes *confluens (CO)* resulting in thickened veins, and

(iii) Another duplication causes *hairy wing (Hw).*

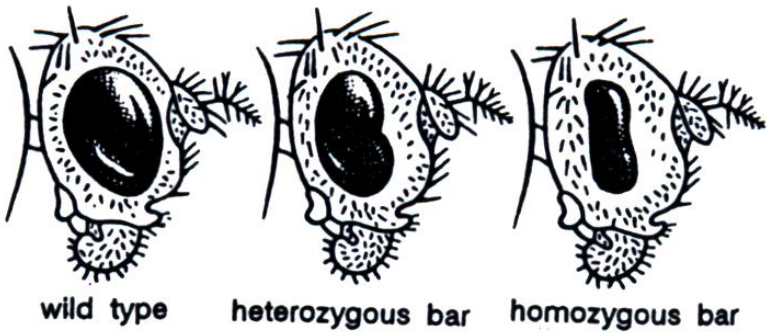


Figure 8.11: Shapes of eyes in normal, heterozygous bar and homozygous bar *Drosophila* flies

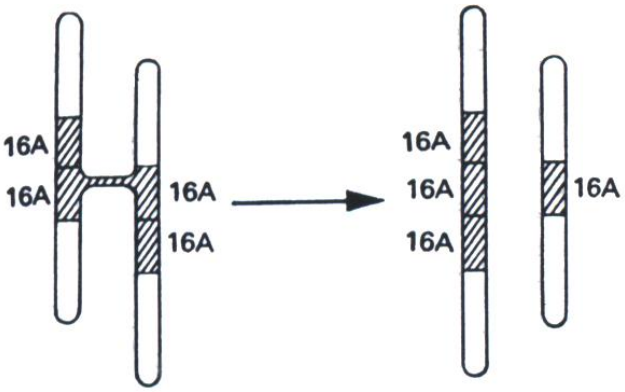


Figure 8.12: An ultrabar condition resulting from 'Bar' due to unequal crossing over

Translocations

Translocations are a broad term including all types of unilateral or bilateral transfer of chromosome segments from one chromosome to another. An important class of translocations having evolutionary significance is known as reciprocal translocations or segmental interchanges, which involve mutual exchange of chromosome segments between two pairs of non-homologous chromosomes (Fig 8.13).

Cytology of a translocation heterozygote

If a translocation is present in one of the two sets of chromosomes, this will be a translocation heterozygote. In such a plant, normal pairing into bivalents will not be possible among chromosomes involved in translocation. Due to pairing between homologous segments of chromosomes, a cross-shaped

(+) figure involving four chromosomes will be observed at pachytene (Fig 8.13). This ring of four chromosomes at metaphase I can have one of the following three orientations:

Alternate

In alternate orientation, alternate chromosomes will be oriented towards the same pole. In other words, adjacent chromosomes will orient towards opposite poles. This will be possible by formation of a figure of eight (Fig 8.13).

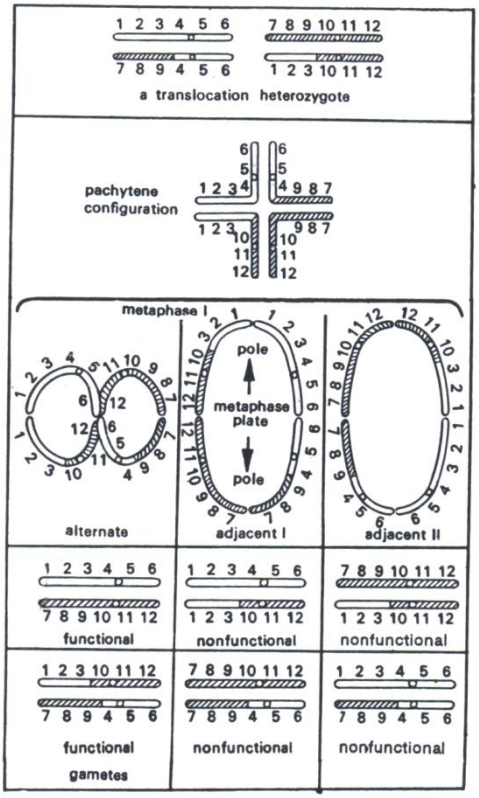


Figure 8.13: Chromosome pairing and different kinds of gametes formed in a translocation heterozygote

Adjacent I:

In adjacent I orientation, adjacent chromosomes having non- homologous centromeres will orient towards the same pole. In other words, chromosomes having homologous centromeres will orient towards opposite poles. A ring of four chromosomes will be observed.

Adjacent II:

In adjacent II orientation, adjacent chromosomes having homologous centromeres will orient towards the same pole. A ring of four chromosomes will be observed.

As shown in Figure 8.13, alternate disjunctions will give functional gametes. Adjacent I and adjacent II disjunctions will form gametes, which would carry duplications or deficiencies and as a result would be non-functional or sterile. Therefore, in a plant having a translocation in heterozygous condition, there will be considerable pollen sterility. A ring of four chromosomes, as described above, is found

under conditions when a single interchange is found. If two interchanges areinvolving three non-homologous chromosomes, a ring of six chromosomes is found, and the size of ring can increase with additional interchanges. More than one ring can also be found if two or more interchanges are independently found, each involving two different non-homologous chromosomes.

The first case of translocation was found in *Oenothera,* which was originally described as a mutation by de Vries while working for his Mutation Theory. *Oenothera*, *Tradescantia* and *Rhoeo* are such cases, where translocations in heterozygous condition are frequently found in nature. In many other crop plants they have been artificially induced.

Breeding behaviour of a translocation heterozygote

Presence of translocation heterozygosity can be detected by presence of semi- sterility and low seed set. This can then be confirmed at meiosis by quadrivalent formation. As shown above only two types of functional gametes are formed which result from alternate disjunction. The functional gametesr will give rise to three kinds of progeny (Fig 8.14) namely:

(i) Normal,

(ii) Translocation heterozygote, and

(iii) Translocation homozygote. These three types would be obtained in 1:2:1 ratio.

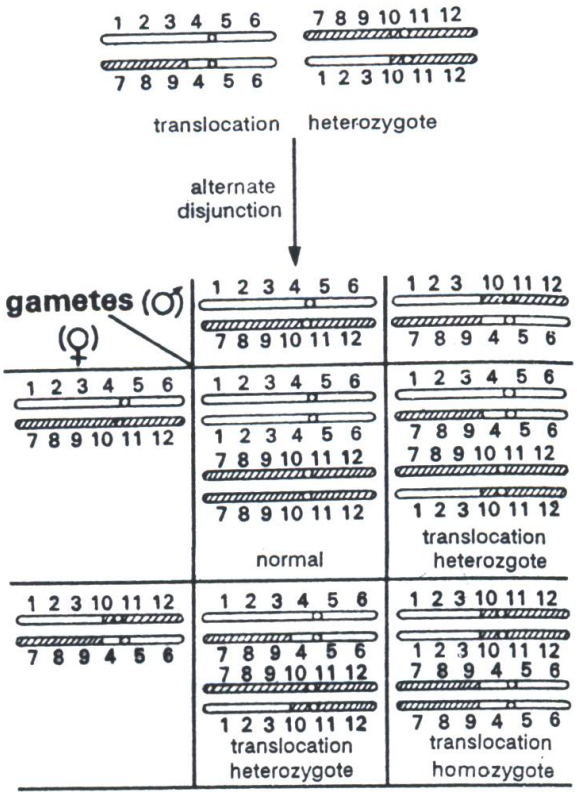


Figure 8.14: Different kinds of progenies obtained due to self fertilization in a translocation heterozygote

Interchange heterozygosity in *Oenothera*

Subgenus *Euoenothera* of genus *Oenothera* has been studied during 1920-1930 and cytogenetic structure leading to evolution in this group was examined. This group has *2n* = 14 and all 7 chromosomes of a haploid complement have median centromeres. Different species in the subgenus *Euoenothera,* can be classified in three groups:

(i) First group is represented by species showing bivalents or small rings at meiosis (e.g. *O. hookeri, O. grandiflora, O. argillicola).*

(ii) Second group is represented by species forming rings of various sizes at meiosis indicating the presence of interchanges. These rings are not permanent but are maintained due to their superiority in adaptive value (e.g. *O. irrigua).*

(iii) The third group is represented by those having permanent translocation heterozygosity involving all chromosomes, so that a ring of 14 chromosomes is regularly formed (e.g. *O. biennis, O. strigosa, (). parviflora).*

In *O. lamarckiana,* a ring of only 12 instead of a ring of 14 chromosomes is observed (Fig 8.15). These three groups also differ in phenotypes like flower size, etc. and can be identified. The members of third category behave like pure lines and are actually permanent heterozygotes. Balanced Icthals and gametic complexes: permanent hybridity in *Oenothera.*Permanent hybridity in some species of *Oenothera* is maintained due to operation of a balanced lethal system, which may function due to gametic lethality or zygotic lethality. Since complete rings are formed and alternate disjunction is a rule, only two types of gametes are formed showing complete linkage between 7 chromosomes. The gametic and zygotic lethality leads to survival of only heterozygotes (Fig 8.16).

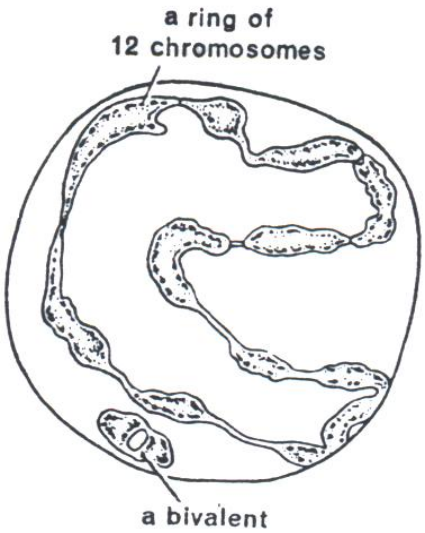


Figure 8.15: Chromosome complex in *Oneolhcra lamarckiana (2n* = 14) showing formation of a ring of 12 chromosomes and a bivalent, as an evidence of multiple interchanges

Balanced Icthals and gametic complexes:

Permanent hybridity in *Oenothera*.Permanent hybridity in some species of *Oenothera* is maintained due to operation of a balanced lethal system, which may function due to gametic lethality or zygotic lethality. Since complete rings are formed and alternate disjunction is a rule, only two types of gametes are formed showing complete linkage between 7 chromosomes. The gametic and zygotic lethality leads to survival of only heterozygotes (Fig. 8.16).It may be noticed that in gametic lethality, only one of the two types of gametes will function on the male side, the other type being functional on the female side, thus giving rise to only one type of progeny, which will be heterozygous. In zygotic lethality on the other hand, both the types of gametes will function on male as well as on female side, but the homozygote progeny due to recessive lethal genes will not survive (Fig 8.16).

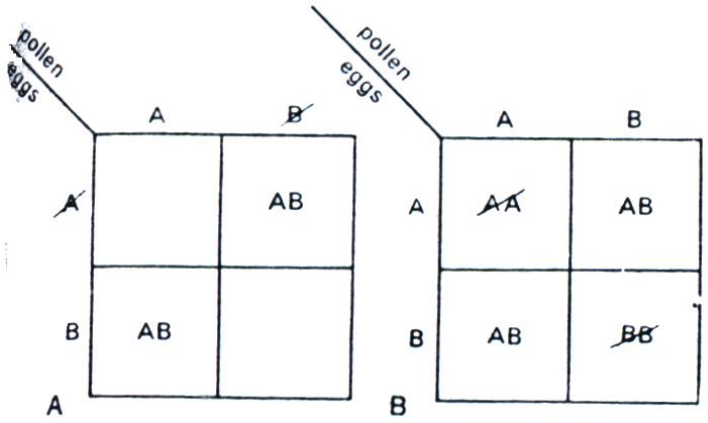


Figure 8.16: Gametic lelhality (A) and zygotic lethality (B) showing balanced lethal systems

Inversions

An inversion is produced when there are two breaks in a chromosome and the intercalary segment reunites in reverse order i.e. the segment rotates at 180°. Let us imagine that a chromosome *1-2-3-4-5-6-7-8* gives rise to another chromosome having the order *1-2-7-6-5-4-3-8*. The segment *3-4-5-6-7* has rotated here at 180° giving an inverted order of genes *7-6-5-4-3*.

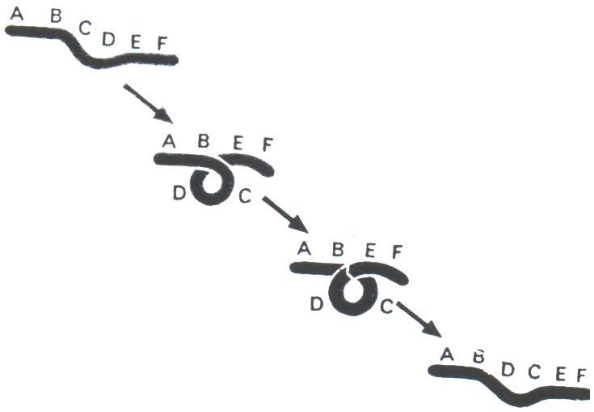


Figure 8.17: One of the possible mechanisms, which may give rise to chromosomal inversion

A similar hypothetical example using a chromosome *ADCDEF* has been shown in Figure 8.17, where due to coiling, breaks occur between *B* and *C* as well as between *D* and *E*. Reunion at broken ends may lead to inversion of the segment *CD* into *DC*. The inversion can be of two types:

(i) Paracentric inversion, and

(ii) Pericentric

Inversion: Paracentric inversions are those inversions, where inverted segment does not include centromere. On the other hand, in a pericentric inversion, inverted segment includes centromere. In order to remember these terms and their meaning, one should bear in mind that pericentric means surrounding the centromere or on the periphery of centromere.

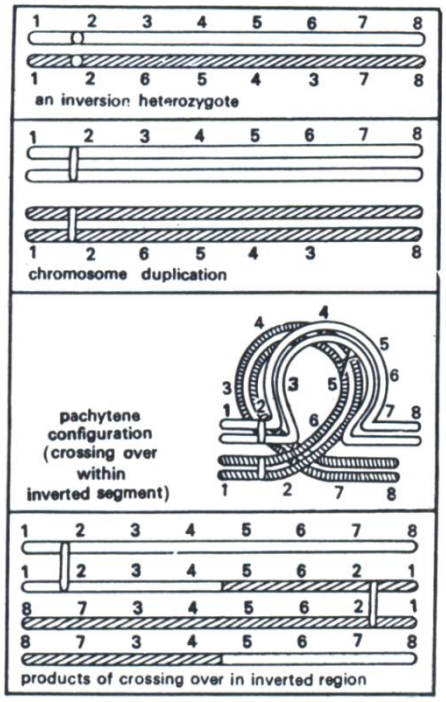


Figure 8.18: Chromosome pairing and products of crossing over in a paracentric inversion heterozygote

Cytology of inversions

Due to an inverted segment in one of the two homologous chromosomes, the normal kind of pairing is not possible in an inversion heterozygote. In order to enable pairing of homologous segments, a shape of loop is formed by each of the two chromosomes as shown in Figures 8.18 and 8.22. This kind of configuration will be observed both in paracentric as well as in pericentric inversions. As will be observed, the products of crossing over and the subsequent stages of meiosis will differ in these two kinds of inversions.

Paracentric inversion

A single crossing over or an odd number of crossovers in inverted region will result into formation of a dicentric chromosome (having two centromeres) and an acentric chromosome (with no

centromere). Of the remaining two chromatids, one will be normal and the other will carry the inversion (Fig 8.18). The dicentric chromatid and acentric chromatid will be observed at anaphase I in the form of a bridge and a fragment (Fig 8.18). Double crossovers and crossovers within and outside inversion will give various kinds of deficiencies and duplications (Fig 8.20). These will also give rise to a variety of characteristic configurations at anaphase I andanaphase II (Fig 8.21)

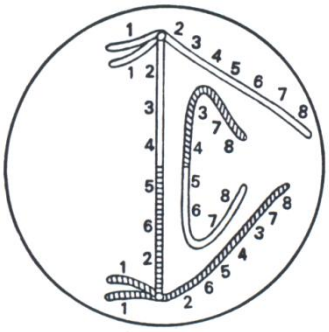


Figure 8.19: Dicentric Bridge and an acentric fragmental anaphase I

Pericentric inversion

Ina pericentric inversion (where centromere is present within the inverted segment), the pachytene configuration observed is similar to the one described above forparacentric inversion (Fig 8.18). However, the products of crossing over and configurations at subsequent stages of meiosis differ. In this case, two of the four chromatids resulting after meiosis will have deficiencies and duplications. However, unlike paracentric inversion, no dicentric bridge or acentric fragment will be observed (Fig 8.22). Consequently, at anaphase I, no bridge or fragment will be seen.However, in pericentric inversion, if two breaks are not situated equidistant from the centromere, this will result in a change in shape of the chromosome. For instance, a metacentric chromosome (with centromere in the centre) may become submetacentric and *vice versa* (Fig 8.23).

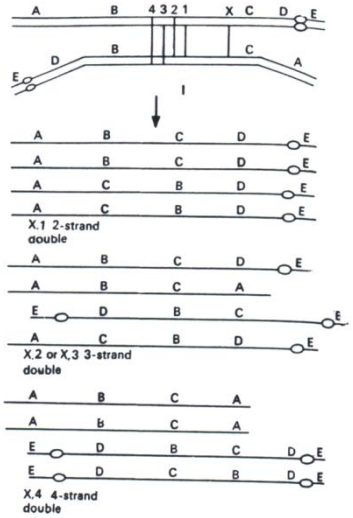


Figure 8.20: Consequences of 2-strands, 3-strands and 4-strands double crossovers within a paracentric inversion

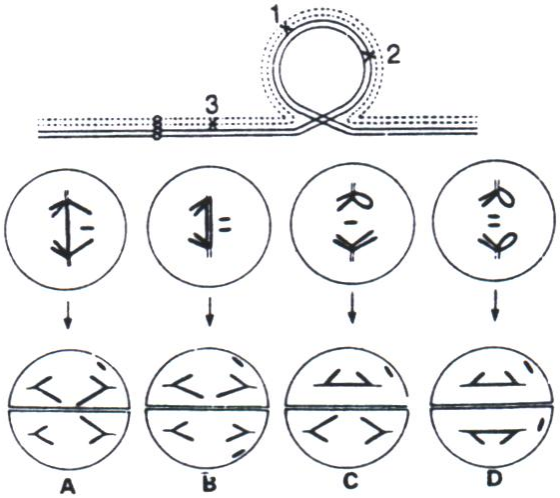


Figure 8.21: On the top is shown inversion loop in a inversion heterozygote. Below are shown anaphase I (AI) and Anaphase II (AII) configurations (A-D) resulting from four different combinations of crossovers within and outside.The inversion loop. A. Single cross over at position 1 or 2; B. 4-Strand double crossovers at postions 1 and 2; C. 3- Strand double cross overs with one crossover at position 1 or 2 within inversion loop and the other crossover out side the loop at positions 3; D. 4-strand triple crossover at position 1, 2 and 3

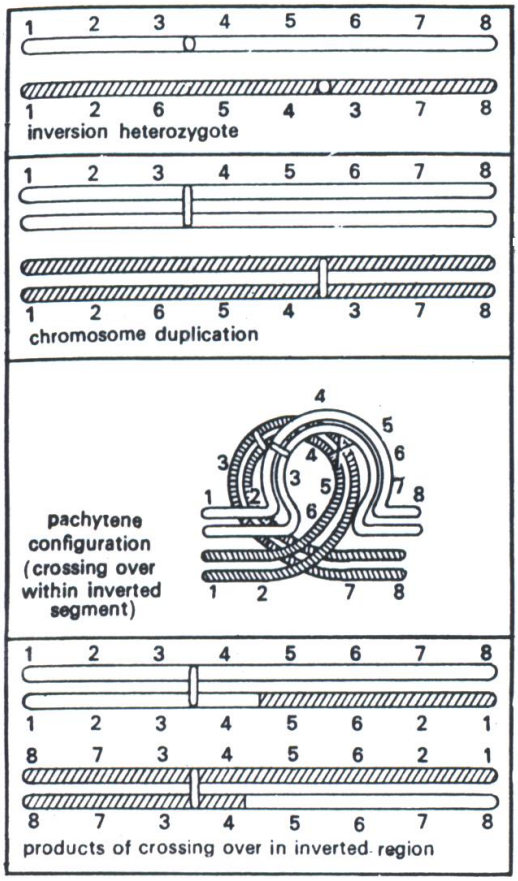


Figure 8.22: Chromosome pairing and the products of crossing over in a pericentric inversion heterozygote

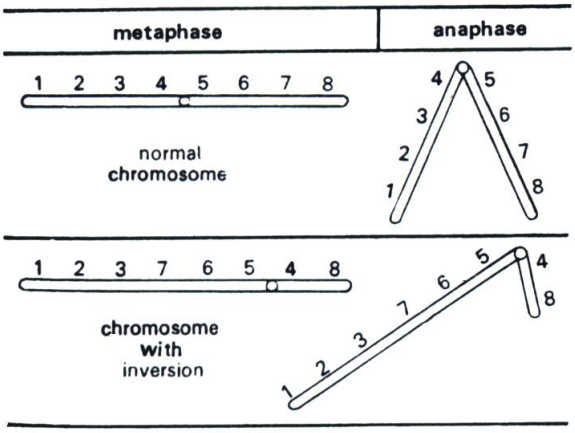


Figure 8.23: Change in chromosopme shape due to pericentric inversion

Genetic consequences of inversion

As discussed in the preceding section on cytology, among four chromatids resulting after crossing over, the two chromatids resulting from crossing over would have deficiencies and duplications. The gametes having these chromosomes will not function. Therefore, there should be considerable gametic or zygotic lethality. In plants, there will be sufficient pollen sterility. However, since the products of single crossover will not function and the only crossovers recovered will be double crossovers, the observed frequency of recombination between any two genes in question will be considerably reduced. Due to this reason, inversions are often called crossover suppressors. This reduction in crossing over is not the actual reduction in cytological crossing over, but is the result of lack of recovery of the products of single crossovers. This property of inversions has been utilized in the production of *CIB* stock, used by H.J. Mullerfor the detection of sex linked lethal mutations.

Overlappingin versions

Sometimes a second inversion is induced in a chromosome which already has one inversion. This result in an overlapping inversion, if the segments involved in first and second inversions contain a common region. The gene orders and meiotic configurations found in an inversion heterozygote of this type are shown in Figure 8.24.

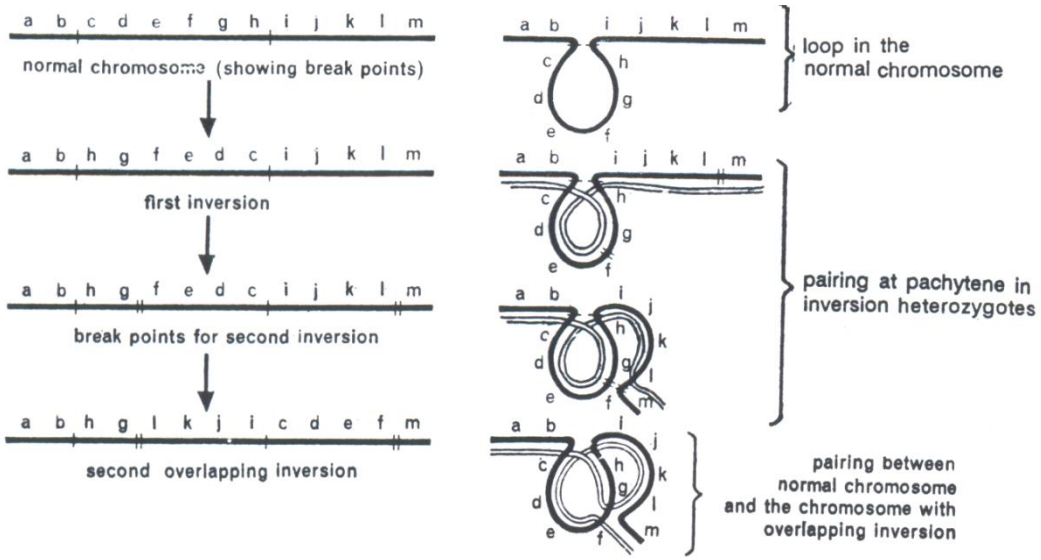


Figure 8.24: Steps leading to overlapping inversions and corresponding pachytene configurations

Inversions in *Drosophila* populations

Inversions are known to have played a significant role in evolution of different species and races of *Drosophila.* This knowledge is particularly available in *Drosophila* due to the ease of identification of inversions in salivary gland chromosomes. They also occur in plants, but can not be so easily worked out in the absence of giant chromosomes. In *Drosophila,* however, it isobvious that inversions occurred spontaneously innature and became established in populations dueto the adaptive benefit they conferred. Due toadaptive value, these inversions are restricted to Figure 8.25.

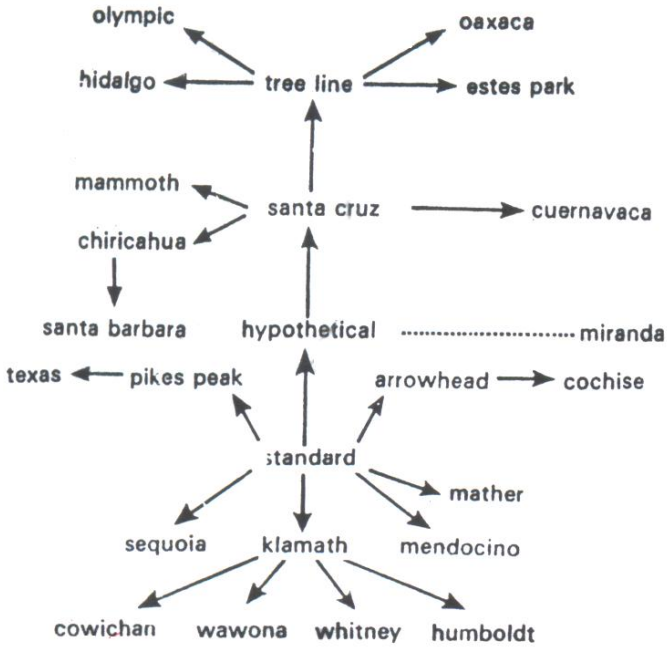


Figure 8.25: An evolutionary tree, showing derivation of a number of races due 10 simple and overlapping inversions in *Drosophila*

Numerical changes in chromosomes

Numerical changes in chromosomes or variations in chromosome number (heteroploidy),can be mainly of two types, namely (i) aneuploidy and (ii) euploidy**.** Aneuploidy means presence of chromosome number which is different than a multiple of basic chromosome number. Euploidy, on the other hind, means that the organism should possess one or more full sets of chromosomes. Let us imagine that 7 is the basic chromosome number *(x)* in a particular class of individuals where diploid number (*2n)* is 14. In this case, chromosome numbers 2n = 15 and *2n* = 13 would be aneuploicls, while those having *2n* = 7, 21, 28, 35 or 42 would be euploids. A classification of different kinds of numerical changes in chromosomes is presented in Figure 8.26.

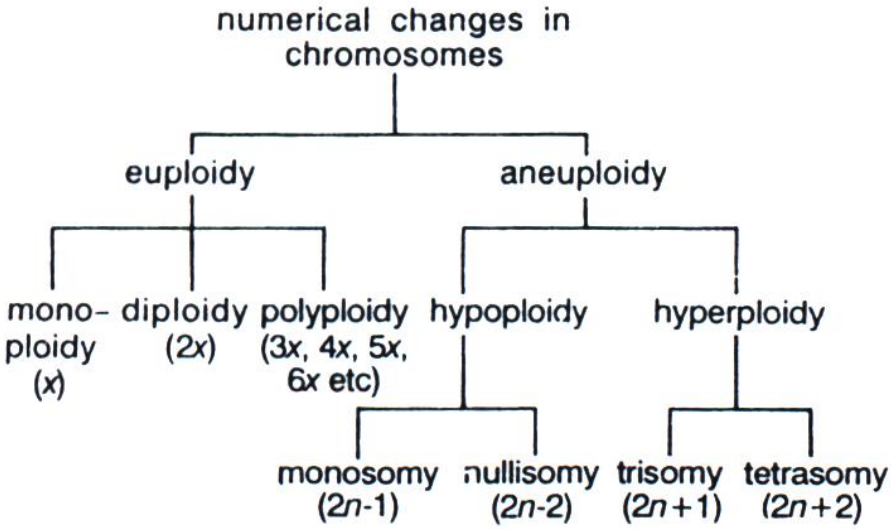


Figure 8.26: Different kinds of numerical changes in chromosomes *(x* = basic chromosome number; *2n* = somalic chromosome number)

Aneuploidy

Aneuploidy can be either due to loss of one or more chromosomes (hypopioidy) or due to addition of one or more chromosomes to complete chromosome complement (hyperploidy).Hypopioidy is mainly due to loss of a single chromosome-monosomy (2*n- 1*), or due to loss of one pair of chromosomes—nullisomy *(2n - 2).* Similarly, hyperploidy may involve addition of either a single chromosome—trisomy *(2n + 1*) or a pair of chromosomes—tetrasomy *(2n* + 2). In representing chromosome number of aneuploids, here we are using *2n* as the euploid chromosome number, even though *2n*actually represents the somatic chromosome number of any organism, whether euploid or aneuploid. It is for this reason that in the preceding paragraph aneuploids are shown as *2n* = 15 or *2n* = 13 and not as *2n+ 1* = 15 or *2n* - 1 = 13.

Monosomy

Since monosomics lack one complete chromosome, such aberrations create major imbalance and cannot be tolerated in diploids. These could be easily produced in polyploids. A polyploid has several

chromosomes of same type and, therefore, this loss can be easily tolerated. The number of possible monosomies in an organism will be equal to haploid chromosome number. In common wheat, since 21 pairs of chromosomes are present,21 possible monosomies are known. These 21 monosomics in wheat were produced by E.R. Sears (who died in 1991) in the variety Chinese Spring and are being used for genetic studies all over the world. Monosomies were also isolated in cotton (2n = 52) by J.E. Endrizzi and his co-workers, and in tobacco (*2n* = 48) by E.R. Clausen and D.R. Cameron.

As indicated above, monosomics are normally found in polyploids and diploids cannot tolerate them. Nevertheless, in tomato (*2n* = 24), which is a diploid, rarely monosomics could be produced. During the last decade surprisingly a complete set of monosomics has also been produced in maize, which is a diploid crop Doublemonosomics *(2n*—1-1) or triple monosomics(*2n*—1—1—1) could also be produced in polyploidslike wheat. Double monosomics mean that thechromosome number is 2n - 2, like that in a nullisomic, but the missing chromosomes are ,non-homologous. Same explanation would apply in case of triple monosomics also.

Monosomic condition for a particular chromosome may be associated with a characteristic morphology. Moreover, in progeny of a monosomic we will get a mixture of disomies (2n), monosomics (*2n*- 1) and nullisomics (*2n*- 2) and a nullisomic will not possess any of the genes located on this specific chromosome. Therefore, by looking on the morphology of monosomies and that of their progeny, genes can be located on specific chromosomes. For a detailed account of monosomicanalysis,which is an important technique for locating genes on chromosomes.

Nullisomy

Nullisomics arc those individuals, which lack a single pair of homologous chromosomes, so that the chromosome formula would be *2n*- 2 and not *2n*- 1 - 1, which would mean a double monosomic. Sears had isolated all the 21 nullisomics in wheat.

Trisomy

Trisomics are those organisms, which have an extra chromosome (2*n* + 1). Since the extra chromosome may belong to any one of the different chromosomes of a haploid complement, the number of possible trisomics in an organism will be equal to its haploid chromosome number. For instance, we know that haploid chromosome number in barley is *n* =7, consequently, seven trisomics are possible. Trisomics, where extra chromosome is identical to twohomologues, are called primary trisomics. Besides these, there are secondary and tertiary trisomics. While a secondary trisomic means that extra chromosome should be an isochromosome (both arms genetically similar), a tertiary trisomic would mean that extra chromosome should be the product of a translocation (Fig. 8.27).

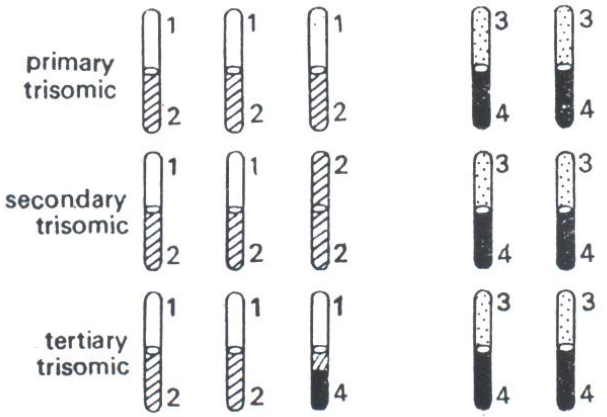


Figure 8.27: Three kinds of Trisomics

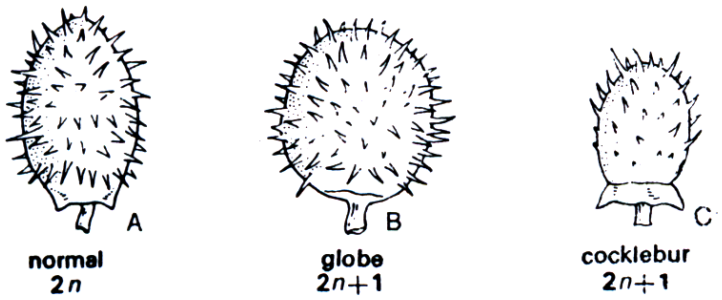


Figure 8.28: Trisomic conditions associated with changes in fruit morphology in *Datura stramonium*

Trisomics were obtained for the first time in *Datura stramonium* (jimson weed) by A.F. Blakeslee and his co-workers. Since haploid chromosome number in this species is n = 12, 12 primary trisomics, 24 secondary trisomics and a large number of tertiary trisomies are possible. Most of the trisomies were identified by size, shape and other morphological features of the fruit (Fig 8.28).One of the most extensively studied trisomic series is that produced and studied by T. Tsuchiva in barley. Trisomies are also known in *Homo sapiens* (human beings). Trisomy for certain chromosomes causes definite morphological abnormalities in human beings.

Mongolism (Down's syndrome)

Itis one such feature, which is common in children and is characterized by mental retardation, a short body, swollen tongue and eyelid folds resembling those of Mongolian races. Other cases of trisomy are also known in a number of different plant and animal species.

Production of trisomics

Trisomies may originate spontaneously due to production of n + 1 type of gametes (Fig 8.29) due to rare nondisjunction of a bivalent. However more often trisomies are produced artificially either by

selfing triploids (produced by crossing diploids and autotetraploids) or by crossing these triploids as females with diploids as male (3x x *2x).* In either case trisomies are obtained in large number and can be identified through phenotypic effects of individual chromosomes.

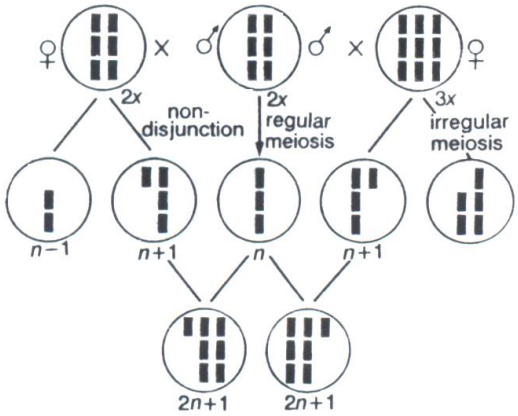


Figure 8.29: Production of trisomies due to formation of *n* + 1 type of gametes in diploid (2x) and triploid (3x) and triploid (3x) individuals

Cytology of trisomics

A trisomic has an extra chromosome which is homologous to one of the chromosomes of the complement. Therefore, it forms a trivalent. This trivalent may take a variety of shapes in primary and secondary trisomics as shown in Figure 8.30. In a tertiary trisomic a characteristic pentavalent is observed.

Table 8.2: Phenotypic ratios obtained from different genotypes of trisomic *(2x* + 1) or triploid *(ix)* individuals (assuming 50% transmission and notransmission of n + 1 type of gametes)

Genotype	Phenotypic ratios			
	Chromosome segregation		Chromatid segregation	
	Selfing	Test cross	Selfing	Test cross
AAA (triplex)	All : 0	All : 0	All : 0	All : 0
AAa (duplex)	17 : 1	5 : 1	14 : 1	4 : 1
Aaa (simplex)	2 : 1	1 : 1	29 : 16	14 : 16
Aaa (nulliplex)	0 : all	0 : all	0 : all	0 : all

Trisomic analysis

Trisomies are also used for locating genes on specific chromosomes. If a particular gene is located on the chromosome involved in trisomy, segregation in the progeny of this trisomic will not follow a Mendelian pattern, but the ratio will deviate from normal 3 : 1 F_2 and 1 : 1 test cross ratios. The expected ratios in trisomics can be worked out, and are given in Table 8.1. In Table 8.1, ratios based

on chromosome segregation as well as those based on chromatid segregation arc given. Chromosome segregation will hold good, when the gene is located very close to centromere permitting no crossing over between the gene and the centromere, so that both sister chromatids will be similar. In chromatid segregation, gene is located away from centromere permitting crossing over between gene and centromere.

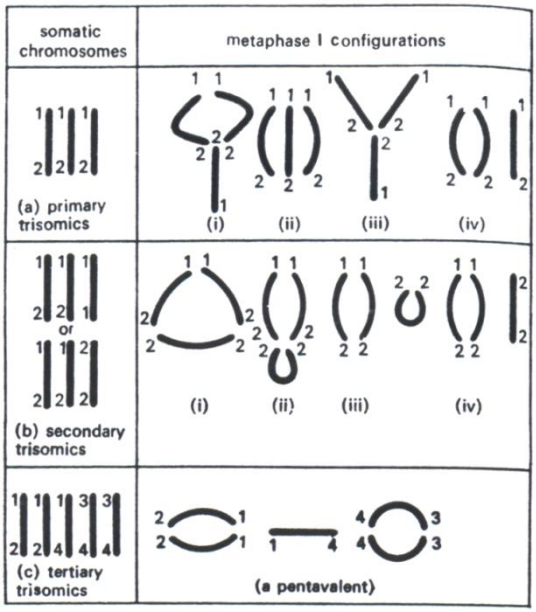


Figure 8.30: Different types of trisomies and their meiotie configurations at metaphase 1

Tetrasomy

Tetrasomics have a particular chromosome represented in four doses. Therefore, general chromosome formula for tetrasomics is *2n*+ 2 rather than *2n*+ 1 + 1, the latter being a double trisomic All 21 possible tetrasomics are available in wheat. Besides these tetrasomics, E.R. Sears was also able to synthesize a complete **set** of compensating nuliisomic tetrasomics *(2n* -2 + 2), where addition of a pair of homologous chromosomes would compensate for the loss of another pair of homologous chromosomes. Such non-homologous chromosomes, which are able to compensate for each other, are considered to be genetically related and are called homoeologous chromosomes.

Euploidy

Euploids can be monoploids, diploids or polyploids (Fig 8.31). A brief account of the two types of aberrations in this class, namely monoploidy and polyploidy will be presented in this section. Since diploids are normal individuals, these will not be discussed.

Monoploidy and haploidy

A distinction should be made between monoploidy and haploidy.Monoploidshave a single basic set of chromosomes e.g. *2n* = *x* = 7 in barley or 2n = *x* = 10 in corn. Haploids, on the other hand

represent individuals having half the somatic chromosome number found in normal individual. Therefore, individuals having *2n = 3x* = 21 in wheat would also be haploids. These latter kind of haploids obtained from polyploids are often called polyhaploidsin order to distinguish them from monoploids.

While reviewing the work on haploids in flowering plants in 1963, G. Kimber and R. Riley of Plant Breeding Institute, Cambridge, England (now, Institute of Plant Science Research or IPSR, Norwich, UK), gave a classification for haploids. They classified haploids in euhaploids and aneuhaploids which as the terms indicate are derived from euploids and aneuploids respectively. A modified classification recently given by K.J. Kasha is presented in Figure 8.31. Euhaploids will include monoploids as well as polyhaploids.

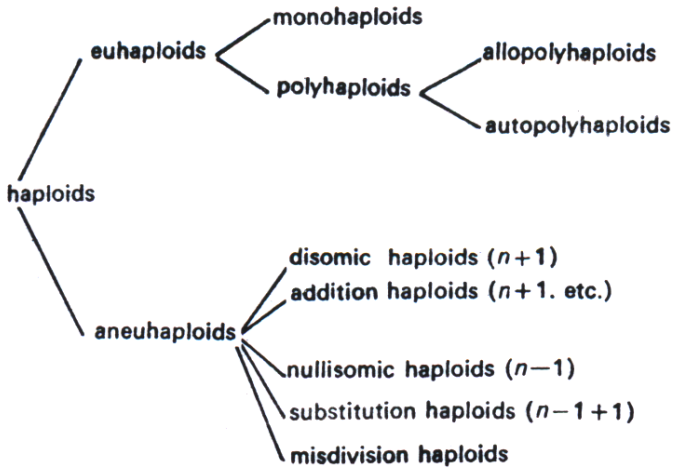


Figure 8.31: Classification of haploids

Origin and production of haploids

Haploids in some cases as in male insects (Hymenoptera) are found as a routine and are produced due toparthenogenesis. In these insects, queen and drones are diploid females. Haploids may also originate spontaneously due to parthenogenetic development of egg in flowering plants. Such rare haploids have actually been obtained in tomatoes and cotton under cultivation. Rarely haploids may originate from pollen tube rather than form egg, synergids or antipodals of embryo sac. These haploids will be called androgenic haploids.Haploids can be artificially produced by any one of the following methods:

(i) X-rays treatment,
(ii) Delayed pollination,
(iii) Temperature shocks,
(iv) Colchicine treatment,
(v) Distant (interspecific or intergeneric) hybridization,
(vi) Anther or pollen culture.

Among different available methods for haploid production as listed above, the most important are distant hybridization and anther culture. Thus haploids can be produced at different points in the life cycle of a flowering plant. The details ofmethods used are given in a life cycle shown in Figure 8.32. It may be noticed that haploids may originate due to:

(i) parthenogenetic or androgenic development of gametes,

(ii) due to chromosome loss in hybrid embryos and

(iii) by pollen culture.

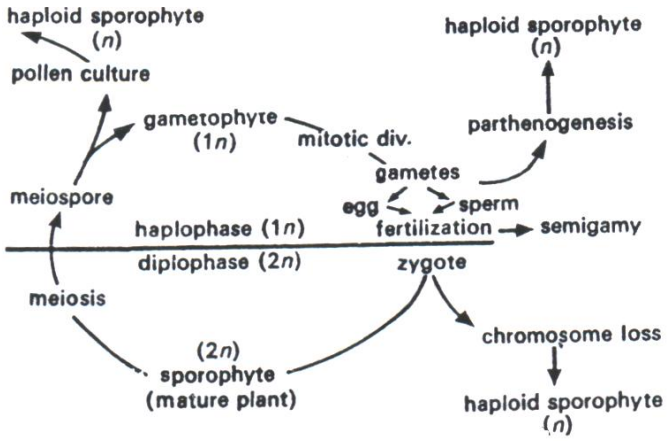


Figure 8.32: Life cycle of a flowering plant illustrating different stages at which haploids can be produced

Interspecific crosses in genera of *Solanaceae,* (e.g. *Solanum* and *Nicotiana)* have been successfully used for production of haploids, both parthenogenetic and androgenic. Haploids by this method have also been produced in potato in large number. K.J. Kasha from University of Guelph, Canada evolved an excellent technique for producing haploids in large number in barley (Kasha and Kao, 1970). He discovered that if the cross *Hardeum vulgare* x *H. bulbosum,* is made, chromosomes of *H. bulbosum* are eliminated in early zygotic divisions, so that a few days after pollination, embryos can be cultured to get haploids. This technique is being extensively utilized now all over the world.

The production of haploids by anther and pollen culture was demonstrated for the first time in the laboratory of S.C. Maheshwari of Delhi University (Guha and Maheshwari, 1970). Subsequently haploids by this technique could be produced in other plant genera e.g. *Oryza* (rice) and *Nicotiana* (tobacco). This technique is now considered to be a very potential source of haploid production.

Morphology of haploids

Haploids are characterized morphologically by a reduction in size of all vegetative and floral parts. Only rarely a haploid may equal or exceed the diploid in size. When haploids were studied in *Nicotiana* by D. Kostoff, it was found that leaves, flowers, and overall plant size were smaller. The seed

size, stomata size and pollen diameter were also generally smaller in haploids than in the diploids. In many cases in haploids, the size of nucleus measured as nuclear volume was half that in diploids.

Cytology of haploids

Since in a haploid set, the chromosomes are non- homologous and have no homologues to pair with, they are found as univalents at metaphasc I of mciosis. Consequently, these univalents distribute at random during anaphase I. For instance a haploid in maize (*2n* = 20) will have 10 chromosomes and the number of chromosomes in a gamete of haploid plant can range from 0-10. Consequently,considerable sterility will be found. Moreover, since univalents are scattered all over the cell, they may constitute a restitution nucleus including all chromosomes and may thus give rise to gametes having a complete haploid set of chromosomes. Haploids (polyhaploids; *2n = 3x* = 21) were used by E.R.

Table 8.3: Crop cultivars derived by haploid systems

Crop	Cultivar	Haploid System
Barley	Mingo	Chromosome elimination
	Gwylan	"
Rapesced	Maris Haploma	Twin seedings
Rice	Hua Be 702	Anther culture
	Hua Yu No. 1	"
	Hua Yu No. 2	"
	Ta Be 78	"
	Shin Shu	"
	Tan Fong No. 1	"
	Tanfeng No. 1	"
Tobacco	Tan Yu No. 1	"
	Tan Yu No. 2	"
	Tan Yu No. 3	"
Wheat	Hua Pei No. 1	"
	Lung Hua No.1	"

Sears for production of monosomies by pollinating the haploid by pollen from a diploid individual (hexaploid; *2n = 6x* = 42). If the egg has a chromosome number, one less than the complete set, this will result into a monosomic.

Uses of haploids

When the chromosome number of a haploid is doubled by colchicine treatment, derived diploid will be completely homozygous for all genes so that the most important use of haploids is in the

production of homozygous diploids. Although this theoretical expectation was earlier used only for the production of experimental material, but during the last few years a number of varieties for cultivation have been produced using this method. A number of such cultivars, were listed by K.J. Kasha in a chapter written in the book *'Cytogenetics of Crop Plants'* published by McMillan India Ltd. in 1983. Some of these varieties produced as homozygous diploids derived from haploids are listed in Table 8.2. The most extensive use of haploids for developing superior cultivars has been made in rice crop in China. The details of some rice varieties developed from pollen in China are presented in Table 8.3.

Table 8.5: Some varieties of rice from pollen plants

Veriety acreage (mu)[a]	**Cultivated (kg/mu)**	**Yield (%)[h]**	**Increase inyield**
Tanyu No, 1 Xin Xion	>5X10^5	—	—
Huayu 1	1X10^5	—	—
Late Keng 76	1X10^5	449	17
Late Keng 959	>5X10^5	300-400	10
Tonghua No.s and 2	>3X10^5	453-513	10-15
Hua Han Zao	20X10^5	—	8.7-27.7
Zhe Keng 79-66	10X10^5	403	8.1
Zhong Hua 8, 9, 10 & 11	20X10^5	487-665	8.8-13.7
Nanhua No. 11[c,d]	>1.5X10^5	408	7.4-10.1
Chao Hua Aic		435-553	18.8-51.7
Huapei Shanyou 63[c, e]	>40X10^5	—	5-7
Hua-03[d,f]		400	—

[a] 1 mu = 1/15 ha; [b] Local rice variety or hybrid rice used as control; [c] Hsien type rice;
[d] From three lines hybrid rice; [e] A new three lines hybrid rice variety by using a purified male-sterile line from anther culture; [f] A high- protein rice.

Polyploidy

There are mainly three different kinds of poly-ploids, namely

(i) Allopolyploids,

(ii) Allopolyploids,

(iii) Segmental allopolyploids.

Let us imagine that A, B_1, B_2 and C are four different haploid sets of chromosomes and that genomes B, and B_2 are related. Different kinds of polyploids using these genomes are derived in Figure 8.33.

Autoopolyploids

Autopolyploids are those polyploids, which have the same basic set of chromosomes multiplied. For instance, if a diploid species has two similar sets of chromosomes or genomes (AA), an autotriploid will have three similar genomes (AAA) and an autotetraploid will have four such genomes (AAAA) as shown in Figure 8.33.

(a) Origin and production of autopolyploids

The autopolyploids may occur in nature or may be artificially produced. When they are found in nature, their autopolyploid nature is inferred mainly by their meiotic behaviour. One of the very common examples of natural autopolyploidy relevant to Northern India, pertains to common 'doob' grass *(Cynodon dactylon).* In U.P. and Bihar, common *'doob'* grass was found to be an autotriploid as inferred from its meiotic behaviour. It is perhapssuccessful due to efficient vegetative reproduction, because, as will be seen, autoeupolyploids are normally triploids and set no seeds. Autotriploids are also known in watermelons, sugarbeet, tomato, grapes and banana, although in several of these cases the polyploids have been artificially produced. Similarly autotetraploids are known in rye *(Secale cereale),* corn *(Zea mays),* red clover *(Trifolium prdtense),* bersecm *(Trifolium alexandrium),* marigolds *(Tagetes),* snapdragons *(Antirrhinum), Phlox,* grapes, apples, etc. In *Oenothera lamarckiana,* an American plant, a giant mutant described by Hugo de Vries was later discovered to be an autotetraploid.

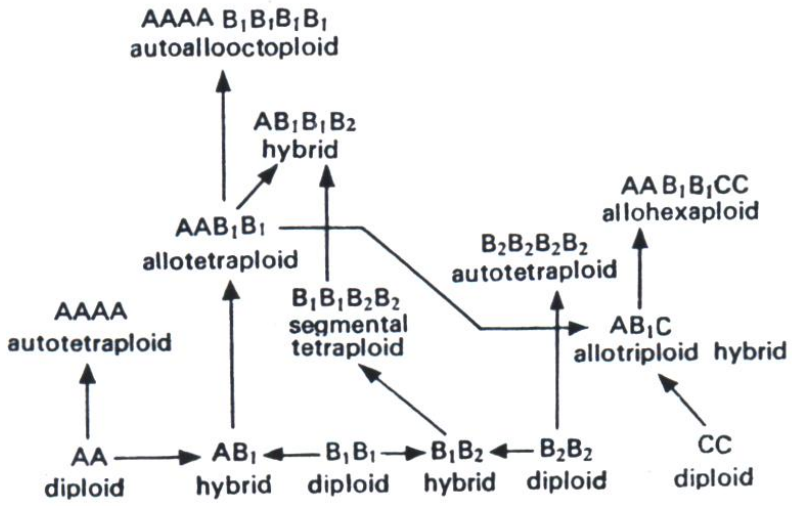


Figure 8.33: Different kinds of polyploids and their derivations

(b) Induced autopolyploids

In many cases listed above, autopolyploidy is artificially induced. Since polyploids are normally larger and more vigorous, their role in crop improvement has been realized and techniques developed for artificial induction of polyploidy. Polyploidy is mainly induced by treatment with aqueous solution of a drug called colchicine. This drug has the property of arresting and breaking the spindle so that a cell division without cell wall formation may be affected leading to doubling of chromosome number. The

concentration of aqueous solution of colchicine may vary from 0.01 % to 0.50% and the treatment may be given in one of the following manners,

(i) Seed treatment may be mainly given by soaking seeds for different durations in aqueous solution of colchicine,

(ii) Injections of colchicine solution may also be given at seeding stage so as to inject solution into cortex tissue with the help of a hypodermic needle,

(iii) Axiliary bud treatment is also effective. Since bud is meristematically active, placingbud and continuous dropping of solution on the bud leads to induction of polyploidy in the branch arising from the treated bud.

(iv) Shoot apex treatment is brought about just like bud treatment and is fairly effective, but the shoot apex should come in direct contact of the solution. In order to facilitate this, young leaves covering the shoot apex may be removed.

Colchicine is an alkaloid extracted from seed and corm of *Colchicum autumnale.* The action of colchicine and its use in inducing polyploidy was first studied in 1930's. The successful doubling of chromosome number was described for the first time by Levan (1938) and by Eigsti (1938-40). It was surprising however, that *colchicine* does not affect *Colchicum,* the plant from which it is extracted. This was attributed to the presence of an anticolchicine'. G.D.H. Bell (1959), who was once the Director of Plant Breeding Institute at Cambridge (England) had suggested that the success of colchicine treatment will depend on three prerequisites, namely

(i) Direct contact of growing tissue with solution,

(ii) Effective concentration of colchicine and

(iii) The optimal stage of development. These three conditions are met with in the methods of treatment described above.

(c) Effects of chromosome doubling

One of the important effects of polyploidy is to produce 'gigantism'. The auto polyploids may be normally larger in size. Sometimes plants may be smaller than diploids, but leaves, flowers and the cellsthemselves may be bigger in size. Some of the important effects of polyploidy arc as follows:

(i) With increase in cell size, water content increases leading to decrease in osmotic pressure. This results into loss of resistance against frost, etc.

(ii) Growth rate decreases due to slower rate of cell division; this leads to a decrease in auxin supply and a decrease in respiration.

(iii) In autopolyploids, time of blooming is delayed and prolonged due to slow growth rate, (iv) At higher ploidy level (autooctoploids or higher), the adverse effects are highly pronounced and lead to death of plants.

(d) Cytology of autopolyploids

In an autopolyploid, there will be more than two sets of homologous chromosomes. This leads to formation of multivalents instead of bivalents as found in diploids. An important difference exists even between autotriploids and autotetraptoids, becausewhile in the latter normal disjunction is possible giving rise to diploid gametes, in triploids it is not possible. In an autotriploid, there are three sets of homologous chromosomes. If these three sets are normally paired, trivalents can not disjoin normally and will either disjoin 2 : 1 chromosomes to two poles or will disjoin 1 : 1 leaving one chromosome as a laggard. The number of chromosomes in gametes of triploid organism, therefore, will vary from *n* to *2n*. Most of these gametes are unbalanced leading to high degree of sterility.

In autotetraploids, since there are four sets of chromosomes, quadrivalents are formed, which disjoin in a normal 2 : 2 manner giving diploid gametes. Rarely however, a quadrivalent may disjoin 3 : i or may leave a chromosome as a laggard at anaphase I. Therefore autotetraploids also have a certain degree of sterility, although it will not be as high as in autotriploids.

(e) Genetics of autopolyploids (trisomic and tetrasomic inheritance

The segregation pattern in polyploids is quite different than what we find in diploids. In a polyploid there are more than one kind of heterozygotes, because there are more than two homologous chromosomes. The different possible genotypes and expected ratios in autotetraploids are given in Table 8.4. Similar ratios for triplqids are given in Table 8.1. The ratios are based on the^assumption that cither the gene is close to centromere leading to what we call chromosome segregation or the gene is away from centromere so that crossing over between gene and centromere will take place and ratios will be modified. In the latter case it will be called chromatid segregation, because segregation is taking place not at chromosome level, but at chromatid level due to crossing over.

Table 8.6: Phenolypic ratios obtained from differentgenotypes of autotetraplold (4x) or tetrasomic *(2x* + 2) individuals due to selfing or testcrosses

Genotype	Phenotypic ratios			
	Chromosome segregation		Chromatid segregation	
	Selfing	Test cross	Selfing	Test cross
AAAA (quadriplex)	All : 0	All : 0	All : 0	All : 0
AAAa (triplex)	All : 0	All : 0	783 : 1	27 :1
AAaa (duplex)	35 : 1	5 : 1	187 : 9	11 : 3
Aaaa (simplex)	3 : 1	1 : 1	559 : 225	13 : 15
Aaaa (nulliplex)	0 : all	0 : all	0 : all	0 : all

It can be seen from Table 8.4, that main effect of allopolyploidy on segregation, when compared with that of diploids is the reduction in frequency $_0$f genotype homozygous at a particular locus. The result is that recessive phenotype is much less frequent as shown by a ratio 35 : 1 as against a disomic ratio of 3 : 1 and so on (Table 8.4). , Tetrasomic inheritance and trisomic inheritance are useful criteria to differentiate between autopolyploids and allopolyploids and for locating genes on specific chromosomes.

(f) Uses of induced polyploidy

Since fertility level and seed set arc low in induced polyploids, their utility in improvement of crop plants will depend on two major considerations, namely:

(i) what is the commercial product, because if seed is not the commercial product low seed set will not be a handicap, and

(ii) whether or not fertility level is important.

Keeping these requirements in mind, seedless fruits can be produced by using triploids as in case of seedless watermelons produced by H. Kihara (who died in 1986) in Japan. These triploids were obtained from seeds raised by a cross tetraploid x diploid, the tetraploids being raised from diploids by colchicine treatment. This method was also used for obtaining triploids in sugarbeet, tomato and grapes.

Autotctraploids could also be used commercially. For instance, among grain crops autotetraploid rye is grown in Germany and Sweden. Tetraploids were also raised in barley and corn, although commercially they could not prove to be very useful. Among fruits, tetraploid apples and grapes have been found to be useful. Several ornamentals including marigolds, snapdragons, lily and *Phlox* are induced autotctraploids. Among forage crops, tetraploid berseem is a very popular crop in Northern India. In all cases of commercial uses of autotctraploids, fertility can be improved by selection, but sometimes unconsciously selection leads to reversion to diploidy and original tetraploid crop becomes diploid again.

Recently D.R. Dewey (1980) in his article 'Some applications and misapplications of polyploidy in plant breeding' published in a book 'Polyploidy : Biological Relevance', emphasized that none of the autopolyploids mentioned above (triploid sugarbeet, tetraploids red clover and alsike clover, triploid watermelons and several ornamentals) lived upto the expectation. Although, if sustained efforts are made, some autopolyploids may proveto have breeding value, but plant breeders may question the time, money and labour spent towards this goal.

Allopolyploids

Polyploidy may also result from doubling of chromosome number in a F_1hybrid which is derived from two distinctly different species. This will bring two different sets of chromosomes in F_1 hybrid. The number of chromosomes in each of these two sets may differ. Let A represent a set of chromosomes (genome) in species X, and let B represent another genome in a species Y. The F, will then have one A genome and another B genome. The doubling of chromosomes in this F, hybrid (AB) will give rise to a

tetraploid with two A and two B genomes (Fig. 8.34). Such a polyploid is called an allopolyploid or amphidiploid.

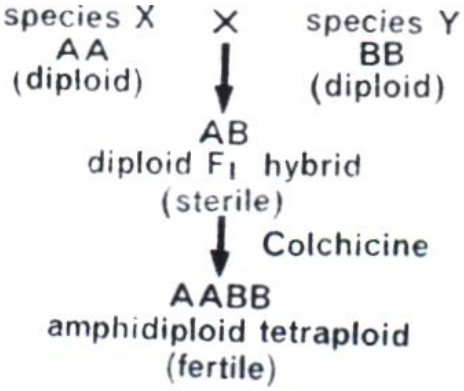


Figure 8.34: Derivation of a tetraploid amphidiploid from two diploid species

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Raphanobrassica is a classical example of allopolyploidy. In 1927, G.D. Karpechenko, a Russian geneticist, reported a cross between *Raphanus sativus (2n* = 18) and *Brassica oleracea (2n* = 8) *to* produce F_1 hybrid which was completely sterile. This sterility was due to lack of chromosome pairing, since there is no homology between genomes from *Raphanus sativus* and *Brassica oleracea.* Among these sterile F_1 hybrids, Karpechenko found certain fertile plants. On cytological examination these fertile plants were found to have *2n* = 36 chromosomes, which showed normal pairing into 18 bivalents (Fig. 8.35).

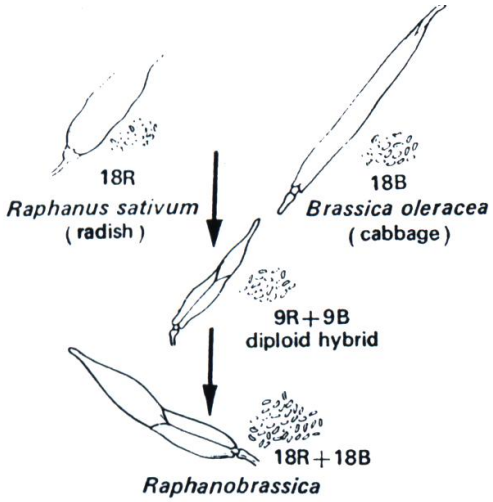


Figure 8.35: Artificial synthesis of *Raphanobrassica*

(a) Evolution of wheat

Common cultivated wheat is another important example of allopolyploidy, although its allopolyploid nature has now been questioned. There are three different chromosome numbers in the genus *Triticum,* namely *2n* - 14, *In*= 28 and *In* = 42. The common wheat is hexaploid with *2n* = 42, and is derived from three diploid species:

(i) AA =-*Triticum aegilopoides (2n* = 14),

(ii) BB = *Aegilops speltoides* ?*(2n* = 14) (in the past evidence wasmade available, showing that *Ae. speltoides* may not be the donor of B genome; it is also believed that the donor of B genome may never be discovered) and

(iii) DD = *Aegilops squarrosa* (2*n* = 14). The hexaploid wheat, therefore, is designated as AABBDD, the tetraploid *(2n* - 28) as AABB and diploid (2*n* = 14) as AA.

There is, however, evidence available now which suggests that A, B and D genomes from three diploid species mentioned above are not much different from one another, so that it is believed that the three diploid progenitors of common hexaploid wheat were derived from a common ancestor. For this reason, the common hexaploid wheat is now considered an autopolyploid rather than an allopolyploid. At best, it may be a segmental allopolyploid.

(b) Synthesized allopolyploids

Certain allopolyploids were artificially produced in order to find out the origin of naturally occurring allopolyploids. Common hexaploidn wheat and tetraploid cotton furnishes two such examples. These two and few other examples will be brieflydiscussed.

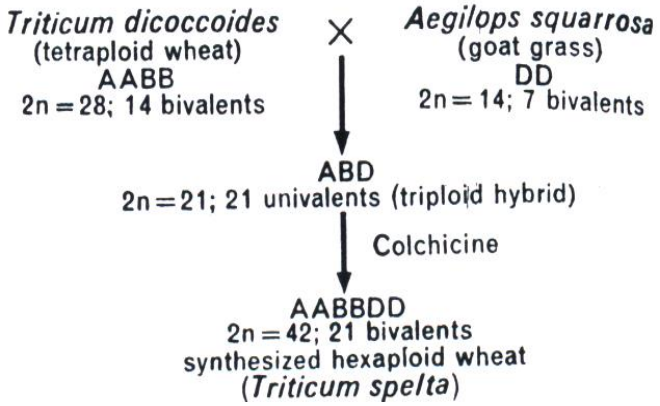


Figure 8.36: Artificial synthesis of Hexaploid wheat

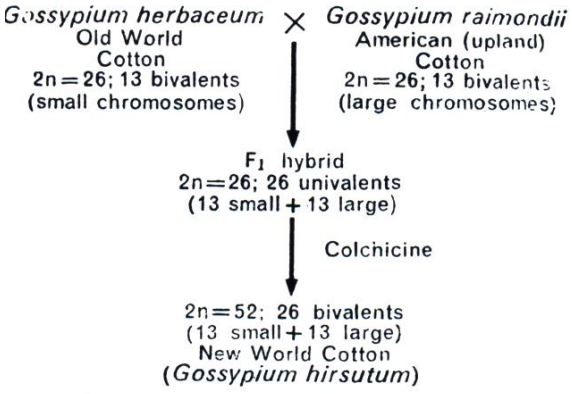


Figure 8.37: Artificial synthesis of new world cotton

(1) ***Triticum spelta,*** a hexaploid was artificially synthesized in 1946 by E.S. McFadden and E.R. Sears, and also by H. Kihara. They crossed an emmer wheat (tetraploid; 2n = 28) with Aegilops squarrosa (diploid; 2n = 14), and doubled the chromosome number in F(hybrid (Fig 8.36). The hexaploid or amphiploid synthesized in this manner was found to be similar to the primitive wheat T. spelta. When this synthesized hexaploid was crossed with naturally occurring T. spelta, F, hybrid was completely fertile and showed normal pairing of chromosomes into bivalents. Therefore, it is suggested that hexaploid wheat must have originated in the past due to natural hybridization between tetraploid wheat and goat grass (Aegilops squarrosa) followed by subsequent chromosome doubling.

(2) ***Gossypium hirsutum,*** popularly known as upland cotton, is another interesting example of amphidiploidy. Old World cotton has 13 pairs of large chromosomes, while American or upland cotton has 13 pairs of smaller chromosomes. The New World cotton, the cultivated long-staple type has 26 paris of chromosomes, 13 large and 13 small. J.O. Beasley crossed the American and Old World cottons and doubled the chromosome number in the F| hybrids (Fig 8.37). The amphidiploid thus produced resembled the cultivated New World cotton and when crossed with it gave fertile Fj hybrids. The evidence presented in Figure 8.37, thus showed that New World cotton, the cultivated *Gossypium hirsutum* (tetraploid) originated from two diploidspecies, namely G. *herbaceum* (2*n* = 26) and *G. raimondii* (2*n* = 26).

(3) Spartina townsendii, commonly called asTownsend's grass and found in Europe, hasa chromosome number, 2*n*= 126. This speciesin its morphology is intermediate to *Spartinaalterniflora* (2*n* = 70), the American marsh grass and *S. stricta (2n* = 56),the European marsh grass. A study ofmorphology and cytology of these threespecies and the fact that *S. townsendii* was fertile, suggested that 5. *Townsendii* originated by natural hybridization of *S. alterniflora* and*S. Stricta,* followed by doubling of chromosome number in the F, hybrid so derived. This is thus an amphiploid (Fig.8.38).

(4) Primula kewensis is an allotetraploid (amphidiploid) with *2n=4x* = 36 and was derived from a 4x branch of a *2x* hybrid between *P. floribunda (n = x =* 9) and *P. verticillata (n = x* = 9). Crosses can beeasily made between *P. floribunda* and*P. verticillata* and the F_1 hybrid with *2n* =18is sterile showing almost complete lack ofpairing. This difficulty is overcome bydoubling of chromosome number (Fig. 8.39).

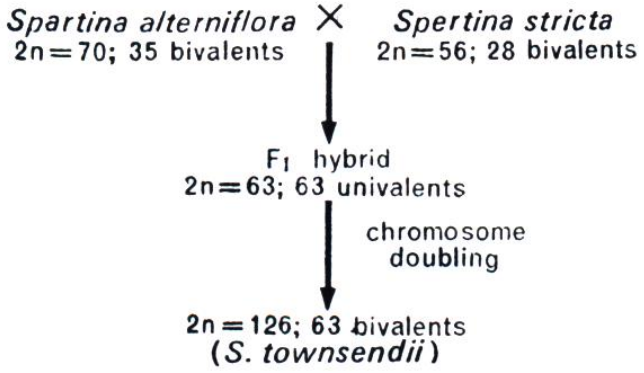


Figure 8.38: Probable origin *of Spartina townsendii*

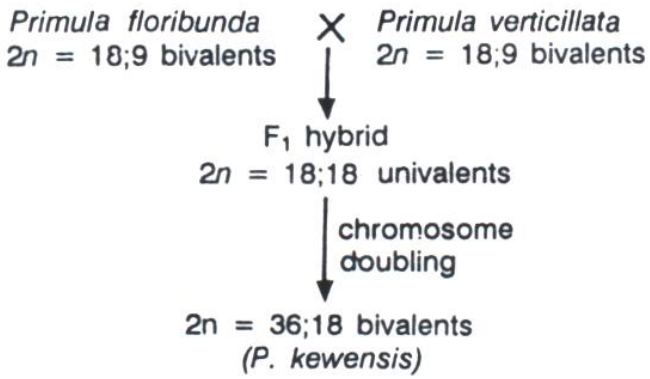


Figure 8.39: Probable origins of *Primula kewensis*

(5) *Galeopsis tetrahit* *(2n* = 32) could be synthesized (Fig 8.40) from crosses involving diploid species G. *pubescens (2n* =16) and *G. speciosa (2n*=16). However, A. Muntzing, who performed theseexperiments suggested that G. *tetrahit* is nota strict amphidiploid, but has a tendency towards allopolyploidy.

The above examples indicate that a number of natural polyploids have originated by a process which can be duplicated in laboratory. Since the products obtained in laboratory resemble those found in nature, the method used in laboratoryindicated evolutionary path which must have beenfollowed in nature in remote past.

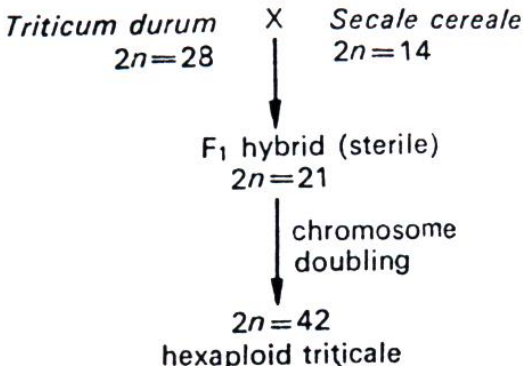


Figure 8.40: Probable origin of tetraploid of *Galeopsis tetrahit*

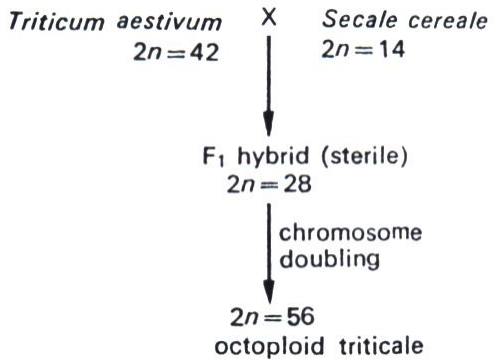


Figure 8.41: Artificial synthesis of (a) hexaploid triticale and (b) octoploid triticale

(ISBN: 978-93-88901-16-1)

Table 8.7: Some hexaploid spring triticale cultivers released

Country	Variety	Year
Australia	Dua	1979
	Satu	1979
	Groquick	1979
	Ningadhu	1982
	Towan	1981
	Tyalla	1979
	Coorong	1980
	Venus	1981
	Currency	1982
Canada	Rosner	1969
	Welsh	1978
	Carman	1981
	OAC Triwell	1981
India	TL419	1982
Italy	Mizar	1979
Kenya	T50	1979
	T65	1979
Mexico	Yoreme 75	1975
	Cananea 79	1979
	Caborca 79	1979
	Alamos 83	1979
	Eronga 83	1913
Portugal	Armadillo	
	Beagle	
	Mapachc	
	Arabian	
	Bacum	
	Borba 1	1982
	Borba 2	1982
	Pantera 1	1982
	Pantera 2	1982
	Tarrugam	1982
	Vedor	1982
	Avis	1982
	Monsanto	1982
Pakistan	NIAB-T-183	1982
Spain	Manigcro	1979
	Balboa	1981
	Badiel	1981
	Aseret	1982
South Africa	Usgen I	
	Usgen 2	
	Usgen 3	
	Usgen 4	
	Usgen 5	
	Usgen 6	
	Usgen 7	
USA	Siskiyou	1979
	Beagie 82	1982
Kramer	1982	

(b) ***Triticale (X Triticosecale)***, a new man made cereal. In recent years, considerable emphasis has been laid on the possibility of utilizing a new man made cereal known as triticale, on a commercial scale. It is already grown in an estimated area of more than one million hectares and research at several places all over the world is in progress to improve this man made crop. Work done in this crop has been reviewed in detail. Several hundred cultivars of triticale have been released during the last 10 years and more cultivars of this crop are being released every year. The area under its cultivation is also fast increasing.

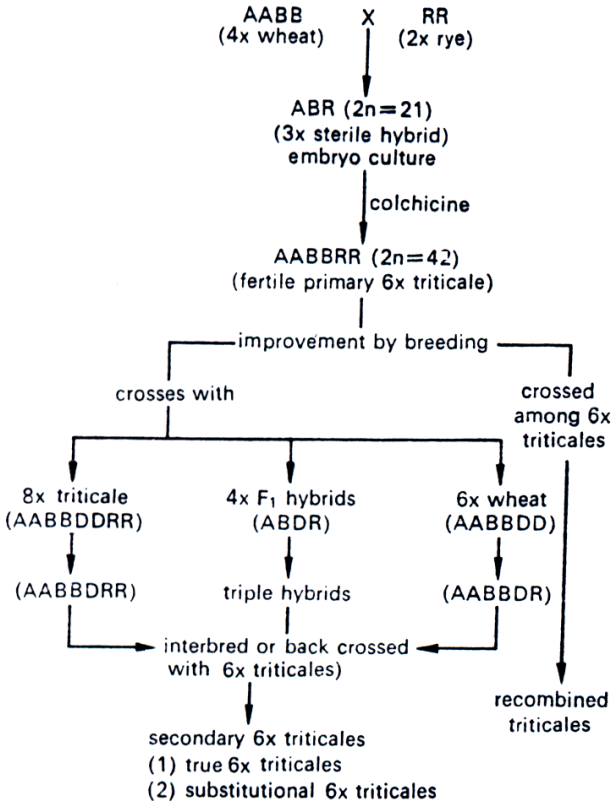


Figure 8.42: Methods of improvement of primary triticales lo obtain secondary and recoinbincd irilicales

Triticale is the first man made crop, in so far as it resulted as an artificial allopolyploid derived by crossing wheat *(Triticum)* and rye *(Secale).* Depending upon whether tetraploid *(2n = 4x* = 28) or hexaploid (2*n* = 6*x* = 42) wheat is utilized for tlte synthesis, one would get hexaploid triticale (2*n* = 6*x* = 42) or octoploid triticale *(2n = 8x* = 56) respectively. In each case, only diploid rye (2*n*= *2x*=14) was used. The derivations of triticales afc shown in Figure 8.41.In a similar manner, tetraploid triticales (2*n* = 28) and decaploid triticales *(2n* = 70) were also produced, but they were not of much significance. In decaploid triticales, tetraploid rye instead of diploid rye wasutiliized. Telraploid trilicales were produced indirectly through crosses between *6x* triticales and2*x* rye at some places. However, at present only the hexaploid triticales *(2x* = 42) are considered to havethe potential of becoming a new crop, although inChina, octoploid triticales are also being cultivated.

The hexaploid triticales, which are initially produced as amphiploids are called primary triticales. These primary triticales have many drawbacks like shrivelled grains, meiotic instability, poor yield and pre harvest sprouting. For further improvement, these primary triticales are regularly intercrossed among themselves and also with either hexaploid wheat or with octoploid triticales followed by backcrosses with hexaploid tritcales. General methods of improvement of these triticales are shown in Figure 8.42. It will be seen that the improved triticales can be either recombined triticales or secondary triticales, the latter resulting due to crosses with *6x* wheat or *8x* tririticales. It is these secondary triticales which are being released as cultivars in different parts of the world. A list of some of these released triticalccultivars is given in Table 8.5. It will be seen that in India also in 1982 a cultivar. TL 419 was released by Punjab Agricultural University (PAU), Ludhiana, on a regional basis. During the last several years, after the release of TL 419, even better strains giving yields higher than TL 419 could be produced and will be released soon.

Segmental allopolyploids

In some allopolyploids, the different genomes which are present are not quite different from one another. Consequently, in these polyploids chromosomes from different genomes do pair together to some extent and multivalents are formed. This mearts that segments of chromosomes and not the whole chromosomes are homologous. Therefore, such allopolyploids are called segmental allopolyploids according to Stebbins (1943, 1950). These segmental allopolyploids are intermediate between autopolyploids and allopolyploids and can be identified by their meiotic behaviour.

It is also believed that most of the naturally occurring polyploids are neither true autopolyploids nor true allopolyploids but are rather segmental allopolyploids. Our common hexaploid bread wheat is also regarded to be a segmental allopolyploid, because the three diploid genomes (A, B and D) are related (homoeologous) to each other.

Gene mutations *(or)* point mutations

Gene is a segment of DNA molecule. The specificity of a gene lies in its sequence of base pairs. Any change in the base pair sequence leads to a shift in the "reading frame' of the DNA molecule, when it is transcribed into a mRNA. This shift generally leads to the formation of a protein different fro-n the original one. Such a prorein may be inactive (or) less active (or) more active than the normal one which leads to an altered phenotype of the organism. Since these mutations include very limited segment of DNA, these are also called Point mutations.

Salient features of gene mutations

1. Gene mutations result from the deletion (or) addition of either a purine (or) pyrimidinenucleotide (or) an entire section of a gene.

2. The addition or deletion of nucleotides determines which amino acids are to be incorporated in to the protein molecule.
3. Gene mutations include *substitutions* and frame shifts.
4. Most of the gene mutations ate deleterious(or) harmful and only a few are beneficial.
5. Usually mutant genes are recessive to the wild (normal) type. In diploid ogamisms, the mutant allele will be expressed only when it occurs in homozygous condition. However, in haploid organisms, recessive allele is expressed immediately.
6. Some times a mutant allele changes back to the original Wild type. Then, it is called *reverse mutation.* The frequency of forward mutations is generally higher than the frequency of reverse mutations.
7. Due to mutation, a gene changes into an alternative form called the *allele.* Some times, a single gene mutates several times giving rise to different forms of genes. These are called *multiple alleles.* The blood groups in man are caused by multiple alleles.
8. Gene mutations are comparatively rare. The genes which have great frequency of mutation are called unstable (or) mutable genes. The genes which mutate less frequently are the stable genes. Emerson found that the R gene (Controlling anthocyanin production) of Maize frequently changes. The R^r gene changes to r^r at the rate of 50 per 100,000 gametes. The presence of mutable genes in somatic cells result in variegation of leaves, petals and endosperm. The colour variegations of *Antirrhinum* (snap dragon) and *Mirabilis falapa* (4'0-clock plant) are due to mutable genes.
9. Sometimes a gene induces other genes to mutate. The gene is called a mutator gene. Mutator genes were observed in Maize, Bacteriophages and *Drosophila;*
10. Sometimes a single mutation in a gene may effect different characters. Such mutations which.have more than one phenotypic effect are described as pleotrophic mutations.

Types of Gene Mutations

Gene Mutation may occur due to frame shifts and substitutions.

Frame Shift Mutations

These mutations result from an addition or loss of one (or) more nucleotides in a gene: 'These are also termed as insertion or deletionmutations respectively. This results in a shift of the reading frame during protein synthesis. Frame shift muta- tions, therefore generally lead to Eton functional proteins. These mutations are thus more severe than those that result from the substitution of one purine or pyrimidine base for another.

Substitution Mutations

These points mutations occur as a result of the substitution of one nucleotide for another in the specific nucleotide sequence of a gene.The substitution mutations are of two types:

(i) Transitions:

In this substitution, one purine base of a codon is replaced by another purine base or one pyrimidine base for another pyrimidine. Thus four different transitions are possible.

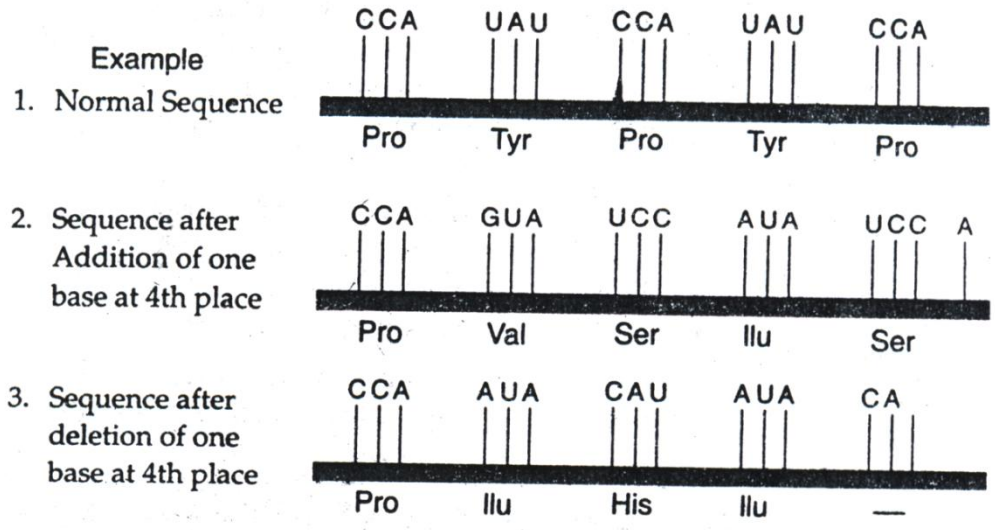


Figure 8.43: Types of gene mutations

(ii) Transversions:

This is a substitution of a purine for a pyrimidine (or) vice versa, i.e., a chage from A=T to C=G.

Certain alkylating agents like ethyl methane sulphonate (EMS) and methyl methane sylphonate (MMS) induce transver sions. These chemicals add alkyl groups to guanine and later seperate the guanine from the DNA chain. This is known as dipurination. The loss of the base $_{r}$pairproduces gaps in the DNA which may be filled up with a wrong base, thus producing mtations. The occurence of transversion was first postulated by E. Freese in 1959.

Transition and Transversion may result in any one of the three kinds of alterations, effecting the translation process.

a) The altered gene triplet produces a codon in the m RNA which specifies an amino acid different from the one present in the normal protein. This mutation is called a missense mutation. A good example of missense mutation in humans is the disease sickle cell anemia.
b) The altered gene triplet produces a chain terminating codon in m RNA. This results in premature termination of protein chain during translation. This is called nonsense mutation. The result is an incomplete polypeptide which is non functional.
c) The altered gene triplet produces a m RNA codon which specifies a synonym for the original codon. This is called a neutral mutation.

Transposable elements (transposons)

DNA is the genetic material, and the specific regions on the molecule that direct the synthesis of polypeptide chains are called genes or genetic elements. The smallest unit of genetic material responsible of the synthesis of a specific polypeptide is called cistron.The movement or shifting of genetic elements between two distinct chromosomes derived from two different sources results in genetic recombination. Generally, recombination events may occur during, meiosis due to crossing over. They also may occur in bacteria by othermeans during transformation, conjugation or bacteriophage infections. Genetic recombination in all these cases occurs by different mechanisms.

Transposons and recombinations

Generic recombination does not result in loss or gain of the genetic material. Genetic Recombination and Transposons are interrelated to one another. So, transposable genetic elements are described always with a special reference to recombination phenomenon.

Three types of recombinations and their mechanisms are known to day. They are

1. Homologous Recombination
2. A site - specific Recombination
3. Non - Homologous Recombination

Transposable generic elements come under nonhomologous. Recombination category.Recombination events between DNA segments, without recognisable gene homology, are described as Non - Homologous recombination type. It involves the addition rather than exchange of DNA, similar to a site specific re-combination type. And alsothis non - homologous type is independent of the rec A gene, unlike homologous recombination which is under the control of rec A gene as in *E. Coli.*

Generally three types of DNA, participate in such non homologous recombinations and they are described as

1. Is elements (Insertion sequences)
2. Tn elements (Transposons)
3. Mutator elements (bacteriophage Mu)

All the above three types belong to a category of specialised genetic elements known as "Transposable elements".

Genes are generally located on the chromosomes at fixed loci or location. There may be shifted to other locations only when chromosomal rearrangement occurs due to crossing over (illegitimate) between incompletely homologous sections of DNA. Experiments with Maize in the later half of 1940, (Barbara Meclintock) showed that certain genetic elements regularly jump to new locations. Such jumping is described as 'Transposition". These "jumping genes" often inactivate the genes with which they become attached. These jumping genes sometimes even disturb the functioning of the resident genes at their

natural location. The nature of transposing elements in E. Coli brought to light by the sophisticated techniques (Recombinant DNA technology and DNA sequencing technique) in recent years. These elements were found to be DNA sequences that code for enzymes which bring about the insertion *of* an identical copy of themselves into a new DNA site.

Transposition involves both recombination and replication processes. It forms two daughter copies of the original transposable element. One copy remains at the parental site, while the other at the new location or target site. This insertion generally disrupts the activity of the target genes. These transposons, sometimes, may even activate dormant genes of the target chromosome.

Definition of transposon

Transposons are small, mobile DNA sequences, that can replicate and insert copies at random sites with in chromosomes. They have nearly identical sequences at each end, oppositelyoriented (inverted) repeats and code for the enzyme, transposase, that catalyses their insertion.The insertion of these elements may produce deletions, inversions and several complicated chromosomal rearrangements. The movement of Transposons is of two types –

(1) Non Replicate transposition
(2) Replicative Transposition (described above).

Types of transposons

Based on Transposition:

Transposons are classified into three classes based on their *"Mechanism* of *Transposition"* like the following –

Class I: Retro transposons

Class II: DNA transposons

Class III: MITEs (Miniature Inverted-Repeat-Transposable elements).

(I) **Retro transposons**

These transposons move by a *"Copy and paste"* mechanism. The copy is made of RNA and not from DNA like in most organisms. These transposons initially copy themselves to RNA (transcription) and this RNA is copied into DNA by reverse transcriptase and inserted back into the genome.

Many retro transposons have long germinal repeats (LTRs) at their ends that may contain over 1000 base pairs each. Retro transposons are similar to retroviruses, like HIV.RetroTransposonsare generally divided into three types like the following:

Type I: ***HIV - 1 (Viral Superfamily)***:

Types that are included under this class are similar to retro viruses (e.g. AIDS virus) and possess Long Terminal Repeats (LTPs). They can encode reverse transcriptase (that transcribes RNA into DNA). The RNA genome of HIV - I contains a gene for reverse transcriptase and integrase.

Type II: *LINEs (Long Interspersed Elements)*:

These are long (~ 5000 base pairs) DNA sequences that represent reverse -transcribed RNA molecules, originally transcribed by RNA Polymerase II. Lines can encode a functional reverse – transcriptaseand/or integrase. These transposons follow copy-paste mechanism in their transposition. On account of this, the number of LINEs can increase in the genome. The human genome contains over 500,000 LINEs, representing about 16% of the genome.

Type III: SINEs (Short Interspersed Elements):

These are short DNA sequences (100 - 500 base pairs) that represent reverse -transcribed RNA molecules, originally transcribed by RNA polymerase III. These transposons do not encode for reverse - transcriptase. Like LINEs their presence in the genome is a mystery and they seem to represent only "Junk" or selfish DNA. Human genome contains about one million copies of SINEs, representing about 11% the total DNA. The most abundant SINEs in the human genome are the *"Alu"* elements.

(I) DNA-Transposons:

These transposons use"cutandpaste"mechanism for transposition instead of copy and paste type as in Retro - transposons. Transposase enzyme helps the process of Transposition. These transposons contain only DNA which moves directly from place to place. Thetransposon is cutout of its location and inserted in to a new location. All activities of transposition (cutting at the target site, cutting out the transposon,ligation) involving target site are helped by the enzymes like transposase, DNA polymerase and DNA ligase.It results in the duplication of target sites.

The above two classes of may lose their ability to synthesis transposase or reverse transcriptase.

(II) MITEs:

MITEs are too small to encode any protein. Just how they are copied and moved to new locations is still uncertain. Probably larger transposons that encode the necessary enzyme and recognize the same inverted repeats.The recent completion of the genome sequence of rice and C. elegans has revealed that their genomes contain thousands of copies of a recurring motif consisting of almost identical sequences of about 400 base pairs flanked by characteristic inverted repeats of about 15 base pairs such as

5' GGCCAGTCACAATGG .. ~ 400 nt..CCATTGTGACTGGCC 3'3' CCGGTCAGTGTTACC.. ~ 400 nt.. GGTAACACTGACCGG 5’

There are over 100,000 MITEs in the rice genome (representing some 6% of the total genome). Some of the mutations found in certain strains of rice are caused by insertion of a MITE in the gene.MITEs have also been found in the genome of humans, Xenopus and apples.

Based on Structure:

Two types of transposable elements have been identified in bacteria based on structural differences-

(1) Simple Transposons;

(2) Complex Transposons.

(1) Simple Transposons:

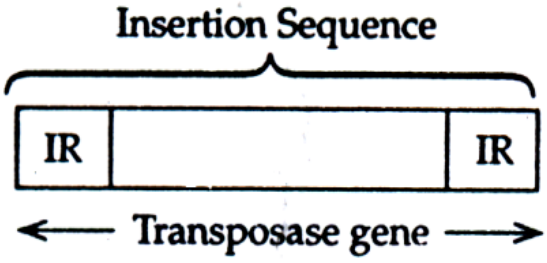


Figure 8.44 Simple Transposan

They are also called insertion elements or sequences (IS). This name is due to their discovery as insertion with in bacterial operons.These simple transposons were first noticed in certain spontaneous mutants of *E. Coli.* These mutants were formed due to the insertion of extraneous DNA (alien DNA). This DNA* segment contains insertion sequences (elements). They occur in chromosomes of bacteria and plasmids. These IS donot carry any genetic information except the transposase genie part (necessary for transposition). The transposase gene region is the coding region for an enzyme transposase, which recognises - the ends. Specific terminal DNA sequences are present at both ends of transposase gene region. These terminals are short and are called inverted repeat sequences (IR) The insertion sequence contain limited base pairs (800-1400). These .base pairs can not regulate or express any phenotypic character.This IS or simple transposon mainly acts as a mutagen on account of its insertion and may cause errors in transcriptional stages.

(2) Complex Transposons:

Simple transposons contain only genes which help in transposition. Where as complex transposons contain additional genes besides transpositional genes.The additional gene part helps to express a phenotypic character or it carries out a specific function.

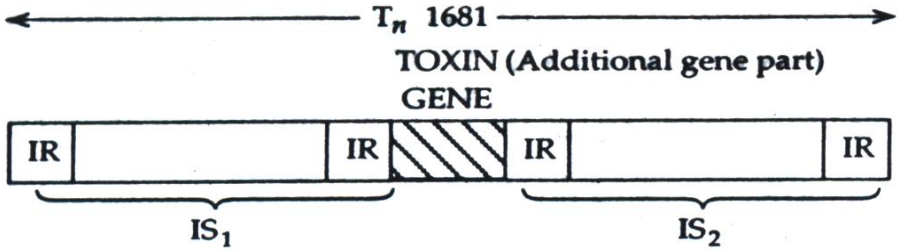


Figure 8.45: Complex TransposonIR = Inverted Repeat sequence

IS_1, IS_2; Insertion sequence; T_n 1681: Complex Transposon

The T_n 1681 transposon carries the gene for a heat labile toxin. The complex transposons may carry or enclose one to several functional bacterial genes. Complex Transposons can be easily recognised (than simple transposons) since they carry genes that determine. recognisable characters such as antibiotic resistance or production of Toxins.

Integration of transposons

The integration of a transposon into a genome may occur in different ways

(1) Plasmid to Plasmid

(2) Plasmid to a bacterial chromosome

(3) Plasmid to a temperate phage

Transposable elements are identified in all organisms. Some of the common Transposons of complex type in *E. Coli* are given in the following table.

Mutator elememts (Bacteriophage Mu):

(a) Structure:

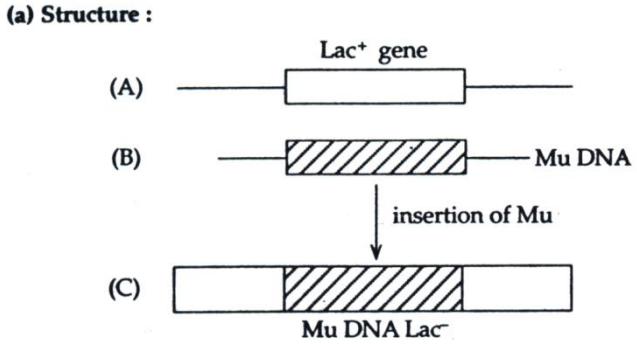


Figure 8.46: Mutator elememts (Bacteriophage Mu)

(b) Properties:

1) It is a temperate bacteriophage and regarded as a Transposon.
2) It shows similar integration behaviour like Other Transposons.
3) Mu has inverted repeats (IR) at the terminal ends like other transposons.
4) Mu can generate many new copies of itself, which can be reinserted randomly in the E. Coli chromosome at random locations.

	Type	**Base Pairs**	**Complex transposans in *E. coli***	
1	Tn 3	4957	5	Ampicillin Resistance
2	Tn 10	9300	9	Tetracyclin Resistance
3	Tn 1681	2100	9	Heat Stable Toxin

Target DNA Repeat (bp)

Known Function

Examples for transposons

Maize, Drosophila and bateria are the classical examples that were employed as primary tools in the past to make fundamental observations and studies to reveal the nature of transposons.

1. Maize:

The first transposons were discovered in maize *(Zea mays)* by Barbara McClintock in 1948, for which she was awarded a Nobel Prize in 1983. She noticed insertions, deletions and translocations, caused by these transposons. These changes in thegenome could, for example, lead to a change in the color of corn kernels. About 50% of the total genome of maize consists of transposons. These transposons come under class II type.

2. Drosophila:

One family of transposons in the fruit fly *Drosophila melanogaster* are called *P elements*. They seem to have first appeared in the species only in the middle of the twentieth century. Within 50 years, they have spread through every population of the species. Artificial P elements can be used to*insert genes into Drosophilia by injecting the embryo.

3. Bacteria:

Transposons in bacteria are also called insertion sequences. They usually carry an additional gene for a function other than transposase, often an antibiotic resistance. In bacteria, transposons can jump from the "regular" DNA to plasmids and back, allowing the transfer and permanent addition of, for example antibiotic resistance, leading to multiresistant strains. Bacterial transposons of this type belong to the Tn family.

The most common form of transposon in humans is the Alu sequence. The Alu sequence is approximately 300 bases long and can be found between 300,000 and a million times in the human genome.

Transposons causing diseases

Transposons are mutagens. They can damage the genome of their host cell in different ways:

1) A transposon or a retroposon that inserts itself into a functional gene will most likely disable that gene.
2) After a transposon leaves a gene, the resulting gap will probably not be repaired correctly.
3) Multiple copies of the same sequence, such as Alu sequences can hinder precise chromosomal pairing during mitosis, resulting in unequal crossovers, one of the main reasons for chromosome duplication.

Diseases that are often caused by transposons include .hemophilia A and B, severe combined immunodeficiency, predisposition to cancer and muscular dystrophy etc. Additionally, many transposons contain promoters which drive transcription of their own transposase. These promoters can cause aberrant expression of linked genes, causing disease or mutantphenotypes.

Evolution of transposons

The evolution of transposons and their effect on genome evolution is currently a dynamic field of study. Transposons are found in all living organisms. They may or may not have originated from a common ancestor, or arisen independently multiple times, or perhaps arisen once and then spread to other kingdoms by horizontal gene transfer. While transposons may confer some benefits on their hosts, they are generally considered to be selfish DNA parasites that live within the genome of cellular organisms. In

this way they are similar to viruses. Viruses and transposons also share features in their genome structure and biochemical abilities, leading to speculation that they share a common ancestor.

Since excessive transposon activity can destroy a genome, many organisms seem to have developed mechanisms to reduce transposition to a manageable level. Bacteria may undergo high rates of gene deletion as part of a mechanism to remove transposons and viruses from their genomes while eukaryotic organisms may have developed the RNA interference (RNAi) mechanism as a way of reducing transposon activity.

Significance of transposons

Apart from giving 'antibiotic resistance' transposons do not seems to be of any value to a cell. Generally a gene that serves no purpose to a cell will ultimately be mutated until it is non - functional and deleted. For sometime, it has been thought transposons are examples of 'selfish' DNA - that is, a DNA segment that has persisted because it has evolved to maintain itself. How ever recent experiments have suggested that a$_v$transposon does not provide selective advantage to an individual cell, but it's presence will in the long run confer advantage to the population. Thisargument is based on reasoning that since evolution proceeds by accumulating rr.utations, process that accelerates mutation, may speed up evolution.It has been proved that transposons induce mutations and cells containing Transposons evolved faster

Biological effects of transposition

1) When an element transposes, it may insert within a gene of the host DNA and there by produces a mutation.
2) Insertion of transposon between a promoter and a coding sequence or between genes of a polycistronic operon, prevents transcription of down stream genes.
3) Insertion of Plasmids into the E. Coli choromosome generate Hfr cells or deletions and inversions.
4) In Eukaryotes transposition is sometimes associated with switching on and off of certain genes.

Transposes in science

Transposons were first discovered in the plant maize. Likewise, the first transposon to be molecularly isolated was from a plant (Snapdragon). Transposons have been an especially useful tool in plant molecular biology. Researchers use transposons as a means of mutagenesis. In this context, a transposon jumps into a gene and produces a mutation. The presence of the transposon provides a straightforward means of identifying the mutant allele, relative to chemical mutagenesis methods.

The insertion of a transposon into a gene can disrupt that gene's function in a reversible manner. Transposase mediated excision of the transposon restores gene function. This produces plants in which neighbouring cells have different genotypes. This feature allows researchers to distinguish between genes that must be present inside of a cell and genes that produce observable effects in cells other than those where the gene is expressed.

Transposons are also widely used as tools for mutagenesis in *Drosophilia melanogaster*, and a wide variety of bacteria to study gene function.

Chapter 9

Gene Expression

Organisation of Gene

The vast amount of knowledge gained in the field of classical genetics and in its latest annexe, the molecular genetics, is making the situation complicated and it is not that easy now to define a gene. However an attempt may be made by saying that a gene is a part of the DNA strand capable of controlling and directing the formation .of a protein or enzyme or polypeptide which can discharge a definite physiological function in the phenotype and is heritable into the offspring. It may appear quite simple but obviously not so when we probe into the details of the gene action.

Mendel was the first m 1 to visualise a gene as a unit of inheritance in 1865 itself. He conceived it as a particulate unit and referred to it as a factor of heredity or element of heredity. A gene is a fundamental biological unit like an atom which is a fundamental physical unit. The term 'gene' was coined by johanssen in 1909 for a single unit of heredity occupying a specific position on any chromosome. It was found later that genes are present in a linear order on the chromosomes and was carried along with them to the daughter cells during the cell division. Even before the rediscovery of Mendel's laws in 1900 it was already established that chromosomes have a definite role in the inheritance because it was found that the chromosomes were the only link between one generation and the next generation. So the gene is a unit of inheritance which is carried from a parent on a chromosome in a gamete and controls the expression of a specific character in the offspring in co-operation with other genes and the environment.

One gene one Character Concept

Earlier workers proposed various hypotheses to explain the nature of genes. De Vries postulated "one gene one character" hypothesis, which says that a particular character of an individual is controlled by a particulargene. Bateson and punnet proposed the presence or absence theory. It says that in a cross such as a tall plant with a short plant, tall becomes expressive because the gene for tall is not only present but it dominates the other trait i.e., the short trait. Such a determiner is not present for the short trait.

Particulate Concept

Morgan had proposed a particulate gene theory in 1926. He considered genes as particles which are arranged in a linear order on the chromosomes and appear like beads on a string. The particulate theory of gene was widely accepted and supported by cytological observations. But the discovery of DNA molecule as the custodian of genetic information has discarded altogether the Morgan's particulate theory of gene: because it was realised that the gene is a segment of DNA having a limited number of nucleotide base pairs in an unique sequence. Different genes have different sequences of nucleotide base pairs. The

specific sequence of bases in a gene forms the code that directs the cell to manufacture a particular protein or enzyme. This situation compells us to have both, the classical as well as modern approach of a gene.

Classical approach of a gene

Classically a gene was assumed to be a genetic unit by the following three criteria.

1) A gene is a Unit of physiological function that occupies a definite locus in the chromosome and is responsible for a specific phenotypic character. Ex:-Red or White colour of eye of *Drosophila* ; Normal or curly hairs of man.
2) A gene is a Unit of segregation and transmission. It can be segregated and exchanged during meiosis through crossing over.
3) A gene is a Unit of mutation. A mutation or change whether spontaneous or induced can give rise to an altered phenotypic expression.

This classical approach of a gene has been revised thoroughly due to the recent knowledge gained in the f ieid of gene action and in the field of molecular genetics.

One gene one enzyme concept

At the time of rediscovery of Mendel's work in 1900, an English physician and biochemist Sir Archibald, E.garrod was studying several congenital metabolic diseases in humans. One of them was the heritable disease alcaptonuria, which is easily detected because of the blackening of urine upon exposure to air. The substance responsible for this blackening is alcapton or homogentisic acid, an intermediary compound in the break down of aminoacids, tyrosine and phenylalanine. (Fig 9.1)

Garrod noted that patients suffering from this disease were offspring of consanguineous marriages. He also concluded that alkaptonuria was a heritable disease which was determined by a recessive gene. He believed that the presence of alcapton or homogentisic acid in the urine was due to a block in the normal pathway of metabolism of this compound. The mutation of the recessive gene results in the failure of formation of the enzyme homogentisic acid oxidase, which would normally degrade the homogentisic acid into acetoacetic acid, fumaric acid, CO_z and H_20. Thus a genetic block at this stage leads to a metabolic block showing, itself in the phenotype in the form of a disease, the alkaptonuria.

The results of Garrod's studies of alcaptonuria and a few other congenital human diseases such as albinism, Phenyl-Ketonuria etc, were presented in detail in the first edition of Garrod's book, Inborn Errors of Metabolism published in 1909. His insight into the generic control of metabolism was so dear that the experiments conducted by many others in the later years proved it to be totally correct. His concept be best stated as one mutant gene - one metabolic block. Garrod's concept was the forerunner to the one gene - one enzyme concept and the latest, one gene - one polypeptide concept. Like Mendel's work Garrod's work also was largely ignored until it was rediscovered again by Beadle and Tarum.

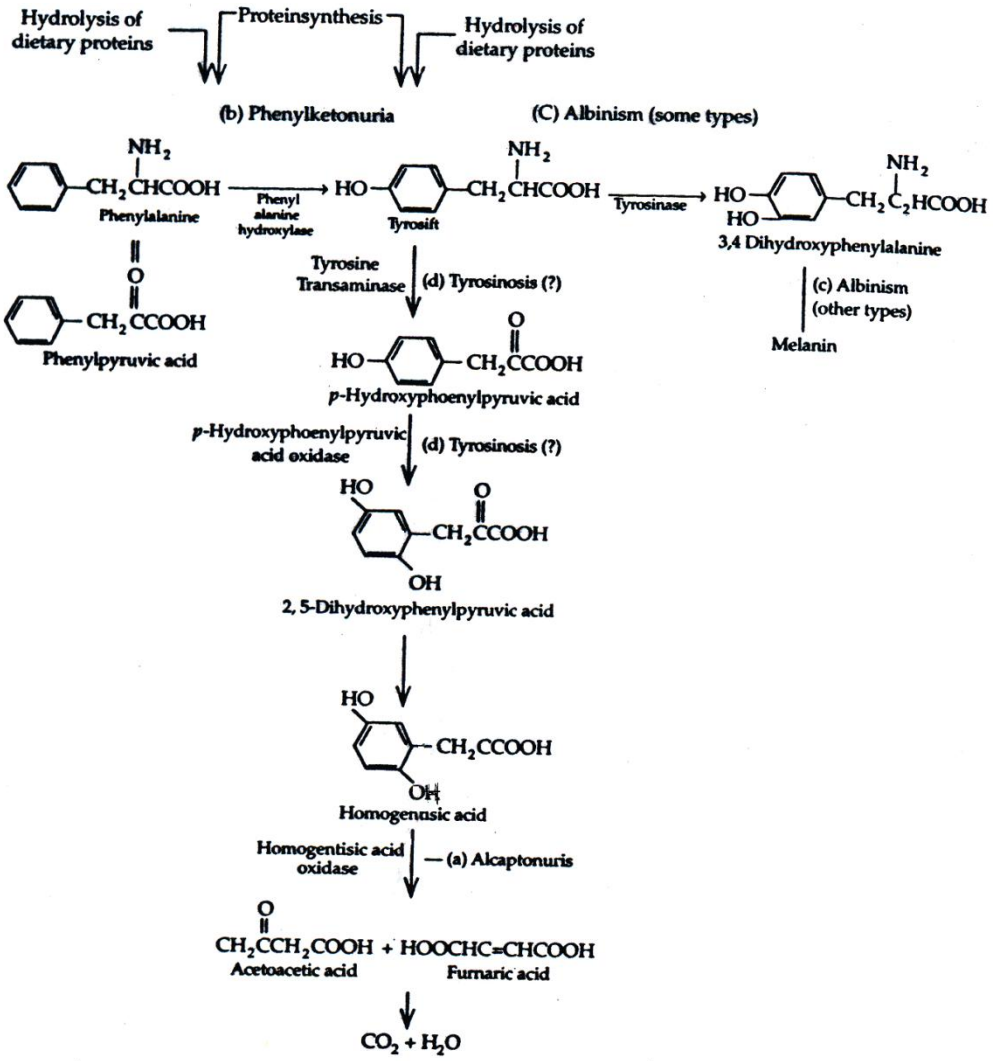


Figure 9.1: Inherited human diseases ("inborn errors") with defects in phenylalanine-tyrosine metabolism, (a) alcaptonuria, extensively studied by Garrod at the t um of the century, which results in blackening and hardening of cartilage and blackening of urine upon exposure to air; (b) phenylketonuria, wf itch results in severe mental retardation if untreated; (c) albinism, the inability to synthesizemelanin pigments, and (d) tyrosinosis, the inability to convert tyrosine to 2, 5- dihydroxy phenyl pyruvic acid, an early stage in the tyrosine degradative pathway.At least two different types of albinism occur in humans. One type is caused bya mutation resulting in the loss of tyrosinase activity. A second type of albinismapparently results from a mutational block in a subsequent step in melaninsynthesis or in the ability to deposit melanin pigment in melanocytes

Experiments of Beadle & Tatum

It is very clearly known that the building blocks of the body, the proteins and the enzymes are certainly formed under the control of genes. It is therefore logical to step up investigations into the relationship of genes and the enzymes. That was what exactly done by g.w.beadle and e.l.tatum on the bread mold *Neurospora crassa*, a fungus in 1941. Like bacteria. N.crassa offered the advantages of

being small having a short generation span and large numbers of progeny. Its haploid nature reduces the complexity by eliminating dominance and recessiveness. Thus there is no masking of expression of genes in the cells of a haploid organism. So any mutations of genes can therefore be immediately detected. (Fig 9.2).

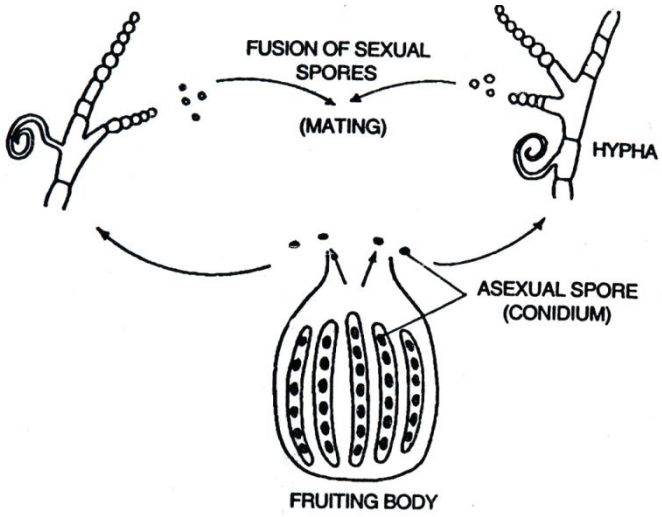


Figure 9.2: Abbreviated life cycle of the bread mold, *Neurospora crassa*.Asexual spores known as conidla ore released from fruiting bodiesand germinate into threadlike hyphae. The hyphoe In turn win form sexualspores which can fuse with spores in fruiting bodies of compatible mating types.The spores undergo metosb and mitosis, and are then released to form more hyphae

Normal or wild type *N* crassa can be grown in a medium containing only the irtinimum number of nutrients necessary for survival, since the fungus is able to manufacture many substances essential for growth through its metabolic reaction such a medium is known as minimal medium. Beadle &Tatum exposed the cultures of wild (normal) *N*.crassa to X-rays and analysed the results of the exposure. After exposure some of the cultures could no longer survive on minimal medium.When they added the supplemental nutrients to the medium, the cells were able to live.

By adding individual supplements to the medium one at a time/Beadle and Tatum found that the mutation always resulted in the inability of cell to synthesize some essential substance. In otherwords a particular metabolic reaction which normally led to the production of an essential substance was blocked resulting in the cell's inability to survive on minimal media (Fig 9.3).

In their analysis Beadle and Tatum felt that this mutation-induced block to a single metabolic reaction reflected a definite relationship between genes and those vital proteins that control the metabolic reaction, enzymes. Quoting their own version,"The development and functioning of an organism consists essentially of an integrated system of chemical reaction controlled in some manner by genes. These genes which are themselves as a part of the system control or regulate specific reactions in the system either by acting directly as enzymes or by determining the specif kites of enzymes."

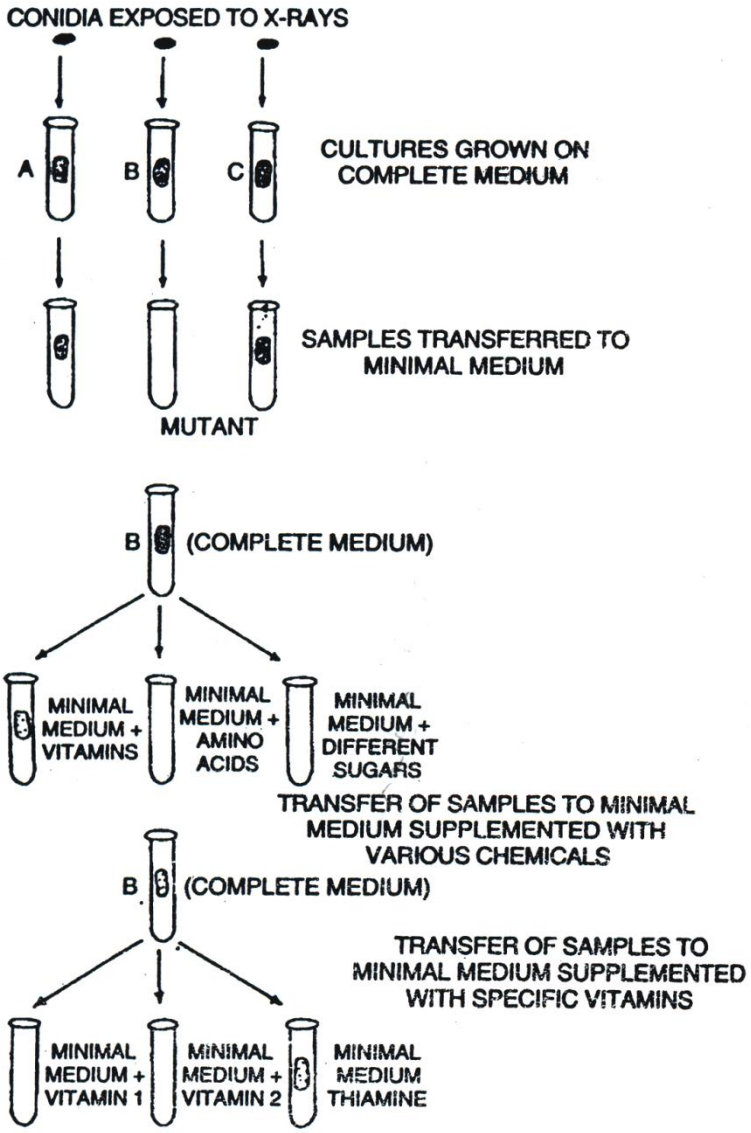


Figure 9.3: The classic experiments pertomed by G. Beadle and E. Tatum, providing evidence for a direct link between genes and enzymes

Thus each mutation obtained through irradiation blocked the system of a particular essential substance because the gene necessary for the production of the enzyme needed for that particular reaction had been changed. From their analysis arose a hypothesis that came to be known as the one gene - one enzyme concept. Although modern genetics has led to the modification of this theory, the close relationship between genes and enzymes has been confirmed on the basis of modern concept of a gene.

One gene one polypeptide concept

After knowing about the relationship existing between the genes and the proteins they code for, doubts have crept in regarding the structure and action of a gene. How many nucleotides are needed for

the formation of a gene? If a change (mutation) occurs in a nucleotide, how does it affect the gene and the consequent protein formed by it?How does this mutation express itself in the phenotype? All these questions have been answered and being answered by the latest advancement in the field of molecular generics resulting in the alteration in the concept of a classical gene, of course the classical concept is also there coexisting with it. These advancements have led to the formation of the latest hypothesis namely one gene one polypeptide concept.

One gene - One polypeptide concept

Haemoglobin is the oxygen transporting macromolecule in all the vertebrates incuuding man. It is a complex protein consisting of four protein globin chains combined with the iron pigment haeme.The human haemoglobin is divided into two types

(1) Foetal haemoglobin or haemoglobin-F

(2) Adult haemoglobin or haemoglobin A.

Each molecule is composed of four polypeptide chains plus a haeme group. Haemoglobin A contains two identical alpha chains and two identical beta chains. Haemoglobin F contains two identical gamma chains besides alfa chians. Each polypeptide, alfa, beta or gamma is coded for by a specific gene. Haemoglobin F of the foetus is replaced by Haemoglobin A within the first six months after the birth.

Each alfa polypeptide consists of a specific sequence of 141 aminoacids. The beta and gamma chains are 146 aminoacids long. Because of the similarities in their aminoacid sequences all the haemoglobin chains are believed to have evolved from a commonprogenitor. Many different variants of haemoglobins have been identified especially in haemoglobin A. Some of them have severe phenotypic effect. Many variants are initially detected by their altered electrophoretic behaviour (movement in an electric field due to charge differences). The haemoglobin variants provide an excellent example of the effects of mutation on the structure and functions of gene products and on the phenotypes of the affected individuals.

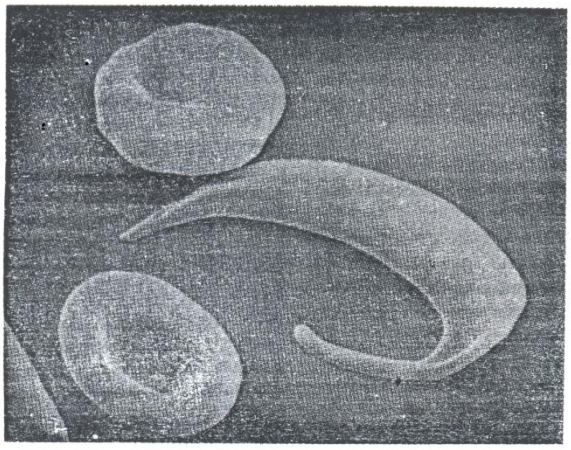


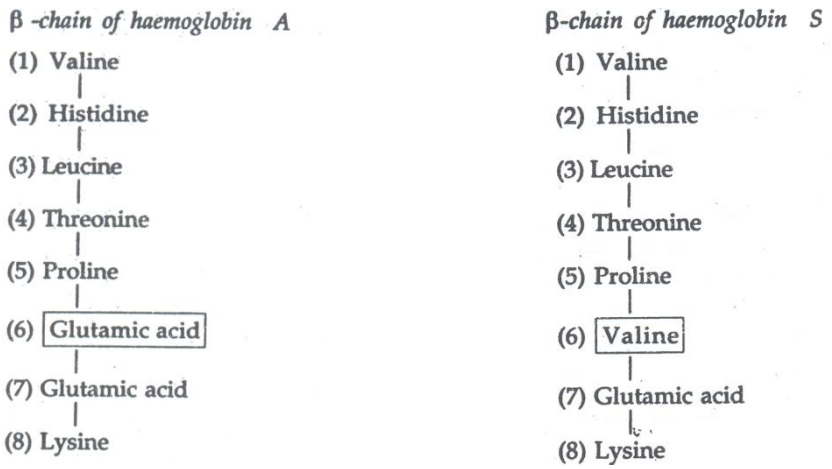
Figure 9.4: Foetal haemoglobin or haemoglobin-F

Haemoglobin (S)

Sickle cell haemoglobin (haemoglobin-S) is one such variant. Individuals homozygous for the haemoglobin Sallele ($Hb_{\beta}^{s}/Hb_{\beta}^{s}$), the P subscript are used because haemoglobin S isa variant of the beta chain, develop severe haemolytic anaemia. The haemoglobin S molecules distort the morphology of red blood corpuscles. They elongate and form sickle shaped cells. These sickle shaped cells clog small blood vessels and cut off oxygen transport to various tissues. It leads to death in childhood itself in many cases. (Fig 9.4)

Linus Pauling and his associates of California Institute of Technology detected a change in the electrical charges of haemoglobin S and the normal haemoglobin, the haemoglobin N or haemoglobin A. He also indicated that the haemoglobin S molecule may be abnormal. In 1958 Vernon M. Ingram and John Hunt' of Cambridge university analysed the complex polypeptide chains of both Haemoglobin A(Normal haemoglobin) and Haemoglobin S and observed their aminoacid sequence. They found out the presence of two glutamic acid residues and one valine at a particular point of the beta chain of normal haemoglobin (Haemoglobin A). In haemoglobin S there were two valines and one glutamic acid at the same point. So there was addition of a valine in a place which ought to have been occupied by the glutamic acid. How had this happend?

They suspected the mistake was due to an error in the arrangement of the base pairs of nucleotides of the DNA which had coded for the Haemoglobin S molecule. They theoriticaily predicted that the erratic DNA strand carried the wrong sequence of the base pairs as, C-G, G-C, G-C; The correct sequence inthis caseshould be C-G, T-A, G-C. So the wrong sequence resulted in the incorporation of another valine in the place of a glutamic acid. The mutation of a gene resulted in the wrong placement of G-C, where T-A must have been there.Thus a small variation in base pair sequence of nucleotides' resulted in the selection and incorporation of a wrong aminoacid (valine instead of glutamic acid), in the beta chain of the haemoglobin molecule, converting it into haemoglobin S. So the erythrocytes became sickle shaped and the individual suffered from the disease, sickle celled anaemia.



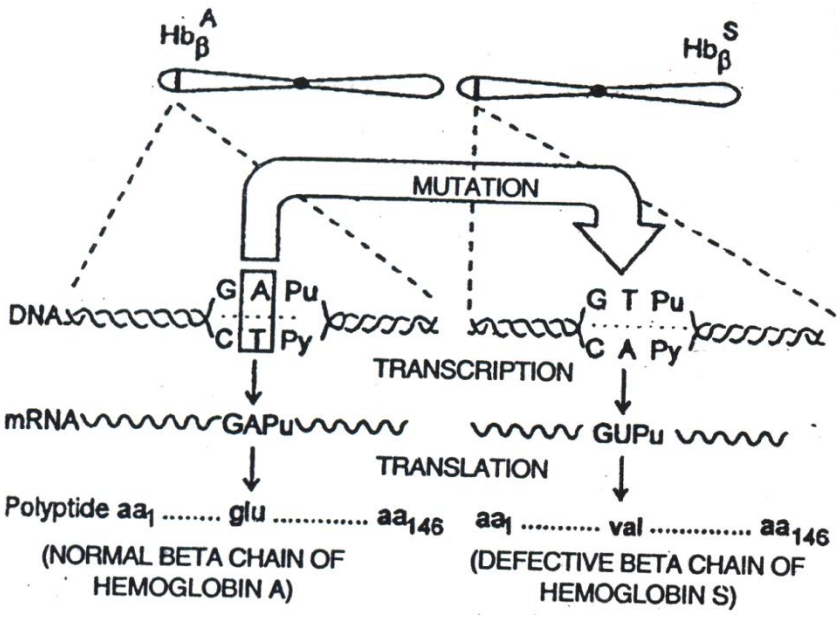


Figure 9.5: Mutational origin of sickle-cell hemoglobin (hemoglobin S).The amino acid sequences of the "normal" (hemoglobin A) betapolypeptide and the sickle- cell beta polypeptide have been determinedby direct chemical analysis (amino acid sequencing). From our knowledgeof the nature of the genetic code and the observed substitution of valine atamino acid position 6 in the beta chain of hemoglobin S, one can deduce thatthe mutation giving rise to hemoglobin S (or, more accuratly, to the Hbs/b gene) is the substitution of an adenine for a thymine in the transcribed strandof DNA

Later investigations proved that in the beta chain ofhaemoglobin *A*, the sixth aminoacid from the amino (NH_2^-) terminal end of the beta chain is glutamic acid (a negatively charged aminoacid) The beta chain of haemoglobin S contains valine (no charge at neutral pH) at that position. Thus changing of a single aminoacid in one polypeptide can have severe effects on phenotype. Although the nucleotide pair sequences of Hb^A_β (beta chain of normal haemoglobin) and Hb^S_β (beta chain of sickle cell haemoglobin) genes are not known the mutational change in the Hbg gene that gave rise to Hbpcan be deduced from our knowledge of genetic rode. (Fig 9.5)

Other Investigations

Over 75 haemoglobin variants -with aminoacid changes in the beta chain are now known. All of them except one differ from the normal beta chain (of normal haemoglobin) by a single aminoacid substitution. Besides these investigations on haemoglobin molecule of humans those conducted on Tobacco Mosaic Virus (TMV) and on E.coli (Activity of tryptophan synthetase) by C.yanofsky ef ai etc proved that a mutation of a gene can lead to the incorporation of a wrong aminoacid in the polypeptide chain. Thus the protein structure gets modified and results in the onset of a disorder in the phenotype. Since one gene mutation can modify one polypeptide, and theconcerned protein, Vernon Ingram

suggested one gene - one polypeptide concept instead of one gene - one enzyme concept of Beadle and Tatum.

Modern approach of a gene

After the study of DNA and the mutations mentioned above geneticists have agreed to investigate into the way of functioning of gene.seymour benzer proposed after his studies on the r II region of T4 phage further division of a gene. He, said the gene cansplit just like the atom depending on the function it is discharging Accordingly he divided the gene into three smaller units *viz.,*

(1) Cistron,

(2) Muton,

(3) Recon.

1) Cistron

The part of the DNA which can specify a single polypeptide chain and code for it is known as a cistron. A cistron may carry some nucleotides (always more than 1). Cistron can be chosen as a synonym for the term, the gene of physiological function. Human haemoglobin therefore requires two cistrons to code for its globin protein fraction, one for the alfa chain & another for beta chain. Each cistron holds the responsibility of coding for one polypeptide chain. The cistron for alfa chain has atleast 141 x 3 = 423 nucleotides and the cistron for beta chain has 146 x3 = 438 nucleotides. Like that number of polypeptides produced by a cell or organism are under the control of so many cistrons.

2) Muton

There are many positions or points within a cistron where a mutation can occur. Therefore the gene as a unit of mutation is smaller and consists of fewer nucleotides than a cistron. Benzer coined that smallest length of a DNA capable of mutational change as muton. So it is a smallest unit of genetic material which, when mutated or changed produces a phenotypic effect. A muton may thus be limited to a single nucleotide or *a* part of it. Different forms 0f mutationally defined genes are called homoalleles. A singlenucleotide pair of bacterium may act as a muton. In the cistron that codes for the alfa or beta chain of haemoglobin the muton may be a single nucleotide. So the induction of valine in the beta chain in the place of glutamic acid is the effect of a muton. Which expresses itself in the form of sickle cell anaemia in the phenotype.

3) **Recon**

Sometimes crossing over or recombination occurs in a cistron and this provides still other subdivisional concept of the cistron namely the recon. Thus a recon is the smallest unit of DNA capable of bringing out a recombination. Since a muton is creating a change in die nucleotide sequence the effect of which is expressed not only in thephenotype, but also it is inherited by the offspring, the recon & muton can be equated as formed by a single nucleotide of the cistron. Thus the haemoglobin S is inherited into the offspring also, subjecting them also to the disorder the sickle celled anaemia, the

recombinationally separable form? Of a cistron are called hetero alleles. Structurally, the recon may consist of one or two pairs of nucleotides.

Cistron Recon and muton are the subunits of a gene in a descending order of size. It is concluded that gene is not synonymus to cistron.Thus a gene can contain several cistrons, a cistron of several recons, and a recon of several mutons. Sometimes Recon and muton may be of the same size; in such condition recon may not contain several mutons.

Co-linearity of gene & protein structure

The nucleotides are arranged *in* the genes (DNA) ina linear manner and so are the aminoacids in the polypeptides (proteins) the linear sequence of nucleotides in a gene corresponds *to* the linear sequence of aminoacids in the polypeptides for which itcodes. This correspondence is called co-linearity of gene and protein structure. Charles yanofsky and associates have proved that an alteration of a specific codon in DNA leads to specific alteration of amino acid in the corresponding location in the protein synthesized by the mutant individual of *E.Coli.*

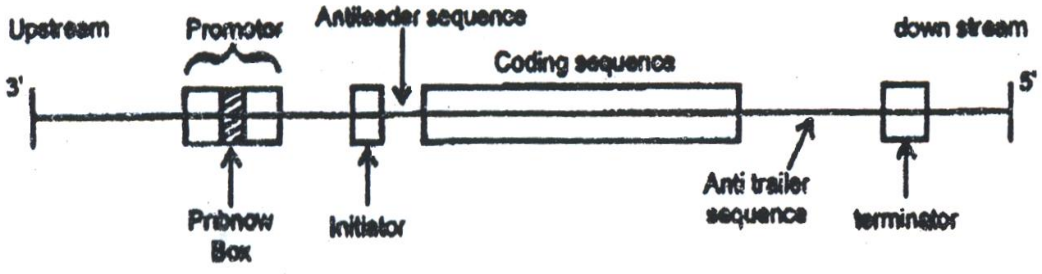


Figure 9.6: Schematic representation of Gene

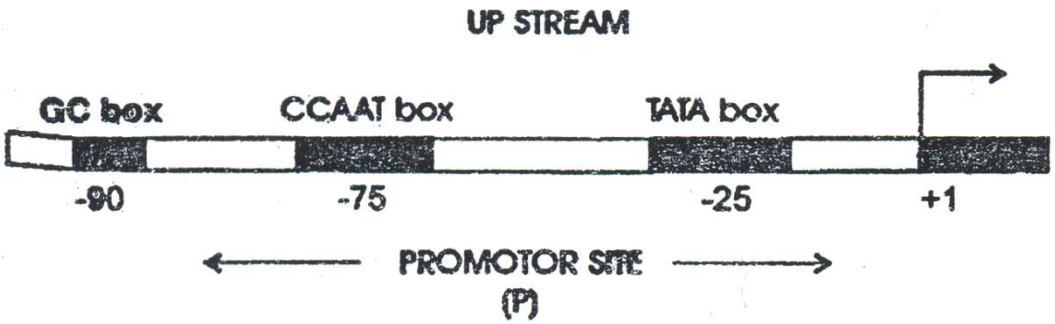


Figure 9.7: Eukaryotic gene: Upstream 3[I]- flank zone

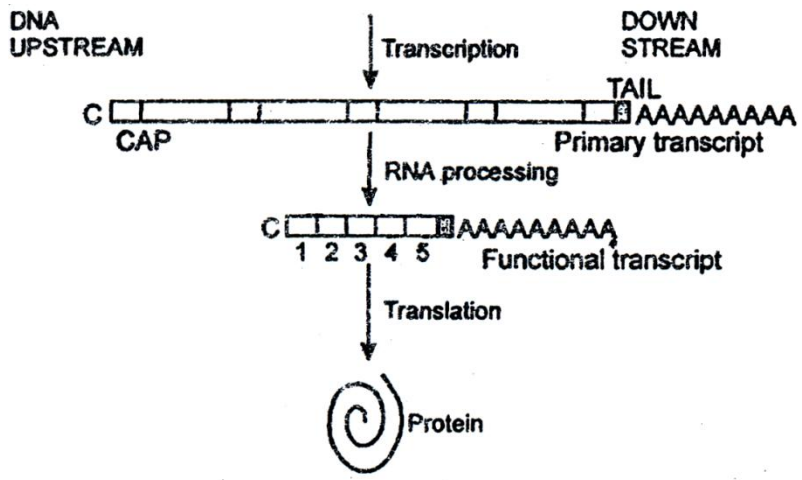


Figure 9.8: Schemati strucyture of a eukaryotic structural gene-over simplified.

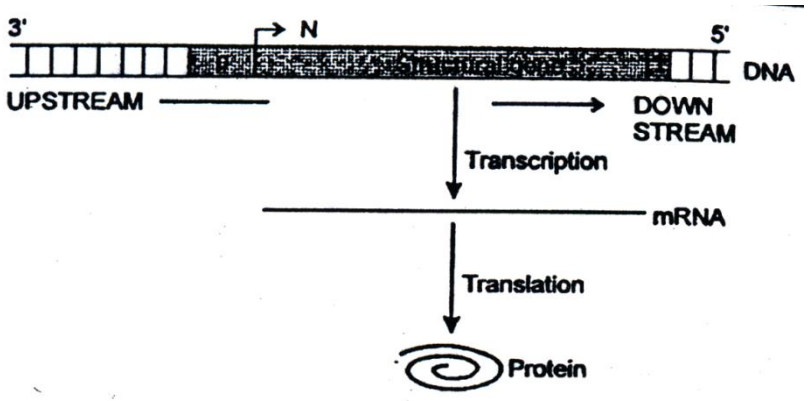


Figure 9.9: Schematic Structure of a Prokaryotic gene - Over simplified

New types of genes

1) Overlapping Genes

According to one of the dogmas of genetic code, each gene is responsible for coding one protein (or) polypeptide. This hypothesis was first proposed by Beadle and Tatum in 1940's and is called the one gene - one protein hypothesis. Barrel and his co workers (1976) first gave evidence that suggests the possibility of split genesin the bacterial virus øxl74. The bacteriophage consists of an icosahedral protein capsid encircling a single stranded circular DNA molecule. It was realized that the total number of proteins syntheesized by øxl74 genome is greater than its coding potential. Further analysis by Sanger and his colleagues (1977) indicated that proteins coded by genes D and E are specified by the same segment of DNA. It was later demonstrated that complete E gene is located within the D gene . Similarly gene B is contained within gene A.

2) Split Genes

It was earlier belived that a gene coding the synthesis of a particular polypeptide had a continuous sequence of nucleotides.However in several eukaryotes, the codon sequence of a gene does

not directly correspond with the amino acid sequence of the protein coded by it. This is due to the fact that a gene may be split into small pieces. A split gene can be explained with the help of the Fig 9.10, where a gene is coding for m RNA.

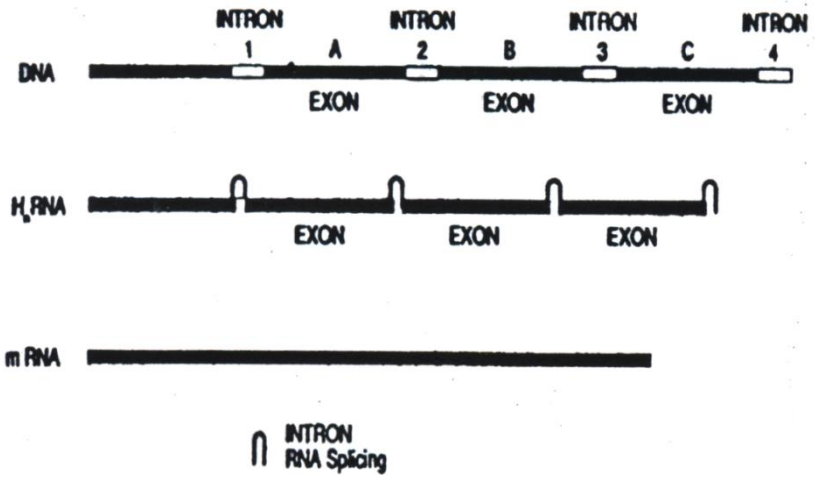


Figure 9.10: Diagramatic representation of a hypothetical split gene

A comparision of the DNA with the m RNA produced by it shows that a number of sequences are not found in m RNA. These sequences which are present in DNA but not in mRNA were called intervening sequences or Introns, whereas the sequences which are found in m RNA are called Exons. Thus a DNA sequence containing a gene consists of alternating introns and exons. Only the exons are theinformation pieces of a gene.The DNA is first completely transcribed forming Hn RNA . Then the sequenes corresponding to the introns are removed by a process called RNA splicing to form m RNA With the exception of archebacteria. Split, genes are absent in prokaryotes.

Gene expression

Living cells contain thousands of genes. All these genes do not function at the same time. Genes function according to the requirement of a cell. Genes control the phenotypic expression of a character thro the production a specific enzyme. The synthesis of a particular protein or enzyme coincides with the requirement of a cell. So there is an on - off system which regulates protein synthesis in all living cells.

The study of this ON - OFF mechanism is called- Regulation of Gene action or Regulation of gene expression or regulation of protein synthesis.Since the genes are expressed thro' proteins the mechanism of gene expression is generally explained thro protein synthesis and its mechanism.

The genetic information for the synthesis of a protein is stored in the polynucleotide chain of DNA. This information from DNA is transferred to mRNA during a process called Transcription. A polypeptide Chain or a protein is formed from mRNA. This is called "Translation".

$$\text{DNA} \xrightarrow[\text{Nucleus}]{Transcription} \text{mRNA} \xrightarrow[\substack{\text{Cytoplasm} \\ \text{(Ribosomes)}}]{\text{Translat ion}} \text{Protien}$$

Transcription

Transcription is the synthesis of RNA using DNA as template. It is intensively studied and relatively well understood especially, in prokaryotic cells. Three kinds of RNA.are produced as a result of transcription. Messenger RNA (m RNA) is produced for coding the aminoacid sequence specified by a gene or set of genes. In *E. coii* about 95% of the chromosome codes messenger RNA while the remaining part codes tor transfer RNA and ribosornal RNA.

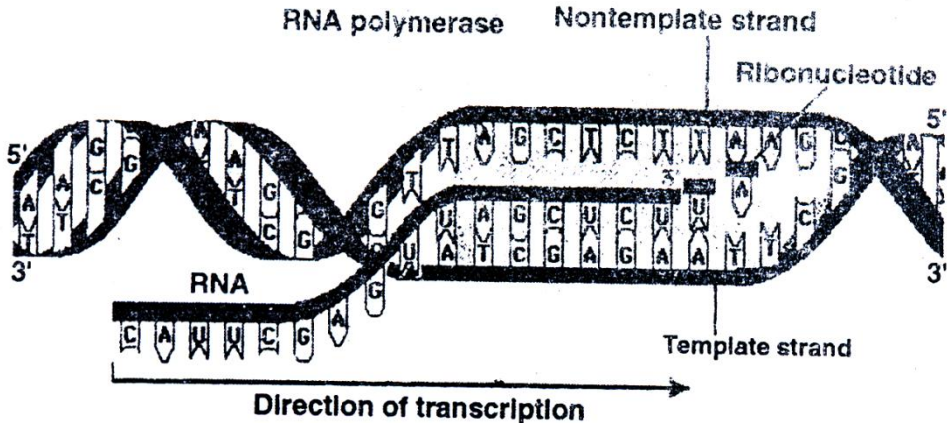


Figure 9.11: Transcription process

In complementary base pairig, A, T, G, and C on the template DNA strand specify U, A, C, and G, respectively on the RNA strand being synthesized.During replication of DNA, the entire chromosome is copied to yield daughter DNA where as in transcription only individval genes or groups of genes are copied.

During transcription, the two strands of DNA separate and one of the strands becomes a template for m RNA. The nucleotides of m RNA are complementary to those of DNA strand. In mRNA uracil (U) replaces thymine (T) of DNA. If the DNA strand has nucleotide sequence ofTTAAAACATA C, the sequence of nucleotides in m RNA would be A A U U U U G U A U G. Thus in the DNA of gene only one strand is the sense strand. The other antisense or non-coding strand is used as a template for the synthesis of messenger RNA.

Messenger RNA is single stranded. If it carries the code for only one polypeptide, the m RNA is *monogenic* or*monocistronic.* If it codes for two or more different proteins, the m RNA is *polygenic* or *polycistronic.* The minimum length of mRNA depends on the polypeptide chain for which it codes. For example polypeptide chain of 100 aminoacids residues requires m RNA of 300 nucleotides.

The enzymes responsible for transcription in prokaryotic and eukaryotic cells are known as DNAdependent RNA polymerases. In prokaryotic cells like E. coli, only one type of RNA polymerase catalyses the synthesis of different RNAs, i.e., mRNA, tRNA and rRNA. On the other hand in eukaryotes, three distinct transcribing enzymes are present in the nucleus.

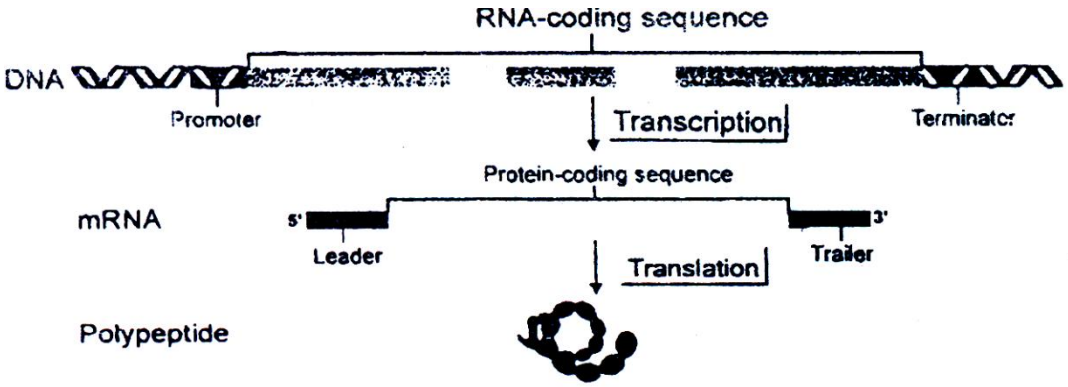


Figure 9.12: m-RNA formation in prokaryotes. The sequence of a prokaryotic protein - coding gene is colinear with the translated mRNA; that is, the transcript of the gene is the molecule that is translated into the polypeptide

RNA polymerase I is located in the nucleolus. It catalyses the synthesis of rRNA. RNA polymerase II is present in the nucleoplasm. It is responsible for the synthesis ofheterogenous nuclear RNA(H_n RNA) which is a precursor to mRNA. RNA polymerase III is located in the nucleoplasm and it synthesises tRNA. The activity of RNA polymerase depends on the presence of nucleoside triphosphates (ATP, CTP, GTP, UTP).

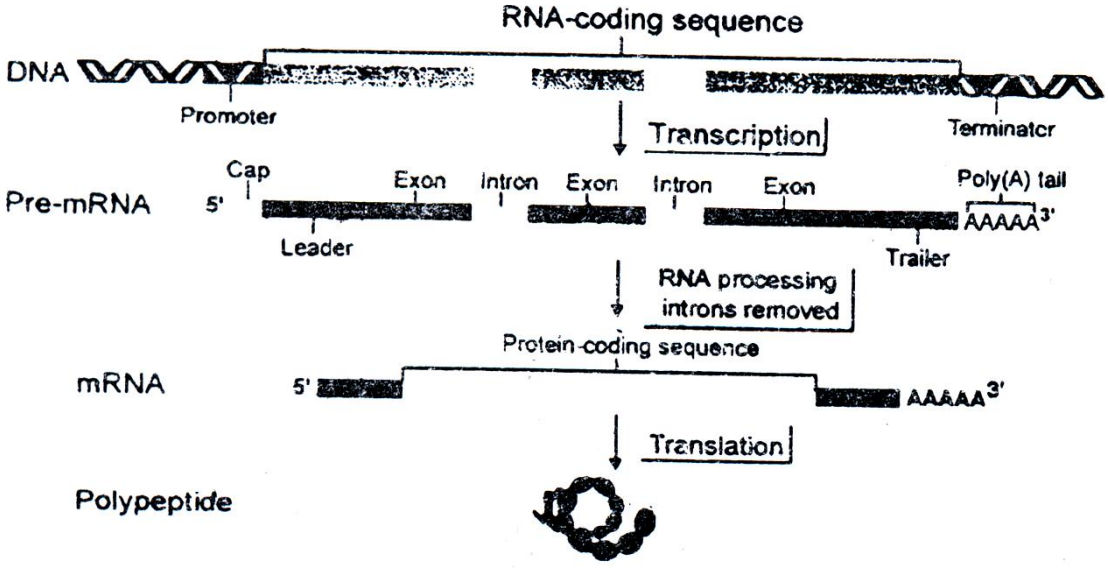


Figure 9.13: m-RNA formation in Eukaryotes

The sequence of a eukaryotic protein - coding gene is typically not colinear with the translated mRNA; that is the transcript of the gene is a molecule that must be processed to remove extra sequences (introns) before it is translated into the polypeptide

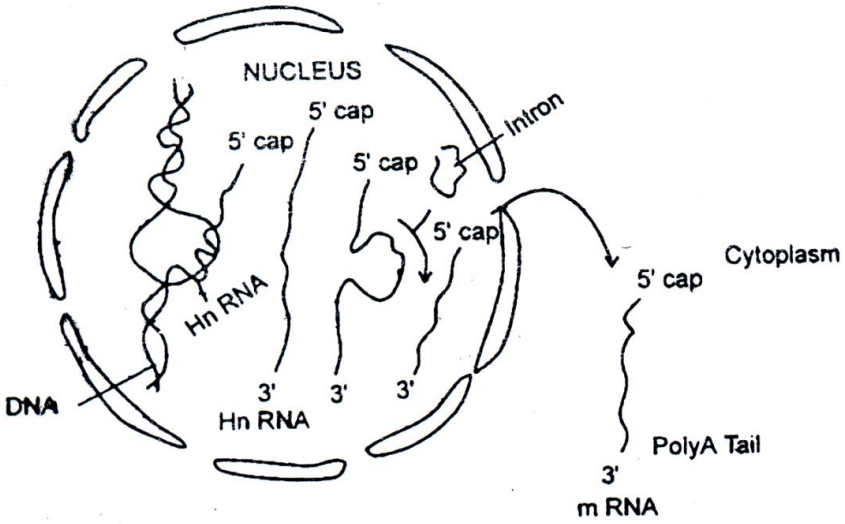


Figure 9.14: m-RNA formation of Eukaryotic cells

Heterogenous nuclear RNA (Hn RNA):

In eukaryotes mRNA is not directly formed from DNA. A precursor to mRNA is formed from DNA and it is called heterogenous nuclear RNA (Hn RNA). It is also known as pre mRNA. While mRNA is present(in the cytoplasm, Hn RNA is present in the nucleus. It consists of very long RNA with diverse nucleotide sequences namely exons and introns. The coding sequences are called exons and non coding, intervening sequences are called introns. The processed mRNA contains only exons. which can code for the amino acids. The introns are removed by RNA splicing. Small nuclear RNA (s_n RNA) splice the intervening sequence or introns. As a result, the excised intron is removed and the two exon sequences of the primary transcript (Hn RNA) are brought together in the same order as in DNA. Thus the nucleotide sequence of mRNA is complementary to the exons but not to the entire DNA of the gene.

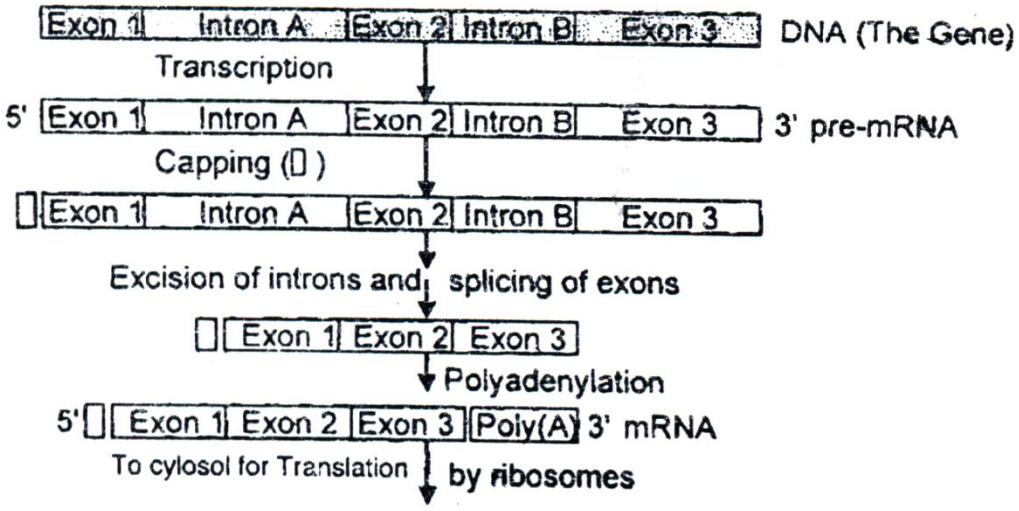


Figure 9.15: The steps of RNA processing

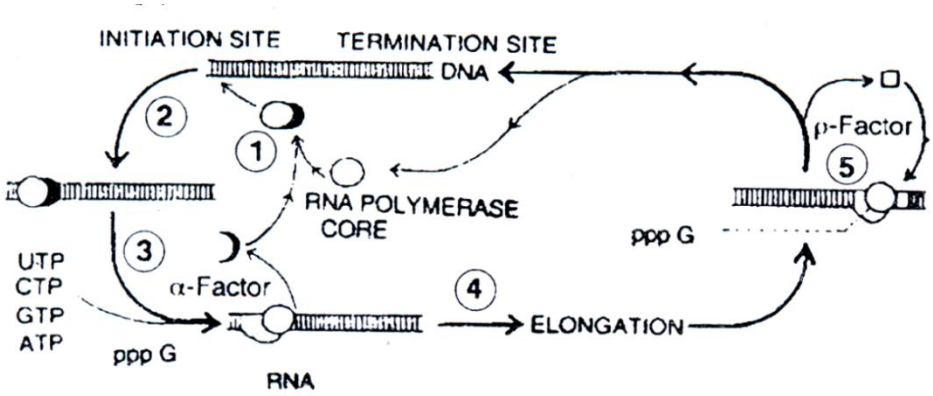


Figure 9.16: Scheme for transcription

It is now well established that mRNA synthesis is initiated at the 5' end and proceeds to 3' end. The RNA polymerase attaches to the structural gene for catalyzing mRNA synthesis. The enzyme contains a sigma factor (a polypeptide) which is required for correct initiation of RNA synthesis at the specific site on the DNA template. As soon as the m RNA is started, the *sigma factor* (σ) is released from the core enzyme. It can be reused in the transcription ofother specific mRNA molecules. The transcribed mRNA is released by a releasing factor, a protein called *rho* factor (ρ). The mRNA crosses the nuclear membrane and reaches the cytoplasm. It is utilized for protein synthesis.

Post transcriptional modifications:

Eukaryotic m RNA_S have special modifications at their 5' and 3' termini known as cap and poly a tail respectively. The 5' triphosphate end of nascent m RNA is modified immediately forming a cap. The last pair of nuclotides at 5' end of m RNA is methylated.The methyl guanine Cap at 5' end of m RNA plays an important role in translation. Cap is essential for binding of 40s sub unit of eukaryotic ribosome to the mRNA.In addition to serving as a ribosomal binding signal, the cap may be involved in protecting m RNA from digestion by ribo nuclease. Poly A tail: Most eukaryotic m RNA contains poly adenylate (poly A) tail at the 3' end. Like the 5' cap, the poly A is also added to the RNA molecule after it has been synthesized. The base sequence of the 3' end contains 50 to 200 nuclotides composed exclusively of adenine these adenosine residues are refered to as poly A tail. Poly A tail has a role in stabilising the m RNA in the cytoplasm, protecting it from premature degradation.

Other types of RNA in protein synthesis

1. **Ribosomal RNA (rRNA):**

Ribosomal RNA is found in ribosomes. It accounts for more than 80% of RNA content in the cell. Major part of ribosomal RNA exists as double stranded hair pin loops. It provides surface to various enzymes of protein synthesis.

2. **Transfer RNA (tRNA)**

Transfer or soluble RNA constitutes 15% of cellular RNA. tRNA is small molecule composed of 75-85 nucleotides. There are twenty amino acids and for each aminoacid there is a specific tRNA. Robert

Holley proposed the clover leaf model.The tRNA transports aminoacids from cytoplasm to the ribosomes where they are assembled into proteins. Some parts of the molecule appear double stranded but it is single stranded. It consists of unpaired CCA at the 3'end. One of the loops opposite to CCA end carries a triplet of nucleotides forming the anticodon.The anticodon has unpaired bases and it is capable of recognizing the codons ofmRNA and forming hydrogen bonds. In the tRNA molecule the anticodon is the most specific end of the molecule that reads the message. Not only the tRNA can read the message as codons of mRNA, it can also recognize the specific aminoacid. Hence it is called an adaptor, molecule.ThetRNA with its attached aminoacid on mRNA at a point where t RNA is in contact with a ribosome. Thus mRNA translates the genetic messge contained; in it into protein synthesis by controlling the sequence of amino acids. This is known as translation.

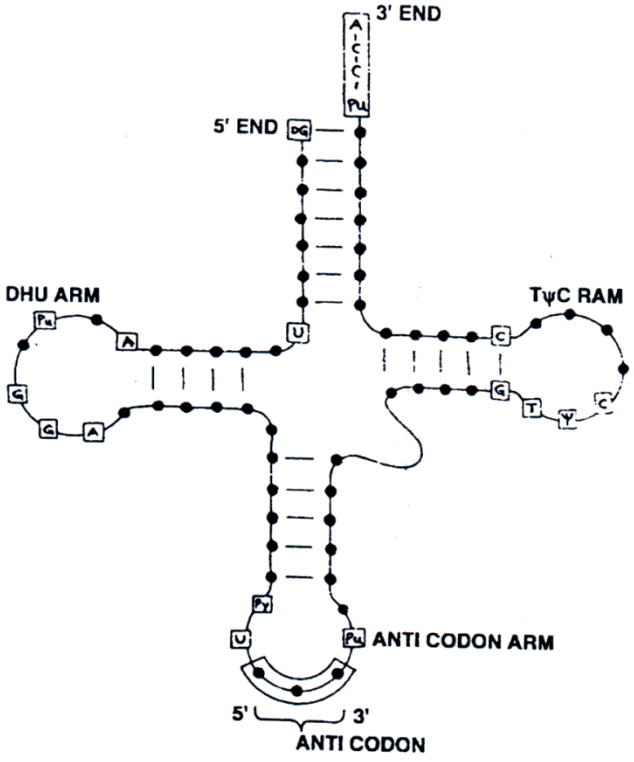


Figure 9.17: Structure of tRNA (clover leaf model)

Genetic code

The mRNA formed in the nucleus carries the genetic information to the surface of ribosomes. The information for the synthesis of a protein molecule is present in the DNA of a gene. There isa colinearity between the nucleotide sequence of DNA and the aminoacid sequence of the protein. As the cell is made up of different proteins, the DNA strand determines the sequence of aminoacids in a protein molecule. A protein may contain a single polypeptide chain of 20 to 2000 aminoacids or several polypeptide chains linked by various bonds.

The nucleotides of mRNA constitute the genetic code. The four nucleotides combine in units of three (a triplet) forming 64 (4^3) *triplets.* Each triplet codes for an aminoacid. As there are only 20 aminoacids, the triplets are more than sufficient to code for all the aminoacids. The genetic code contains 64 codons (triplets). A codonis the sequence of nucleotides in mRNA which code for a particular aminoacid. Nirenberg, Matthei, Ochoa and H. G. Khorana contributed greatly in deciphering of the genetic code. Out of 64 codons, 61 codons code for 20 aminoacids. The remaining three triplets (UAA, UGA, UAG) which were previously called nonsense codons are now recognised as terminating codons. In the genetic code there are few tRNAs and each tRNA can recognize several codons. The first two bases of the codon form base pairs with corresponding bases of the anticodons and they are specific. But the third base is less important in coding. It is known as wobble. It is flexible in pairing with bases that are not complementary. For example G in this wobble position of anticodon can pair with U or C of mRNA while U can pair with A or C.

An inspection of the genetic code reveals that in a number of cases, more than one triplet code exist for a given amino acid. This is referred to as degeneracy of the genetic code.Few generalizations about the genetic code are mentioned below:

1. The code is universal. It is applicable to all organisms.
2. The code is comma less and non overlapping.
3. The code is degenerate *i e.*, more triplet codes than the number of aminoacids.
4. The polypeptide chain is initiated by initiating codon AUG. .
5. The polypeptide chain is terminated by chain terminating codons UAA, UGA and UAG.

Translation

Protein synthesis is the most complicated synthetic activityoccurring in the cell. While other molecules are manufactured as aresult of straight forward enzymatic reactions, the assembly of proteins requires a large number of macromolecules. These include:

1. tRNA s with attached aminoacids;
2. ribosomes
3. aminoacid activating enzymes
4. a number of proteins involved in polypeptide chaininitiation,elongation and termination
5. Mg^{2+}
6. GTP

Protein synthesis requires the incorporation of each of the 20 different aminoacids in precisely the proper sequence as dictated by the coded message. Although it is a highlv complex process, proteins are made at rapid rates. In *E coli* a complete polypeptide chain of 100 aminoacid residues is made in 5 seconds. It takes about 1 minute to synthesize haemoglobinchain consisting of 150 aminoacids. However the process consumes lot of energy. In *E.coli* 90% of cellular energy is consumed for protein synthesis.

The synthesis of polypeptide chain requires 5 stages. They are

1. Activation of amino acids
2. Initiation of polypeptideChain
3. Elongation
4. Termination and release
5. Folding andprocessing.

1. Activation of aminoacids:

This stage occurs in cytoplasm. In this stage 20 different aminoacids are activated and are covalently attached to 20 different tRNA molecules. The aminoacid is activated by re with ATP in the presence of $Mg^{2+}$and. an activating enzyme. Itresults in aminoacyl adenosine monophosphate complex (aa AMP) and inorganic phosphate.

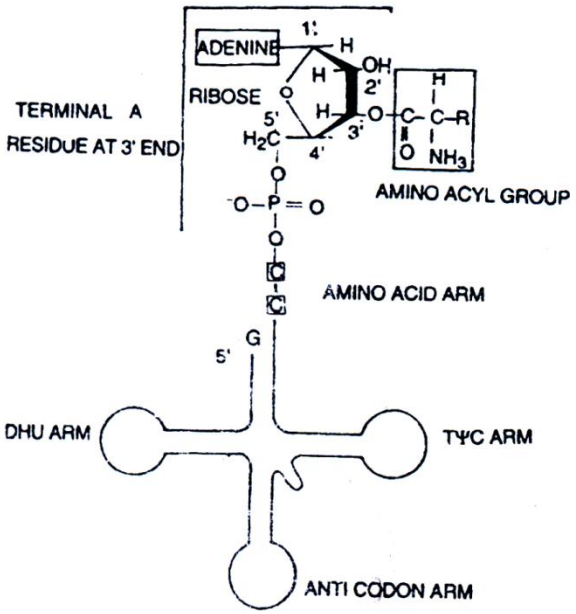


Fig 9.18: Aminoacyl tRNA

Aminoacid + ATP ---→Aminoacyl AMP + Pi .... (1)

Aminoacyl AMP+ tRNA----- →

Aminoacyl tRNA + AMP + Enzyme .... (2)

The aminoacid activating enzymes are called aminoacyl tRNAsynthetase. There is one specific enzyme for each aminoacid. In the second step the activating enzyme catalyses the transfer of the amino acid to a tRNA molecule to form aminoacyl tRNA.Transfer RNA (tRNA) carries the activated aminoacid to the mRNA on the rlbosome. On the ribosome the amino acid is transferred to the growing peptide. Guanosine triphosphate (GTP) drives the transfer and tRNA is liberated.

a. Ribosome- The site of Translation:

The translation process occurs on ribosomes. Ribosomes are considered as work benches of protein synthesis, as they are provided with machines and tools needed to make a polypeptide. It is made up of RNA and protein. Ribosome is made up of [r] large and a small subunit.In the absence of Mg ,

the ribosome dissociates into two subunits. In eukaryotes, the ribosome is of 80 s type and it dissociates into 60 s and 40 s. Whereas in prokaryotes, the ribosome is of 70 s type and it dissociates into 50 s and 30 s (s is the rate of sedimentation during centrifugation. It is expressed as svedbergunits). These two oddly shaped subunits join together during initiation of translation and dissociate at the end of the process.

Ribosome has two sites for binding the aminoacyl tRNA. A or aminoacyl site and P or peptidyl site. The A or aminoacylsite binds the incoming aminoacyl tRNA and P or peptidylsite is the site from which the empty tRNA leaves and towhich peptidyl tRNAisattached.

b. Polysomes or Polyribosomes:

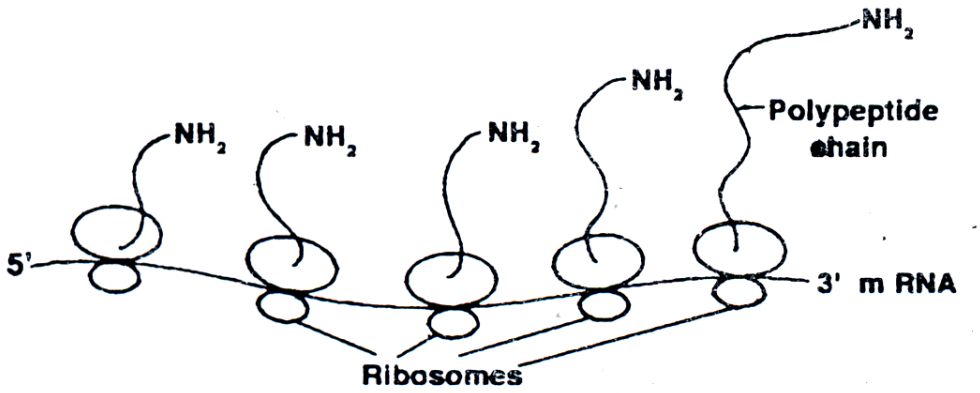


Figure 9.19: Polyribosome

When protein synthesis is taking place several ribosomes get attached to mRNA like beads ona string. This is known as *polyribosome* or polysome. Generally polypeptide synthesis takes place on polyribosome and rarely on a single ribosome. GTP supplies energy for movement of ribosomes along mRNA. As several ribosomes are held together, translation takes place simultaneously and mRNA is efficiently utilized. The synthesis of polypeptide chain begins at the amino terminal end and proceeds to the carboxyl terminal end. At any given time polysome contains both nascent polypeptide chain and already synthesised polypeptide.

2. Initiation of Polypeptide Chain

In prokaryotes, the polypeptide chain is initiated by the aminoacid N formyl methionine.This is regularly coded by the codon AUG and rarely by GUG. These are called initiating codons.The formyl group or the aminoacid is enzymatically removed after the synthesis of polypeptide. Its specific RNA is known as tRNAf met.In eukaryotes the initiating aminoacid is methionine and its specific tRNA is known as tRNA m. It is also recognised by AUG. AUG acts as the starting signal.The initiating factors($IF_{1r}IF_2$, If_3) are required during initiation. Out of these IF_3 is used for binding of 30 s subunit to mRNA while the other two factors IF_1, IF_2 are required for binding of 30 s - mRNA - $tRNA_f$ met.

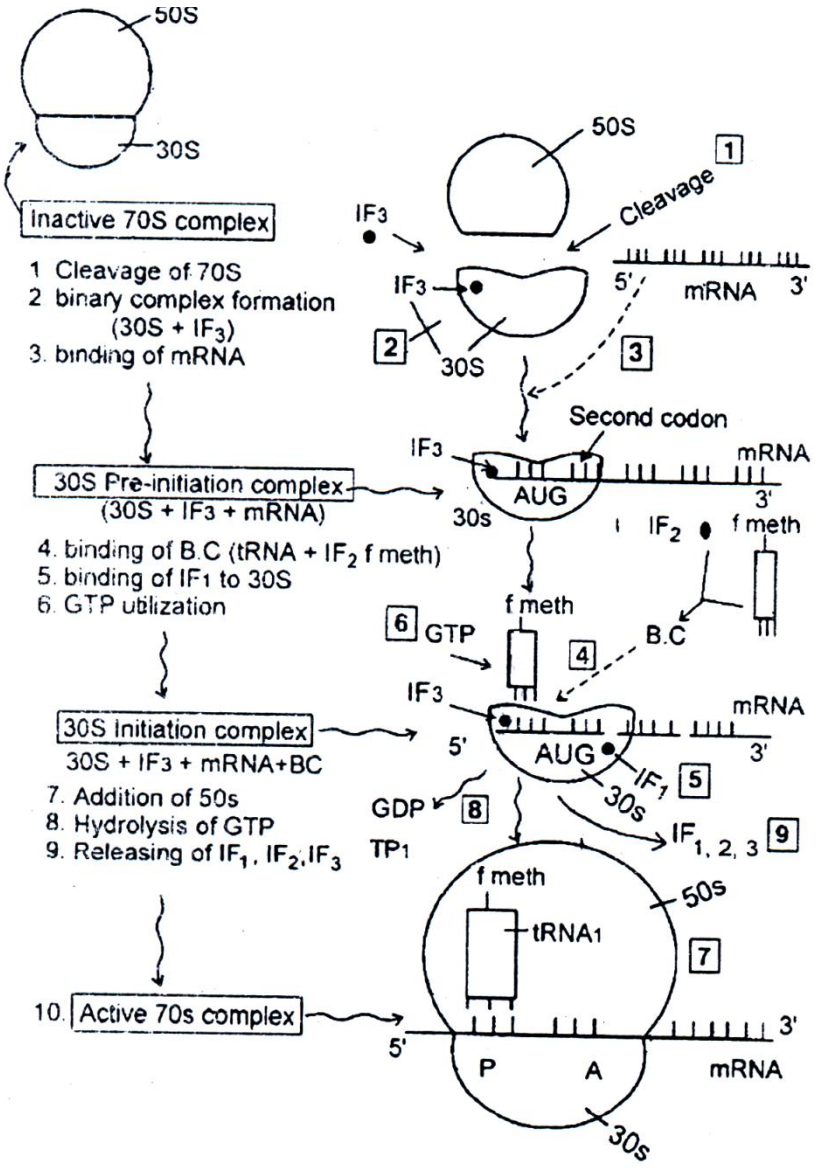


Figure 9.20: Chain intiation

The energy required for formation of this complex is derived by hydrolysis of GTP to GDP. In the next step 50s subunit joins the 30s subunit so that 70s ribosome is assembled on mRNA. This elaborate initiation process is required to ensure that the initiating aminoacyl tRNA is bound at the peptidyl site and positioned at the initiation codon for starting translation at the correct point on mRNA. The initiation factors are released. These are used again and again to start new chain.

The process of initiation in eukaryotes is basically similar to that of prokaryotes with the following variations.

1. The initiation of chain is brought about by special fRNA known as tRNA m
2. The ribosomal subunits are 40 s and 60 s
3. There are at least 9 initiating factors and the role of these factors is not yet known.

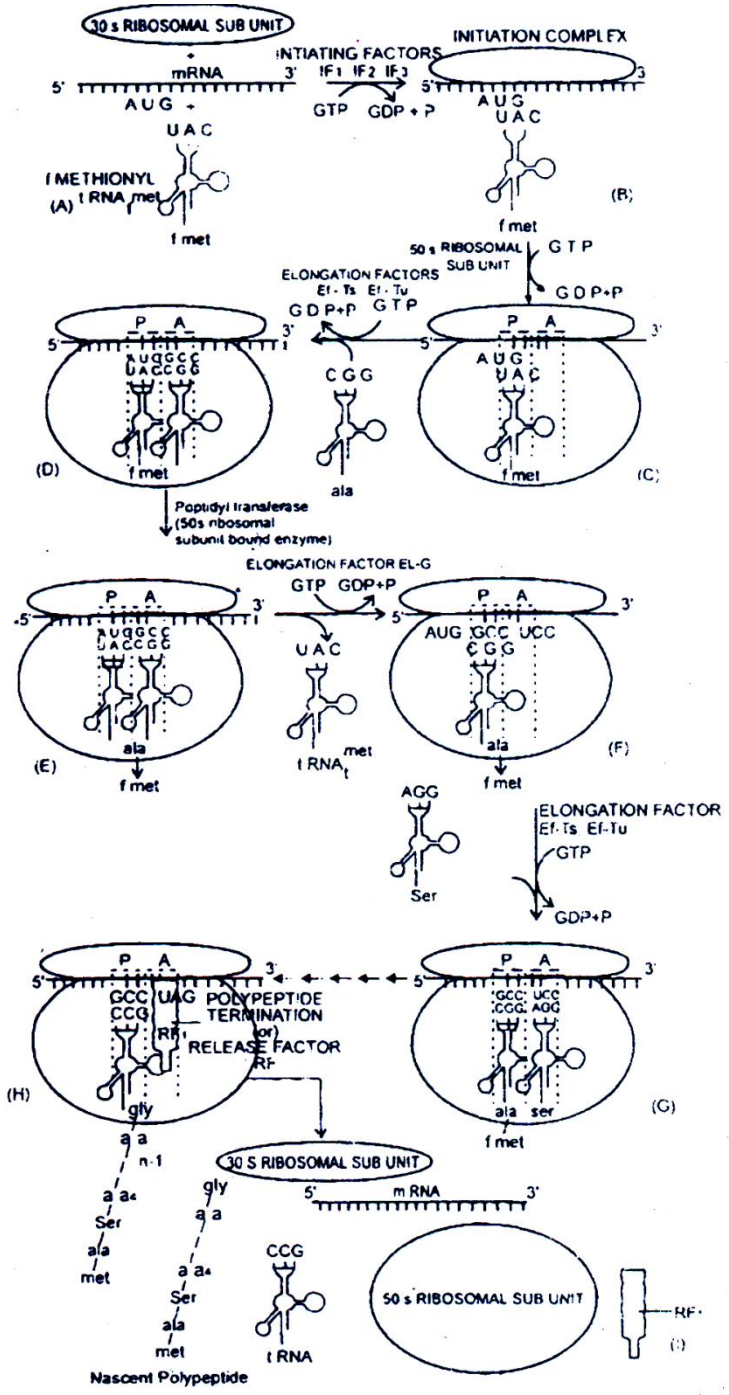


Figure 9.21: Scheme of steps involved in Translation (A to I) – Overall view

Mechanism of Gene Expression

The mechanism of Regulation of gene expression is generally explained in detail with reference to prokaryotes and Eukaryotes.

(A) Regulation in Prokaryotes

(B) Regulation in Eukaryotes

Even in prokaryotes there is considerable regulation of gene activity. For example a bacterium has enough DNA to code for 3000 different proteins at any given moment. A bacterial cell contains

nearly 10 million protein molecules some of which are as few as 10 copies while others are as many as 50,000 copies. Metabolic needs of a cell are related to which genes shall be active and to what extent. The question is how this regulation is achieved? There are some answers from the studies of prokaryotic systems. However eukaryotic mechanisms of gene control are less well understood.

The process by which the expression of a gene is turned on in response to a substance in the environment is called induction. Genes whose expression is so regulated are called inducible genes. Their products, if enzymes are called inducible enzymes. The substances or molecules responsible for induction are called inducers. Enzymes that are involved in catabolic (degradative) pathways such as lactose, Galactose arabinose utilisation are inducible. Induction occurs at thelevel of transcription. Induction is different from enzyme activation, in which the binding of a small molecule to an enzyme increases the activity of the enzyme (but does not affect the rate of its synthesis)

E .Coli has five genes coding for enzymes that are required in the synthesis of tryptophan. If it is growing in a medium which is.devoid of tryptophan, all these five genes become expressed. Suppose it is present in a medium where there is abundant of tryptophan, these genes get shut off automatically. So tryptophan synthesis inside the bacterium stops. The process of "turning off the expression of sets of genes is called repression. A gene whose expression has been turned off in this way is said to be repressed. When its expression is "turned on" again it is called derepressed.

Enzymes involved in biosynthetic pathways are frequently repressed by the addition of the end product. This phenomenon is known as co-ordinate repression. Repression also like induction occurs at the level of transcription. Repression should not be confused with feed back inhibition. In which the binding of an end product to the first enzyme in a biosynthetic pathway inhibits the activity of the enzyme (but does not affect its synthesis) Feed back inhibition is also known as end product inhibition.

LAC OPERON (Regulation in bacteria- *E. coli*)

F. Jacob and j. Monad of *pasteur Institute Paris*,-provided the first well documented system of gene regulation in microorganisms in 1961. They were awarded the Nobel prize in 1965. They proposed the OPERON MODEL to explain the regulation of genes coding for the enzymes required for lactose utilisation in *E.coli*. If bacteria are grown in media containing glucose, enzymes for the breakdown of lactose are present in very low levels. If they are grown in media without glucose but with lactose, the level of lactose enzymes increases a thousand fold. Jacob and Monad selected this system as a clear example of gene regulation based on metabolic needs of the cells.

1. Structural genes:

Jacob and Monad identified three genes which determine the structure and synthesis of the three relevant enzymes, which take part in the breakdown of Lactose These three genes were named the *structural genes* and were designated 'Z' (b -galactosidase) 'Y' (b - galactoside permease) and 'a' (b - ga'lactoside transacetylase).

2. Regulator gene:

Through a series of skillful experiments areas of the DNA near the structural genes were found to have regulatory function controlling transcription of structural genes. Connected to the structural genes is a region of DNA now known as the operator region, which does not code for any protein? Its function is purely to control, it does this by interacting with a protein molecule whose structure is determined by a *regulator gene*, designated as 'i'. The protein acts as a repressor molecule and binds to the operator region (o). As a result transcription enzymes cannot produce m RNA s from the structural genes i.e., transcription does not take place. This happens when *E.coli* cells have good amount of glucose than lactose in the medium. The result is that levels of enzymes for the breakdown of lactose are low. (Fig 9.22 A)

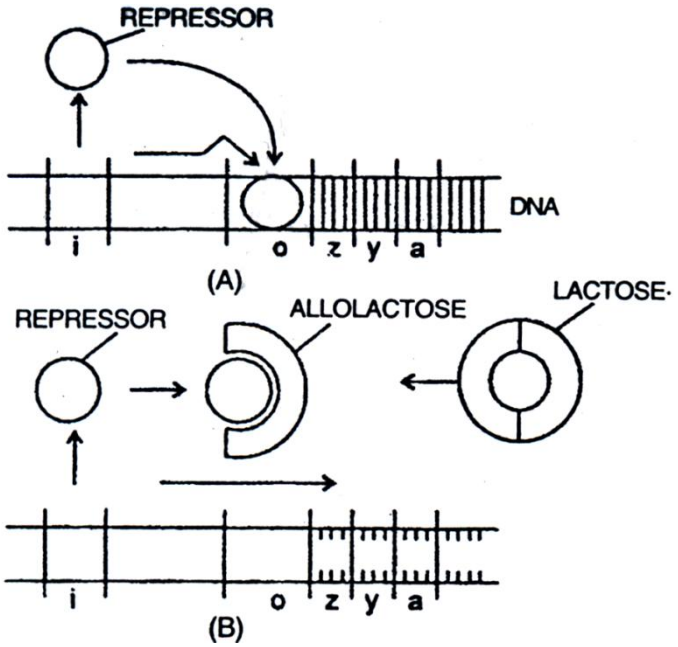


Figure 9.22: Induction of lac enzyme production

(A) In the absence of lactose, the repressor molecule, encoded for by theregulator *(i)* gene, interacts with the operator region (o) to block transcription(dark arrow) of structural genes *z,y,* and a.

(B) When lactose is present, it isconverted into allolactose, which interacts with the repressor. Absence ofrepressor-operator binding allows transcription of the structural genes toproceed.

When lactose is present in the medium, the low levels of enzymes break down the lactose into a somewhat simpler sugar known as allolactose.The allolactose interacts with the repressor molecules and prevents them from binding to the operator region. So the transcription of structural genes follows. (Fig 9.22 B) Thus when lactose is absent no lactose enzymes are produced, when lactose is present the lactose enzymes are produced. As Jacob and Monad had guessed, this system clearly reflects the regulation of gene activity based on needs of cell. (*E.coli*)

As the presence of lactose can induce the cell to synthesize lactose breakdown enzymes, this system is known as an *inducible system.* The allolactose is called the effectorsubstance. The effect of repressor - operator interaction shutting down transcription is known as negative regulation.

3. Promotor region:

Between the operator region and T gene, there is yet another region of DNA which is involved in the regulation of lactose (lac) gene activity. This is known as the promoter region.The term promoter now refers to the areas of DNA which interact with RNA polymerase, the enzyme which transcribes precursor m RNA from genes in the DNA. What is special about promoter DNA is not clear, but it is known that RNA polymerase binds to the DNA at promoter sites and moves on to the next neighbouring structural genes. The reason for the variation in the regulatory function of promoter region in inducting the lactose enzymes differently is again linked to the cell economy. Glucose is the simple form of sugar, which is used by cells for energy. Lactose must be broken down to allolactose and then to glucose before it can be used. Therefore if glucose is available it would the unnecessary to use lactose. How is this controlled?

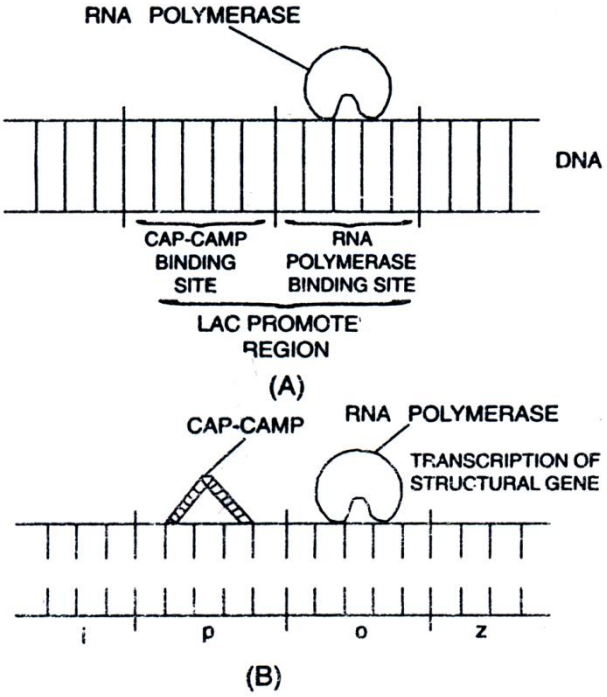


Figure 9.23: Thetwo areas of *the lac* operonpromotor region

(A) Without CAP-CAMP interaction. RNA polymerase cannot transcribe structural genes because the DNA remains a double helix

(B) With the CAP-cAMP Interaction, the DNA structureIcosene up, so thattranscription can proceed

It is found that a complex protein called catabolic activator protein, CAP binding with cyclic AMP (CAMP) must interact with part of the promoter region, which is nearest to the T gene. This interaction is important for RNA polymerase to interact with the part of the promoter nearest the operator

and thus initiate transcription of structural genes. The CAP - CAMP interaction with the promoter must occur for transcription, even if there is no repressormolecule binding to the operator region. The effect of CAP - CAMP on transcription is known as positive regulation. (Fig 9.23) The presence of glucose in medium is known to reduce the level of CAMP in bacteria. The fewer the CAMP molecules are, the less CAP -CAMP complex that is formed. The result is a decrease in positive regulation and a decrease in the transcription of lac enzymes.

Thus a dual control over the synthesis of enzymes for the breakdown of lactose exists. The repressor - operator and CAP -CAMP promoter interactions. Both controls are determined by the presence or absence of glucose and presence of lactose. Thus this system illustrates a beautiful control over the gene activity by themetabolic needs of the cells (Fig 9.24). The compound cyclic AMP (CAMP) was discovered in animal cells in 1960 was thought to have no role in bacteria. However in 1965, Makmanand Sutherlandnoted the presence of cyclic AMP in *E. coli* cells. The concentration of CAMP varied depending on the metabolic state of the cells, especially the concentration of glucose which greatly depressed the concentration of CAMP.

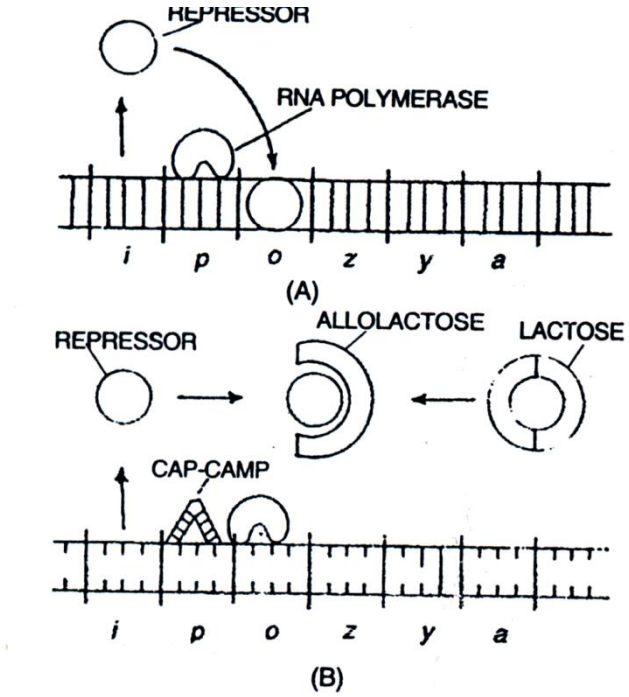


Figure 9.24: A summary of the dual control over lac operon genes

***(A)* in the presence of glucose;**

***(B)* in the absence of glucose and presenceof lactose**

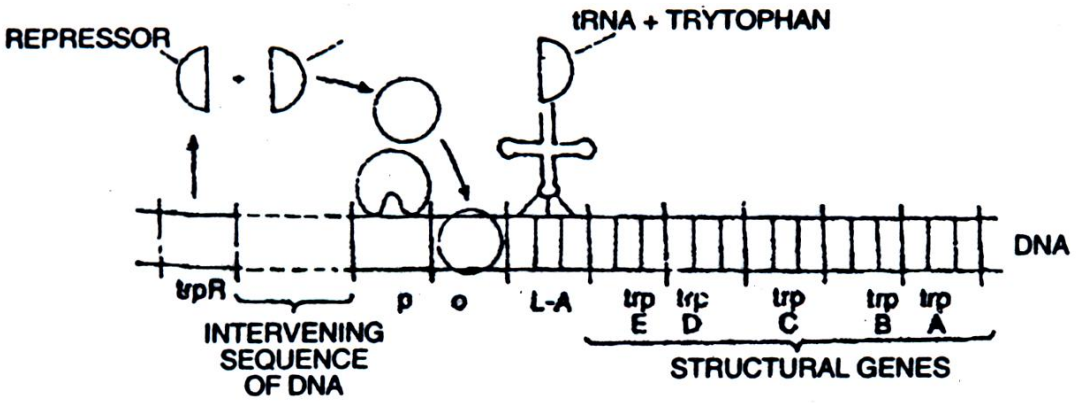
Thus the concept of operon was advocated by Jacob and Monad, with the *lac operon* worked out in the bacterium E. coli, now operon is a commonly used term referring to genes in prokaryotes that control a particular metabolic process. Other bacterial operons dealing with the breakdown of large sugars have since been studied although there are aspects that are unique to each, the basic dual system of regulation involving positive and nagetive regulation has been found to be similar to that of the lac

system. However, furtherinvestigations are necessary to understand completely, how the operator & promotor regions interact with various substances in various operons. One more operon by name tryptophan operon is (trp operon) described below. The trp *operon* is an attenuator type.

Tryptophan operon

The tryptophan operon is different from lac operon, because it is concerned with the synthesis by the cell of a substance namely the tryptophan, an aminoacid. The lac operon deals with the production of enzymes which ultimately degrade the lactose, a sugar, to form the end products.

Some detailed studies have been made on genes encoding enzymes for the synthesis of the aminoacid tryptophan by yanofski and his associates. It is known, the *trp operon.* The *trp operon* carries a promotor and o orator region situated next to a number of structural genes which code for enzymes needed for a rather complex series of reactions leading to tryptophan formation there is a promotor region which interacts with RNA polymerase. The operator interacts with a repressor molecule, which when bound to tryptophan, shuts down the transcription of structural genes. In otherwords when tryptophan is present some molecules of the aminoacid interact with the *trp operon* repressor. This combination then binds to operator region of DNA and shuts down transcription, again an example of economy by the cell. (Fig 9.25)



Fugure 9.25: The trp operon genes

The repressor molecule interacts with tryptophanand the resulting complex binds to the operator region to block transcription.P = promoter region; o = operator region; L-a = leader - attenuator region

The region of DNA between the operator region (o) and the first structural gene *trp E* is unique in *trp operon.* This region is constantly kept transcribed and is called *trp L* (for leader) region. The transcription of trp L region occurs even if repressor molecules are bound to the operator. Interaction of t RNA charged with tryptophan with a short stretch of bases at the end of leader region now referred to as attenuator region (Fig 9.25) seems to be stopping the transcription before it proceeds to the structural gene. If the t RNA is not charged with tryptophan, it cannot prevent transcription from proceeding to the structural genes. However details of this interaction are still under study (Fig 9.26)

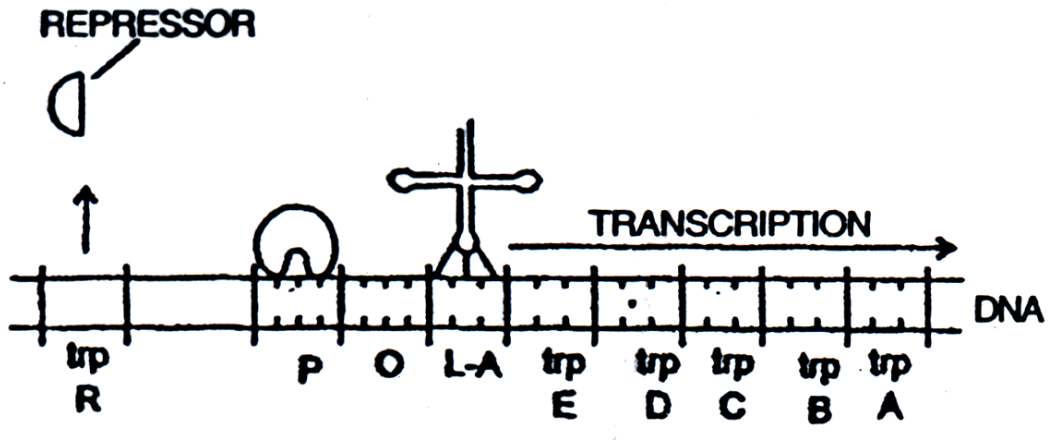


Figure 9.26: In the absence of tryptophan, the repressor molecule can no longerbind with the operator region, and the uncharged tRNA does not blocktranscription at the attenuator region. The structural genes are transcribed, and the enzymes produced catalyze the synthesis of tryptophan in the cell

Thus, in the trytophan operon the transcription of structural genes is regulated in two ways. Which are depeding on the availability of tryptophan in the cell. In the absence of tryptophan, there will be no tryptophan repressor substance to interact with the operator region. Tryptophan charged t RNA also will not be there to interact with the attenuator region. So transcription of structural genes takes place, and the enzymes to synthesize the tryptophan become available to the cell. Once the aminoacid is formed sufficiently, some of it interacts with repressor molecules and t RNA, which results in shutting down of the transcription.

Two other bacterial operons involving the synthesis of aminoacids (histidine and phenylalanine) have been found to have an attenuator type of regulatory mechanism. Whether this system is common to all genes involved in amino acid synthesis is a problem yet to be investigated. It can be pointed out here that interaction between proteins and DNA has been found in all operons in bacteria. Transcriptional control by proteins can be expected to be one characteristic of gene regulation universal in all prokaryotes.

(ISBN: 978-93-88901-16-1)

Chapter 10

Extra Nuclear Genes

Extra nuclear genome

It is well known that normally chromosomes contain DNA that behaves as the sole genetic material and stores the genetic information. But several discoveries in the past revealed the existence of DNA in cell organelles like plastids and mitochondria. These two cell organelles located in the cytoplasm, carry DNA, like that of chromosomes in the nucleus. These cell organelles control extra chromosomal activities and inheritance thro' their DNA which carries genetic information. All types of genes, found out side the nucleus, together are referred to as "Extra Nuclear Genome". The DNA in chloroplast and mitochondra together forms the part of extra nuclear genome. The inheritance ie carried an by such extra nuclear genes, is called extra nuclear or chromosomal inheritance.

The DNA which is present in chloroplasts is called chloroplast DNA and depicted as ct or Cp DNA and the DNA in mitochondria is depicted as mt- DNA traditionally. Both ct/Cp DNA and mt DNA play important roles in inheritance and in certain cell functions of their own. But their regulation over /inheritance and cellular activities, is always limited and / restricted. Besides this the chromosomal DNA of the respective cell organelles in its turn, partially controls the functions of ct DNA and mt DNA. The Two DNAs *i.e.*, ct DNA and mtDNA.are together described as cytoplasmic DNAs. And the genes in them are called plasma genes or extra nuclear genes.

Differences

The nuclear DNA and Cytoplasmic DNA show the following differences.

	Nuclear DNA	**Cytoplasmic DNA**
1.	Present only in chromosomes.	Present in chloroplasts and mitochondria as ct/cp DNA and mtDNA.
2.	It is circular in prokaryotes and Linear in Eukaryotes.	Mostly circular in shape (except some protozoa).
3.	Synthesis occurs only in interphase during cell division.	Synthesis is a continuous process in cell cycles. Replicates in chloroplast and mitochondria.
4.	It replicates in the chromosomes.	Mostly, inheritances occur in a Non -Mendelian fashion.
5.	It is inherited in a Mendelian fashion.	
6.	Recombinations are quite common	Recombinations are rare.
7.	Biparental inheritance is common	Mostly uniparental that too maternal inheritance is common.

Plastid DNA

The DNA which is present in the chloroplasts is called plastid DNA or plastid genome or chloroplast DNA or cp DNA or ct DNA. It is also called extranuclear genome or cytoplasmic DNA. The ct/cp DNA is smaller in size compared to the plant chromosomal DNA, which is generally 10^4 to 10^5 times larger than ct DNA.Most higher plants have approximately 50 to 100 choroplasts per leaf cell and each chloroplast has about 10 to 100 copies of the chloroplast DNA genome. The chloroplast DNA (ct DNA). contains genes that encode a number of important and special proteins, ct DNA can code only limited number of proteins and the rest of the required proteins are encoded by nuclear DNA.

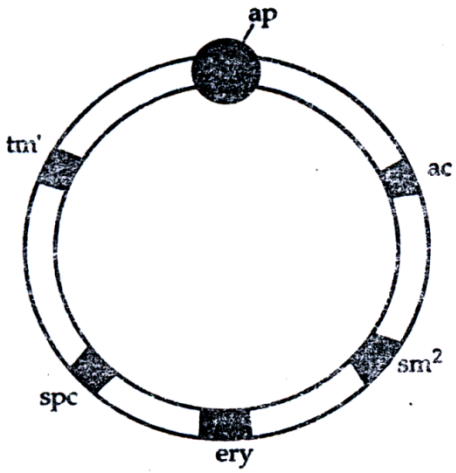


Figure 10.1: Map of chloroplast Genome in *Chiamydomonas* ap = Attachment point

RIS and plaut (1962) first observed the occurrence of DNA in chloroplasts. sager (1972) first reported the presence of several gene pairs on chloroplast genome and proposed a circular genetic map for the chloroplast genome in Chlamydomonas, etc.

ct DNA in higher plants

Chloroplasts of all higher plants and lower eukaryotes contain DNA (ct DNA). Chloroplast genomes are relatively large ranging from 120 kb to 210 kb (kilobase pairs) in size (except in Acetbularia (400 kb). As mentioned earlier each chloroplast contains several circular DNA molecules (ct DNA). The percentage of ct DNA, compared to the total cellular DNA, is always low. But, lower eukaryotes show higher percentages (about 14%) compared to higher eukaryotes.

For example,

Organism	Ct DNA %	Cp DNA (kb pairs)
1. Chlamydomonas reinhardi	14%	195
2. Higher plants	variable	120 - 210
3. Euglena	3%	135

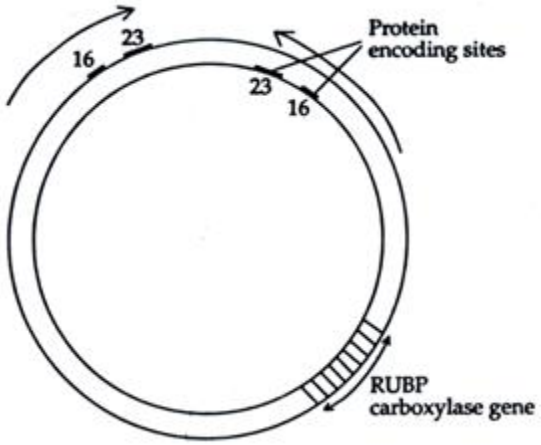


Figure 10.2: Map of circular ct- DNA of *Zea mays*

ct DNA, generally contains genes in their genome which control some specific characters or can synthesis some specific RNAs and proteins. Specific genes which encode rRNAs and tRNAs are also seen located on the ct DNA in several plant species. The genes on ct DNA can also synthesize mRNA which can encode certain proteins.Ribulose biphosphate carboxylase (rubisco) is the most common protein or enzyme, that is normally encoded by mRNAs in green leaves of many plants, (Rubisco). A smaller portion of this enzyme is synthesized by mDNA.

DNA molecules of the chloroplasts are present in the stroma part. The ct DNA is much larger than mt DNA and therefore codesfor a large number of proteins relatively, ct DNA exists as a large protein DNA complex associated with inner membrane and called plastid "nucleoid".

Protien synthesis in chloroplasts

The ct DNA exhibits semi - genetic autonomy. The ct DNA alone is not capable of synthesizing all proteins. It partly depends on nuclear DNA for other required proteins.Plastid genes in higher plants are transcribed by two distinct RNA - polymerases called PEP and NEP. Of which, the first one ie PEP is called plastid - encoded eubacterial type of enzyme (with multiple subunits). The second one is NEP is called Nuclear -encoded - phage type enzyme. In higher plants, a small family of nuclear genes encodes the plastid sigma factors, required for its transcription.

The studies in vitro, relating to the transcriptional systems (homologous to plastids) revealed the role of individual plastid sigma factors in plastid transcription, (eg. tobacco plastid genome).

Organelle inheritance (**inheritance – Involving cell organelles**)

Plastids and mitrochondria, the two important cell organcells that are located in cytoplasm which carry DNA, control extrachromosomal inheritance in many cases through their DNA (ct DNA and mt DNA). This type of inheritace is also described as "true cytoplasmic inheritance"to distinguish it from that of common nuclear inheritance. Cytoplasmic inheritance normally involves plastids

(chloroplasts) and mitochondria and occurs in a non - mendialan fashion. This inheritance is of two types namely.

1. Plastid inheritance
2. Mitochondrial inheritance

These two types though differ in certain aspects, show some common features like the following. Both types exhibit.

1. Differences in reciprocal crosses in F_1
2. Segregations thro' mitotic as well as meiotic divisions.
3. Maternal effects in their inheritances.
4. Non - Mendelian pattern of inheritance.

Plastid inheritance

The first case of cytoplasmic or plastid inheritance wasreported by Correns in1909 in Mirabilis jalapa (Four 'O' clock plant) for leaf colour.In Mirabilis jalapa, three kinds of branches namely green, white and variegated are present with reference to the occurrence of plastids. Correns (1909) made crosses and reciprocal crosses like the following and observed the results.

Corren's Experimental results of crossing flowers on variegated Four – o' Clock plants

Phenotype of branch bearing egg parent	Phenotype of branch bearing polln parent	Phenotype of progeny
White	White	White
White	Green	White
White	Variegated	White
Green	White	Green
Green	Green	Green
Green	Variegated	Green
Variegated	Green	Variegated, Green, or White
Variegated	White	Variegated, Green, or White
Variegated	Variegated	Variegated, Green, or White

The results of the above crosses revealed that whatever be the color of the branch of the Male parent, the color of the progengy would be after the colour of the branch of the female flower establishing the inheritance pattern as maternal, occuring through plastids (ct DNA).It is observed that in Mirablis jalapa the variation in colour of leaves, branch or whole plants is due to two kinds of plastids (normal and mutant white or albino). The distribution of these plastids to their daughter cells during cell divisions will be a chance process- Egg cells of the female parents may contain green, white or a mixture of both green and white plastids. Plastids, mostly, are transmitted through egg cytoplasm.

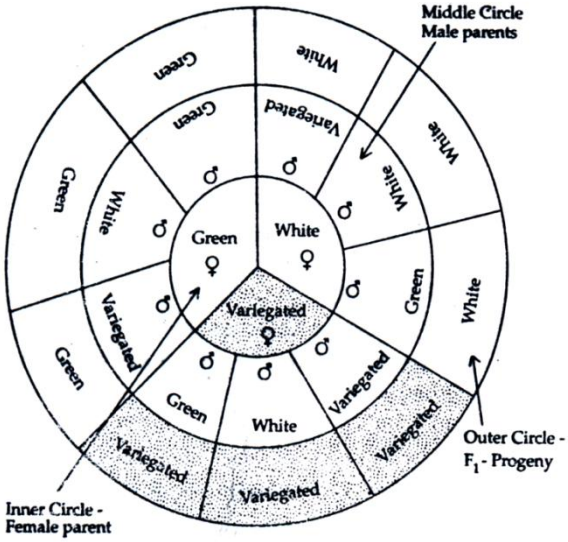


Figure 10.3: Plastid inheritance in *Mirabilis jalapa* - Simplified over view

And plastid transmission thro' pollen is a rare phenomen. Consequently, characters, depending on plastids, exhibit cytoplasmic inheritance independent of nuclear genes as in *Mirabilis jalapa*. So the phenotype of the progeny depends upon the nature of female gametes which may carry either green, white plastids or both. It means the phenotype of the female parent decides the character of the progeny in F_1

Iojap inheritance in Maize:

This is another example for plastid inheritance through cytoplasm or by plastid genes. It is similar to *Mirabilis jalapa* in-its mode of inheritance. In Maize, three kinds of leaves namely Green, white, and Iojap are present. Iojap leaves show green and white stripes, (striped condition). Crosses between green female and iojap male produced all green individuals in ¥*l*and a Green and iojap in 3:1 ratio in F_2. But a reciprocal cross between iojap female X green male produced green, white and iojap (striped) phenotypic progeny in F_1.

The dominant allele Ij is responsible for green plastids and its recessive allele is for iojap (striped) characters. So generally. Ij Ij is green and ij ij is iojap. Female gametes from ij ij may carry green, or white plastids or both.The crosses and reciprocal crosses clearly proved that male parent does not contribute cytoplasm as well as plastids to the zygote and the iojap inheritance depends on the phenotype of the female parent. Further crossing also proved that the Iojap, a striped phenotype, may have any three possible genotypes like Ij Ij, Ij ij and ijij.

Iojap inheritance in maize - results of different crosses

Iojap inheritance in Maize - Results of different Crosses

Cross	Female Parent ♀		Male Parent ♂		F_1 Progeny	
A	Pheno type	Geno type	Pheno type	Geno type	Pheno type	Geno type
	Iojap	ij ij ×	Green	Ii Ij	Green, White, Iojap	Ii ij
Eggs carry	Green plastids or white plastids or both (white and Green)		Male gametes do not carry plastids		After Female parent	No effect
Nature of Zygosity	Homozygous Recessive		Homozygous Dominant		[G/W/Iojap]	Hetrozygous Dominant
	♀ Parent		♂ Parent		F_1 Progeny	
B	Pheno type	Geno type	Pheno type	Geno type	Pheno types	Geno type
	Iojap	Ij ij ×	Green	Ij Ij	Green, White Iojap	Ii Ij/Ii ij
Eggs carry	Green, White or Both		Male gametes without plastids		After female parent	
Nature of zygosity	Heterozygous Dominant		Homozygous Dominant		(G/W/Iojap)	Homo and Heterozygous
	Female parent		Male parent		F_1 Progeny	
C	Pheno type	Geno type	Pheno type	Geno type	Pheno types	Geno type
	Green	Ii Ij	iojap	ijij	Green	Ii,ij
Eggs carry	Only Green plastids		No contribution of plastids from Male gamets		After female paerent	
Nature of zygosity	Homozygous dominant		Homozygous Recessive		Homozygous dominant	

From the above observations it is understood that the iojap character once inherited thro' female parent, it survives in the cytoplasm whatever be the genotype of the respective female parent

Inheritance of plastids-current view

Most of the plants inherit plastids from only one parent (uniparental). Angiosperms generally inherit plastids from the mother (as referred to already), while many gymnosperms inherit plastids from the father (paternal inheritance). Algae also show uniparental inheritances. The plastid DNA of the other parent is completely lost in such cases.In normal intraspecific crossings, the inheritance of plastid DNA appears to be a strictly 100% uniparental. But in case of inter specific hybridizations, the inheritance of plastids appears to be different. There are many reports of hybrids of flowering plants

that contain plastids of the father deviating from its normal maternal inheritance. Paternal transmission of ct DNA has been established in many plants by the application of PCR Techniques and using specific molecular markers.

Origin of plastids

The origin of plastids is normally explained by the widely accepted endosymbiotic theory. Plastids are thought to have originated from cyanobacteria. The chloroplasts during the course evolution lost phycobillins, (except in Red, Blue green algae etc) and retained structural components like stroma and grana thylakoids. It is also viewed that the origin of complex plastids might be by secondary endosymbiosis; in which eukaryotes engulf a red or green alga and retains the algal plastid.

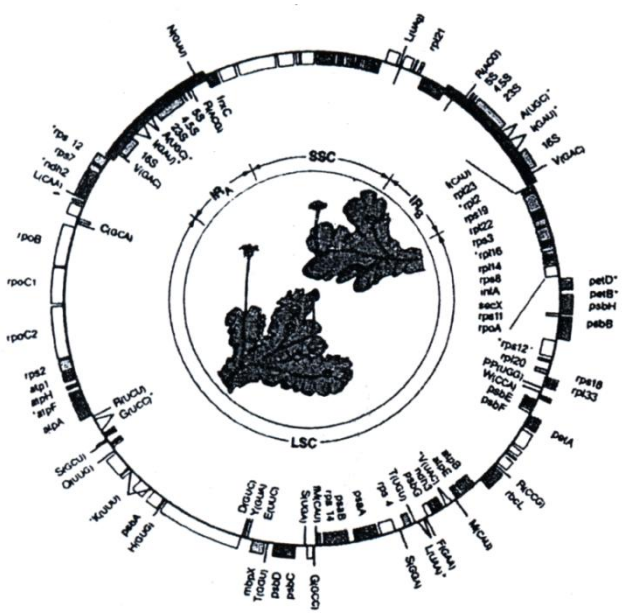


Figure 10.4: An overview of the Chloroplast Genome

Characteristic features

1. Typical size ranges from 120 - 220 kb
2. ct DNA is ten times bigger than mt DNA
3. Typical Number of genes -140 (4 rRNAs, 30t RNAs, 90 protein Coding genes of which 20 code for Photosynthesis and electron Transport).
4. Each chloroplast contains multiple ct DNA genomes
5. Most of the genes required for ct DNA are nuclear encoded.
6. ct DNA contains domains specific to transcription, translation, photosynthesis, electron transport and aminoacids etc.
7. These domains posses genes, enzymes or proteins and machinery to carryout **a** specific function of their own.
8. The respective control units of the domains are indicated by code letters or symbols. Few of them are cited below.

Important Symbols

Translation units

1. rps = 30S ribosmal proteins
2. rpl = 50S ribosmal proteins
3. trnt → RNAs
4. 4-5S, 5S, 16S, 23S ⟶ r RNAS.
5. inf A = initiation factor
6. Sec X = 50S ribosomal protein

Transcription unit

1. rpo = RNA polymerase and mbpx = Permease

Photo Synthesis and Electron Transport units

1. rbc = Rubisco
2. psa = PSI
3. PSb = PSII
4. atp = ATP synthase,
5. pet = Cyt_{borf}
6. ndh = NADH

Each Aminoacid is indicated by single capital letter.

The above information through insufficient, it can provide little insight and an outline view about the structure of ct DNA at micro-level.

Mitochondrial DNA

Mitochondrial DNA (mt DNA) is DNA which is not located in the nucleus (extranuclear) but in the mitochondria. Mitochondria are in the main cell organelle that generates energy in the form of ATP, which drives the machinery of the cell.

Mitochondrial DNA (mt - DNA) was discovered in the 1960's. Arid the advent of r - DNA (recombinant) technology, made it possible, to analyse mt - DNA in great detail. The complete nucleotide sequences of mt DNA of many different species have now been determined.The mitochondria have their own DNA and are assumed to have evolved seperately.

Mitochondrial DNA (mt DNA) is present in mitochondria as a circular molecule and in most species codes for 13 or 14 proteins. These proteins along with extra proteins encoded by nuclear DNA (nu DNA) regulate the electron transport chain of the respiration.mt DNA is typically passed on only from mother, during sexual reproduction (material inheritance) and there is little change in the mt DNA during inheritance from generation to generation, unlike nuclear DNA which changes by 50% each generation.

The circular mtDNA lies in the mitochondrial matrix and occurs in 2 or more copies attached to the inner membrane of the mitochondrion, mt DNA is also found in Ribosomes of its own and can synthesize its own proteins.

Number size, shape and structure

Most mt DNA molecules are circular but in *Chlamydomonas reinhardti* and *Paramecium aurelia* they are "Linear" mt DNA molecules vary greatly in size from 6kb in plasmodium to 2500 kb in some of the flowering plants. Each mitochondrion appears to contain several copies of DNA, and the number of mt DNA molecules per cell is very large because cells usually contain several mitochondria. Invertebrate oocytes, that as many as 10^8 copies of the mt DNA are present. But contrastingly, the somatic cells contain fewer copies ie less than 1000.

The mt DNA genome is a small circular molecule about 16 -18,000 bp (base pairs) in Circumference enclosing 37 distinct genes in most vertebrate species. These genes appear as 13 protein coding regions, two r RNA genes and 22 t RNA genes with a replication control region. In human beings, the mt DNA is 16,659 base pairs long and contains 37 genes like in other vertebrates but with a different genetic organization.

In Fungi the mt DNA is considerably larger than in animals. (19 to 176 kb). Yeast mt DNA contains 75 kb pairs and larger than animal mt DNA. By comparative structural studies of mt DNA through DNA sequence techniques it has been established that plants mt DNA, are generally bigger in size than other organisms. A major fraction of the genetic material in mtDNA, in yeasts and in mammals, is represented by Unidentified FramesURFs).

Most of the yeast URFs are present in introns and code for mRNA splicing proteins and URFs in mammalia code for hydrophobic proteins on the inner membranes.

Table showing structural details of mt DNA in selected organism

Organism	mt DNA (kb pairs)	No. of genomes per organelle
Human (Hela cells)	16.6	2.6
Drosophila	18.4	Unknown
Yeast	75.0	4
Cabbage	218.0	1
Zea mays	570.0	6
Cucumis melo	2500	-

Expression of mt DNA genes (orreplication, transcription and translation)

Mitochondrial replication is controlled by chromosomes in the nucleus. Mitochondrial polymerase is used in the copying of mtDNA during replication. The two strands (heavy (H) and light,

(L)) in the circular mt DNA, have different origins of replication. One strand (H) begins to replicate first displacing the other strand (L). This continues until replication reaches the Origin of replication (ori) on the other strand (L) at which point, the other strand (L) begins replication in the opposite direction. It results in two new mt DNA molecules. The particulars of the above replication can be clearly known by the following diagram of Human mt DNA (showing replication).

During replication, the position of the origin of synthesis of H - Strand is marked by displacement loop (D - Loop). The replication of mt DNA is always unidirectional. It is also described as a symmetric type.In Human mt DNA, H(heavy) strand contains 22 genes for rRNA, 14 genes for t RNA, and 12 genes for phosphorylase enzymes. L (Light) strand contains 8 genes for t RNAs and one gene for phosphorylation enzyme. The two larger transcripts undergo cleavage that separates the tRNAs for rRNAs and mRNAs. Finally mRNA is translated into a polypeptide.Translation steps are similar to typical cytosolic translations, except that some of the codons have a different meaning. The translation mechanism of mt DNA particularly with reference to codons, shows some exceptions, deviating slightly from the Universal Genetic code working pattern.

Genrtic code differences

mRNAs	**In nu DNA (Universal)**	**In mt DNA (Exceptional)**
1) Termination Codons	UAA, UAG,UGA	AGA and AGG
2) AUA codon codes	Isoleucine	Methionine
3) UGA	Acts as a Terminating Codon (Stop Signal)	Codes tryptophan
4) AGA, AGG	Codes Arginine	Act as Termination codons

There are many other variations among the codes used byother mt RNAs.Anotherpeculiarity of plant mt DNA gene expression is mat many mt RNA transcripts undergo editing. Some of the nucleotides may undergo changes in the Post Transcri-ptional stages. The most frequent changes is "C to 'U' and occasionaly 'U' changes to C Thus RNA editing alters the composition of codons in plant mt DNA transcripts.

Yet another peculiarity of mt (DNA) gene expression is the formation of some mRNAs by the process of "trans - splicing". Where some times, certain independently formed transcripts may Join by exons (Splicing or linking).Evidences suggest that the genetic material in mitochondria can also undergo fusion and exchange (like crossing over) Plant mtDNA. In some plants (eg Brassica campestris) the mitochondrial genes may become seperated on to different circular molecules byintra - molecular - recombination method. It is common in mt DNA with repetition sequences. By the above method the MASTER mt DNA circle transforms into two smaller circles as shown in fig 10.6.

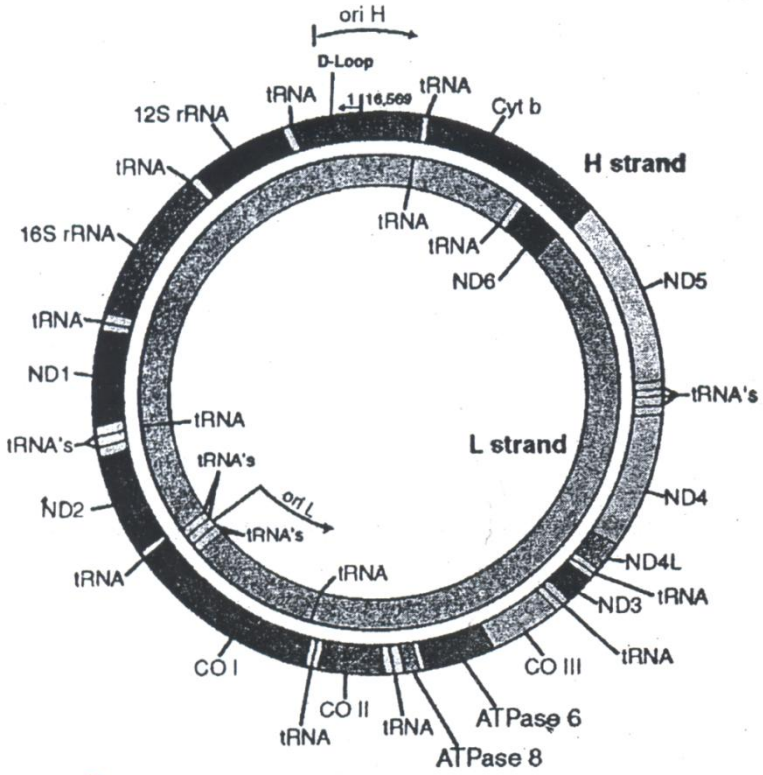


Figure 10.5: Gene map of human mitochondrial DNA, showing the genestranscribed from the heavy (H) and light (L) strands, transcriptionproceeding counterclockwise on H and clockwise on L. Ori H, Ori L,origins of replication; ND1-6, NADH dehydrogenase subunits; Cytb,cytochrome b; COI-W, cytochrome oxidase subunits; tRNA, transferRNA; rRNA, ribosomal RNA

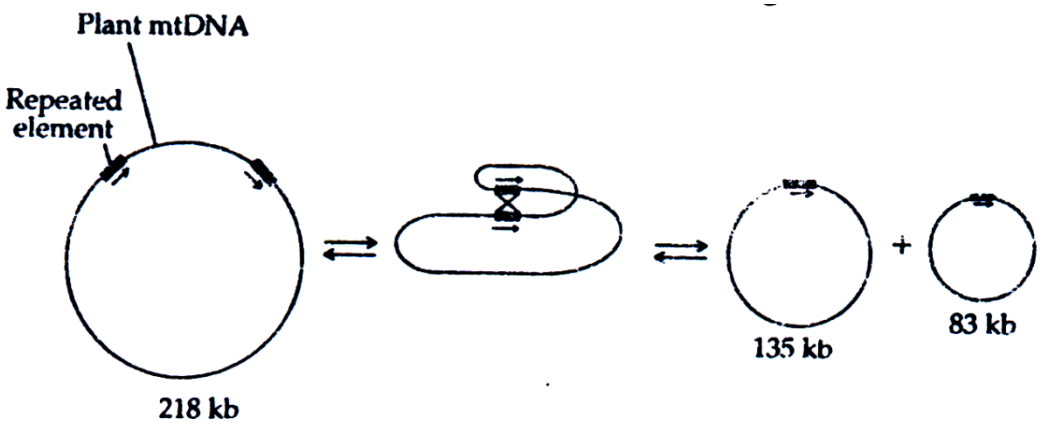


Figure 10.6: Plant mtDNA (Varations)

Mitochondrial inheritance

In mammals, 99.99% of mitochondrial DNA (mt DNA) is inherited from the mother. It means that mutations of mt DNA can be passed from mother to child.The genetic role of mt DNA is revealed now in several organisms like plants and human beings. The studies on various mutants in yeast,

Neurospora etc. helped to understand the mt DNA inheritance. Generally 'Petite mutants in yeast and poky mutant in Nurospora are taken as classical examples to explain mt DNA inheritance.

Petite mutants in yeast fail to grow on Glucose medium and they have a defective aerobic respiratory mechanism on account of the absence (P°) or presence of defective (P") rho (P^+) factor.Poky mutants in Neurospora are without cytochromes a and b types and cytochrome is found in excess compared to that of its wild type. Poky mitochondrial character shows maternal inheritance.

Evidences suggested that the two above mentioned mutant characters are located in mt DNA.Generally Mutations in mt DNA particularly in Mammalia, do cause genetic diseases or genetic illness. These disorders are normally more servere in ageing patients rather in young persons.The one common well known, genetic illness in humans is described as KSS (Kearns - Sayre - Syndrome), which causes a person to lose hill function of their heart, eye and muscle movements.

DNA in mitochondria is used to track certain genetic diseases and used to trace the ancestry of the organisms that contain Eukaryotic cells. The tendency of mt DNA.ie material inheritance pattern, helps to study the above.

Origin of mtDNA

Endosyirsbiotic origin of mt DNA has been suggested on the basis of comparinons of 'protein synthesizing Machinery' and genome sequences of some microbes with those of the cell organelles in question. The endosymbiotic origin of mitochondria from α-proteobacteria appears to be the currently accepted view of many scientists.

Plasmids

J. Lederberg (1952) first proposed the term plasmid" for all extra chromosomal hereditary determinants.Plasmids are self replicative, double stranded, circular DNA molecules that are maintained as independent extra chromosomal entities in bacteria. All bacterial genera virtually have plasmids.

Plasmids are small circular, extrachromosomal DNA molecules found in bacteria. Some times rarely seen in Eukaryotic organisms (eg. 2 - micrometer - ring in *Saccharomyces cerevisiae*).Plasmids are small sized DNA molecules and their size varies from 1 to 400 kilobase pairs. They usually carry only one or few genes and possess a single origin of replication. Plasmids are replicated by the same machinery that replicates the bacterial (Host) chromosome Plasmids are found in numbers ranging from one per cell to hundreds per cell (it is called copy number). Based on this number, plasmids are identified as high (10 - 100 copies per host cell) and low (1 to 4 copies) copy number types.

Some plasmids show specificity to the species of host cell. Plasmids from different incompatibe groups can be maintained together in the same host cell. e.g..seven different plasmids have been found in E. Coli, that are coexisting in a single host cell.

Classification of plasmids

Plasmids are classified into 6 types mainly based on their functional behaviour.

1. Conjugative plasmids:

These types contain 'tra - genes' which perform the process of conjugation which involves the sexual transfer of plasmids to another bacterium e.g F. plasmids. These have high mole. Weight and are present as 1 to 3 copies per cell.

2. Non - Conjugative plasmids:

These types can net initiate conjugation. They can only be transferred with the assistance *of* conjugative plasmids 'by accident'. These have low molecular weight and are present in multiple copies (20 - 25).

3. Resistance Plasmids(R plasmids):

These types contain genes which can build resistance against antibiotics or Toxins.

4. Col - plasmids:

They contain genes that code for colicines, proteins that kill other bacteria.

5. Degrative or Degradative Plasmids:

They contain genes which code for enzymes that degrade toxic substances eg toluene, salicylic acid eg Tol palsmid of Pseudomonas putida.

6. Virulence plasmids:

These turn or convert the normal bacterium into a pathogen (ie they provide pathogenecity) eg. Ti-plasmid of Agrobacterium tumefaciens.

The above classification is arbitrary because some plasmids show properties of more than one functional types of plasmids mentioned above. For example, R - plasmids of some bacteria exhibit drug resistance as well as fertility regulation (ie conjugative property).

It has been noticed that many bacteria have special plasmids with no definite function. Such plasmids are described as 'Cryptic' plasmids.E. Coli and other similar bacteria contain plasmid like structures called Episomes. Episomes are also circular double standed DNA molecules like plasmids. But plasmids and episomes differ from one another in many properties. The major difference is that plasmids replica ie independently in the host cell, where as episome replicate only after its integration in to the host chromosome.

All plasmids do not show the same properties, though they exhibit few common properties like the following.

(1) Smaller size

(2) Circular nature

(3) Containg DNA as genetic elements

(4) Self - replication capacity

(5) Existing as a separate entity from the host chromosome

The other properties *o*fplasmids that are not common to all plasmids are

(1) Control of bacterial conjugation

(2) Transferability nature

(3) Reversible insertion in bacterial chromosome.

Bacteriophages are not included under the category of plasmids on account of their unrestricted replication. But some phages like lambda, (λ) which share many properties of plasmids, are included under plasmid types by some.

Structure of typical plasmid

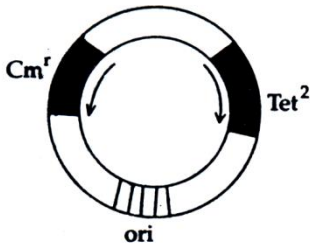


Figure 10.7: p ACYA 184

The following figure is an example of a typical plasmid, ailed pACYC 184 which is 4,240 base pars (or 4.24 kb).This plasmid has 3 'marked' regions on it. Two of them are antibiotic resistance genes, one for Tetracycline resistance *(Te^r)* and rhe other for chloromophenicol resistance (Cm^r). The arrows indicate the direction path of the genes that are transcribed. (5 - 3' directions). The other marked region is the origin of replication (denoted as 'Ori') where the DNA replication machinery assembles an a plasmid.

Genetic map of the plasmid - p rp 4

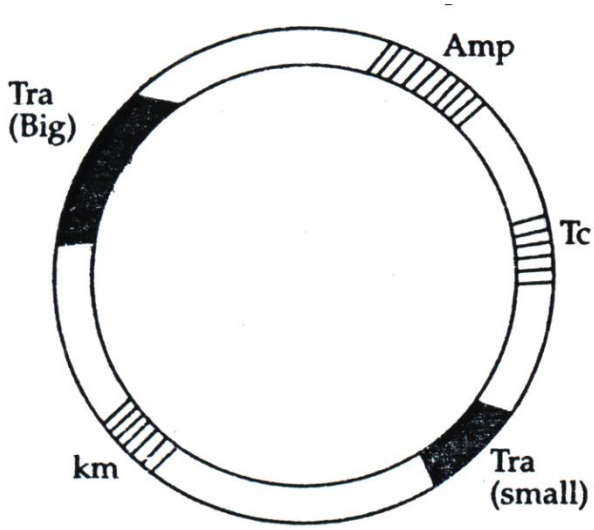


Figure 10.8: Tra - Genes responsible for (Big and small) for Conjugation

Amp = Ampicillin geneKm = Kanamycin geneTc = Tetracyclin gene

Characters of certain important types of plasmids

(1) F - Plasmid:

It is a conjugative type of plasmid. It is also called Fertility factor or Sex Factor. It is described generally with reference to the conjugation process in E. Coli.F - Plasmid gives the character maleness to E. Coli Cells,. When F - plasmid is present as a distinct body, the cells are male (F^+). In the absence of F - Plasmid, the cells are female (F~). Male cells (F^+) are capable of transferring its genes into female cells. F - plasmid is a circular, double stranded DNA molecule containing genes responsible for fertility. F- Factor transfersfrom donor to recipient cell by cell to cell contact. About 1/3 of F plasmid contains 19 transfer genes involved in the transfer of male genetic material into female cells. Among these, some of them are responsible for the formation of sex pili.

F - Plasmid also contains insertion sequences. These are transposable elements coding for the enzyme Transposase.

F - Plasmid also contains genes associated with

i) fertility inhibitor (fih),

ii) phage inhibitor (Phi),

iii) incompatability (inc),

iv) replication (rep),

v) immunity to lethal zygosis (ilz) and

vi) origin of replication (ori) zones.

(2) **R - Plasmid**:

These plasmids confer bacteria resistance to several antibiotics such as streptomycin. Tetracycline chloramophenicol, etc. Sometimes, R - plasmid contains two parts, one part containing genes for resistance and the other responsible for transfer of plasmids. In some cases like penicillin resistance, resistance is not associated with transfers.

(3) **Col - plasmids**:

This plasmid is responsible for the production of toxin colicine (E. Coli, Shigella, Solmonella) which kills other bacteria. Col. plasmid can transfer from one bacterium to the other, like F- Plasmid. Transfer of col. plasmid. can take place between two F - Cells, one carrying col and other lacking it. Some col. plasmids required the aid of F. plasmid for their transfer. Like R - factor, some col - factors have the ability to mobilise the transfer of bacterial chromosome.

(4) **Virulence - Plasmids**:

These plasmids give pathogenecity to the bacteria. Ex Ti - plasmid, *Agrobacterium tumifaciens*.The size of the Ti - plasmid ranges from 150 - 230 kb pairs. A, Ti plasmid, has a T. DNA segment, two border sequences, tragene, a sequence for opine catabolism, and vir genes. T. DNA has a sequence for tumour induction, with two border sequences on either of it. Border sequences are essential for tumour induction. T. DNA contains genes for the synthesis of auxins and cytokinins. Due to these

genes infected tissue goes on dividing producing tumour. During infection T. DNA part is transferred and inserted into the chromosomal DNA of the plant cell.

Agrobacterium tumefaciens causes crown gall disease in several dicot plants Disease causing genes are present in vir -plasmids.Crown of gall formation is the consequence of the transfer, integration and expression of genes of a specific segment of bacterial plasmid DNA - called T. DNA into the plant genome.

Expression of plasmid genes (replication, transfer and recombination)

Like that of Bacterial gene, the DNA plasmid also replicates in a semiconservative fashion. Plasmid genes show control only on the initation phase of replication. The other following phases genes. Plasmid DNA can transfer from one bacterium to other as a single strand that can replicate inside the recepient cell.

Plasmid DNA is also capable of undergoing recombinations either with another plasmid or with bacterial DNA. Homologous insertion sequences in bacterial DNA and plasmid DNA help in recombinations.

Important plasmids

pBR322 and Ti plasmids are the two best known plasmids.

pBR 322:

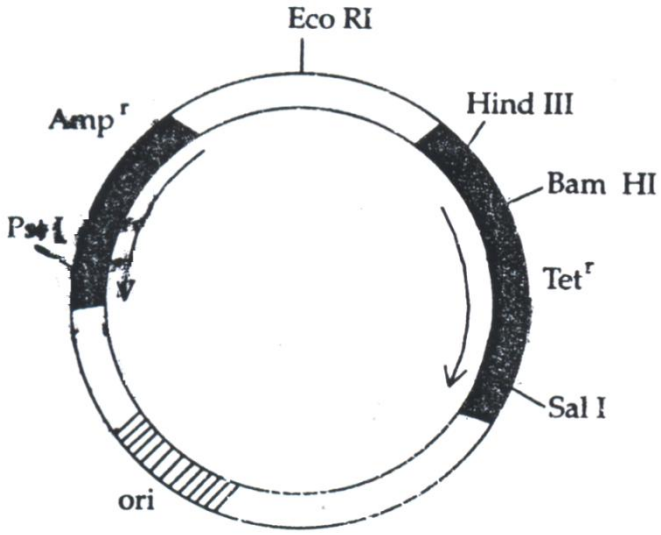


Figure 10.9: Genetic map of PBR^{322}

AMP^{r} = Ampicillin resistance; Tet^{r} = Tetracyclin resistance; E. CoRI = Restriction Endinuclease; ori = Origin of replication; PSA I, Hind III, Base HI, Sal I are recognition sites

This plasmid carries genes for resistance to ampicillin and tetracyclin which act as markers for the selection and identification of clones carrying the plasmid. pBR 322 contains sites for restriction

endonucleases. These allow the insertion of DNA fragment. This plasmid has low molecular weight and 4.3 kb pairs long. This plasmid is mainly used as a vector in gene cloning techniques. Only modified forms of pBR 322 types are employed as cloning vehicles.

Ti plasmid:

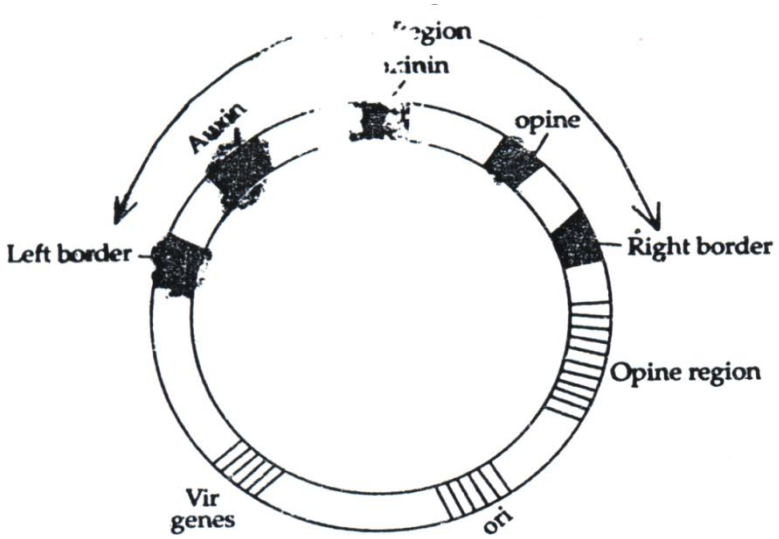


Figure 10.10: Ti plasmid – Schematic Representation

These are found in tumour cells produced by Agrobacterium tumifacience. Ti plasmids act as natural gene vectors, which can transfer, incorporate and express the genetic information in the plants.

Most of the genes with in the T - DNA region are activated only after T- DNA is inserted into the plant genome.The two plasmids (pBR322, and Ti plasmids) that are described before are used in genetic engineering as vectors. They are used to transfer genes from one organism to another.

Role of plasmids

Several plasmids, in addition to the above two, serve as important tools in genetics and biochemistry. They are used to multiply or express genes of interest.Another major use of plasmids is to make large number of copies of a particular gene by which several desired products can be generated, eg. Insulin, antibiotics.

The development of techniques of DNA transfer to plant by using Ti plasmid of Agrobacterium in particular and other plasmids as vectors.in general, is an important land mark in plant genetic engineering.

Plasmids which contain a fragment of DNA including Co3 sites (cohesive termini) are called cosmids. The cosmids are used as vectors and they are considered as excellent tools for cloning" large DNA fragments.

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Cell Biology and Cytogenetics: Volume I)

Book at Glance

Cytogenetics, in cell biology, field that deals with chromosomes and their inheritance, particularly as applied to medical genetics. Chromosomes are microscopic structures found in cells, and malformations associated with them lead to numerous genetic diseases. Chromosomal analysis has steadily improved
in precision and resolution, and that has led to improvements in the diagnosis of various genetic diseases in all areas of medicine. The study of chromosomes begins with the extraction of intact chromosomes from live cells.

The book is written in simple language so that the students can easily grasp the matter. Some important terms has been incorporated, so that the students may search the useful related for competitive examinations. In the recent years included in the syllabus of almost all Indian Universities in various subjects of Biology or Life Sciences as an independent evergreen subject. Exponential growth in many areas of basic fundamentals made it necessary in some cases to write several chapters on the same topic which was covered in a single chapter in the earlier book. Similarly, in the present volume, separate new chapters have been written on topics which in the earlier title either did not figure at all or were each covered very briefly as a part of a chapter.

This book is intended to be an accessible introduction to the cell biology and cytogenetics for junior or senior undergraduate students who have already had an introduction to biological sciences. This engaging and stimulating text focuses on detailed aspects in cell biology.

About the Author

Dr. M. Venkateshwarlu working as an Assistant Professor CUC , KU at University Post Graduate College, Department of Botany Kakatiya University, Warangal, Telangana since 2001. He is also serving his academic guidance at Diploma in Sericulture Botany and Integrated Chemistry of Kakatiya University, Warangal. He completed his Ph. D. in Botany in the year 1999 from Kakatiya University, Warangal. He has 20 years of research experience in the area of Plant Tissue Culture, Plant Biotechnology, Cytology, Genetics, Mutation Breeding and Molecular Biology. Dr. M. Venkateshwarlu published more than 90 research articles in various national and international journals. He attended and presented his research work in more than 70 national and international conferences. He is life member of various societies like Academy of Plant Sciences, Indian Botanical Society, Jaipur and Honorary fellow of Society for Sciences. He is working as editorial member of many journals in India and abroad. He was special interest in social work especially in plantation.

Bhumi Publishing

ISBN 978-93-88901-16-1