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THE PHYSICAL BASIS OF HEREDITY

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Course Design Committee

Prof. M.S. Nathawat
Former Director, School of Sciences
IGNOU, Maidan Garhi, New Delhi-110068

Prof. S. S. Hasan (Retd.)
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Prof. Jaswant Sokhi (Retd.)
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Dr. A.K. Bali
Associate Professor in Zoology
Bhaskaracharya College of Applied Sciences
Sector 2A, Dwarka, New Delhi-110075

Dr. S.K. Sagar
Associate Professor in Zoology
Swami Shraddhanand College, Alipur Village
University of Delhi, Delhi- 110036

Dr. H.S. Pawar
Scientist D, NIMR,
Sector 8, Dwarka, New Delhi-110077

Dr. Ranjana Saxena
Associate Professor in Zoology
Dyal Singh College, Lodhi Road, New Delhi-110003

Prof. Sarita Sachdeva
Head, Department of Biotechnology
Manav Rachna International Institute
of Research and Studies, Faridabad, Haryana-121004

Prof. Neera Kapoor
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Prof. Amrita Nigam
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Dr. Nisha
Consultant, School of Sciences
IGNOU, New Delhi-110068

Block Preparation Team

Prof. Sarita Sachdeva
Head, Department of Biotechnology, Manav Rachna
International Institute, of Research and Studies
Faridabad, Haryana-121004 (Units 5 and 7)

Prof. Abhilasha Shourie
Department of Biotechnology
Manav Rachna International Institute of Research and
Studies, Faridabad, Haryana-121004 (Unit 6)

Dr. Archana Bahuguna
Scientist E, Zoological Survey of India
Dehradun-248195 (Unit 8)

Dr. A.K. Bali
Associate Professor in Zoology
Bhaskaracharya College of Applied Sciences
Sector 2A, Dwarka, New Delhi-110075 (Unit 9)

School of Sciences

Prof. Neera Kapoor (Units 5 to 9)
Prof. S.S. Hasan (Units 5 and 8)
Dr. Nisha (Units 6, 7, and 9)

Course Coordinator	:	Prof. Neera Kapoor
---------------------------	---	---------------------------

Course Editor	:	Prof. P.C. Joshi Professor & Head, Department of Zoology Gurukul Kangri University, Haridwar Uttarakhand-249404
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Production

Mr. Hemant Kumar, SO (P), MPDD, IGNOU

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BLOCK 2: THE PHYSICAL BASIS OF HEREDITY

In the first block you have studied the fundamental concepts of genetics. The main emphasis being on the principles and processes governing heredity. By now, we presume you are well equipped to study and comprehend more specific aspects of heredity that will be the subject matter of this block.

This block consists of five units, Units 5 to 9, dealing mainly with the elements controlling the inheritance process. These have been discussed in the light of transmission and molecular genetics.

The chromosome aberrations, that is, abnormalities in chromosomal structure and number are described in Units 5 and 6 respectively. In recent years such studies have gained utmost importance as more and more evidence indicate their involvement in a number of human inherited disorders.

Unit 7 explores further one of the major exceptions to Mendelian genetics – sex linkage. This is discussed by considering several examples in inheritance of genes located on the X and Y – the sex chromosomes. The dosage compensations mechanism that equalises the X-linked gene activity in both the sexes is also discussed. The genetic basis of development with respect to the differentiation of sexes is described in Unit 7. It explains the role of genetic information in early development in normal instances, and also reviews the occurrence of developmental mishaps leading to a path of anomalous development.

In Unit 8 we begin the study of a branch of genetics that has revolutionised the science of biology in its entirety – the molecular genetics. The chemical nature and structure of genetic material were proposed by James Watson and Francis Crick in 1953 in a two page paper they published in *Nature* under the title “Molecular structure of nucleic acids”. The deduction of the structure and chemistry of DNA, along with the input of countless insights and discoveries of molecular nature, transformed the science of genetics.

In Unit 9 we discuss the mechanism that generates variability in genetic materials, namely the mutations. Mutations along with recombinations form the source of variability from which the selection process promotes adaptations to suit specific environments. In this unit we discuss the different types of mutations. There is a discussion on the rates at which the mutations occur. Many mutations are spontaneous and random events. Mutations can also be induced by using mutagenic agents. The unit on ‘Mutations and Mutagenesis’ further describes the various agents that would induce mutations in the genetic material.

Objectives

After studying this block, you should be able to:

- describe the techniques employed to study the various aspects of human chromosomes,
- describe the normal human chromosome at morphological as well as molecular level,
- recognise and explain the various kinds of structural chromosomal anomalies in humans and their resultant effects,
- recognise and explain the different forms of numerical chromosomal anomalies in humans and their resultant effects,

- discuss the nature and structure of genetic material,
- describe the mechanisms of recombination and complementation in a bacteriophage,
- describe the different types of mutations that arise in the genetic material and the rates at which they occur, and
- distinguish between the spontaneous and induced mutations and list the various mutagenic agents that would induce mutations.



UNIT 5

STRUCTURAL ABNORMALITIES IN CHROMOSOMES AND THEIR EFFECTS

Structure

- | | |
|---|---|
| 5.1 Introduction | 5.4 Duplications |
| Objectives | Duplications are less Harmful than Deletions |
| 5.2 Structural Abnormalities In Chromosomes | Unequal Crossing Over in Human Red-Green Colour Blindness |
| Natural Variation Exists in Chromosome Structure | Duplications Provide Additional Material for Gene Evolution |
| Classification of Chromosome on Basis of Location of Centromere | 5.5 Inversion |
| Mutations can alter Chromosome Structure | Inversions do not have any Phenotypic Consequences |
| 5.3 Deficiencies/Deletions | Inversion Heterozygotes can Produce Abnormal Gametes |
| Deficiencies tend to be Detrimental to an Organism | 5.6 Translocations |
| Detection of Deficiencies using Cytological, Genetic and Molecular Techniques | 5.7 Summary |
| Isochromosomes | 5.8 Terminal Questions |
| Ring Chromosomes | 5.9 Answers |

5.1 INTRODUCTION

In the Unit 5 you have learnt about Chromosome Mapping. As you know, in a given cell of an organism, the number of chromosomes is generally fixed. It ranges from one in bacteria to hundreds in some plants and animals. Most organisms are diploids ($2n$) since their somatic cells have a chromosome

complement consisting of two homologous sets. However, naturally a few species have only one set of chromosomes, and they are called haploid or monoploid ($1n$). Gametes produced in diploid organisms as a result of meiosis and gametogenesis also contain the haploid number of chromosomes. In plants, surprisingly, condition of polyploidy has been observed i.e.; complete sets of chromosomes exceed two. On the other hand, there are also conditions called aneuploidy, wherein cells or individuals have one, two, or a few chromosomes either absent or in excess of the basic number for the given species. There are several important reasons to study chromosomal variations. Geneticists have found the variations in structure of chromosomes that have led to cause major effect on phenotype in an organism. Also, an individual can show normal phenotype and yet possess chromosomal rearrangement with high probability of producing offspring with genetic abnormality. Finally, changes in the chromosomal structure have been an important force in evolution of new species. We are, therefore, to discuss structural abnormalities in chromosomes and their effects on the organisms.

Objectives

After studying this unit, you will be able to:

- ❖ explain the normal variation that exists in chromosome structure,
- ❖ describe the concepts underlying the structural abnormalities,
- ❖ analyse the influence of various anomalies that affect genes and phenotype,
- ❖ distinguish among different structural abnormalities, and
- ❖ explain the mechanisms leading to structural abnormalities.

5.2 STRUCTURAL ABNORMALITIES IN CHROMOSOMES

Variations in the structure and number of chromosomes have been observed in natural populations and can occur in variety of ways. These differences are due to chromosome anomalies and one or more individual chromosomes can be structurally altered into a variety of rearrangements. Such changes are called **chromosome aberrations**. In addition, deviations from haploidy in sex cells, diploidy in somatic cells, and normal chromosome structure are not rare. In history of genetics, chromosomal abnormalities were originally discovered through their genetic effects, which might be confusing at first but were investigated later on and were confirmed subsequently by observing under the microscope. Not surprisingly, even small changes in the chromosome segments, have profound consequences on normal gene expression, leading to severe developmental and physiological abnormalities.

5.2.1 Natural Variation Exists in Chromosome Structure

Before we try to understand as to how chromosome structure is altered, it is important to have a reference point for a normal set of chromosomes. In

order to determine what the normal chromosome of a species look like, cytogeneticists have microscopically examined the chromosome from several species. In most cases, two phenotypically normal individuals of the same species have the same number and types of chromosomes.

Chromosomal composition of a species can be determined by karyotyping the chromosomes in actively dividing cells. A **karyotype** is a micrograph in which within a single cell, all chromosomes have been arranged in a standard fashion. By studying a karyotype, one can understand that for any given species, there is considerable variation in size and shape. There are various ways to classify and identify chromosomes. The three most commonly used features are:

- Size
- Location of the centromere and
- Banding patterns revealed on staining

By convention, the chromosomes are numbered according to their size, with the largest chromosomes having the smallest number (Fig. 5.1). However, an exception to this system are the sex chromosomes, that are designated with letters (for humans X and Y).

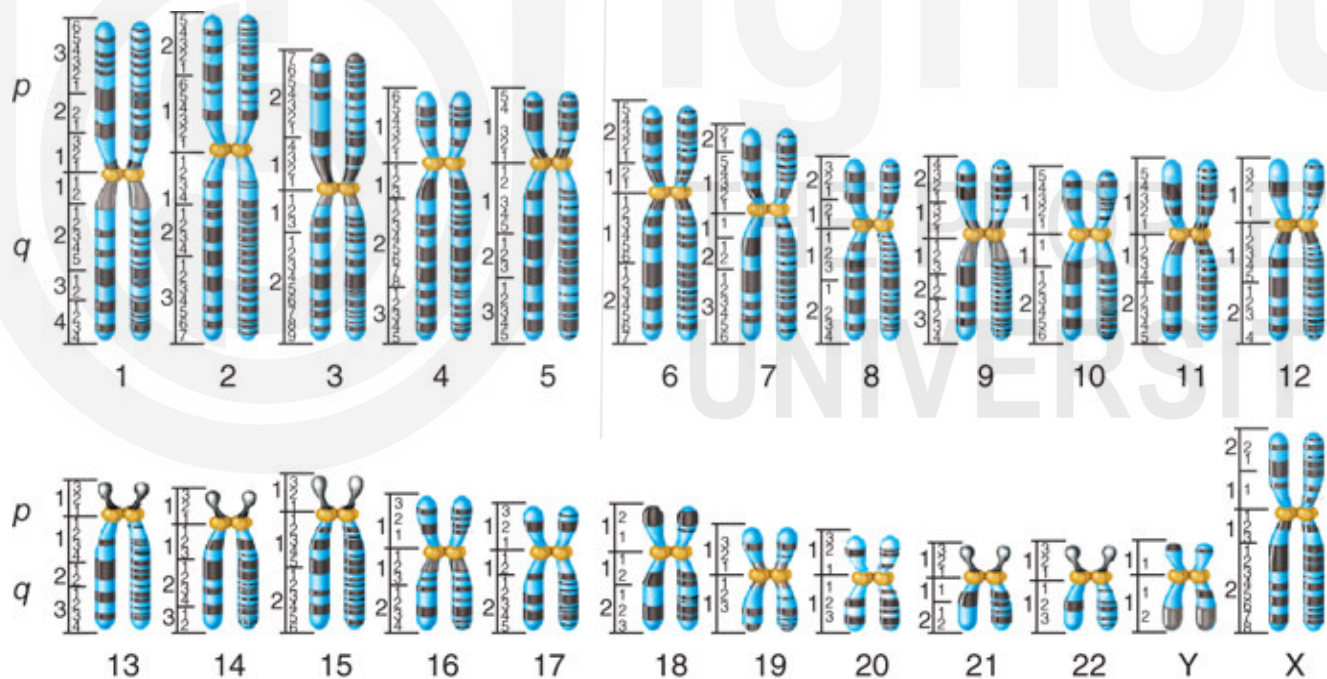


Fig. 5.1: Conventional numbering system of G bands in human chromosomes.

5.2.2 Classification of Chromosome on the Basis of Location of Centromere

In eukaryotic chromosomes, a distinguishing feature is the location of the centromere and accordingly they are classified as:

- **Metacentric** – In this type, centromere is in middle, which means p and q arms are of comparable length e.g. chromosomes 1, 3, 16, 19, 20.

- **Submetacentric** – In this type, there is centromere off-centre, leading to shorter p arm relative to q arm e.g. chromosomes 2, 4 - 12, 17, 18, X.
- **Acrocentric** – In this type, centromere is severely off-set from centre, leading to much shorter p arm e.g. chromosomes 13 - 15, 21, 22, Y.
- **Telocentric** – In this type, centromere is found at the end of chromosome, meaning no p arm exists and such chromosomes are not found in humans.

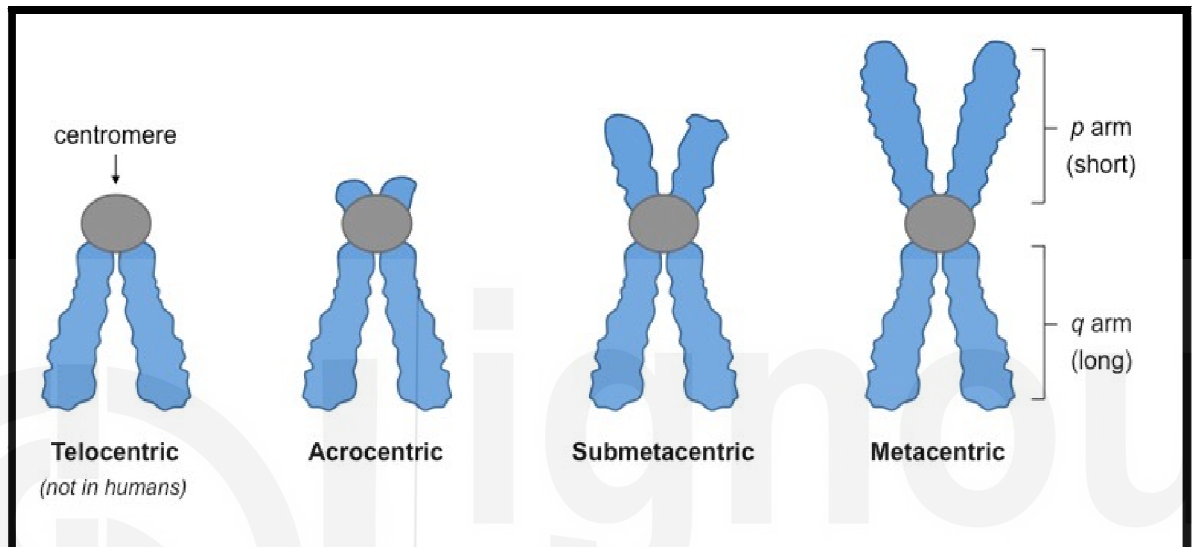


Fig. 5.2: Chromosome classification based on centromere location.

It is noticed that the location of the centromere on the chromosome (Fig. 5.2), is not exactly the centre, each chromosome has a long arm designated with letter “q” (**q stands for-queue**) and a short arm designated with the letter “p” (**p stands for petit**). But in case of telocentric chromosomes, the short arm may be almost nonexistent. Hence, when we prepare a karyotype, the chromosomes are aligned with the short arms on top and the long arms on the bottom. Since different chromosomes often have at times similar sizes and centromeric locations (for example, in humans, chromosome 8, 9 and 10). Therefore, some additional methods to accurately identify each type of chromosome is need of the hour. This is taken care of by staining the chromosomes with chemical dyes e.g. **Giemsa stain** to produce characteristic banding patterns. The stain binds heavily and produces a dark band in some regions and in others it hardly binds at all, producing a light band. **This alternating banding pattern known as G bands, is a unique feature for each chromosome (Fig. 5.3).**

The banding pattern of eukaryotic chromosome is useful as individual chromosomes which can be distinguished from each other, even if they have similar sizes and centromeric locations. Banding patterns are also used to detect changes in chromosome structure. Also chromosome banding may reveal evolutionary relationships among the chromosomes of closely related species.

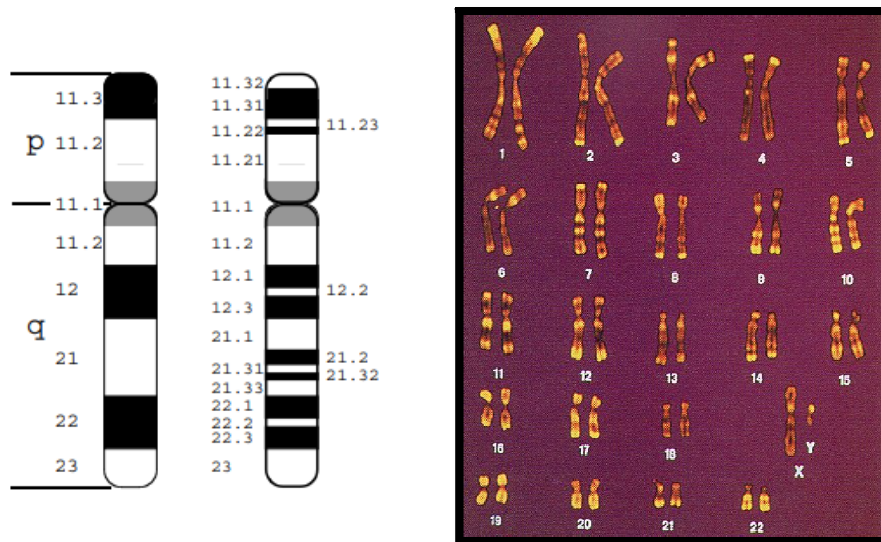


Fig. 5.3: Banding Pattern and Giemsa staining of Human chromosomes.

5.2.3 Mutations can alter Chromosome Structure

Once we understand that normal chromosomes can exist in a variety of shapes and sizes, there is a need to understand how the structure of normal chromosomes can be altered or modified. **A chromosome mutation is a substantial change in the structure of the chromosome and accordingly it stands altered.**

There are several principal types of structural aberrations, each of which has characteristic genetic effects. However; there are two primary ways in which the structure of chromosomes can be altered:

1. The total amount of genetic information in the chromosome can change in the following ways i.e.:

Decrease: Deficiencies and Deletions.

Increase: Duplications and Insertions.

2. The genetic material may remain the same, but is rearranged as in **Inversions** and **Translocations**.

In this section it is important to consider that how these changes occur, how these changes are detected experimentally and as to how they affect the phenotypes of the individuals who inherit them.

SAQ 1

Fill in the blanks:

- i) Chromosomal composition of a species can be determined by the chromosomes in actively dividing cells.
- ii) The location of the on the chromosome is not exactly the centre.

- iii) Banding patterns are used to detect changes in structure.
- iv) Chromosome may reveal evolutionary relationship among chromosomes of closely related species.
- v) A chromosome is a substantial change in the structure of the chromosome.

5.3 DEFICIENCIES/DELETIONS

Changes occur in the total amount of genetic material within a single chromosome. When a deficiency occurs, a segment of chromosomal material is missing i.e. in other words it means, affected chromosome is deficient of significant amount of genetic material (Fig. 5.4).

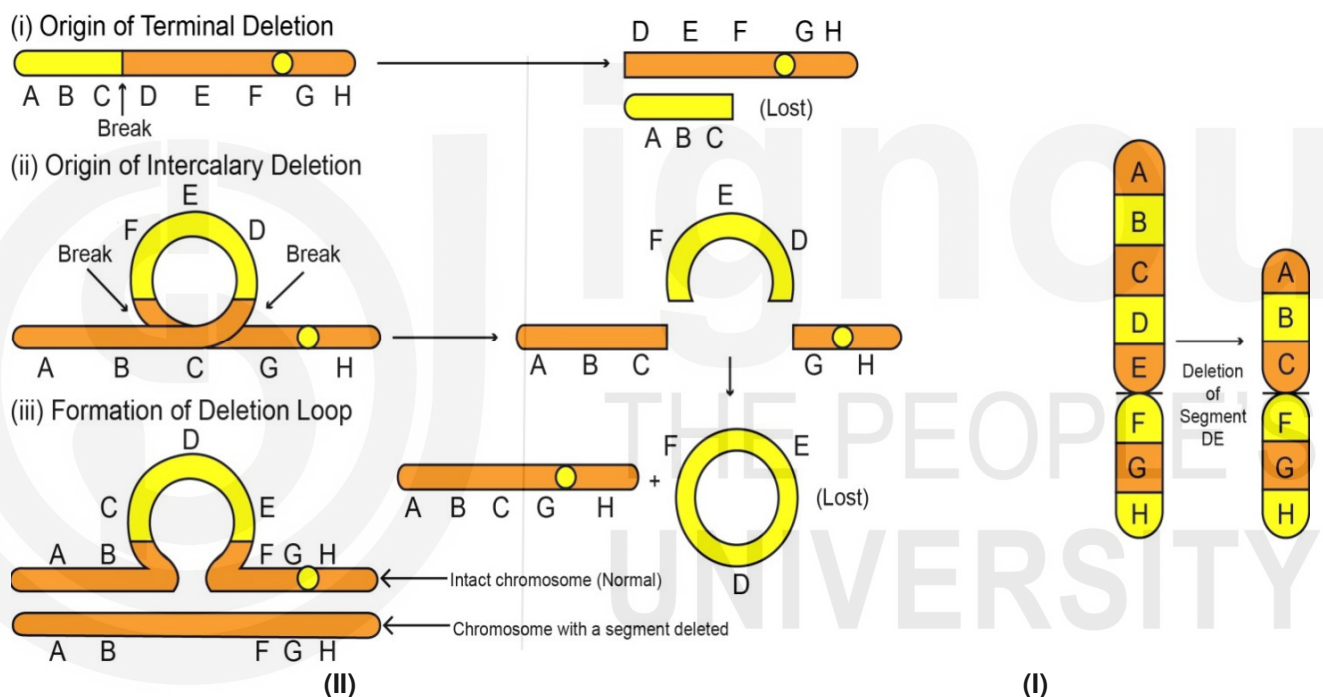


Fig. 5.4: (I) A chromosome showing deletion of a segment, that is, D, E. (II) Chromosome showing Terminal (i) and interstitial (ii) deletions (iii) Formation of deletion loop.

5.3.1 Deficiencies tend to be Detrimental to an Organism

A chromosomal deficiency will take place when a chromosome breaks in one or more places and a fragment of it is lost. The piece without the centromere is the one which will be lost and degraded, therefore, this event produces a chromosome with **terminal deficiency**. Further, if a chromosome gets broken at two places, it will produce three chromosomal fragments, the central fragment may become lost and get degraded, and the two outer pieces may get re-attached to each other. This process will create a chromosome with **interstitial deficiency**. Further deficiencies can also be created when recombination takes place between two homologous

chromosomes at incorrect locations and is called **ectopic recombination**, this results in products with one chromosome having deficiency and another chromosome with duplication.

The phenotypic consequences of this type of event depend on size of deletion and also whether it includes genes vital to the development of the organism. Hence when deletions have a phenotypic effect they are usually harmful to the organism and the usual rule is that larger the deletion, the greater is the harm. Very large deletions are usually lethal even when heterozygous with a normal chromosome. **Small deletions are often viable when they are heterozygous with a structurally normal homolog, because the normal homolog supplies gene products that are essential for the organism to survive. At the same time even small deletions can be homozygous lethal i.e. when both members of a pair of homologous chromosomes carry the deletion.**

Many examples are known where deficiencies produce phenotypic influences. In humans a genetic disease known as ***cri-du-chat syndrome*** involves a deficiency in a segment of the short arm of human **chromosome 5**. Individuals who carry this abnormal chromosome (single copy) along with normal chromosome 5 display an array of abnormalities which include severe mental retardation, facial anomalies and an unusual cat like cry. Some other human genetic diseases are **Angelman syndrome** and **Prader-willi syndrome** and are due to deficiency in **chromosome 15** (Fig. 5.5).

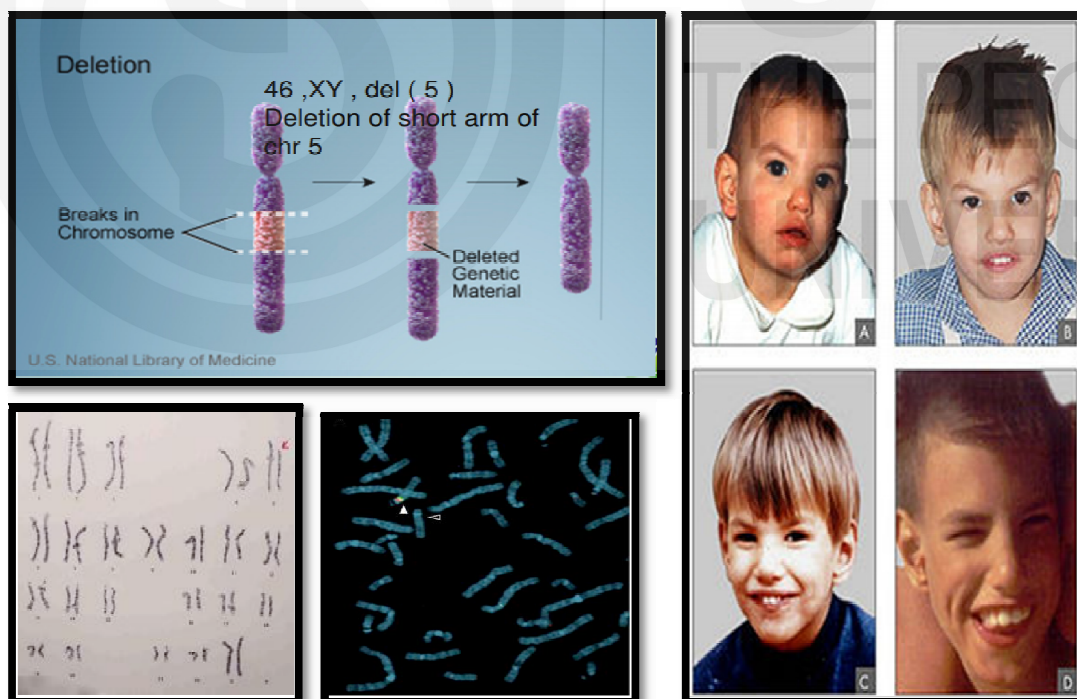


Fig. 5.5: Deletion: Cri-du-chat syndrome, Angelman syndrome & Prader-willi syndrome.

Prader-Willi syndrome (PWS)

Prader-Willi child is often talkative and friendly; and is especially prone to stubbornness, argumentativeness, irritability, and verbal and physical aggression. Other features include perseveration (the repeated and often

uncontrolled repetition of a phrase or gestures), mild obsessive-compulsive rituals, intolerance of a change in daily routine, and sleep problems (PWS people often require several naps during the day).

PWS children usually develop insatiable appetites by age of 3 to 7 years and are doubly disadvantaged because they require fewer calories than normal to gain weight. They often spend considerable time foraging for food and sequestering large amounts of it, and result in life-threatening obesity. Cognitive development is delayed. The average IQ is around 65, PWS can result in anything from severe mental retardation to IQ well within the low normal range.

Angel man syndrome (AS)

These cases are often normal appearing at birth, the disorder being diagnosed after the child fails to develop appropriately. AS cases are characterized by hyperactivity, attention problems, unusual happiness, and a failure to speak. Infants often express persistent social smiling as early as the first trimester after birth. Soon they begin to laugh, often uncontrollably, at the proverbial drop of a hat and, in many cases, for no discernible reason. Such cases exhibit a striking disparity in their understanding versus expression of language. Even as adults few AS people have a vocabulary exceeding ten words, and they often use their few words indiscriminately and without symbolic use. Adults may develop nonverbal communication skills by pointing, gesturing, and signing, with rudimentary communication and enjoy socializing and participate in the daily activities of their families.

In humans, many types of cancers are also associated with chromosomal deficiencies.

Generally genes have two or more alleles. If both alleles of a diploid organism are **same**, the organism is homozygous at that locus. If they are different, the organism is **heterozygous** at that locus. If one allele is missing, it is **hemizygous**.

5.3.2 Detection of Deficiencies using Cytological, Genetic and Molecular Techniques

There are varieties of techniques used to detect deletion within a chromosome. Microscopic techniques are relatively quick and easy and can detect chromosomal deficiencies large enough to be seen within a light microscope. However, small deletions which involve only one or few genes cannot be detected with simple microscopy but high end microscopy techniques such as *in situ* hybridization can detect such small deletions. In addition, at times deletions can be revealed by a phenomenon known as **pseudodominance**. This occurs when one copy of a gene has been deleted from a chromosome and the remaining copy of a recessive allele on the homologous chromosome is phenotypically expressed. **In such cases the individual is hemizygous for the recessive allele.**

5.3.3 Isochromosomes

Isochromosome is a chromosome with two identical arms. Instead of one long arm (q) and one short arm (p), an iso-chromosome has two long or two short arms thereby having an extra copy and missing copy of other genes.

In maize, Creighton and McClintock have found that small deficiencies are viable even in the homozygous state. A special kind of single break occurs through the centromere of a metacentric chromosome giving rise to two isochromosomes with terminal telomeres (Fig. 5.6). Such an event is also called **misdivision of centromere**.

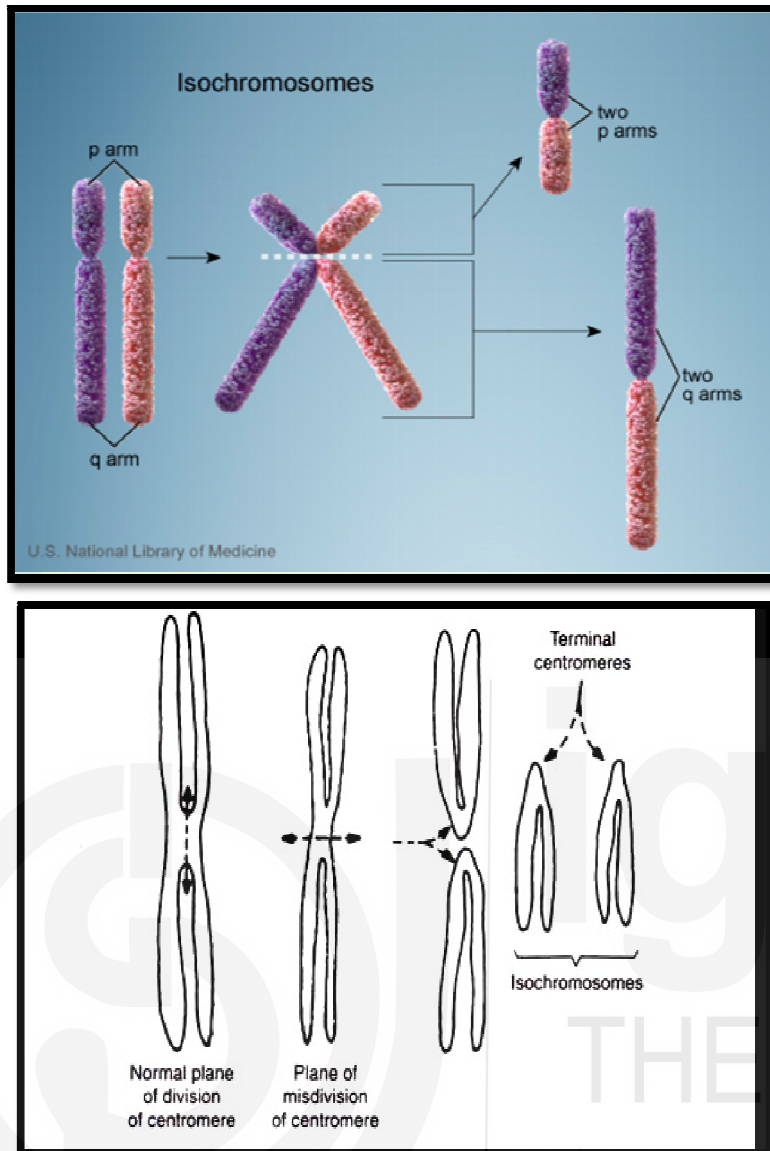


Fig. 5.6: Isochromosomes: 46, X, I (Xq) in Turner Female and mechanism.

5.3.4 Ring Chromosomes

Ring chromosomes are uncommon, however, inheritance has been reported, but the majority of ring chromosomes are *de novo*. They have been reported for all human chromosomes and are known to cause multiple congenital anomalies and mental retardation. It is a general belief that they result from **distal breakage of the short and long arm of a chromosome and rejoining of the ends**. The ring may or may not include the chromosome's constriction point (centromere). In many cases, the genetic material near the ends of the chromosome is lost. There are also reports with no apparent deletion of the telomeric ends, thus resulting in complete ring chromosomes. Individuals with ring chromosomes often exhibit a general overlap in phenotype, due to which the term '**ring syndrome**' has been coined (Fig. 5.7).

The formation may occur through breaks in the chromosome arms and fusion of the proximal broken ends, leading to loss of distal material. Rings may also be formed by telomere dysfunction triggering fusion of reactive chromosome ends without major loss of genetic material.

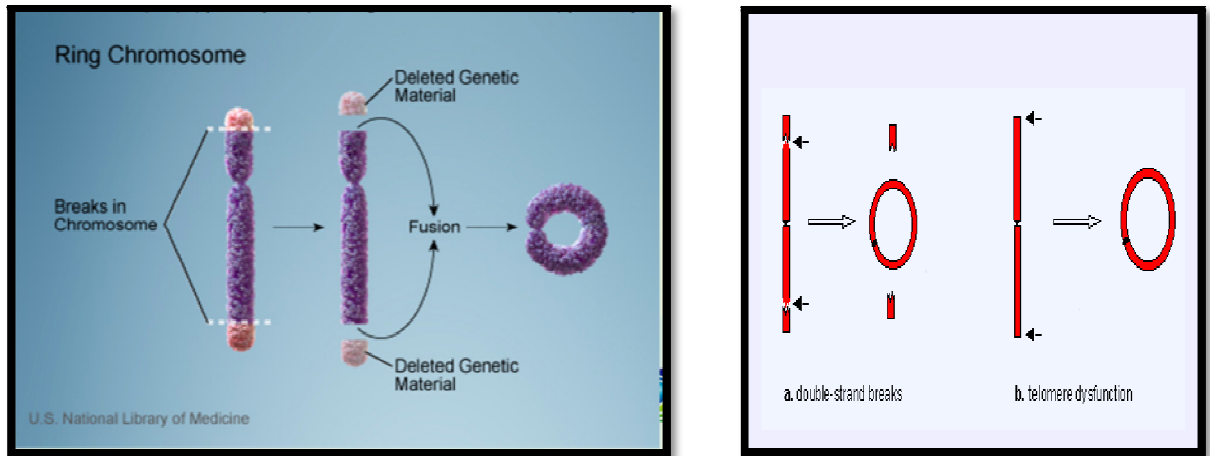


Fig. 5.7: Ring Chromosome formation, mechanism & syndrome.

Growth failure with no or minor anomalies is found to be the major abnormality in patients with complete ring chromosomes and is thought to be the result of cell death during development. Indeed, sister chromatid exchange during the cell cycle may cause mechanical interference of the cell division because of disruption, breakage, entangling or doubling of rings, resulting in aneuploidy and possible death of the daughter cells. These chromosomes come to clinical attention either in association with developmental anomalies at the beginning of life or with telomere shortening in ageing and neoplastic cells.

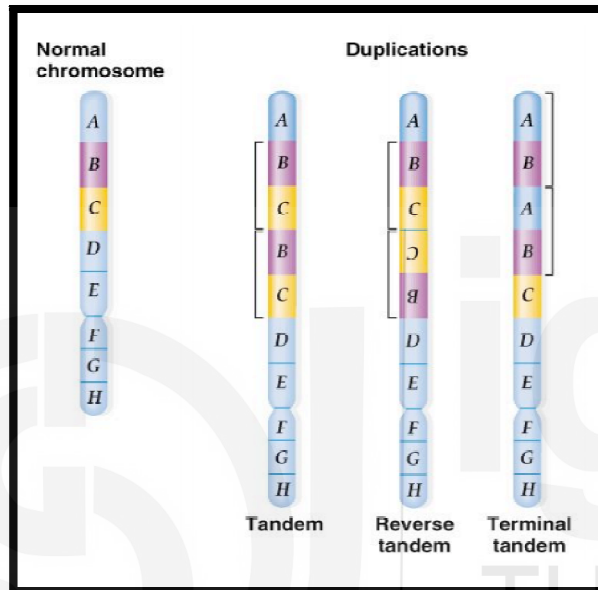
SAQ 2

Chose the correct option given in the parentheses.

- i) When a (deficiency/addition) occurs a segment of chromosomal material is missing.
- ii) Some (animal/human) genetic diseases like Angelman syndrome and Praderwilli syndrome are due to deficiencies in chromosome.
- iii) (PWS/AS) children usually develop insatiable appetites by the age of 3-7 years.
- iv) Even as adults few AS people have a vocabulary exceeding more than (ten/twenty) words.
- v) Isochromosome is a chromosome with two (identical/dissimilar) arms.
- vi) Individuals with (ring/iso) chromosomes often exhibit a general overlap in phenotype.

5.4 DUPLICATIONS

Duplications are the result of addition of genetic material due to abnormal events during recombination crossing over. Under normal circumstances this occurs at analogous sites between homologous chromosomes. On rare occasions the crossover can occur at two different sites on the homologues. This creates one chromatid with an internal duplication and another chromatid with a deletion. In such events the chromosome with extra genetic materials carries gene duplication because the number of copies of a particular gene has been increased from one to two. In most cases this is rare and sporadic event during evolution of species (Fig. 5.8).



- Tandem duplications are adjacent to each other.
- Reverse tandem duplications result in genes arranged in the opposite order of the original.
- Tandem duplication at the end of a chromosome is a terminal tandem duplication

Fig. 5.8: Duplication and its types.

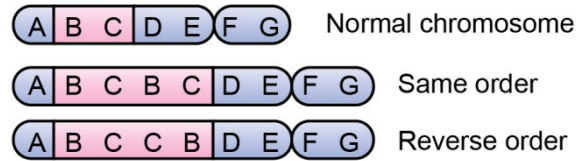
5.4.1 Duplications are Less Harmful than Deletions

Duplications can also produce phenotypic consequences if they involve a large piece of chromosome. In general, small duplications are less harmful. Duplication of chromosome segment can be brought about by addition at any one of the following positions (Fig. 5.9):

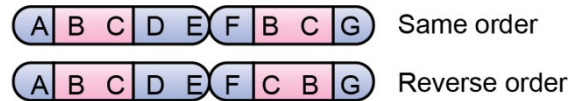
- i) In adjacent region
- ii) At a displaced position of the same arm
- iii) On different arm of the same chromosome or
- iv) On a different chromosome

Sometimes the duplications may be found as a reverse repeat.

Tandem duplications



Nontandem (dispersed) duplications



Chromosome breakage can produce duplications

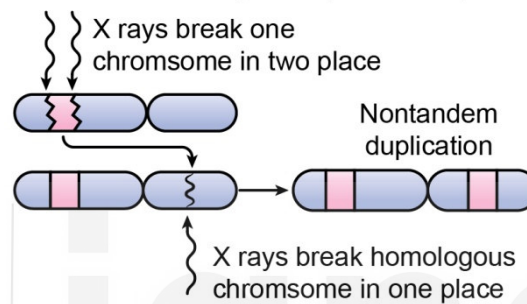


Fig. 5.9: Generation of Duplication.

A classical example of duplication was observed from studies in *Drosophila* (fruit fly) involving a trait that affects the number of facets in the eye. It was found that the fly had a reduced number of facets and this was called Bar eye. A genetic analysis of the trait has revealed that it is X-linked trait which shows incomplete dominance (Fig. 5.10). Bar eye is a character where eyes are narrow as compared to normal eye. By studying the structure of gigantic polytene chromosomes it could be demonstrated that “**Bar**” character was due to duplication in the region 16A of X chromosome and is a case of **tandem duplication** which is when duplicated segment is present in the same orientation immediately adjacent to the normal region in the chromosome.

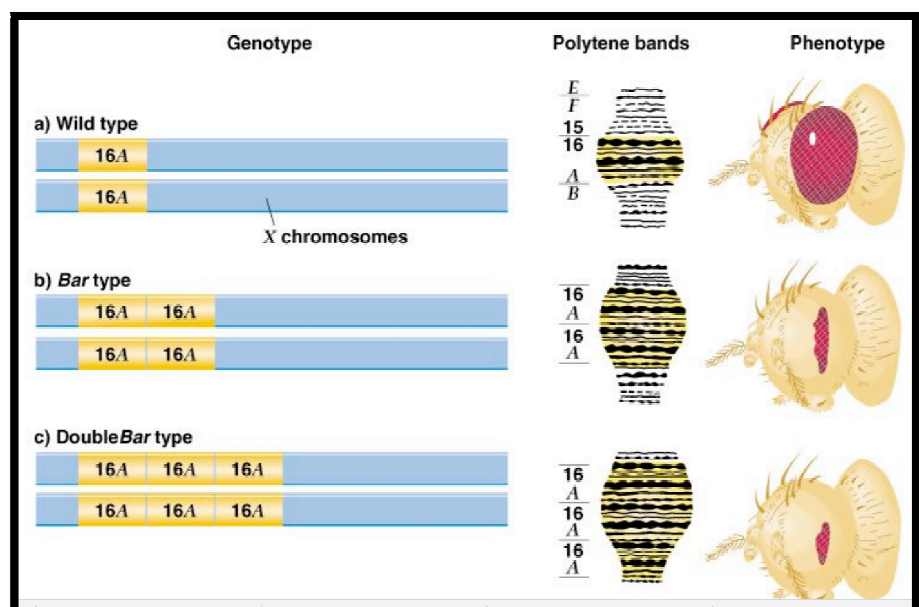


Fig. 5.10: 16A of X chromosome, *Drosophila*- Bar Eye.

5.4.2 Unequal Crossing Over in Human Red-Green Colour Blindness

Human colour vision is mediated by three light sensitive protein pigments which are present in the cone cells of the retina. Each pigment is related to *rhodopsin* which is the pigment present in rod cells and brings about vision in dim light. The light sensitivities of the cone pigments are towards blue, red and green. These are the pure colours which are detected by our visual pigments. Humans perceive all other colours as mixtures of blue, red and green. Because red and green pigments arose from the duplication of a single ancestral pigment gene and still 96% identical in amino acid sequence, the genes are similar enough to pair up and undergo unequal crossing over and is the genetic basis of red green colour blindness. Approximately 5% of males have some form of red green colour blindness. Affected males have normal sons and carrier daughters and carrier daughters have 50% carrier sons and 50% carrier daughters.

5.4.3 Duplications Provide Additional Material for Gene Evolution

Small chromosomal duplications with no phenotypic effect are still very important because they provide raw material for addition of more genes into a species chromosomes. Over the generations this can lead to the formation of **gene family** consisting of two or more genes that are similar to each other but not identical. During evolution this type of event can occur several times creating a family of many similar genes. **When two or more genes are derived from a single ancestral gene then such genes are called homologous.**

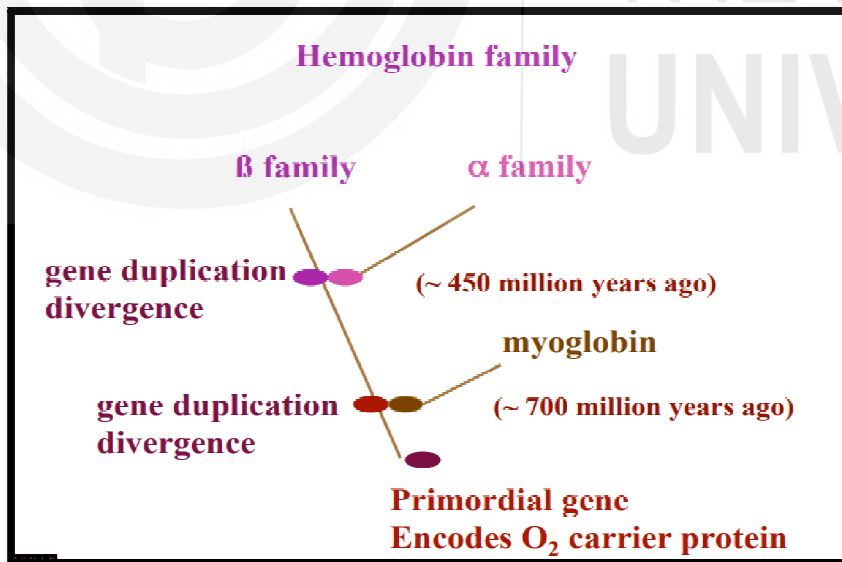


Fig. 5.11: Globin gene family in Humans.

A well studied example is of Globin gene family found in humans. The Globin genes encode for polypeptides that are subunits of proteins which function in oxygen binding. According to evolutionary analysis the single ancestral Globin gene duplicated about 800 million years ago. Since then, additional duplication and chromosomal rearrangements have produced current number of fourteen genes on three different human chromosomes. Gene

families have been important in evolution of traits. Even though all of the Globin peptides are subunits of oxygen binding protein, the accumulation of different mutations in various family members has created Globins with special function e.g. *Myoglobin* is better at binding and storing oxygen whereas *haemoglobin* is better at binding and transporting oxygen via red blood cells. Also these different Globin genes are expressed during different stages of human development (Fig. 5.11).

5.5 INVERSIONS

Let us now focus our attention to the changes in chromosome structure that involve re-arrangement in genetic material. An inversion is produced when there are two breaks in a given chromosome and the intercalary segment reunites in reverse order i.e. the segment rotates at 180 degree. The inversions can be of two types according to the location of the centromere:

- i) **Paracentric inversions** - are those where inverted segment **does not include centromere**.
- ii) **Pericentric inversion** – are those where inverted segment **includes centromere** (Fig. 5.12).

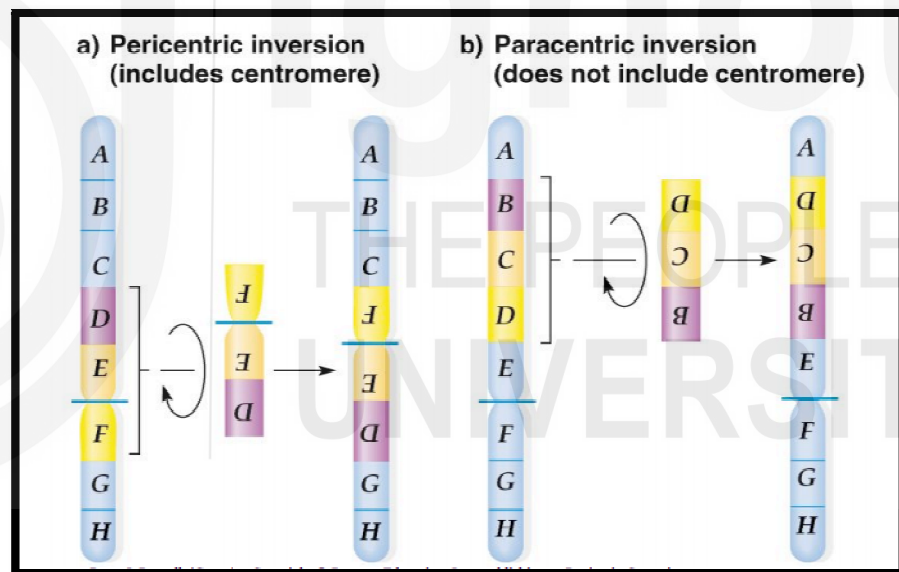


Fig. 5.12: Inversion and its types.

5.5.1 Inversions do not have any Phenotypic Consequences

When an inversion occurs in a chromosome, the total amount of genetic material remains the same as in a normal chromosome. Great majority of inversions for this reason do not have any phenotypic consequences in the individuals who carry them. But in rare cases, phenotype of an individual can be altered due to inversion. This occurs or not is related to the boundaries of the inverted segment. For inversion to happen the chromosome is broken in two places and the ends flip around. If either break point occurs within a vital gene region the function of such a gene gets disrupted producing a phenotypic effect e.g. X-linked inversion that has inactivated a gene for Factor VIII, a blood clotting protein leading to hemophilia type A.

In other cases an inversion can re-position a gene on a chromosome wherein its normal level of expression stands altered. This is also known as **position effect** and leads to decrease in the expression of the gene as for example, in *Drosophila* where such inversion influences red versus white eye pigmentation. Inversion seems to be unusual genetic phenomenon yet surprisingly they are found in human populations in quite significant number.

About 2% of human populations carry inversions, however, in most cases individuals are phenotypically normal and live normally without knowing about this abnormality. But there is a possibility that such individuals may produce offspring with genetic abnormalities.

5.5.2 Inversion Heterozygotes can Produce Abnormal Gametes

An individual who carries one copy of inverted chromosome and one copy of normal chromosome is known as an inversion heterozygote.

Such individuals though phenotypically normal may have a high probability of producing abnormal gametes. The size of the inverted segment decides the frequency of abnormal gametes. An inversion heterozygote with large inverted segment can produce sizable number of abnormal gametes. The underlying fact of abnormal gametes is crossing over within the inversion region.

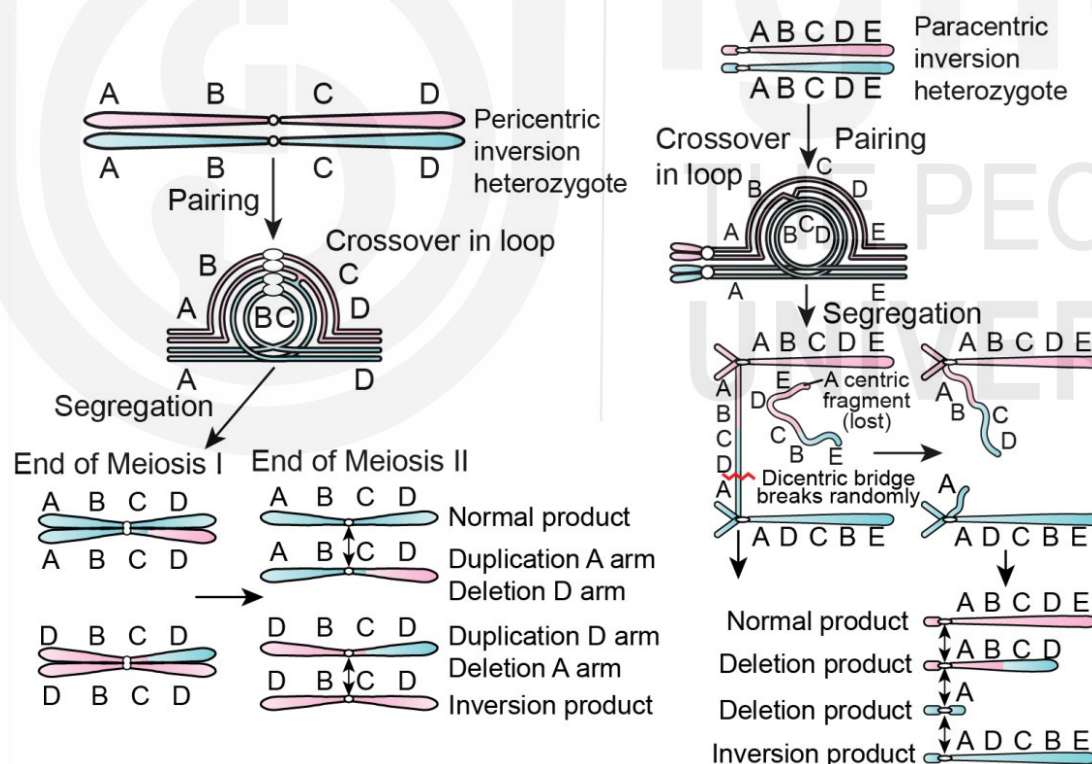


Fig. 5.13: Gametes produced from pericentric and paracentric inversions are imbalanced.

For the normal chromosome and inversion chromosome to synapse properly during meiosis I a loop called inversion loop must form to permit homologous genes to align next to each other on both chromosomes despite the presence of inverted sequence. If crossing over occurs within the inversion loop highly abnormal chromosomes will be produced. The consequences of this type of crossover depend on whether the inversion is pericentric or

paracentric. The net result of crossing over is to produce two normal chromosomes and two chromosomes that consist of deletions and duplications. The gametes having the latter chromosomes will not function leading to zygotic lethality. Due to this reason inversions are often called **crossover suppressors** (Fig. 5.13).

5.6 TRANSLOCATIONS

There are all types of unilateral or bilateral transfer of segments from one chromosome to another. Translocations which involve mutual exchange of chromosomes segments between two pairs of non homologous chromosomes, have evolutionary significance and are called **reciprocal/balanced translocations** or **segmental interchanges** (Fig. 5.14). Like inversions, they are usually without any phenotypical consequences because the individual has a normal amount of genetic material. However, in few cases, position effects can be observed.

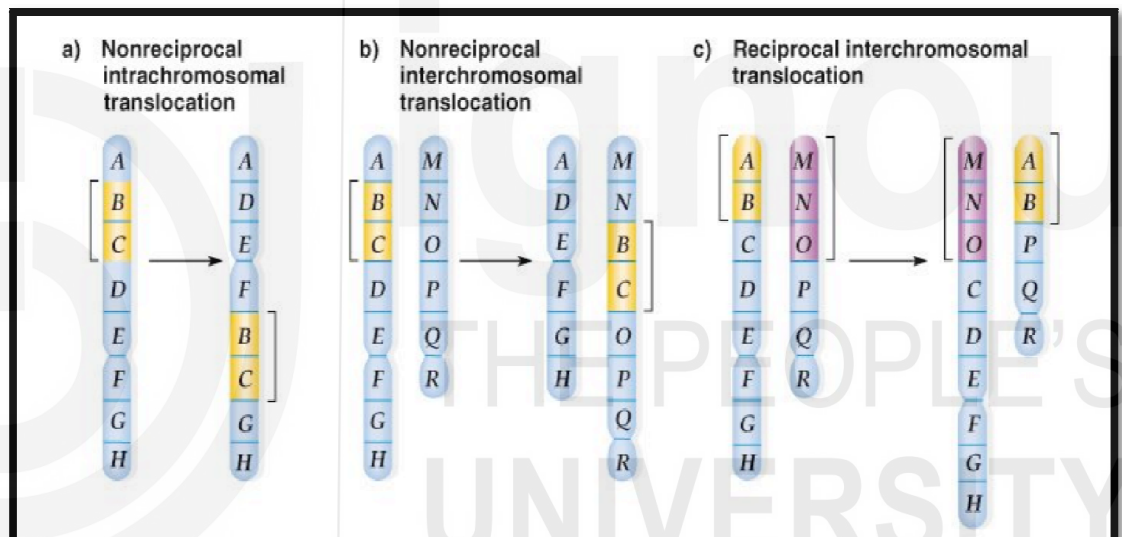


Fig. 5.14: Translocations and its types.

Simple translocation inheritance in which one segment of chromosome is attached to another chromosome can result in a **unbalanced translocation** or **Robertsonian Translocation**.

This translocation occurs as follows:

- Breaks occur at the extreme ends of the short arms of two non-homologous acrocentric chromosomes.
- The larger fragments fuse at their centromeric regions to form a single chromosome.
- The small acrocentric fragments are subsequently lost.
- This type of translocation is the most common type of chromosomal rearrangement in humans.

Robertsonian translocations are confined to chromosomes 13, 14, 15, 21 (Fig. 5.14).

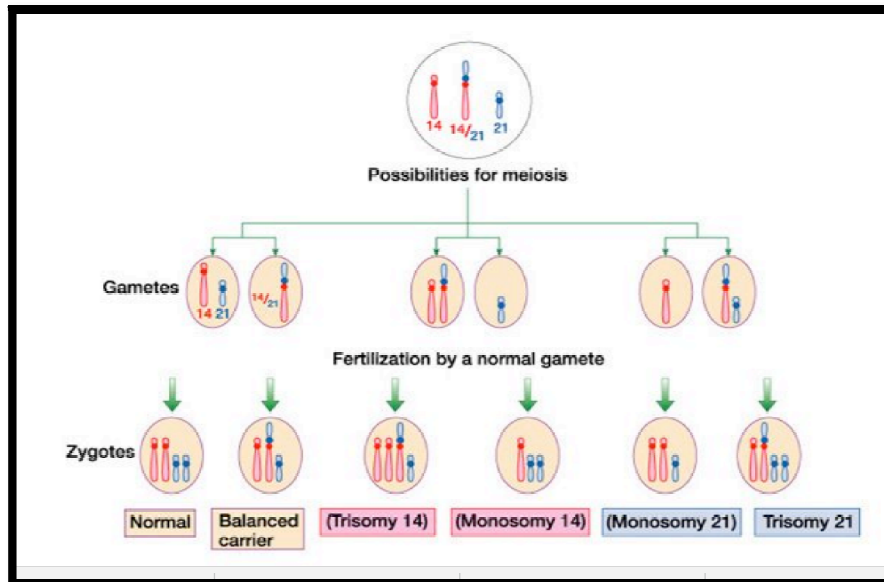


Fig. 5.15: Meiosis in Robertsonian Translocation.

These are generally associated with phenotypic abnormalities and even lethality e.g. **Familial Down syndrome**. In this condition a large segment of chromosome 21 is translocated to chromosome 14. This individual exhibits characteristics very similar to Down syndrome.

SAQ 3

Match the following:

Column A	Column B
i) Deletion	a) Re-arrangement in genetic material
ii) Duplication	b) Unilateral or bilateral transfer of segments of chromosome to another
iii) Inversion	c) Addition of genetic material
iv) Translocation	d) Affected chromosome is deficient of genetic material

5.7 SUMMARY

Let us summarise what we have learnt so far:

- Variations in chromosome structure are classified in four major categories as deletions, duplications, inversions and translocations. Deletions and duplications involve changes in the total amount of genetic material; inversions and translocations are genetic rearrangements.
- Deletions are detrimental to the individual who undergo these changes, although this certainly depends on the size and location of these deletions. In comparison to deletions, duplications are less harmful and provide raw material for the evolution of gene families.

- Inversions and translocations often do not affect the individual who carries them as they are chromosomal rearrangements. But if the breakpoint is within a vital gene or there is position effect, that alters gene expression, then, of course, phenotype is affected. They are associated with fertility problems with higher percentage of producing abnormal offsprings.
- In population, most of the mental retardation and developmental disabilities are due to chromosomal abnormalities. The phenotypes are complex, that are the result of a gain or loss of multiple, dosage-sensitive genes in the altered segments. The characterization of these complex phenotypes permits identification of genes causing particular features of the syndrome.
- The use of high-resolution technologies, such as micro-arrays, has permitted for the identification of new syndromes.
- Cytogenetics is no longer in its infancy, and has emerged a “new” genome science. With the use of new technologies, now it is possible to establish the causes of mental retardation, developmental disabilities, and birth defects in our population.

5.8 TERMINAL QUESTIONS

1. Define karyotype. How do we create a karyotype? What is its significance?
2. What changes in chromosome structure lead to change in total amount of genetic material and why?
3. Why do deletions and duplications have less detrimental effect on the individual's phenotype?
4. What are the Gene families and how are they produced over time to contribute to evolution?
5. Why an individual who carries one copy of inverted chromosome and one copy of normal chromosome produces abnormal gametes?
6. What are isochromosomes and how are they formed?
7. How does presence of ring chromosomes affect an individual?
8. What is Robertsonian Translocation? How does it occur? Give its characteristics.

5.9 ANSWERS

Self Assessment Questions

1. i) karyotyping, ii) centromere, iii) chromosome, iv) banding, v) mutation.
2. i) deficiency, ii) human, iii) PWS, iv) Ten, v) identical, vi) ring.
3. i) d, ii) c, iii) a, iv) b.

Terminal Questions

1. Refer to Section 5.2.
2. Refer to Section 5.3.
3. Refer to Section 5.3 and 5.4.
4. Refer to Sub Section 5.4.3.
5. Refer to Section 5.5.
6. Refer to Sub Section 5.3.3.
7. Refer to Sub Section 5.3.4.
8. Refer to Section 5.6.

FURTHER READINGS

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3. Genetics: Principles and Analysis, 4th edition. D.L. Hartl, D.W. Jones. Pub: Jones and Barlett Publishers
4. Genetics, 9th revised multicolor edition. P.S. Verma & V.K. Agarwal. Pub: S. Chand & Co.
5. Human Heredity by Michael Cummings ©2006 Brooks/Cole-Thomson Learning
6. Human Molecular Genetics, 3/e, Garland Science, 2004

Acknowledgement of Figures

- Fig. 5.1: G. Bradley Schaefer, James N. Thompson, Jr.: Medical Genetics: An Integrated Approach Copyright © McGraw-Hill Education. All rights reserved.
- Fig. 5.2: <http://ib.bioninja.com.au/standard-level/topic-3-genetics/32-chromosomes/chromosome-types.html>.
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UNIT 6

NUMERICAL ABNORMALITIES IN CHROMOSOMES AND THEIR EFFECTS

Structure

- | | | | |
|-----|--|-----|-----------------------------|
| 6.1 | Introduction | 6.6 | Sex Chromosome Aneuploidies |
| | Objectives | | XXX – Trisomy X Syndrome |
| 6.2 | Numerical Abnormalities in Chromosomes | | XYY - Double Y Syndrome |
| 6.3 | Euploidy | | XXY - Klinefelter Syndrome |
| | Monoploidy | | XO – Turner Syndrome |
| | Polyploidy | 6.7 | Summary |
| | Types of Polyploids | 6.8 | Terminal Questions |
| 6.4 | Aneuploidy | 6.9 | Answers |
| | Hypoploidy | | |
| | Hyperploidy | | |
| 6.5 | Autosomal Aneuploidies | | |
| | Trisomy 21 – Down Syndrome | | |
| | Trisomy 18 – Edward Syndrome | | |
| | Trisomy 13 – Patau Syndrome | | |

6.1 INTRODUCTION

In the previous unit you have studied the origin and phenotypic effects emanating from structural chromosome aberrations. In this unit you will deal with the genetic effects of numerical chromosome abnormalities. As you know,

the genetic material in eukaryotes is packaged into chromosomes and every organism has a fixed number of chromosomes, characteristic of its species. Chromosomes show specific behavior during cell division and play an important role in balanced distribution of genetic material in the daughter cells. During mitosis each chromosome is replicated and equally divided into two daughter cells so that each of them receives same number of chromosomes and are identical to parent cell. During meiosis the chromosomes arrange to form homologous pairs, each consisting of one paternal and one maternal chromosome. The homologous pairs of chromosomes exchange some genes through crossing over and then segregate. Meiosis results into formation of four daughter cells, each of which receives half the number chromosomes from parent cell. Meiosis occurs only during gamete formation so that each daughter cell is haploid and is ready to participate in the process of fertilization to restore the diploid condition.

Numerical anomalies in chromosomes lead to serious defects in individuals. These anomalies can be either due to a missing chromosome or due to the presence of one or two extra chromosomes. This results into unbalanced karyotype causing physical, physiological and developmental abnormalities. In proceeding section you will learn about different types of numerical anomalies in chromosome sets of organisms.

Objectives

After studying this unit, you will be able to:

- ❖ define various terms related to ploidy levels in organisms,
- ❖ comprehend various numerical abnormalities found in organisms,
- ❖ discuss about the consequences of presence or absence of one or more chromosomes,
- ❖ explain the inheritance of ploidy related genetic conditions, and
- ❖ differentiate between the autosomal and sex chromosome aneuploidies.

6.2 NUMERICAL ABNORMALITIES IN CHROMOSOMES

Change in chromosome number is more frequently observed among the live born children. A numerical change is also cytologically easy to observe. Basically there are two types of numerical changes in cell chromosome number. These are *euploidy*, in which there is an addition or deletion of entire set(s) of chromosomes, and *aneuploidy*, which involves the addition or deletion of individual chromosomes within a set. Thus both euploidy and aneuploidy alter gene dosage in the affected individuals. Different types of numerical anomalies in chromosome sets of organisms can be summarised in Figure 6.1.

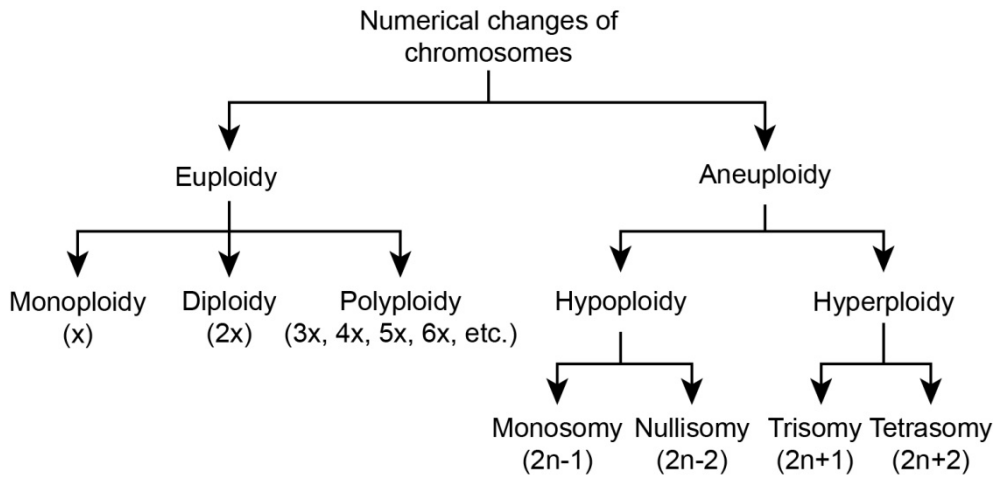


Fig. 6.1: Types of Numerical Changes in Chromosomes.

There are a number of cellular events during which these numerical changes occur. The mechanisms by which numerical anomalies arise are-

i) Meiotic non-disjunction

Non disjunction during meiosis may lead to unequal distribution of certain chromosomes in gametes (Fig. 6.2). These can be autosomes or sex chromosomes. Unbalanced gametes, when participate in fertilization, result into numerical anomaly in the chromosome set of the offspring. Presence of one or more extra autosomes causes conditions like trisomy and tetrasomy. Conversely, absence of one or more chromosomes may lead to the conditions like monosomy and nullisomy. Trisomy and monosomy of sex chromosomes also occur, which are discussed in later section of this unit.

Homogeneous numerical anomalies: Mechanisms of formation

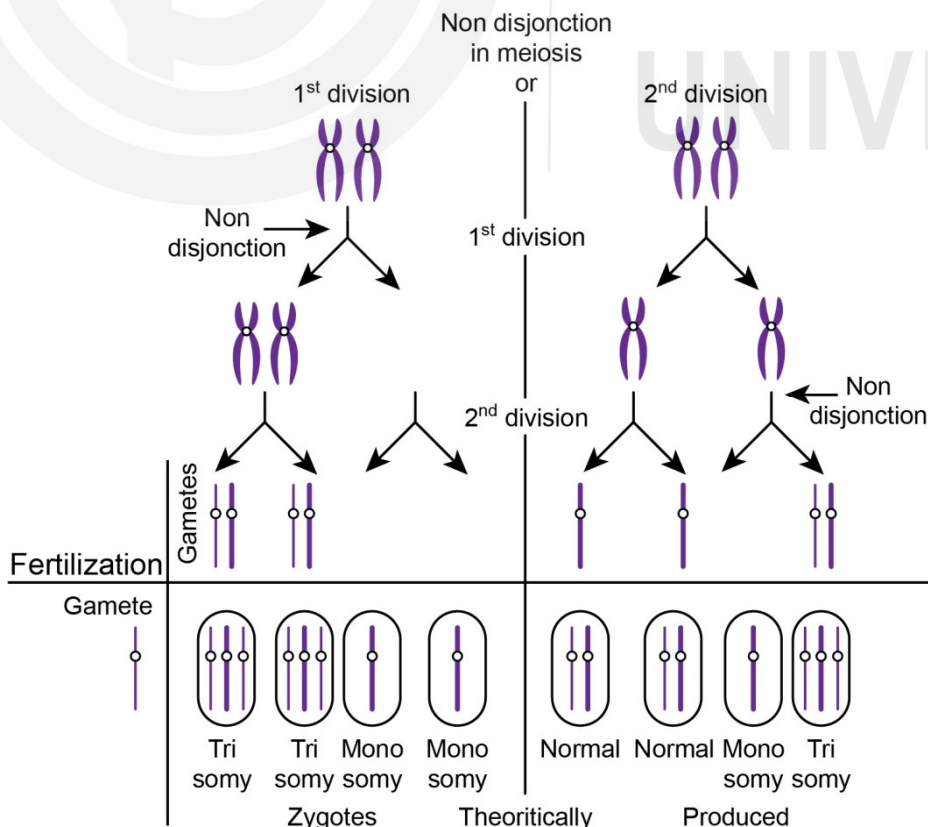


Fig. 6.2: Non-disjunction of chromosomes during Meiosis.

ii) Anomalies occurring during Fertilization

When unreduced gametes having diploid set of chromosomes ($2n$) are involved in fertilization, the resultant zygote restores triploid or tetraploid condition. If the female gamete is diploid, it is called **digyny**, if the male gamete is diploid it is called **diandry**. Thus such kind of anomalies produces polyploids in the population.

iii) Mosaicism

Mosaicism arises in an individual due to numerical anomaly of chromosomes only in some cells of the body. This usually occurs due to non-disjunction of chromosomes after fertilization, i.e. in during early development of an embryo.

SAQ 1

- i) Unequal distribution of chromosomes in gametes occurs due to.
- ii) Numerical anomaly of chromosomes found only in some cells of the body, while other cells are normal, is called.

6.3 EUPLOIDY

Euploidy is characterized by the presence of homologous chromosome sets in multiple of the basic chromosome set (monoploid set) or the genome. For example if in an organism, the monoploid set consists of 12 chromosomes, its diploid species will have (12×2) i.e. 24 chromosomes, triploid will have (12×3) i.e. 36 chromosomes and so forth (Table- 6.1). Thus euploids may range from monoploids, diploids, triploids..... to polyploids. Let us take a look into different ploidy levels.

6.3.1 Monoploidy

Monoploidy refers to the presence of only one complete set of chromosomes, designated as 'x'. This set is basic and can be multiplied into other copies to form different ploidy levels, as explained above. Individual consisting of monoploid set of chromosomes is therefore called a monoploid. Occurrence of monoploids is rare in most species mainly because recessive lethal mutations express in them as they do not have any corresponding dominant alleles to mask their effect. **Some insects like bees, wasps and ants are monoploid as they develop from unfertilized eggs.**

6.3.2 Polyploidy

Polyploidy refers to the occurrence of multiple copies of a complete set of chromosomes in an organism. One complete set of chromosomes is called monoploid (n), thus repetitive presence of the set is referred to as diploid ($2n$), triploid ($3n$), tetraploid ($4n$) and so forth. **Polyplody is very common in plant species; about 40-45 percent of flowering plants are polyploid.** This condition often gives rise to improved phenotypes in most cases such as robust appearance, enhanced vigour and larger size, which has

indeed led to the selection of economically useful varieties of crops such as wheat, cotton, sugarcane, banana etc. *Chrysanthemum* is a beautiful example of polyploidy as the ploidy level ranges from monoploid to decaploid in this plant. Other ornamental plants like roses and tulips are also polyploid. The large variety of sizes and colours of flowers are due to diverse polyploidy status in these plants.

However, polyploidy is not much common in vertebrates, as it interferes with appropriate segregation of chromosomes during sexual reproduction. For example, if sexual reproduction occurs between in tetraploid organisms sex determination of progeny (such as XXXY) comes into question due to pairing of inappropriate sets of chromosomes (Table 6.1)

Table 6.1: Progeny obtained from two tetraploid parents.

Parents	Female	Male
Tetraploid Parent Genotype	XXXX	XXYY
Gametes	XX	XX, XY
Progeny	XXXX	XXXY

6.3.3 Types of Polyploids

There are following types of polyploidy based on the sets of chromosomes.

A) Triploids

Organisms having three identical sets of chromosomes are known as triploids. Triploidy is common in plants. Triploid plants possess vigorous characteristics, due to which many cultivated plant species are triploid. Triploids are usually sterile, therefore, are vegetatively propagated. The most common example is seedless banana. Thus triploids have high economic value and are very useful in agriculture and horticulture.

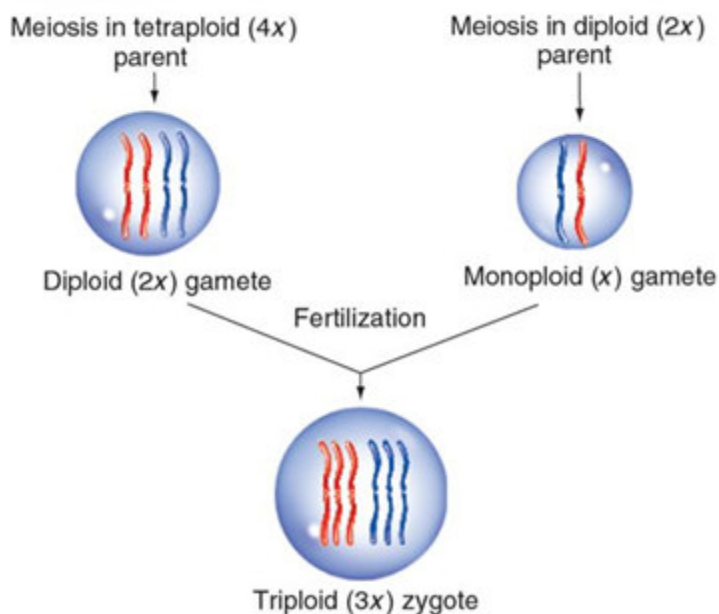


Fig. 6.3: Triploid Production.

Triploids can be produced by four ways:

- i) If a parent organism produces a diploid gamete due to defective meiosis and its fertilization occurs with a normal haploid gamete, the resultant zygote is triploid.
- ii) Triploids may also be produced through sexual hybridization between a diploid and a tetraploid parent ($2x \times 4x$) (Fig. 6.3).
- iii) The only naturally occurring triploid tissue in angiosperms is endosperm. It results from double fertilization in which one male nucleus fuses with egg cell to form zygote while other male nucleus fuses with the diploid secondary nucleus to form endosperm mother cell ($3n$). Endosperm mother cell then undergoes mitotic divisions to form triploid endosperms. The *in-vitro* culture of endosperm can further yield triploid plants.

B) Tetraploids

Organisms having four sets of chromosomes are called tetraploid. Tetraploid condition is obtained due to nonseparation of chromosomes during cell division. Endoreduplication results into tetraploidy since the genetic material replicates during cell cycle but M phase is skipped. It is very common in plants as the tetraploid species are stable and can produce gametes by equal segregation of chromosomes during meiosis. However, gametes in such case are diploid; if fertilizes with a haploid gamete it forms a triploid, or if fertilizes with another diploid gamete it forms a tetraploid. **Self fertilization in tetraploid plants retains the chromosome number in progeny so that the continuity of tetraploid species is maintained.**

C) Hexaploids

Hexaploid species possess six sets of chromosomes. Most common example is wheat (*Triticum aestivum*) having 42 chromosomes ($6x = 42$ or AABBDD genome). Hexaploids may be formed by diploidization of chromosomes in a triploid or as a result of cross fertilization between a tetraploid and an octaploid species. Other examples of hexaploidy are *Chrysanthemum*, *Triticale* and oat.

On the basis of composition of the genome, polyploids can also be classified as autopolyploids or allopolyploids.

i) Autopolyploidy

Autopolyploidy refers to the presence of multiple sets of chromosomes of same genome. Autopolyploids come into existence due to fusion of unreduced gametes. Most tetraploid plants such as potato and coffee and triploid species of banana occur naturally as autopolyploids because they originate due to spontaneous chromosome doubling and exhibit enhanced vigour. Many ornamental plant varieties like *Chrysanthemum* and tulips are autopolyploids. In normal meiotic prophase, chromosomes pair with one another throughout their length. But in situations, when four similar chromosomes are present, they usually pair with different chromosomes, at different places along their length. This complicates the meiotic process and results in the nonviable gametes. Further, sterility in autopolyploids also results due to unequal disjunctions. *Chrysanthemum* is a well known example of autopolyploidy. In

this genus, the basic number is 9. Its polyploidy species are known to have 18, 36, 54, 72 and 90 chromosomes—all multiples of 9. Potato or *Solanum tuberosum* is another example. Its basic chromosome number is 12. Its autopolyploid forms, with chromosome number 24, 36, 48, 60, 72, 96, 108, 120 and even 144, are available.

ii) Allopolyploidy

Allopolyploidy is the condition in which different sets of chromosomes in a polyploidy species come from genomes of different species. They originate from hybridization of organisms with distinct genomes either by fusion of unreduced gametes or by chromosome doubling of fertilization product of two. Allopolyploidy has played major role in speciation. The cultivated hexaploid species of bread wheat (*Triticum aestivum*) is an example of allopolyploid.

Allopolyploidy is the most common natural form of polyploidy that has occurred in various plant groups. Many of the present-day plants have resulted from this kind of hybridisation. The polyploid species, that are established in nature, have genomes that correspond more or less completely to the combined chromosome complements of two different but related diploid plants (Fig. 6.4). In the allopolyploids, since two homologues of each specific chromosome are present, meiosis may occur normally and fertilization may successfully propagate the tetraploid sexually. These allopolyploids, also known as the *allotetraploids* or *amphidiploids*, have undergone hybridization somewhere in their ancestral history. Allopolyploidy thus represents a method by which new species may be formed almost immediately, whereas the autopolyploids result in meiotic anomalies and “dead ends” with reference to evolution.

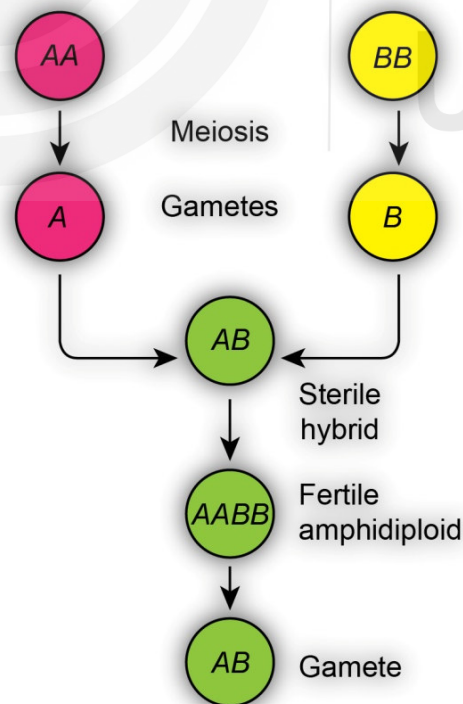


Fig. 6.4: The production of a fertile allotetraploid (AABB) by crossing the diploids AA and BB, and then doubling the chromosomes of the sterile hybrid (AB).

iii) Induced Polyploidy

Polyploids may arise naturally or may be artificially induced. Anything that interferes with spindle formation during mitosis results in a doubling of chromosomes. Induced polyploidy was first demonstrated by subjecting growing plants to a higher than usual temperature. Maize and some other plants responded to such treatment with an increase in chromosomes number in their cells. Application of the alkaloid colchicines, derived from the autumn crocus (*Colchicum autumnale*) disturbs spindle formation. You will study the use of this chemical in karyotype preparation further in this course. The cell division proceeds as usual, but since the spindle formation is inhibited, the cytoplasmic phase of the cell division does not occur. Instead, restitution nuclei with the double number of chromosomes are produced in the treated tissue. Subsequent nuclear divisions are normal, so that the polyploid cell line, once initiated, is maintained. Polyploidy may also be induced by other chemicals (acenaphthene and veratrine) and also by exposure to cold temperatures.

iv) Sterility in Polyploids

Polyploidy often leads to sterility due to inappropriate chromosome segregation. Additional sets of chromosomes, other than diploid set, cause serious defects in gamete formation through meiosis. For example in a triploid organism in which there are three sets of chromosomes, homologous pairing is affected during meiosis. There may be different possibilities of gamete formation, thus the chromosome status of such gametes may range from 0 to 3. If two sets of chromosomes form a bivalent, the third set remains univalent and gametes with $2n$ and n set of chromosomes are formed. If all the three sets of chromosomes remain together, gametes with n and zero set of chromosomes are formed. Even if such gametes are able to perform fertilization, the zygote so formed is often not viable, therefore most of the triploid organisms are sterile.

Gamete formation in species having even number of chromosome sets such as tetraploid, hexaploid or octaploid is comparatively more feasible as the segregation of chromosomes during cell division is easy. Polyploid species of plants often reproduce asexually or through various methods of vegetative propagation.

Cultivated wheat is an excellent example of allopolyploidy. Cultivated bread wheat is a hexaploid with 42 chromosomes constituting a complete diploid genome of 14 chromosomes from each of the three ancestral species. The 42-chromosome allopolyploid is thought to have originated through hybridisation as shown in Fig. 6.5.

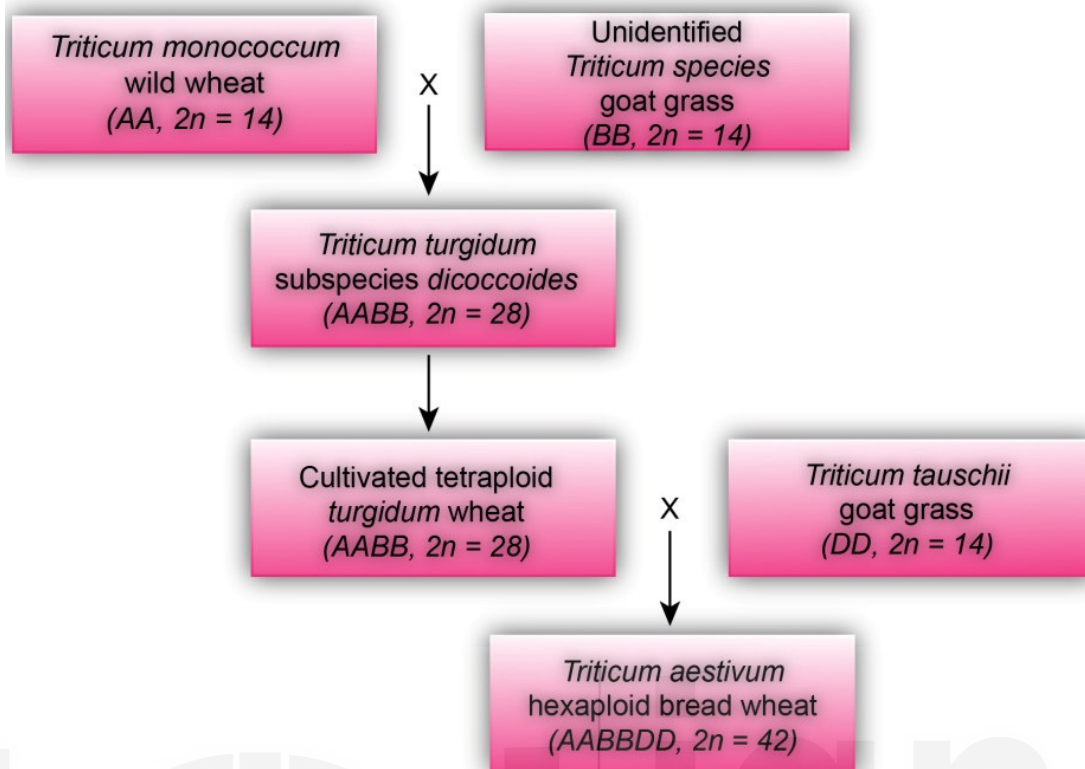


Fig. 6.5: Hybridisations that occurred in the ancestry of cultivated bread wheat (*Triticum aestivum*), which is an allohexaploid containing complete diploid genome (AA, BB, DD) from the ancestral species.

Another allopolyploid – *Raphanobrassica* was experimentally synthesised by G.D. Karpechenko crossing two common vegetables that belong to different genera. These are radish – *Raphanus sativus* and cabbage – *Brassica oleracea*. Both the species have 9 pairs of chromosomes. The diploid hybrid has 18 chromosomes, 9 from each parent, but is sterile and cannot perpetuate itself, largely because of the failure in pairing between the unlike chromosomes in meiosis. When the chromosomes of the F_1 hybrid were doubled, a fertile polyploid, named *Raphanobrassica*, was produced with 18 radish and 18 cabbage chromosomes. Because two sets of chromosomes were now present from each parent, pairing was quite regular and hence normal gametes were produced. This experiment suggested the possibility of incorporating desirable genotypes from two different species into a new polyploid species. Seed capsules of the parent, the sterile hybrid, and the tetraploid plants are shown in Figure 6.6: Unfortunately, from the practical standpoint, *Raphanobrassica* has a foliage of a radish and the roots of a cabbage. But nevertheless, this experiment has shown a novel way of perpetuating the sterile hybrids.



Fig. 6.6: Seed pods of (a) radish, (b) cabbages, (c) sterile hybrid, (d) fertile tetraploid resulting from chromosomes doubling.

v) Applications of Polyploidy

Bread Wheat – Without polyploidy we would have a very different diet. Among the food we would miss bread which is made from one of several species of wheat (*Triticum*). Wheat is the most important rain crop as over 20% of calories consumed by human beings come from wheat. Presently, several varieties of wheat are grown throughout the world.

The genus *Triticum* consists of a polyploid series of wheat. The diploid species, *T. monococcum* or *einkorn* wheat $2n = 14$, was the first to be used for food about 10,000 - 12,000 years ago, about the time civilization began. At that time, seeds of wild plants were collected for food and subsequently brought in cultivation. The *einkorn* wheat is hulled and is small as compared to its polyploid relatives.

The *emmer* and *durum* wheat belong to the tetraploid species *T. urgidum* ($2n = 28$).

These wheats originated from a naturally occurring wild tetraploid variety, whose grains are larger than einkorn wheat. The *emmer* wheat is hulled, but the durum wheat is particularly desirable because of its free threshing quality, that is, the hull is easily removed by threshing; and unlike hulled wheat it does not need to be ground for human consumption.

Varieties of bread wheats *T. aestivum* are agriculturally the most important. Bread wheat is hexaploid, $2n = 42$, derived from hybridisation of tetraploid *emmer* wheat with a diploid relative, the wild grass *Aegilops squarrosa* ($2n = 14$). Bread wheat is especially desirable, because it is free-threshing and has large grains, and also has a high content of proteins called the gluteins. The gluteins are sticky and elastic, allowing wheat breads to rise without collapsing when leavened, i.e., when mixed with yeasts they produce carbon dioxide gas.

Tobacco Resistance – Induced polyploidy has been exploited to a great extent. Practical applications may become more common as additional data has accumulated. By artificially inducing polyploidy, disease resistance and other desirable qualities have been incorporated into some commercial crop plants. Tobacco, *Nicotiana glauca* is susceptible to the tobacco mosaic virus (TMV), whereas another species, *N. glauca*, appeared at first observation to be resistant. Further investigation, however, showed that in *N. glauca* the virus killed the cells that were invaded, and the virus particles became isolated in the dead cell. The apparent resistance was due to hypersensitivity. When the two tobacco species were crossed, the hybrid was found to be 'resistant' to the virus, but was totally sterile. So, when the chromosomes were doubled, it was possible to secure a fertile polyploid resistant to the virus.

Polyloid Fruits and Flowers

Many varieties of plants that fulfil human needs have been found to be polyploids. The polyploids were selected for cultivation because of their large size, vigour and ornamental value. In *McIntosh apple* trees, some twigs produced extra large fruits.

These twigs were known as 'giant sports' and were found to be tetraploid ($4n$). These 'giant sports' were propagated into whole trees, which produced extra fruits. Similar sports with giant fruits are also seen in *Bartlett pears*, several varieties of *grapes* and *cranberries*. These giant sports developed naturally. But with colchicine treatment, a number of polyploids have been developed artificially. This technique has provided a way to explore the mechanism involved in the polyploid formation. The tetraploid *maize* is more vigorous

than the diploid form and contains about 20 per cent more vitamin A. Polyploid *watermelons* have been developed from colchicine treatment. The triploid with 44 chromosomes is large and commercially useful. The triploid watermelons with 33 chromosomes have great market potential because they are sterile and lack seeds. Several tetraploid flower varieties, such as in *marigolds* and *snapdragons* are widely cultivated.

SAQ 2

- i) The presence of only one complete set of chromosomes which is referred as 'x' is called.
 - ii) If there are multiple copies of a complete set of chromosomes in an organism, the genetic condition is called.
 - iii) Polyploidy is rare in animals, yet some tissues in the bodies of some diploid animal species show evidence of polyploidy. Why do numbers above $2n$ persist in somatic tissues when they do not occur in the whole animal?
 - iv) How does colchicine treatment result in chromosome doubling in plants?
-

6.4 ANEUPLOIDY

Aneuploidy is a condition characterized by change in normal chromosome numbers. If the number of chromosomes is less in the complete set, it is called hypoploidy, conversely, if there are additional chromosomes present, it is called hyperploidy.

The aneuploid individuals have unbalanced chromosome sets, that is, with extra or missing chromosomes. Their chromosome complement contains at least one more or one less chromosome than the normal diploid number. If one homolog of the normally diploid complement is present three times, the condition is called **trisomy**, and an individual of this type is called a **trisomic** and the condition can be designated by the symbol $2n+1$. **Tetrasomy** ($2n+2$) indicates that one chromosome of the complement is present four times. A $2n+1+1$ individual would be **doubly trisomic** for two different homologs and the $2n+2+2$ individual would be **double tetrasomic** and so forth. The reciprocal situation involving loss of a chromosome from a pair is called **monosomy** ($2n-1$), and loss of a whole pair of chromosome is known as **nullisomy** ($2n-2$).

The primary cause of aneuploidy is **nondisjunction** of homologs during meiosis (see Box 6.1). Nodisjunction is the failure of chromosomes to separate during cell division (Fig 6.7). If one pair of homologs fails to disjoin, then $n+1$ and $n-1$ gametes will result. And if these gametes are fertilised with a normal gamete, then $2n+1$ and $2n-1$ individuals would be formed instead of the normal $2n$. The chromosome constitution of the zygote is critical to normal development, because any chromosome abnormality present in the zygote will be passed on through each succeeding generation of cells (the single cell line produced by meiotic nondisjunction).

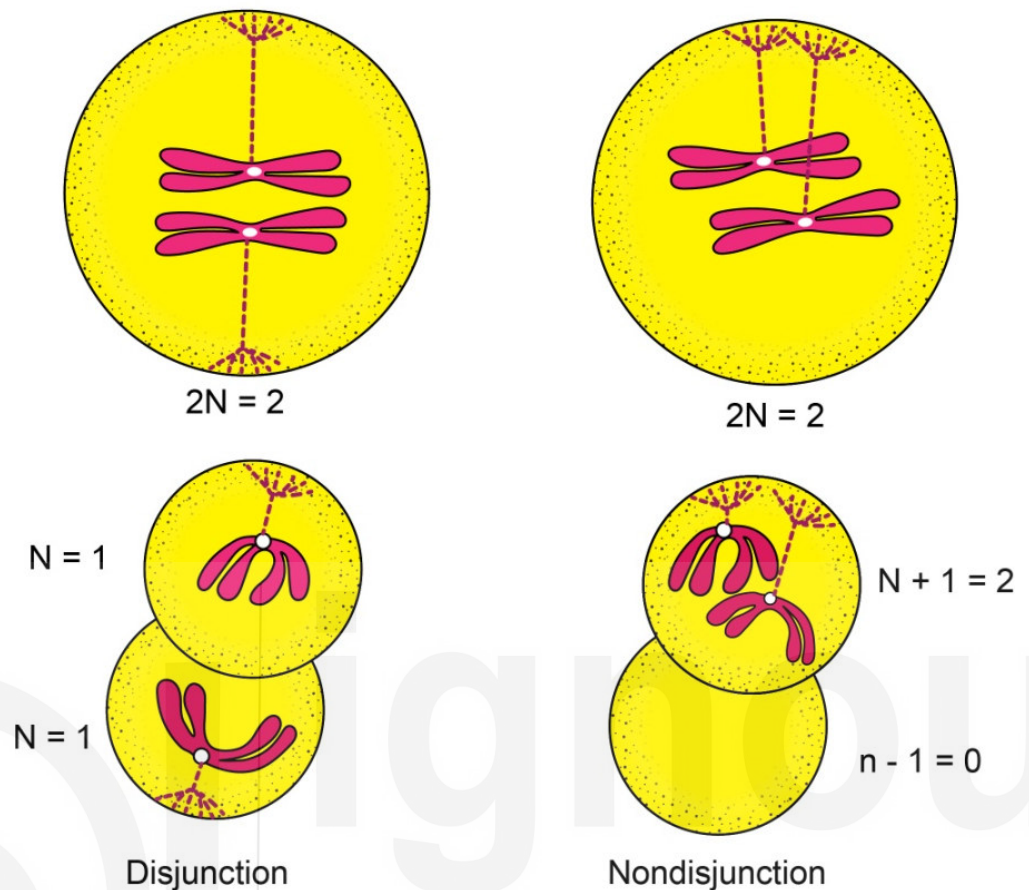


Fig. 6.7: The cell on the left shows a pair of homologous chromosomes during meiosis, with the centromere of each chromosome attached to its closest pole. Normal separation or disjunction converts the diploid ($2n$) to two haploid cells (n). On the right, each of the two homologs is attached to the same pole of the cell. This converts the diploid ($2n$) into nondisjunctional haploid cells: ($n+1$) and ($n-1$), one containing both homologs and one lacking both homologs respectively.

All forms of aneuploidy have serious consequences in meiosis. In the following paragraphs, you will study some well-known examples of aneuploidy.

Box 6.1: Meiotic nondisjunction

If chromosomes nondisjunction occurs during meiosis I, it is referred to as first division nondisjunction (Fig. 6.8a). At meiosis II, it is called second-division nondisjunction (Fig. 6.8b). In the first-division nondisjunction, a pair of homologous chromosomes, (*either autosomes or sex chromosomes*) fail to separate from each other between first metaphase and first anaphase. In second-division nondisjunction, sister chromatids fail to separate from each other, between metaphase II and anaphase II. In either case, nondisjunction results in some gametes (sperms and eggs) having two of a given chromosome, while the companion gametes lack the given chromosomes. When such aneuploid gametes are involved with a normal gamete, in fertilization, the resulting zygote would be monosomic or trisomic for the given chromosome.

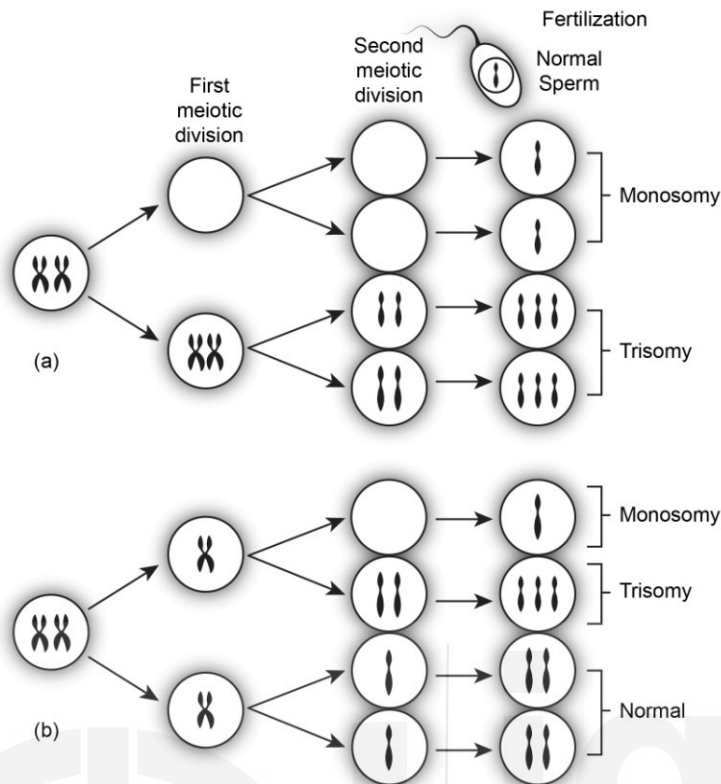


Fig. 6.8: First (a) and (b) second division meiotic nondisjunction. The diagram shows occurrence of nondisjunction in the egg, which is then fertilised by a chromosomally normal sperm. The reverse is also known to occur giving similar results.

6.4.1 Hypoploidy

Loss of one or more chromosomes from the diploid set in an organism is referred to as hypoploidy. Different types of hypoploidy are:

i) Monosomy

Hypoploidy occurring due to loss of one chromosome is called monosomy and is given as $(2n-1)$. Monosomics usually arise from participation of defective $(n-1)$ gametes in fertilization. Monosomy is further categorized on the basis of number of non-homologous chromosomes lacking in an aneuploidy. Single monosomics lack only one chromosome, double monosomic lack two chromosomes $(2n-1-1)$, each from a different homologous pair. Triple monosomics lack three chromosomes $(2n-1-1-1)$ in the similar manner. The maximum number of monosomies in any organism may extend upto the haploid number of chromosomes. Normally, in diploids the monosomy may be deleterious or may have potential ill effects, however in polyploids such ill effects are milder.

ii) Nullisomy

The condition in which there is loss of one complete homologous pair of chromosome is called nullisomy $(2n-2)$. Usually nullisomics arise by selfing of monosomic organisms. To the maximum, nullisomies in an organism may extend upto the haploid number of chromosomes.

6.4.2 Hyperploidy

Occurrence of one or more extra chromosomes in the set is referred to as hyperploidy. If an organism, normally having 24 chromosomes in the diploid set, possesses 25 chromosomes, then the condition is said to be hyperploidy ($2n+1$). Hyperploidy is further classified on the basis of additional number of chromosomes present in an organism for example trisomy and tetrasomy.

i) Trisomy

This condition is characterized by the presence of one extra chromosome and is designated as ($2n+1$). Although very rare, double trisomy ($2n+1+1$) is also reported in nature. Trisomics are characterized with weaker physical features, poor vigour and lesser or no fertility. The number of possible trisomies in an organism is equal to the haploid chromosome number. Trisomy comes into existence due to participation of abnormal ($n+1$) gamete in fertilization, resultantly (n) and ($n+1$) gametes fuse to form ($2n+1$) zygote which may develop in a trisomic. The ($n+1$) gametes are formed due to non-disjunction of chromosomes during meiosis in a diploid organism (Fig. 6.9).

Trisomic organisms are often sterile because due to the presence of an extra chromosome, homologous divalent pairing is hampered, instead a trivalent may be formed. This causes irregularity in meiosis. Even if a trisomic is able to form gametes, they are abnormal and further give rise to another chromosomal abnormality.

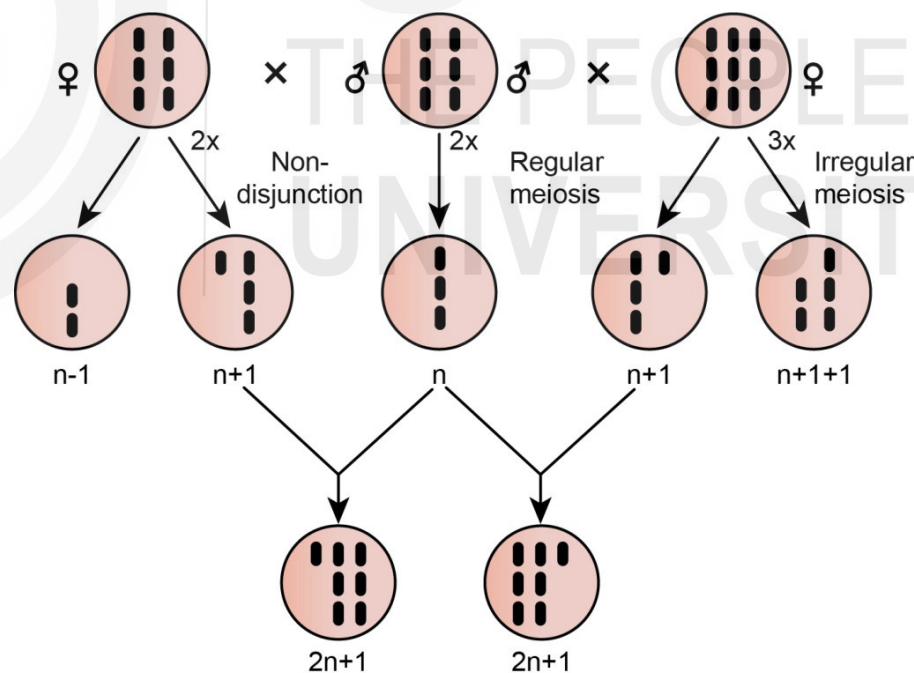


Fig. 6.9: Origin of Trisomics.

Types of Trisomic conditions

The types of trisomy conditions (Fig. 6.10) found in nature are:

- Primary trisomy:** In this condition an exact extra copy of a complete chromosome is present, in other words, the trisomic chromosome is present in three identical copies.

- ii) **Secondary trisomy:** In this case, the extra chromosome has two identical arms, i.e. the extra chromosome is an isochromosome in which one arm is duplicated.
- iii) **Tertiary trisomy:** The extra chromosome is the product of translocation in which copies of arms of two different chromosomes are present.

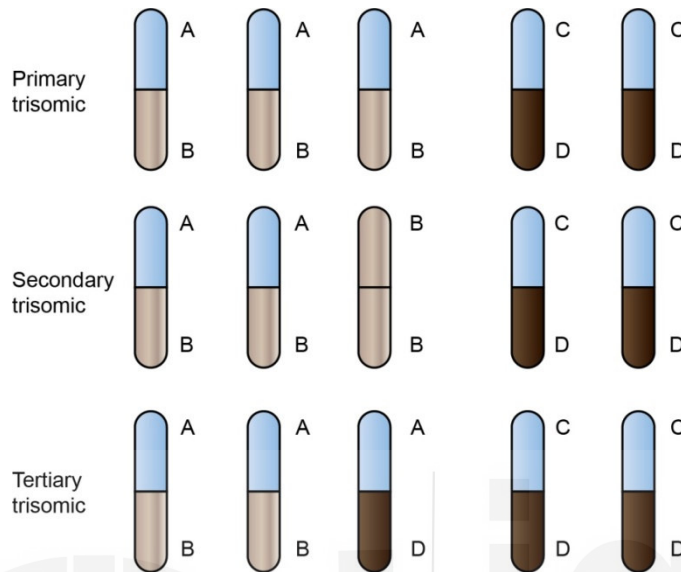


Fig. 6.10: Types of Trisomic conditions.

Trisomy in Jimson Weed

The jimson weed (*Datura stramonium*) shows a considerable amount of morphological (Fig. 6.11) variation in many traits, particularly the fruits. The normal chromosome number of this plant is $2n = 24$. Blakeslee and Belling (1924), who studied this system extensively showed that each of the particular morphological variants had 25 chromosomes. In each variant, one of the 12 kinds of chromosomes was found to be in triplicate. That is, the somatic cells were $2n+1$, and it is a case of trisomy. These people thought, since the jimson weed has 12 pairs of chromosomes, 12 recognisable trisomics should be possible. And as expected, they could collect all the variants for the 12 chromosomes.

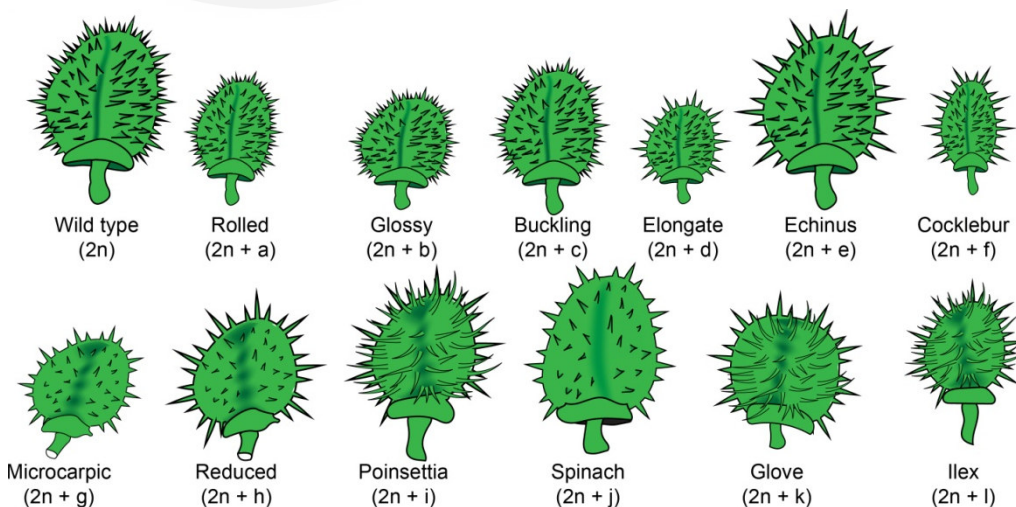


Fig. 6.11: Capsule phenotypes of the fruits of *Datura stramonium*. As compared with the wild type, each phenotype is the result of trisomy of one of the twelve chromosomes (denoted here by the alphabets a-l) characteristic of the haploid genome (After Blakeslee, 1934. J. Hered. Vol. 25, pp. 80-108).

ii) Tetrasomy

Tetrasomy is characterized by presence of two extra copies of a chromosome ($2n+2$), i.e. any one of the chromosomes is present in four copies in a tetrasomic individual. The possible types of tetrasomics in a species is equal to the number of its haploid chromosomes. Tetrasomy is common in several plant species such as wheat. Selfing among trisomic plants yield tetrasomics. In humans tetrasomy leads to various syndromes pertaining to autosomal tetrasomy or sex chromosome tetrasomy.

SAQ 3

Give one word for each of the following:

- i) Double monosomics that lack two chromosomes each from a different homologous pair are designated as:
- ii) The chromosome that has two identical arms and lead to partial trisomy of certain part of genetic material is called:

6.5 AUTOSOMAL ANEUPLOIDIES

After you have learnt the aneuploidy, its origin and classic example of jimson weed let us now discuss some commonly found syndromes due to autosomal aneuploidy in men. The most common aneuploid conditions seen with appreciable frequency in live born children are trisomies **13**, **18** and **21**. In order of inoreasing frequencies, these trisomies are **Patan**, **Edward** and **Down syndromes** respectively.

6.5.1 Trisomy 21 - Down Syndrome

Down syndrome is a genetic condition in which an extra copy of chromosome 21 is present in an individual. It was initially observed and described by a British Doctor John Langdon Down in 1866 and thus was called 'Down syndrome' after his name. However, at that time he could not describe the reason of its occurrence and it was only later in 1959 when trisomy 21 was identified to be its cause.

Down syndrome is characterized by specific facial appearance, muscular hypotonia, cognitive delays, and intellectual disabilities (Fig. 6.12). Cognitive delays lead to abnormal behavior and slower development of speech and language skills. Down syndrome is identified through prenatal screening. **Parents with balanced translocation involving chromosome 21 are potential carriers of Down syndrome** and require genetic counseling to prevent the occurrence of this disorder. Maternal age is highly correlated with the occurrence of Down syndrome in child; the risk increases with the increase in age of mother. The chances are 1 in a 1000 at the age of 30, 1 in a 100 at the age of 40, while it is 1 in a 30 at the age of 45.

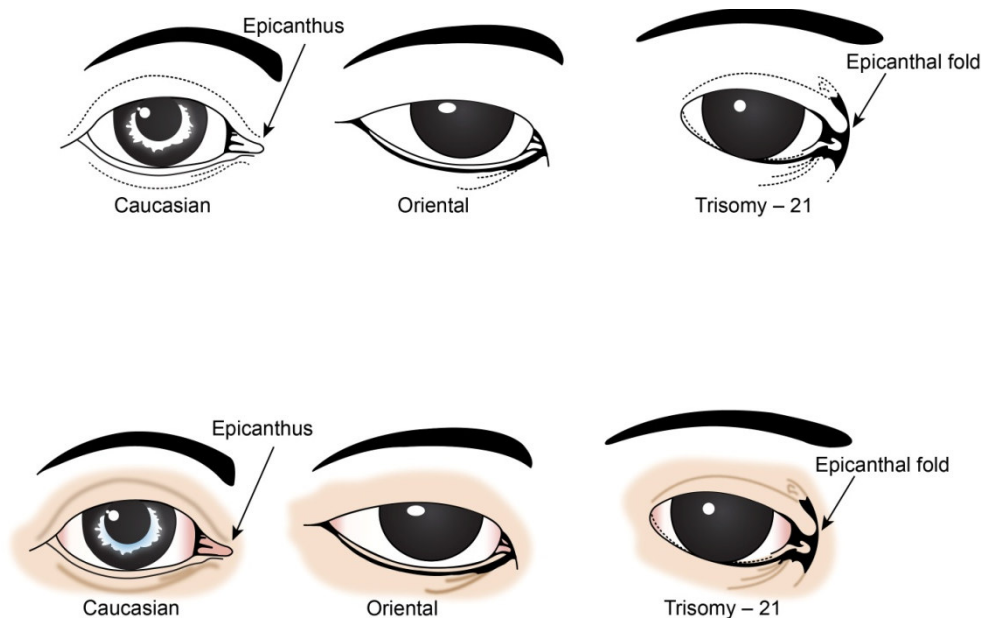


Fig. 6.12: Trisomy – 21 and the epicanthal fold. The junction where the eye folds come together is called the canthus. In trisomy-21, a vertical fold of skin near the basal ridge (the epicanthus) pulls and tilts the eye slightly toward the nostrils. Note that the upper eyelid in the normal caucasian eye runs parallel to the skin in fold below the eyebrow. In Orientals, this skin fold covers a major portion of the upper eyelid. In caucasians with trisomy-21 the epicanthal fold does not cover a major portion of the upper eyelid, making their eyes distinct from the Orientals. The eyes of persons with Trisomy-21 frequently show light patches on the iris. These spots are not found in the normal individuals.

Trisomy 21 is mainly caused by abnormalities during cell division, which can be of three types. In complete Trisomy 21, a whole chromosome 21 is present as an additional copy, i.e. instead of two, three chromosomes are present in an individual. Of the total trisomies 21, 90-93% cases are of Complete Trisomy 21. This extra copy comes from an abnormal gamete in which chromosome 21 has failed to separate during meiotic division. **Mosaic Down syndrome** or Mosaic Trisomy 21 is a rare condition found in <3% cases in which some cells of the body have an extra copy of chromosome 21, while others are normal and have only two of its copies. This forms a mosaic of cells in the body of organism. This mosaicism appears as a result of abnormalities in cell division in the zygote, leading to nondisjunction of sister chromatids to separate during mitosis.

Translocation Trisomy 21 is a type of trisomy which is found in only 2-4% cases. This condition arises due to translocation of some part of chromosome 21, usually the long arm, to another chromosome, most probably chromosome 14. The parent is a carrier in such case and is normal in physical and mental aspects. In such individuals no symptoms of Down syndrome appear as they consist of all the genes despite having 45 chromosomes (only one copy of chromosome 21). However, during formation of gametes in such a parent, the extra part of chromosome 21 is received by a gamete after meiosis, which on fertilization gives rise to the offspring with Down syndrome (Fig. 6.13).

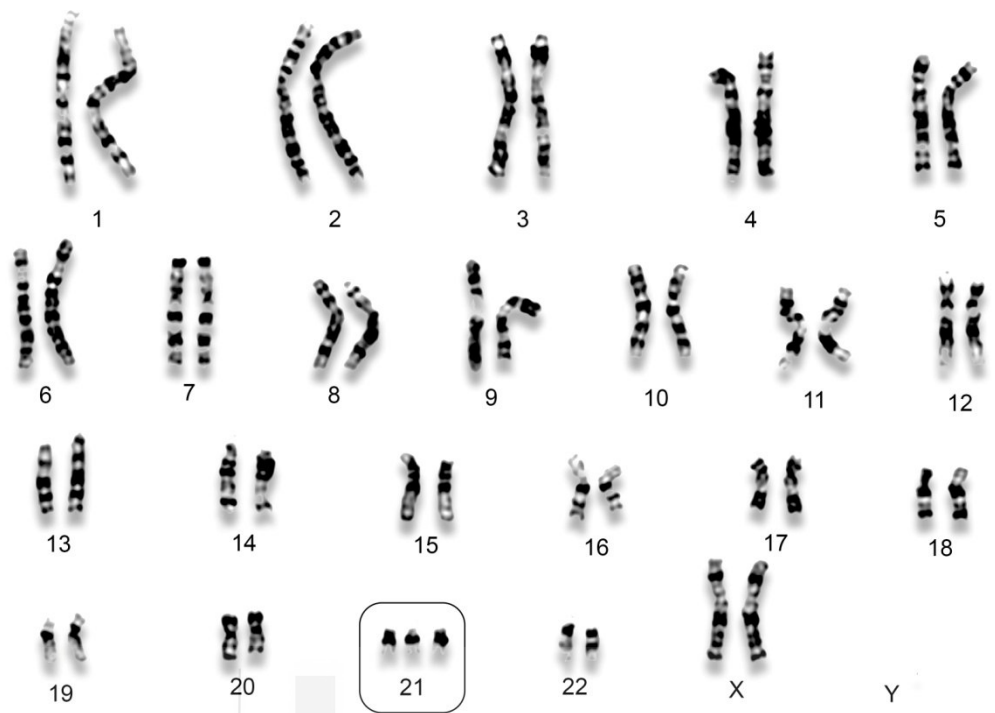


Fig. 6.13: Trisomy 21 or Down Syndrome.

The Down Syndrome (DS) Phenotype: Some three hundred phenotypic characteristics have been associated with Down syndrome. A few dozen of these traits are present in most children suffering from this disorder, and many of them appear as siblings at first glance. A child with DS ages rapidly, has an IQ range of 25-70, and has a flat facial profile and a broad 'saddle' nose (see Fig. 6.14).

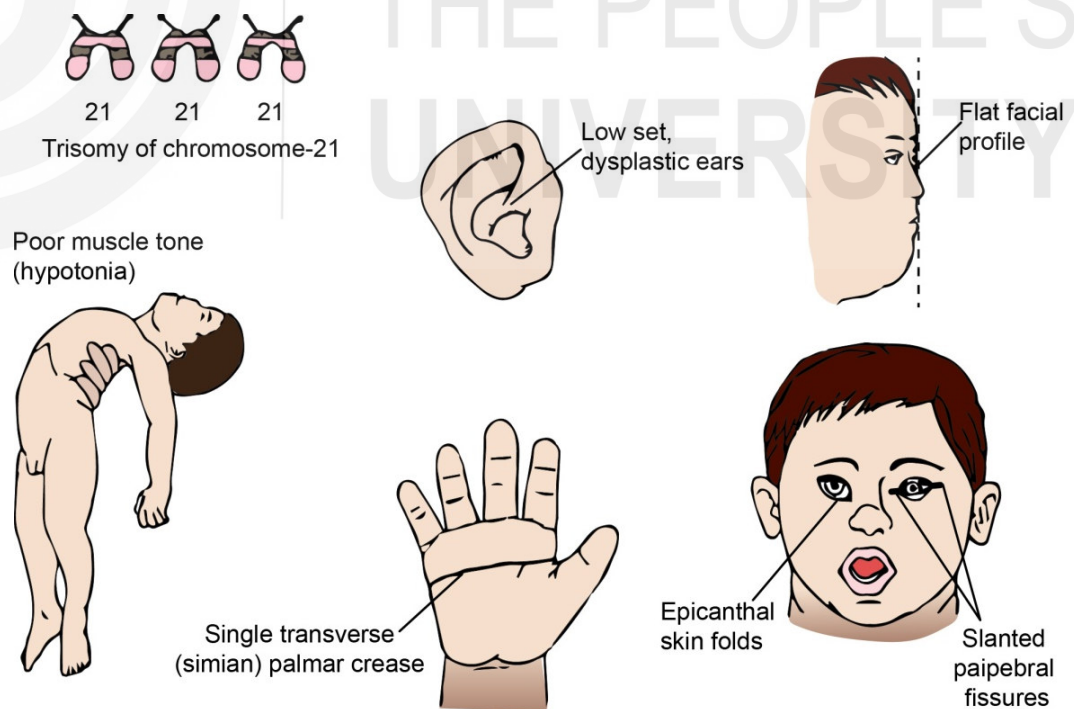


Fig. 6.14: Down syndrome phenotype, Infants suffering from this syndrome have lax and hyperextensible joints. The flat facial profile, low-set, rounded ears, presence of simian crease in the palm and eyes slanted with epicanthal skin fold, are some of the characteristic features of this syndrome.

The ears are small and round; the tongue is large and flat. The child has a protruding lower lip; a broad short skull; thick, short hands with stubby fingers; and thick, short feet and body. As you have seen in Fig. 6.14, DS children usually have light-coloured specks in the iris of their eyes, referred to as *Brushfield spots*. About 30 per cent have a simian crease, and 70 per cent have heart trouble. Hernias and a marked susceptibility to infections are associated with the syndrome. They are usually retarded in physical development and are short- statured.

For example, a six-month old baby with DS often acts like a baby of about two months: slow to sit up, crawl and walk. Sometimes does not talk until the age of 6 years or more. In extreme cases some never develop speech. DS patients have a tendency to be diabetic and to develop leukemia than are persons in the general population. Thirty per cent of DS children die within the first year; 50 per cent within five years; very few reached the age of twenty before antibiotics were introduced. Presently, with the use of antibiotics, life span of DS children has been stretched into the forties.

Origin of Down Syndrome: One of the main ways in which this trisomic condition originates is through **nondisjunction of chromosome 21** during meiosis. Failure of paired homologs to disjoin during anaphase I or II can result in male or female gametes with the $n+1$ chromosome composition. Following fertilisation with a normal gamete, the trisomic condition results. Chromosome banding studies have shown that, while the additional chromosome may be derived from either mother or father, defective ovum is most often the source.

Maternal/Paternal Age and the Down's Syndrome: Clearly, DS syndrome more than any other syndrome, shows marked relationship to maternal age (see Fig. 6.14). A female is born with all the oocytes she will ever produce – nearly 7 million. These remain in an arrested prophase I of meiosis from birth until ovulation, generally at the rate of one per menstrual cycle (about 28 days) after puberty. A given oocyte may remain in this state of suspended development for some 12 to 45 years or so. It has been observed that the accuracy of meiotic division decreases with age and after thirty the frequency of nondisjunction associated with chromosome 21 increases substantially. For women over the age of 45 years the probability of producing a DS child is about one in forty.

Higher than normal frequencies of children suffering from DS have also been discovered for mothers under the age of seventeen (Fig. 6.15). The exact reason, however, is not clear as yet. A convincing theory that may explain the relatively high incidence of DS for both older and younger mothers relates the frequency of nondisjunction to estrogen levels. Estrogen helps to control the rate of meiosis in Primary oocytes. As estrogen levels decrease with age, a slowing of the meiotic process may make nondisjunction more likely. Estrogen levels also do not stabilise following puberty until about the age of twenty. The DS children produced by very young mothers thus may reflect fluctuations in hormone levels. If age or age-related factors increase the likelihood of nondisjunction, then trisomies involving chromosomes other than chromosome 21 should also be more common in older mothers and even in younger than mothers. In fact, the incidence of trisomies 13 and 18 is higher in older women. Other trisomies also be common in these women, but because they are lethal, they are difficult to detect.

Microcephaly is a condition in which a baby's head is significantly smaller than expected, often due to abnormal brain development.

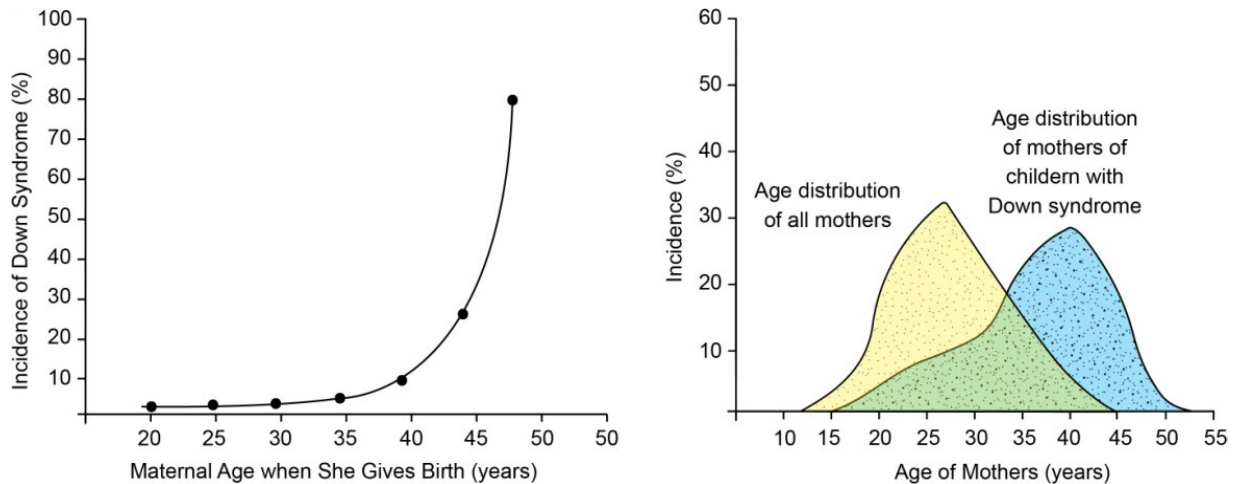


Fig. 6.15: Down syndrome and age of the mother. The incidence of DS is about 1 in every 600-700 live births. (a) Among women below age 17, and over the age of 35 years, however, the incidence of delivering a child with DS increases. (b) The correlation between maternal and DS risk is striking when the age distribution for all mothers is compared to that of mothers of DS children.

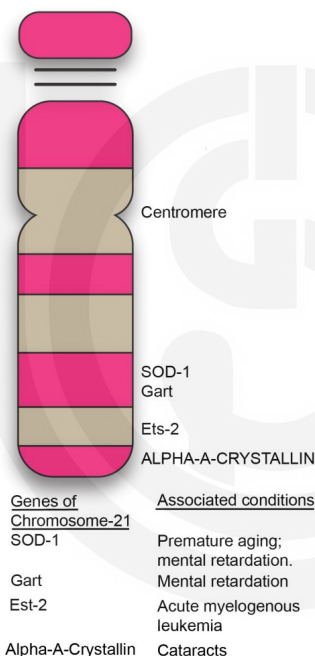


Fig. 6.16: A map of chromosome 21 and some of the genes that may cause phenotypic abnormalities associated with Down syndrome.

In the 1970s improved techniques for chromosomal banding analysis showed that in a considerable number of cases of DS, the extra chromosome is derived from the father also. The probability of a father having an affected child increases with age, as it does for mother. The rate of increase is relatively low until the age of fifty and steep thereafter.

This finding, thereby, implies that the chromosomes of both parents should always be investigated in trying to pinpoint the source of the genetic problem. Older mothers who have produced DS children have often borne an unnecessary burden of guilt, assuming they were the “cause” of their child’s defect and misery.

Chromosome 21 – This chromosome has been referred to several times in this above discussion. Now, let us discuss some details of this chromosome. It is one of the smallest in the human genome and contains about 45 million nucleotide base pairs or 1.5% of the total nuclear DNA. Because of its small size and its association with several important genetic conditions, it is now one of the best known chromosomes. About 1,500 genes are thought to be located on chromosome 21. Only about 20 of these genes have been mapped so far. These genes give sufficient clues about the phenotypic aspects of DS and related conditions (Fig. 6.16).

6.5.2 Trisomy 18 – Edward Syndrome

Another chromosomal abnormality referred to as **Edwards syndrome** (Fig. 6.17) is caused due to **Trisomy 18**. It is manifested by abnormalities in different body parts and organs. Trisomy 18 affects the foetus from the time of its development into the uterus, thus the growth of child remains retarded. New born babies have low birth weight and often defects and deformities develop in several organs. Most common abnormalities appearing during intrauterine development are cardiac and nervous system anomalies leading to defects in heart and shape of head (microcephaly). Due to these reasons,

survival rate of affected individuals is often low; many die either before birth or in early infancy stages. Only about 10 % children are able to survive longer than a year, that too with mental disabilities, malformations in body parts and developmental delays. With this survival rate, the frequency of occurrence of Trisomy 18 is about 1 in 5000 births, since most fetus with this disorder are not able to complete the term. The chances of this genetic disorder are also correlated with mother's age.

Edward Syndrome is characterized by the presence of an extra copy of chromosome 18 in addition to the two members of the homologous pair. The additional chromosome 18 can be present in an individual either as a complete copy or as some part of its genetic material. The extra genetic material appears before fertilization, i.e. it comes from either of the two gametes. Usually it is the result of abnormal meiotic division that takes place during gamete formation. The chromosomes fail to segregate and nondisjunction of chromosomes lead to appearance of 24 chromosomes (additional chromosome 18) in the resultant gamete instead of 23. Participation of such gamete in fertilization results into a zygote with trisomy 18 and 47 chromosomes in all.

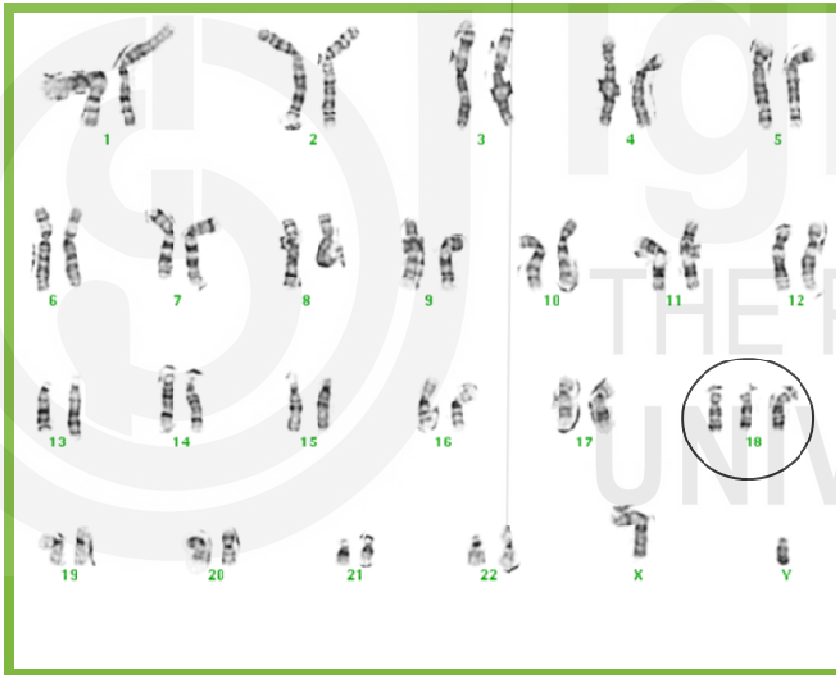


Fig. 6.17: Trisomy 18 or Edward Syndrome.

Mosaic Edward syndrome occurs due to the presence of an extra copy of chromosome 18 in only some cells of the body and not all. This type of trisomy occurs during early stages of embryo development due to defects in cell division, so that only some cells are trisomic and rest are diploid. This condition is therefore not inheritable. The severity of disorder depends upon the number and types of cells that are trisomic in the body.

There is another form of Edward syndrome called **Translocation Trisomy 18**, in which certain part of chromosome 18 gets translocated and attached to another chromosome, so that the number of chromosome 18 remains as two but additional genetic material of chromosome 18 is present on another

chromosome. This causes the appearance of complexities of Edward syndrome in an individual. However the degree of severity ranges from low to high due to partial trisomy, depending upon the extent of translocated genetic material. Translocation of chromosome 18 also occurs usually during gamete formation due to meiotic defects.

In 1960, John Edward and his colleagues reported on an infant trisomic for a chromosome in the E group, now known to be chromosome 18 (Fig. 6.18) designated as 47, 18+. Incidence is about 1 in 4,000 live births. Eighty per cent of trisomy 18 cases are due to meiotic nondisjunction; 10 per cent are due to translocations; and 10 per cent are the result of trisomy mosaics. This syndrome occurs three times more frequently in females than in males. Of the live born, survival beyond one year is rare.

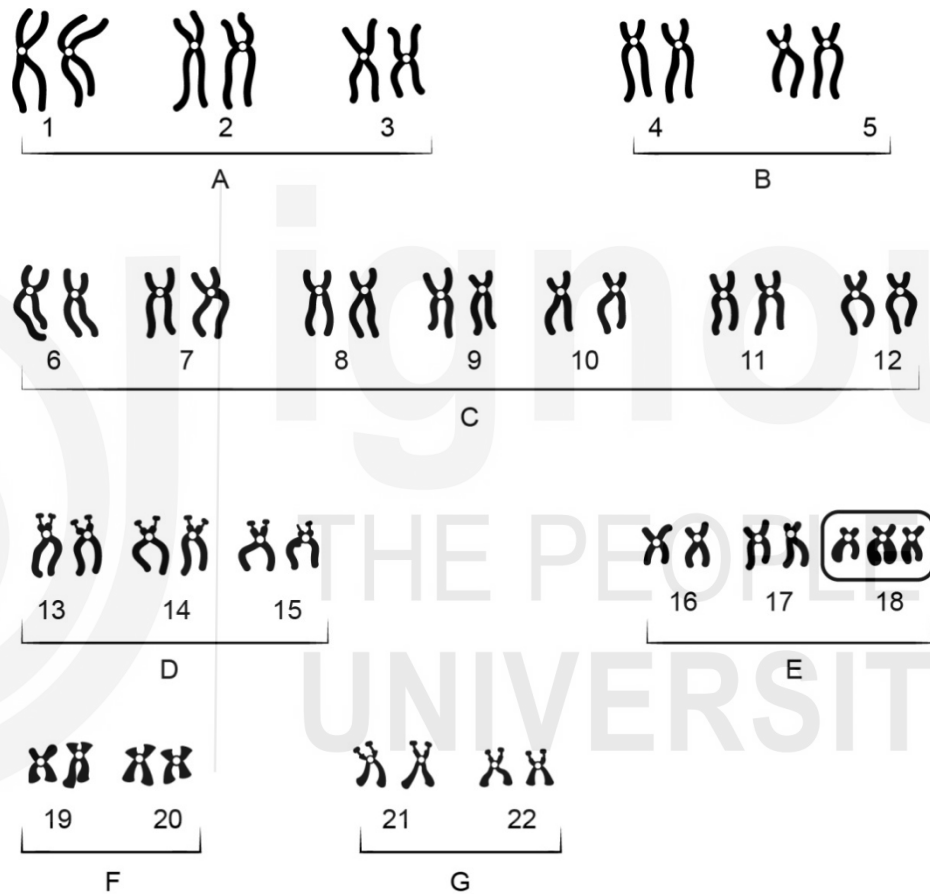


Fig. 6.18: Karyotype of a child with Edward syndrome.

Characteristics of trisomy 18 children are highly variable and pronounced. They demonstrate severe mental retardation, odd-shaped head, webbed-neck, short thumb, low-set ears, small mouth, receding chin, the index finger overlapping the middle and ring fingers, rocker bottom feet and clefts in the lip and palate. Heart defects are found in nearly all trisomy 18 births as well. Survival time is less than four months on the average. Death is usually caused by pneumonia or heart failure.

As in the Down syndrome, there is a pronounced maternal age effect. On plotting frequency against maternal age, a bimodal curve is obtained (Fig. 6.19). A secondary peak in the early 20s reflects the normal maternal age group of maximum births, whereas the pronounced peak from 35 to 45 years is clearly related to the greater age of the mother.

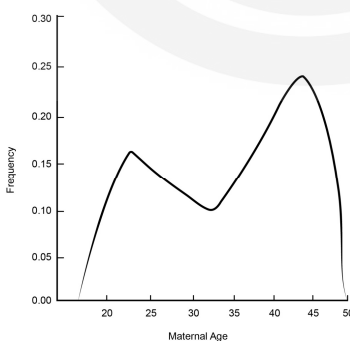


Fig. 6.19: The effect of maternal age in Edward's syndrome. Note the peak between maternal age 35 and 45 indicating the age group associated with maximum production of affected children.

6.5.3 Trisomy 13 – Patau Syndrome

Trisomy 13 is also known as **Patau syndrome**. It is a genetic condition arising due to the presence of an extra copy of chromosome 13 (Fig. 6.20). The manifestations of this condition include cardiac anomalies, defects in nervous system, hypotonia or weaker muscle tone, deformities in physical features and intellectual disabilities. The survival rate of such affected individuals is very low due to multiple serious abnormalities and defects and most children fail to live beyond few weeks or months. The occurrence of Trisomy 13 is just 1 in about 16 thousand cases and the chances increase fairly with the maternal age.

The extra genetic material may be present in form of a complete chromosome 13, i.e. 3 copies of chromosome 13 are present. In such case the severity of anomalies is high, lowering the chances of survival of affected individual. In mosaic condition, an extra copy of chromosome 13 is present only in few cells of the body and not all, therefore, its effects are less severe. If the extra genetic material of chromosome 13 gets translocated to another chromosome, it is called Translocation Trisomy 13. This usually happens during gamete formation or sometimes during early stages of embryo development.

Translocation trisomy 13 is inheritable; a parent with a balanced translocation is a potential carrier and is likely to produce a child with trisomy 13.

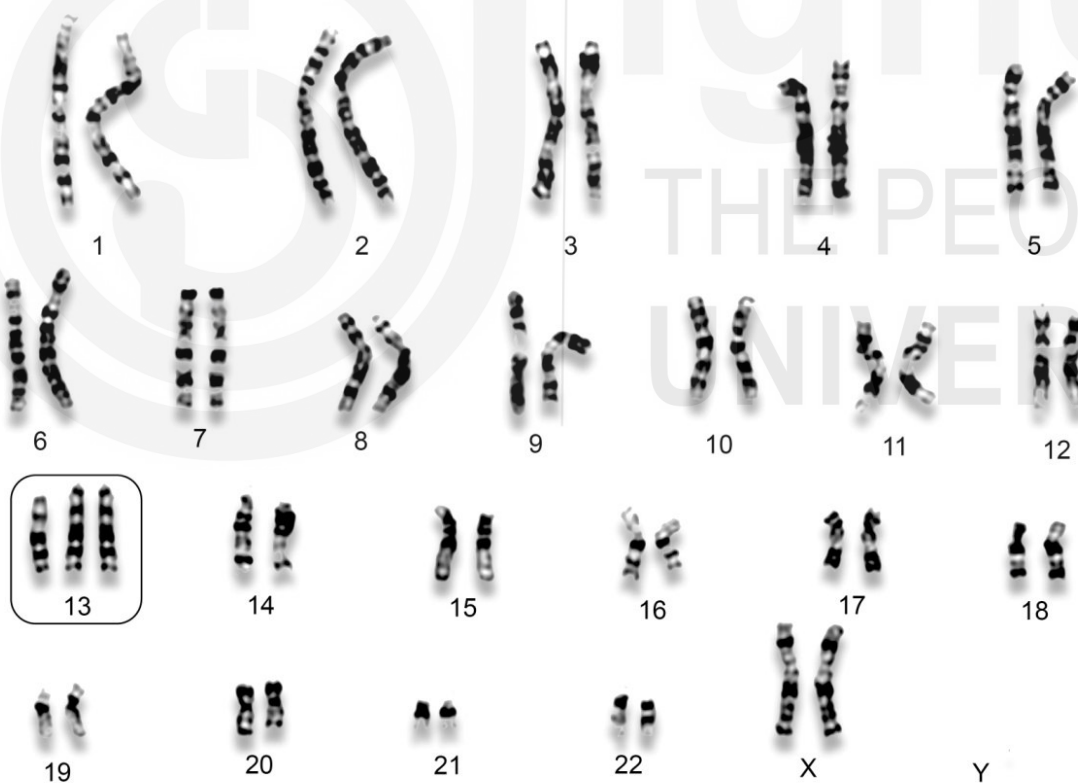
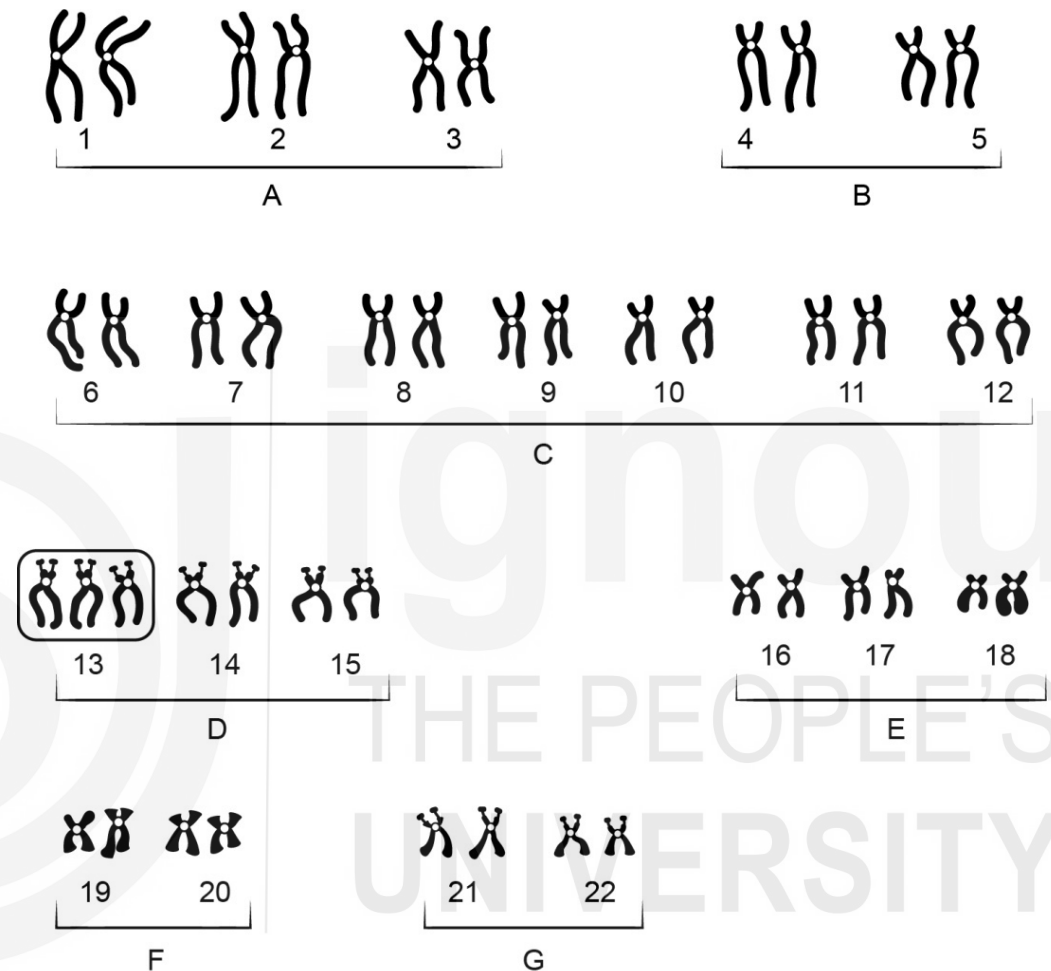


Fig. 6.20: Trisomy 13 – Patau Syndrome.

In 1960s Klaus Patau and his associates observed an infant with gross developmental malformations with a karyotype of 47 chromosomes (Fig. 6.21). The additional chromosome was medium-sized, one of the acrocentric D group. The G and Q banding techniques have identified the extra D chromosome as number 13. The trisomy 13 condition has since been described in many new borns, and is called Patau syndrome, being

designated as 47, 13+. Seventy percent of the trisomy 13 cases are caused by meiotic nondisjunction; 10 per cent of the cases are trisomy mosaics; and 20 per cent are partially trisomic due to translocation. As the chromosome 13 contains considerable active genetic material, most chromosome 13 trisomies abort. Of those that are live born, 90 per cent die during their first year of life. Those few that survive beyond the age of two years suffer from severe mental retardation.



Simian crease is an older name for a condition now more commonly called as **single transverse palmar crease** (STPC). It refers to a single crease across the palm of the hand. In most individuals, there are two slightly offset creases across the palm.

Fig. 6.21: Karyotypes of a child with Patau syndrome.

The characteristics of trisomy 13 include seizures, moderate microcephaly (small head), wide-spaced eyes, beaked nose, clefts of the lip and palate, low-set ears, scalp defects, and characteristic 'trigger thumb' (the thumb and index fingers overlap the third finger). Trisomy 13 babies display a simian crease (one continuous crease across the palm of the hand), as in Down syndrome, as well as polydactyly (extra fingers and toes), congenital heart disease, kidney disorders and malformed genitalia.

The average maternal and paternal ages of parents of affected infants are higher than the ages of parents of normal children, but paternal ages are not as high as the average maternal age as in cases of Down syndrome. Both male and female parents average about 32 years of age when the affected child is born. Because the condition is so rare, it is not known if the origin of the extra chromosome is more often maternal, paternal or arises equally from either parent.

SAQ 4

Give one word for each of the following:

- i) Down syndrome is caused due to:
 - ii) Trisomy 18 that affects the intrauterine development of foetus and retards the growth of child is called:
 - iii) Presence of three copies of chromosome 13 in an individual leads to:
-

6.6 SEX CHROMOSOME ANEUPLOIDIES

Aneuploidies of the sex chromosomes are found more commonly as compared to other aneuploidies, one in a thousand people may exhibit such aneuploidy. Although sex chromosome aneuploidies prevail in population, but are usually not obvious and often remain undiagnosed. The specific genetic mechanisms responsible for such anomalies are also not very well studied. These aneuploidies are usually characterized by peculiar physical appearance and developmental patterns.

As in other types of aneuploidies, these conditions also arise due to non disjunction of chromosomes, either during meiotic process of gamete formation or during early stages of embryonic development. The aneuploidy may appear in all the cells of body or in some cells only resulting into a mosaic.

6.6.1 XXX – Trisomy X Syndrome

This syndrome is also known as trisomy X, XXX or 47, XXX syndrome (Fig. 6.22). As the name suggests it is due to the presence of an extra X chromosome in females, in addition to 46,XX. Such female individuals may show somewhat greater height than normal, however no prominent physical abnormality is seen. Their sexual development is normal and they are able to give birth to children. In some affected individuals the presence of an extra X chromosome may cause developmental delays and intellectual disabilities, the intensity of which may vary from person to person. The frequency of occurrence of Trisomy X is 1 in a thousand females.

Triple X mosaicism is also found in which extra copy of X chromosome is present only in some cells of the body and rest of the cells are normal with 46,XX. This syndrome is not inherited. The extra copy of chromosome X may arise due to non-disjunction of sex chromosomes during meiosis and one of the gametes may receive two X chromosomes. Such gamete after fertilization will contribute the additional copy of X chromosome leading to Trisomy X.

The diagnosis of this condition is usually done through cytogenetic tests like **Fluorescence In situ hybridization (FISH)** and karyotyping or through molecular diagnostics involving **DNA sequence analysis, targeted variant analysis** or **tests for homozygosity**.

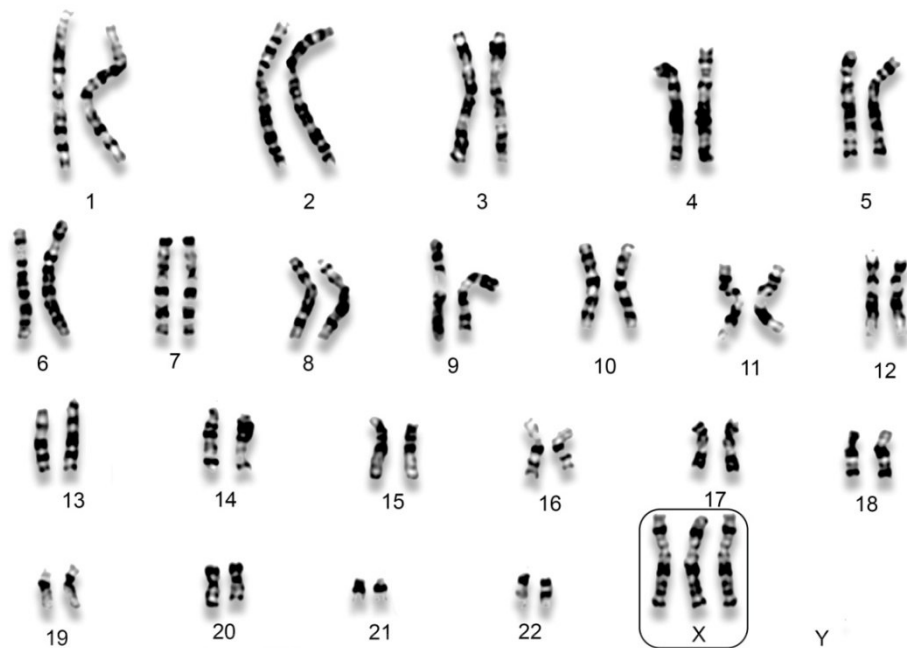


Fig. 6.22: XXX – Trisomy X Syndrome.

6.6.2 XYY - Double Y Syndrome

This syndrome is called 47,XYY syndrome as it happens due to the presence of an extra copy of Y chromosome in males (Fig. 6.23). The individuals having this condition are taller than normal males. Besides this condition may give rise to learning disabilities, delay in cognitive and motor development, weaker muscle tone, curved finger and curvature in spine. Delayed development may also lead to depression and anxiety resulting into emotional imbalances. Such people may thus face difficulty in socializing. Sexual development of 47,XYY males is normal and they are fertile. The frequency of occurrence of such males is 1 in a thousand.



Fig. 6.23: XYY - Double Y Syndrome.

The occurrence of an extra Y chromosome is attributed to random events happening during cell division and sperm formation. Non disjunction of chromosomes during meiosis is seen as the main reason for 47,XYY condition. Sperms with two Y chromosomes may participate in fertilization to produce double Y syndrome in the offspring. In certain cases, an extra Y chromosome may also appear due to mitotic disorders during early developmental stages of embryo.

Diagnosis of double Y syndrome is made through genetic testing or karyotyping. Prenatal diagnosis is done by amniocentesis and chorionic villi sampling.

6.6.3 XXY - Klinefelter Syndrome

Klinefelter syndrome is also called XXY syndrome or 47,XXY (Fig. 6.24). This condition is characterized by the presence of an extra X chromosome in males instead of usual male genotype XY, and is associated with several physical and developmental abnormalities. The affected males have small testes and produce less testosterone due to which their sexual development is highly affected and they develop several feminine characteristics. They grow taller than average and develop enlarged breast (gynecomastia), broader hips, lesser body hair, oligospermia and infertility. Genital defects may also include undescended testes and under developed penis (micropenis). Children with Klinefelter syndrome show delay in cognitive development, learning disabilities and difficulties in speech and language development. The frequency of occurrence of Klinefelter syndrome in a population is about 1 in a 1,000 male births.

Klinefelter syndrome was identified symptomatically by Harry Klinefelter in 1940, however it was in 1956 when its cause was attributed to the presence of an extra copy of X chromosome. The intensity of symptoms of Klinefelter syndrome vary among individuals. Other variants of Klinefelter syndrome also exist, though very rare, which may be characterized by 48, XXXY or 49, XXXXY. In such cases, the symptoms are more severe causing developmental delays, intellectual disabilities, physical abnormalities and increased risk of diseases.

The appearance of extra X chromosome is due to meiotic abnormalities occurring during gamete formation. A gamete with extra X chromosome may contribute to the additional copy of X in zygote on fertilization with normal gamete. All the cells of an embryo will then receive this extra copy and the male child will develop Klinefelter Syndrome. Mosaicism is also seen in this syndrome, in which the extra X chromosome comes into existence due to abnormal cell division during embryonic development. This results into 47,XXY condition only in some cells, other body cells being normal (46, XY).

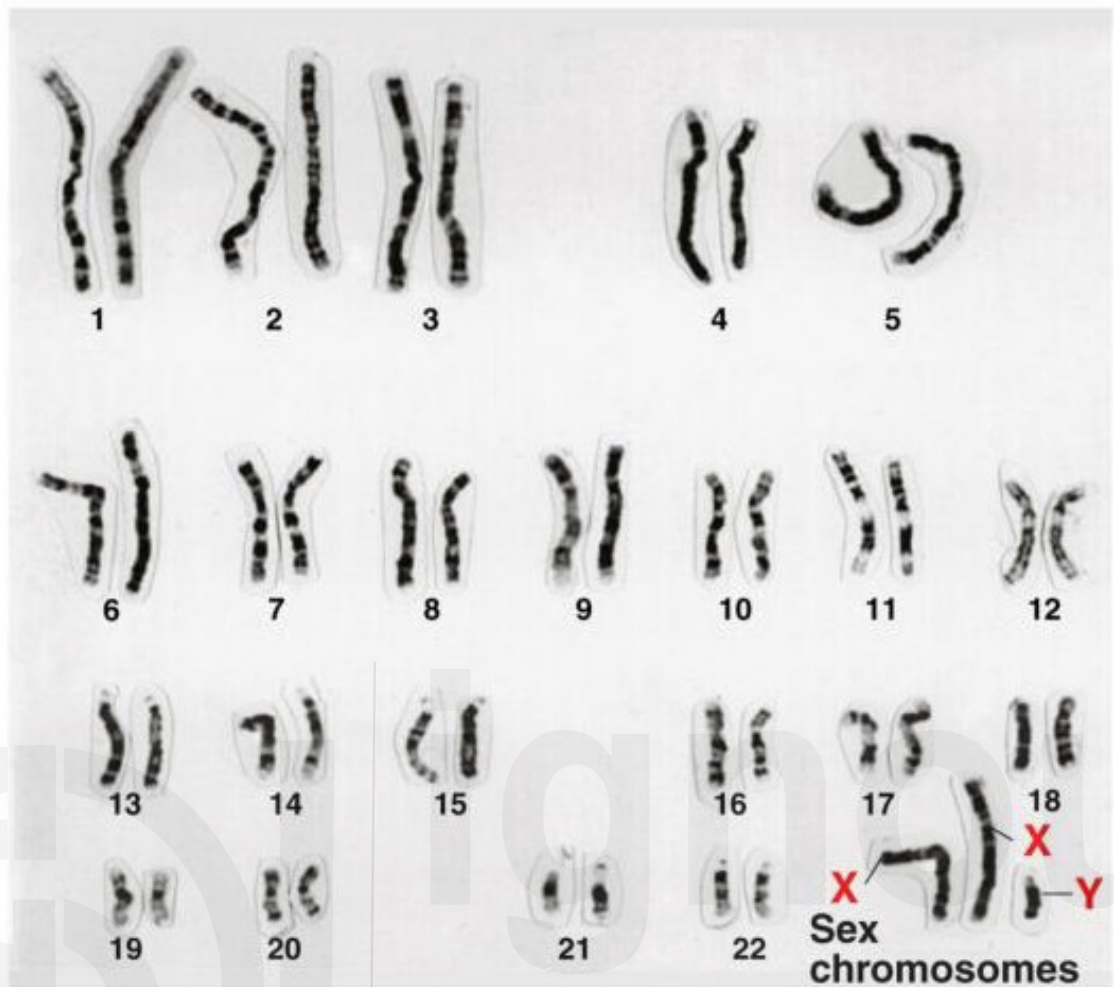


Fig. 6.24: XXY - Klinefelter Syndrome.

6.6.4 XO – Turner Syndrome

Turner syndrome occurs due to absence one X chromosome, i.e. the genetic constitution of affected females is XO (Fig. 6.25). This condition is characterized by short stature and many physical deformities. Many females develop skeletal anomalies, have swollen hands and feet and a webbed neck. Defects in heart and kidneys may also appear, which may turn into serious complications. Congenital heart diseases are commonly observed in such cases. Turner syndrome also hampers ovarian development leading to its hypo-functioning. This affects the onset of puberty and subsequently the appearance of secondary sex characters in girls. Most females suffer from infertility. Some girl children with Turner syndrome may encounter delays in cognitive development and learning disabilities.

Turner syndrome is found in about 1 in 2,500 newborn girls, however, a large number of such fetuses are not able to complete their term and die prematurely.

The genetic defect XO arises from non disjunction of chromosome due to defective meiosis during gamete formation, usually during sperm formation. In most cases of Turner syndrome, the monosomic X chromosome is contributed by mother. X chromosome mosaicism may also be observed rarely.

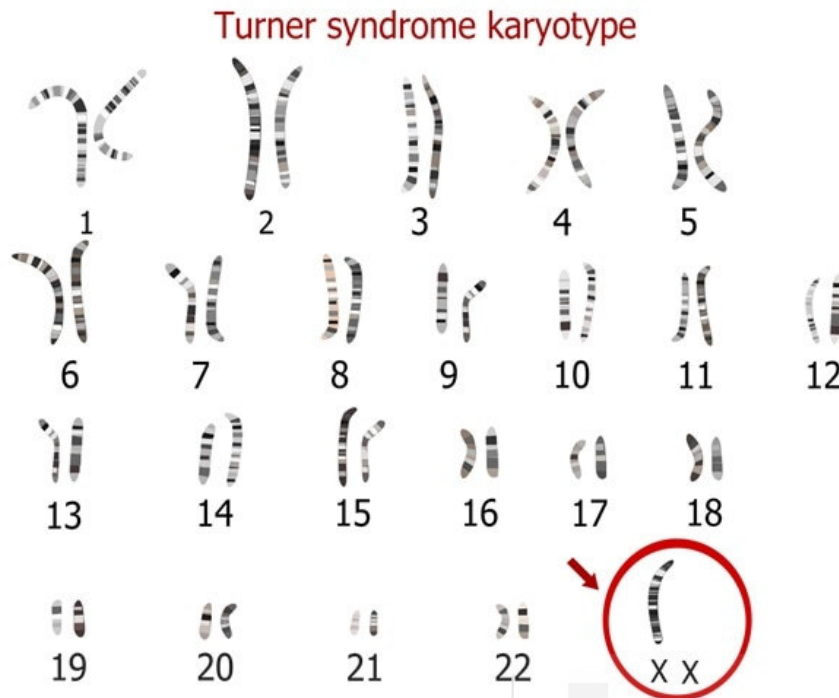


Fig. 6.25: XO – Turner Syndrome.

SAQ 5

Give one word for each of the following:

- i) Cytogenetic tests that can help to detect sex chromosome aneuploidies are.
- ii) Syndrome in which affected males develop feminine characteristics is.
- iii) Absence of one X chromosome in females is called.

6.7 SUMMARY

Let us summarise what you have learnt so for:

- Numerical anomalies in chromosomes lead to serious defects in individuals. These anomalies can be either due to a missing chromosome or due to the presence of one or two extra chromosomes.
- Non disjunction of chromosomes during meiosis forms unbalanced gametes, which on fertilization give rise to numerical anomalies.
- Mosaicism is the condition in which numerical anomaly of chromosomes occurs only in some cells of the body, other cells being normal i.e. diploid.
- Monoploidy refers to the presence of only one complete set of chromosomes, while Polyploidy is the occurrence of multiple copies of a complete set of chromosomes in an organism.
- Repetition of the chromosomal sets lead to occurrence of triploid ($3n$), tetraploid ($4n$), hexaploid ($6n$) conditions and so forth. Polyploidy is very common in plants but rare in animals. Some invertebrates demonstrate the polyploidy condition.

- Polyploids are often sterile due to inappropriate chromosome segregation during meiosis and formation of defective gametes.
- Autopolyploidy refers to the presence of multiple sets of chromosomes of same genome, while Allopolyploidy is the condition in which different sets of chromosomes in a polyploidy species come from genomes of different species.
- Any change in normal chromosome numbers due to loss or gain of one or more chromosomes is called Aneuploidy. Loss of a single chromosome ($2n-1$) is called monosomy and loss of one complete homologous pair of chromosome is called nullisomy ($2n-2$).
- Presence of one or more extra chromosomes in the set is called hyperploidy. Presence of one extra chromosome ($2n+1$) is referred to as trisomy and of one extra copy each of two different chromosomes is called double trisomy ($2n+1+1$). Tetrasomy is characterized by presence of two extra copies of a chromosome ($2n+2$).
- Down syndrome or Trisomy 21 is a genetic condition in which there is an extra copy of chromosome 21 in the genome. It is characterized by specific facial appearance, muscular hypotonia, cognitive delays, and intellectual disabilities.
- Edwards syndrome or Trisomy 18 occurs due to the presence of an extra copy of chromosome 18 in the genome. It gives rise to several abnormalities during foetal development leading to retardation of growth and physical deformities like microcephaly.
- Patau syndrome or Trisomy 13 occurs due to the presence of an extra copy of chromosome 13. The manifestations of this condition include cardiac anomalies, defects in nervous system, hypotonia or weaker muscle tone, deformities in physical features and intellectual disabilities.
- Trisomy X, XXX or 47, XXX syndrome is a sex chromosomes aneuploidy in which an extra X chromosome is present in females. Usually such females are taller than normal, besides no abnormalities are seen in physical or developmental aspects.
- Double Y Syndrome, XYY, or 47,XYY syndrome occurs due to the presence of an extra copy of Y chromosome in males. Such males are taller than normal and this condition may cause abnormalities like weaker muscle tone, curved finger and curvature in spine. Other effects may include learning disabilities as well as delay in cognitive and motor development.
- Klinefelter syndrome, XXY syndrome or 47,XXY is characterized by the presence of an extra X chromosome in males. The affected males have small testes and produce less testosterone due to which their sexual development is highly affected and they develop several feminine characteristics.
- Turner syndrome or XO syndrome occurs due to absence of one X chromosome, i.e. the genetic constitution of affected females is XO. Such females have short stature and many physical deformities such as skeletal anomalies and defects in major internal organs.

6.8 TERMINAL QUESTIONS

1. Differentiate between autopolyploids and allopolyploids.
2. Explain why sterility is common in polyploid organisms?
3. What is trisomy? By what mechanisms does it come into existence?
4. Describe the characteristic symptoms, types and inheritance of Down syndrome.
5. Discuss the appearance of mosaic aneuploidy.

6.9 ANSWERS

Self Assessment Questions

1.
 - i) Nondisjunction during meiosis.
 - ii) Mosaicism
2.
 - i) Monoploidy
 - ii) Polyploidy
 - iii) The polyploid tissues grow through cell divisions that can occur regularly among polyploid cells. Sexual reproduction of animals requires gamete formation, fertilisation, and sex determination. Many irregularities associated with polyploidy result in inviability and sterility in animals.
 - iv) Colchicine treatment interferes with spindle formation during cell division. Double number of chromosomes are thus included in the daughter cells.
3.
 - i) $2n-1-1$.
 - ii) Isochromosome .
4.
 - i) Trisomy of chromosome number 21.
 - ii) Edward Syndrome.
 - iii) Patau Syndrome.
5.
 - i) Fluorescence in situ hybridization (FISH) and Karyotyping.
 - ii) Klinefelter Syndrome or XXY syndrome.
 - iii) Turner Syndrome or XO syndrome.

Terminal Questions

1. Refer to Sub-Section 6.3.3 (a) and (b).
2. Refer to Sub-Section 6.3.3 (d).
3. Refer to Sub-Section 6.4.2.
4. Refer to Sub-Section 6.5.1.
5. Refer to Sub-Section 6.5.1.

FURTHER READINGS

1. Principles of Genetics by Gardner, Simmons, Snustad, 8th edition – John Wiley and Sons, Inc., 2003.
2. Genetics: a Conceptual Approach, 3rd edition, Peter J. Russell. Pub: WH Freeman & Co.
3. Genetics: Principles and Analysis, 4th edition. D.L. Hartl, D.W. Jones. Pub: Jones and Barlett Publishers
4. Genetics, 9th revised multicolor edition. P.S. Verma & V.K. Agarwal. Pub: S. Chand & Co.

Acknowledgment of Figures

Fig. 6.8: <http://www.biologydiscussion.com/chromosomes/aneuploidy-meaning-forms-and-importance-cell-biology/38894>

Fig. 6.12: <https://shsgdp.wikispaces.com/down+syndrome+2>

Fig. 6.16: <https://mydoctor.kaiserpermanente.org/ncal/specialty/genetics/resources/conditions/trisomy18.jsp>

Fig. 6.17: <https://www.larasig.com/node/1735>

Fig. 6.23: Pearson Education. inc. 2009

Fig. 6.24: <https://www.news-medical.net/health/Turner-Syndrome-Signs-and-Symptoms.aspx>

UNIT 7

SEX DETERMINATION

Structure

- | | | | |
|-----|--|------|---------------------------------------|
| 7.1 | Introduction | 7.6 | Sex-Limited and Sex-Enfluenced Traits |
| | Objectives | | Sex-Limited Traits |
| 7.2 | Genetic Basis of Sex Determination | | Sex-Influenced Traits |
| | Genic Type | 7.7 | Dosage Compensation |
| | Chromosomal Type | | In Man |
| 7.3 | Chromosomal Sex Determination Mechanisms | | In <i>Drosophila</i> |
| | The XX-XY System | 7.8 | Summary |
| | A Variation: The ZZ-ZW System | 7.9 | Terminal Questions |
| | The XX-XO System | 7.10 | Answers |
| | Sex Determination by Ploidy Level | | |
| | The Compound Chromosome System | | |
| | The Transfer Gene | | |
| 7.4 | The Chromosome Tneory of Inheritance and Sex-Linkage | | |
| 7.5 | Sex-Linked Inheritance | | |
| | X-Linked Traits in Humans | | |
| | Y-Linked Traits in Humans | | |

7.1 INTRODUCTION

One of the probable deductions from your study of Mendel's laws of inheritance and their extensions and modifications in Units 1 and 2 may be that contribution to inheritance is equal from both the parents. But sex linkage is a major exception to it. Sex linkage occurs when a gene controlling a trait is located on the sex chromosome. The sex chromosome bears several genes in

addition to those directly concerned with sex determination. The inheritance of these genes follows a characteristic pattern which is different from that seen in the examples of monohybrid and dihybrid inheritance, that you have studied so far. The unique sex-linked pattern(s) of inheritance of any particular trait can be easily recognised and studied by pedigree analysis.

Sex linkage forms the main theme of this unit. But we begin by a brief discussion on mendelian factors that we now know are the genes and are located on the chromosomes. Then you will study examples of genes located on the sex chromosomes and their mode of transmission to the next generation. These would be explained with examples of X-, and Y-linked genes. This is followed by a subsection dealing with sex determination in eukaryotes, which involves both environmental and genetic factors. Next, you would study the genetic basis of sex determination, which is either of genic type or of chromosomal type.

Objectives

After studying this unit you would be able to:

- ❖ distinguish between the genic and chromosomal type of sex determination,
- ❖ describe the chromosomal sex determination mechanisms,
- ❖ relate the chromosome theory of inheritance to sex linkage,
- ❖ distinguish the mode of inheritance between the X-linked genes and Y-linked genes,
- ❖ explain with the help of examples the transmission of recessive and dominant X-linked traits,
- ❖ explain with examples the transmission of Y-linked genes,
- ❖ discriminate between sex-limited and sex-influenced genes, and enumerate their role in the control of secondary sexual characters,
- ❖ explain the importance of dosage compensation mechanism,
- ❖ differentiate between the type of dosage compensation in mammals and *Drosophila*, and
- ❖ describe the existence of female mosaics with respect to X-linked traits.

7.2 GENETIC BASIS OF SEX DETERMINATION

In most organisms, the sex determining mechanism is under genetic control, free of environmental factors. In the genome of higher organisms, there are certain genes or chromosomes which regulate sex determination and thus sex is determined at the time of fertilisation.

7.2.1 Genic Type

In some organisms, certain **independent genes located on different chromosomes** are responsible for sex determination. Most species of fish illustrate this mode. The male may be represented as AA^1 for the sex determining genes, and the female as AA (Fig. 7.1). According to this assumption, maleness is determined by the gene A^1 which is dominant over A .

In a parasitic wasp, called *Habrobracon*, genes are responsible for sex determination in a different way. The sex determining gene has multiple alleles (X^a , X^b , X^c , X^d). When two alleles in a zygote are different or heterozygous (X^aX^b , X^aX^c , X^bX^c , etc.) a normal, fertile female is formed. If the alleles in the zygote are in homozygous (X^aX^a , X^bX^b , etc.) or hemizygous (X^a , X^b , etc.) condition males are formed.

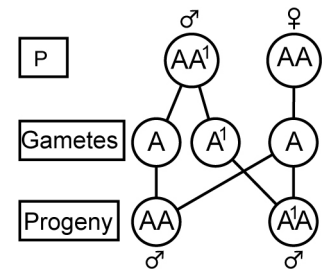


Fig. 7.1: Sex determination in fish at genic level.

7.2.2 Chromosomal Type

It refers to the condition where the genes involved in sex determination are located on specific chromosomes known as the sex chromosomes.

First, we shall see how the genic type of sex determination evolved into a chromosomal type of sex determination mechanism. In the primitive forms, the only difference between the two sexes was in their gametes. Later in evolution, morphological or phenotypic difference developed in the two sexes of a species.

In primitive forms, sex determination was due to genes on autosomes (the genic type). In the process of evolution, gradually, the genes responsible for sex determination got localised on specific chromosomes - the sex chromosomes. These chromosomes were designated as 'X' and 'Y' or 'Z' and 'W' and they can usually be distinguished morphologically from each other. The remaining chromosomes of the complement are known as 'autosomes' and are designated 'A'.

The X and Y chromosomes differ from each other in many respects. This is because, there is accumulation of sex determining genes on the respective sex chromosomes. Also, there is negligible crossing-over between the X and Y chromosomes. This helps to preserve gene combinations favouring distinct sexual differences. The consequence is that the Y chromosome bears mostly the genes essential for the male determination while all the other genes become inert. These regions got reduced in size in some species and are completely lost in others. This was how the heteromorphic sex chromosome evolved.

7.3 CHROMOSOMAL SEX DETERMINATION MECHANISMS

In most of the higher plants and animals, the chromosomal sex determination mechanisms are prevalent. Basically, five types of chromosomal mechanisms exist. These are XX-XY, ZZ-ZW, XX-XO, ploidy level, and compound chromosome system.

7.3.1 The XX-XY System

This is a common mode of sex determination in animals including man and some plants like the angiosperm genus *Lychnis* (see details in subsection 3.5.1). Both the sexes have equal number of chromosomes, of which one pair is of sex chromosomes. In females, the two sex chromosomes are similar and are called the X-chromosomes. Thus, the female is the **homogametic** sex ('homo' meaning same). Males in contrast usually possess an X chromosome, and one chromosome is dissimilar in morphology from all others and is known as the Y chromosome. Because the male sex chromosomes are different, the male is called the **heterogametic** sex ('hetero' meaning different).

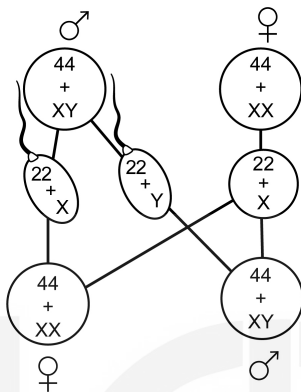


Fig. 7.2: The XX-XY system of sex determination in man.

In humans, the characteristic diploid chromosome number is 46 (Fig. 7.2). The females have 22 pairs of autosomes (AA) and a pair of X chromosomes (AA + XX). The males have 22 pairs of autosomes (AA) along with an XY pair (AA + XY). The sperms formed are either X-bearing or Y-bearing. This sex is determined by the sperm because all eggs are similar and X-bearing. Studies on sex chromosomal abnormalities in man, helped in understanding the crucial role of Y-chromosome in determining maleness. It has been observed that a single Y chromosome, irrespective of the number of X-chromosomes present in the zygote, causes an individual to develop into a phenotypic male. And in the absence of a Y-chromosome, the zygote leads to femaleness. Genes on the Y chromosome direct differentiation of the embryonic gonad to a testis, whose hormonal products then induce a male phenotype. Genes on the Y chromosome also control spermatogenesis. Thus, the presence of the Y chromosome determines maleness, and without a Y chromosome, the female phenotype develops.

7.3.2 A Variation: The ZZ-ZW System

Under this system, the *male is the homogametic sex* and the *female is heterogametic*. In order not to confuse this type of sex determination with the XX-XY mechanisms, the male chromosomes are labelled ZZ, the female ZW. In this system the ovum determines the sex of the resultant offspring, because all sperms carry similar chromosomes. Other than the reversal of homo-, and heterogametic sexes, the ZZ-ZW system functions similar to the XX-XY system. This mode of sex determination has been observed in birds including domestic fowl (see Fig. 7.3), butterflies and moths, some fishes, reptiles and amphibians, and in a plant species *Frageria orientalis*.



Fig. 7.3: The ZZ-ZW system of sex determination in domestic fowl.

7.3.3 The XX-XO System

In some species, the two sexes have different numbers of chromosomes. The difference often involves the sex determination mechanism. This phenomenon is called the XX-XO system (O indicates the absence of one sex chromosome). The female has two sex chromosomes just as in the XX-XY system, but the male has only one and is thus designated XO. In the species exhibiting this system, the diploid number of the chromosomes in male is one less than that of the female as a result of the absence of one sex chromosome. Consequently, the number of chromosomes is odd in males and even in females. The grasshopper (Fig. 7.4) is a good example of this mode of sex determination. The cricket and the beetle also exhibit a similar chromosomal basis of sex determination.

7.3.4 Sex Determination by Ploidy Level

In many species of hymenoptera (bees and wasps) and some mites and ticks, sex is determined by the number of sets of chromosomes or ploidy of the individual. Females are diploid, producing haploid gametes via meiosis. Most eggs are fertilized by haploid sperm from males, but a few are not. The fertilised eggs become females that show biparental inheritance. While the unfertilised eggs develop into haploid males (by parthenogenesis) that inherit their genes exclusively from their mother (Fig. 7.5). Haploid individuals, of course, cannot undergo normal meiosis, so males produce gametes via mitosis.

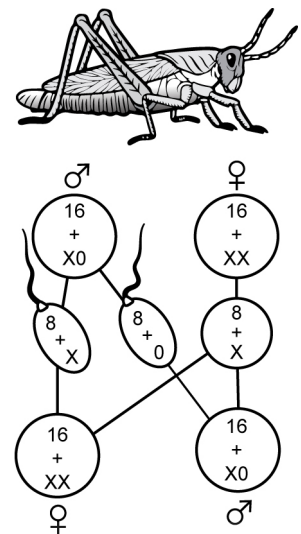


Fig. 7.4: The XX-XO system of sex determination in grasshopper.

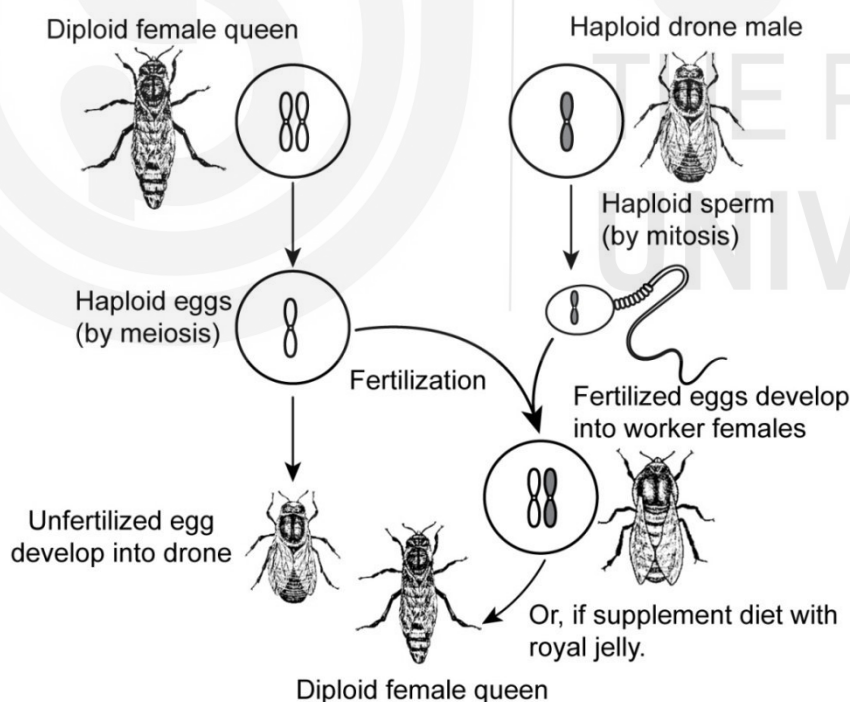


Fig. 7.5: Sex determination by ploidy level in honeybee.

Because males do not undergo meiosis for gamete production, all the sperms from one individual are genetically identical to each other and to the male parent. This has the interesting consequence of increasing the gametic relatedness of a male's daughters. Remember in this system, a male produces no sons - only daughters. On an average, all daughters of one mated pair of bees share 75% of their genes, rather than the normal 50% relatedness of

offspring of most species. The daughters are identical for all the 50% of the genes received from their father, plus one-half of the 50% of the genes from their mother, this makes a total of 75% genetic relatedness.

In some vertebrates also unusual degrees of ploidy are associated with a particular sex. Some species of lizards consist of mostly triploid (3N) females and few males. In fact, males are superfluous, because the females develop *parthenogenetically*. A triploid female produces a triploid egg through mitosis, which undergoes complete development without being fertilised. Haploid males too develop parthenogenetically in the similar manner.

7.3.5 The Compound Chromosome System

Although the X is most commonly found as a single chromosome or single homologous pair or chromosomes, some species have another variation – compound chromosomes. These are named so because a group of chromosomes (e.g. 8X, 12X, 6Y etc.), at the time of meiosis unite end to end and behave as single unit. In such species, there are large differences in the number of chromosomes in males and females. For example, in the nematode *Ascaris incurva* there are 8X chromosomes and 1Y. This species has 26 autosomes. The diploid number of chromosomes in males is 35 (i.e., 26A + 8X + Y), and in females is 42 (or 26A + 16X).

In the above example, X chromosomes exist as a compound chromosome. There are instances where both, the Y chromosomes and the X chromosomes form compound groups., One such example is of *Blaps polychresta*, where the male has 12x3 and 6Y's in addition to 18 autosomes..

7.3.6 The Transfer Gene

One additional complicating factor in sex determination in *Drosophila* is worth examining briefly. This shows that the sex chromosomes (X and Y, Z and W) are not the only ones involved in sex determination, but in addition numerous autosomal genes also come into play. In *Drosophila* a recessive allele, *tra*, on the third chromosome (an autosome), when homozygous, “transforms” normal diploid females (**AAXX**) into sterile males. The *tra* gene has no effect in heterozygous condition. The XX *tra tra* flies have many sex characters of males (external genitalia, sex combs (see Fig. 7.6), and male type abdomen), but are sterile. So are XXY *tra tra* flies. The XY *tra tra* males, however, are normal and fertile.

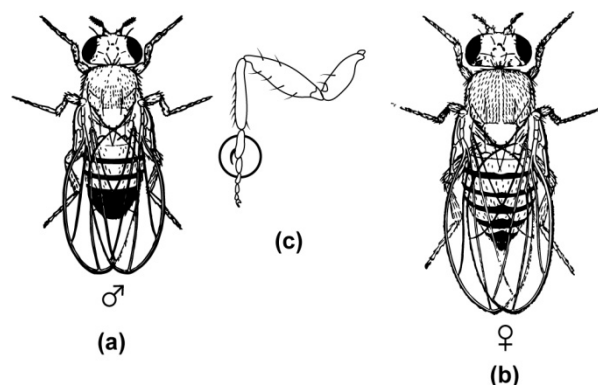


Fig. 7.6: *Drosophila melanogaster*, (a) male, (b) female. Two distinguishing features are: the merging of the posterior bands in the male versus their distinct separation in the female, and the longer more tapering abdomen of the female. (c) shows details of a male leg with sex comb (arrow).

7.4 THE CHROMOSOME THEORY OF INHERITANCE AND SEX-LINKAGE

Soon after the discovery of Mendel's work in 1900, most geneticists accepted the particulate nature of genes. Mendel had predicted that each gamete contains only one allele of each gene instead of two. This prediction was based on the fact that there is reduction in the number of chromosomes by one-half at the time of meiosis during gamete formation.

This notion, that chromosomes carry genes is the **Chromosome Theory of Inheritance**. The credit for the Chromosome Theory of Inheritance goes to Walter Sutton and Theodor Boveri. In 1902, these investigators independently recognized that the behaviour of Mendel's genes during production of gametes in peas precisely paralleled the behaviour of chromosomes at meiosis. The following parallels were drawn between the two: i) genes are in pairs, so are the chromosomes, ii) the members of gene pair segregate equally into gametes, so do the members of a pair of homologous chromosome, and iii) the different gene pairs act independently, so do the different chromosome pairs.

The proposition of the Chromosome Theory was a crucial new step in genetic thinking at that time. No longer were genes just disembodied factors, now they were a part of the observable entities in the cell nucleus. Some geneticists, particularly, Thomas Hunt Morgan remained skeptical of this idea. Ironically it was Morgan himself who in 1910 provided the first definitive evidence for the Chromosome Theory based on his studies on sex linkage.

Morgan worked with fruit fly *Drosophila melanogaster*. When he mated red-eyed flies $R \parallel R$ (dominant) with white-eyed flies $r \parallel r$ (recessive), the F_1 progeny were red-eyed. Furthermore, when Morgan mated red-eyed males of the F_1 generation with their red-eyed sisters, they produced about 1/4 white-eyed males, but no white-eyed females. In other words, the eye colour phenotype is X-linked. X-chromosome and eye colour are transmitted together because the genes governing this character are located on the X-chromosome. In a diploid individual, we know, the autosomes occur in pairs but as regards to X-chromosomes the female fly has two copies and the male has just one. However, Morgan was reluctant to draw this conclusion until he observed sex-linkage with two more characters - miniature wings and yellow body in the fruit fly. That was enough to convince him and other geneticists of the validity of the chromosome theory of inheritance.

7.5 SEX-LINKED INHERITANCE

The inheritance of genes located on the sex chromosomes follows a characteristic pattern which is different from those located on the autosomes. Here, we are elaborating on the XX-XY system as it pertains to man and most of the examples cited are aimed at human context.

7.5.1 X-Linked Traits in Humans

Let us first examine traits determined by genes on the X-chromosome, or the *X-linked traits*. The human X chromosome contains many genes that are required in both the sexes, whereas the Y chromosome contains only a few genes, principally the genes for maleness. More than two hundred traits have been found to be X-linked and only a few are known to be Y-linked. The traits controlled by genes located on the X-chromosome are also referred to as **sex-linked**. That is, the terms X-linked and sex-linked are used synonymously. It is more appropriate, however, to refer to these as X-linked traits, since they follow the pattern of transmission of the X-chromosome.

The X-linked traits have a unique mode of inheritance because females have two doses of X-linked genes, while males have only one. Males are thus hemizygous for X-linked traits.

X-Linked Dominant Genes

Dominant X-linked genes are always expressed in both the sexes just as in autosomal traits. One dose of X-linked dominant allele produces its effects in males as well as females. The hemizygous male transmits the gene to all its daughters but none to his sons. There is no father to son transmission. The heterozygous females transmit the trait to half their children, irrespective of their sex. On the other hand, females homozygous for the dominant allele produce all affected children. For example, a form of vitamin D-resistant rickets is inherited as an X-linked dominant trait (Fig. 7.7).

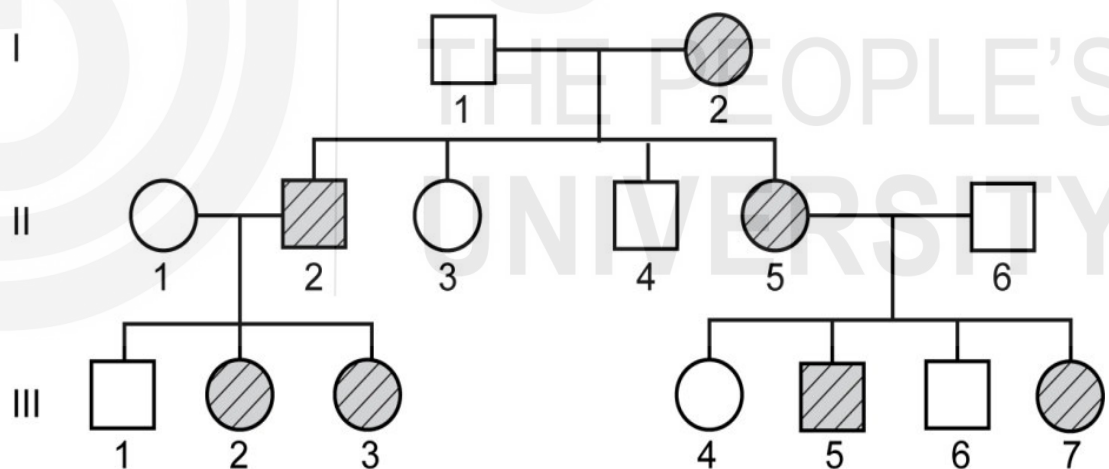


Fig. 7.7: Pedigree demonstrating X-linked dominant trait. The affected progeny are shaded.

X-Linked Recessive Genes

The opposite is true for recessive alleles. Males being hemizygous, always express the **recessive X-linked alleles**. Females, however, express recessive alleles only when they are homozygous. Thus, the **frequency of X-linked recessive traits is always lower in females than males**.

Most X-linked genes are recessive alleles, and they are discovered when their deleterious effects appear in males. Males transmit their X chromosome to every daughter, and their Y chromosome to every son. *Recessive X-linked traits thus show a pattern of inheritance, in which the phenotype is usually*

expressed only in males of alternate generations. A male bearer transmits the recessive allele to daughters, who does not express the allele because it occurs in the heterozygous condition. However, each of her male offspring has a 50% chance of receiving that allele and expressing the phenotype. The trait should thus appear in 1/4 of her offspring (1/2 of her offspring are expected to be male and 1/2 of her sons receive the recessive allele: $1/2 \times 1/2 = 1/4$). The heterozygous female is a carrier of the allele. The X-linked allele is often said to show a **criss cross** pattern of inheritance. In this pattern of inheritance, the allele is transmitted from male to female, female to male, and the trait is expressed only in males in alternate generations (see Fig. 7.8). Well-known examples of X-linked recessive alleles include Red green colour blindness, Haemophilia, Glucose-6-phosphate dehydrogenase deficiency (G-6PD), Congenital hyperuricemia, Duchenne muscular dystrophy, and Ichthyosis.

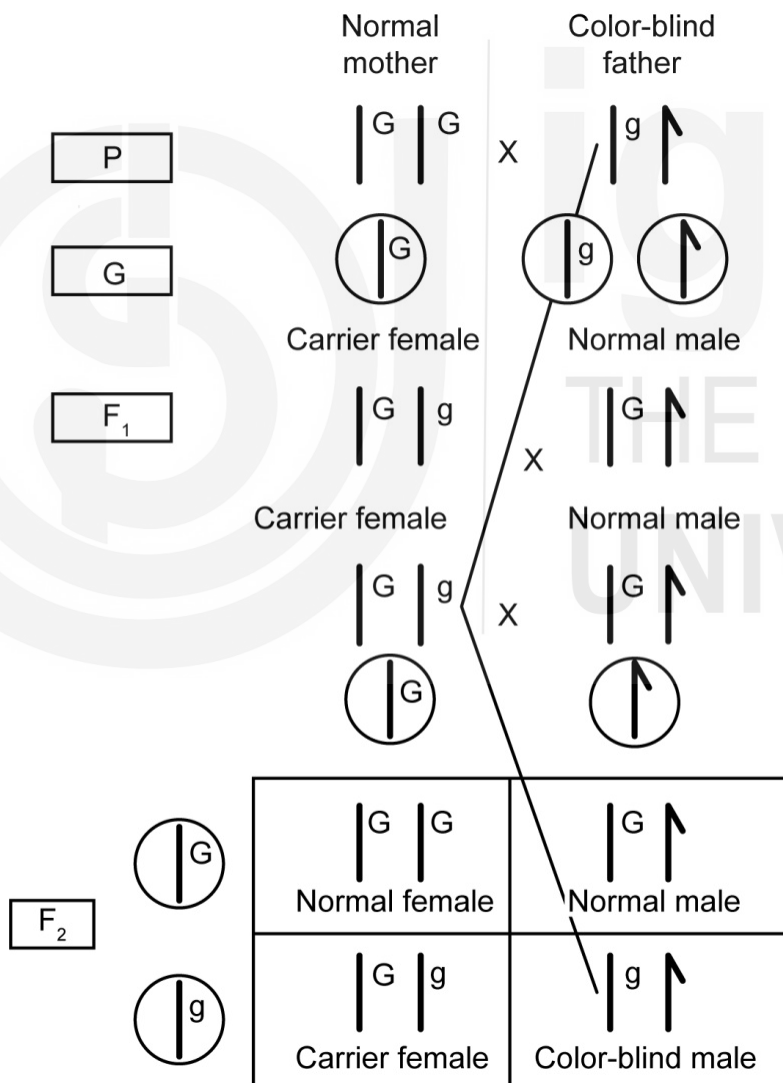


Fig. 7.8: Criss cross inheritance, i.e., the inheritance of a character from father to daughter grandson. It is characteristic of a sex-linked gene. Genes are shown on the chromosomes illustrating a cross between a woman with normal vision and a green-colour defective man. The symbol g represents the sex-linked recessive gene for green colour defective vision, and G the normal condition.

SAQ 1

A husband and wife are normal although both their fathers have a trait which is X-linked recessive. What is the probability that their first child will be:

- a) A normal son?
- b) a normal daughter?
- c) a son with the trait?
- d) a daughter with the trait?

Red Green Colour Blindness: Many persons cannot perceive certain colours. The most common such defect is an inability to distinguish red from green. This condition is also called **partial colour blindness**. Colour perception is controlled by the cone-shaped cells in the retina of the eye. Three types of cone cells, each containing a specific light absorbing pigment (whose nature is protein), that perceives a specific portion of the visible spectrum (see Fig. 7.9) have been identified. These three types of cone cells are referred to as red-absorbing, green-absorbing and blue-absorbing cone cells.

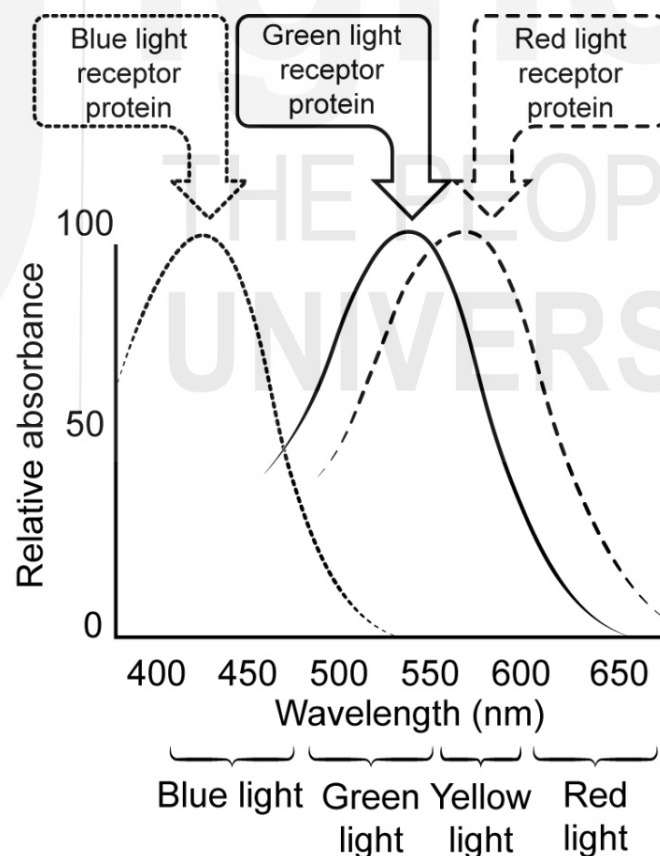


Fig. 7.9: Absorption spectra of the blue, green and red proteins present in the cone cells of the retina of the human eye. The ability of humans with normal colour vision to distinguish colours throughout the visible spectrum depends on the presence of all three proteins. Defective colour vision results from the absence of, or a defect in, one or more of these proteins. [After Nathans, K 1989. *The Genes for: colour vision*, *Science*, 260(2): 42-49].

By 1986, the genes that encode the above three light-absorbing pigments of the retina were isolated and their nucleotide sequence was determined. The sequences have been used to find out the amino acid sequences of the three light absorbing proteins. These light-absorbing proteins were found to have very similar structures. **The genes that encode the green-, and red-receptor proteins are located on the X-chromosomes**, thus the sex-linked patterns of inheritance are observed for defects in green and red colour vision. And the gene encoding the blue receptor protein was found to be located on chromosome-7, that is, an autosome. Let us now examine some crosses (see Fig. 7.10) and assess the pattern of inheritance of this defect. For the sake of simplicity we are only considering here one gene, that is, gene causing defects in the green receptor protein, as a single sex-linked recessive allele. Since the Y chromosomes carried no colour vision locus, the single allele is expressed causing colour blindness. Stop here for a minute and carefully study the five crosses in the figure. The symbol G denotes normal receptor pigment, and g is for the defective receptor pigment causing colour blindness.

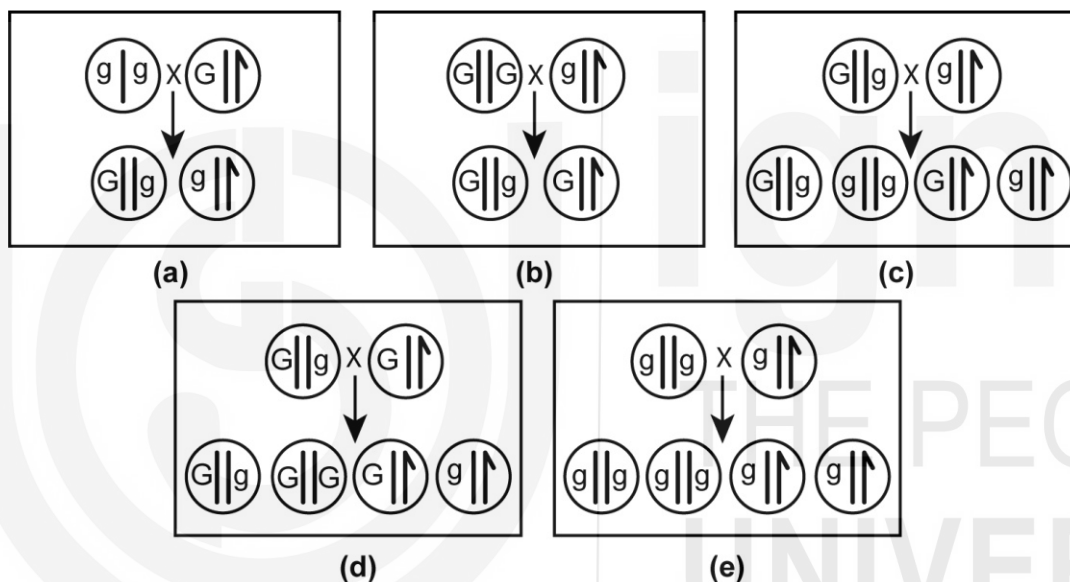


Fig.7.10: Five possible crosses involving inheritance of sex-linked recessive trait – partial colour blindness: a) normal male $\times$ colour blind female; b) colour blind male $\times$ normal female; c) colour blind male $\times$ normal female who is a carrier; d) normal male $\times$ normal female who is a carrier; e) colour blind female $\times$ colour blind male.

In the above crosses have you noticed that sex-linked inheritance does not conform to the Mendel's laws of inheritance? Fig. 7.10 shows the results of reciprocal matings of affected and unaffected parents. The normal male and colour blind (recessive, homozygous) female produce normal but heterozygous daughters, but all sons have the disease (cross a). The reciprocal cross (b) demonstrates criss cross inheritance. A colour blind male (hemizygous) with a normal (homozygous) female produces no affected offspring, but the daughters are carriers (cross b). A colour blind male and a carrier female result in 50% colour blinds (see cross c). Another possibility, a normal male mated with a carrier female, produces all normal, female offspring but 50% affected male offspring (see cross d). The mating of two colour blind individuals result in all colour blind offspring (see cross e) if they have the same colour blindness.

Haemophilia: Haemophilia is a disease in which one of the factors required for the normal clotting of blood (see Fig. 7.11) is deficient. As a result, the blood fails to clot or clots very slowly. Thus even minor injuries can cause profuse internal and external bleeding which can lead to death.

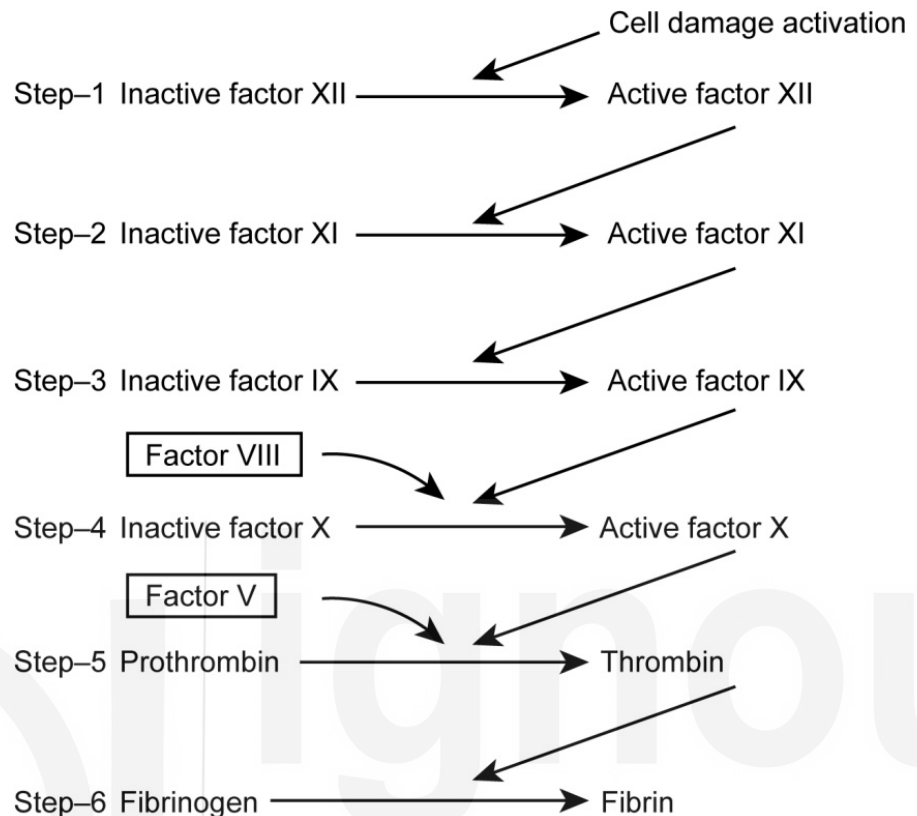


Fig. 7.11: Blood clotting is the end result of a series of reactions requiring various enzymes and cofactors, leading to the formation insoluble fibrin. Many of the steps in the chain entail the conversion of a gene product to its active enzymatic form by the enzymatic action of the active product a previous step. Cofactors (e.g., factor VIII and V) are required to work along with some of the active enzymes. Factor VIII, absent/defective in haemophilia A, is a cofactor required along with factor IX to activate factor X. If factor VIII is absent as a result of a sex-linked recessive condition, the sequence is interrupted and the end result is defective clotting. In haemophilia B, factor X is deficient.

Haemophilia is one of the earliest known diseases. According to Talmud, the Hebrew book of law, when excessive bleeding occurred during circumcision of two male infants of a mother, future male offspring were exempt. When sons of three sisters exhibited bleeding, sons of other sisters were also exempt. However, sons of brothers were not exempt, implying an understanding of criss cross pattern of inheritance.

Three forms of haemophilia controlled by three different gene loci are known. One of them is rare and is controlled by an autosomal recessive gene, while two forms, that is, haemophilia A and B result from recessive alleles at two X-linked loci. Haemophilia B also known as *Christmas disease*, comprises about 20% of all haemophilia and is caused by the deficiency of factor IX (see Fig. 4.7, step 3).

Haemophilia A, classical haemophilia is caused by an abnormality or deficiency of a protein cofactor known as factor VIII is located on X-chromosome. Factor VIII is needed for the activity of one of the enzymes - factor IX, in the series of events leading to the activation of thrombin. Absence of functional factor VIII interrupts the steps leading to the activation of thrombin, and consequently fibrin cannot form. Until recently, haemophilia A was untreatable and only about 25% of the affected males reached age of twenty five. Treatment with factor VIII now results in a longer life span.

The frequency of haemophiliacs is about one in ten thousand males, but is much lower in females, about one in one hundred million or less. A female haemophiliac can result from the mating of a heterozygous female with an affected male. Such a mating, is highly unlikely because very few male haemophiliacs survive long enough to reproduce. Haemophiliac females are also believed to die at the onset of menstruation.

Haemophilia A has been called the “Royal disease” because it affected males in the royal families of Europe. Queen Victoria, a carrier of the haemophilia allele had nine children (Fig. 7.12). Her eighth child, Leopold was a haemophiliac who died at the age of thirty one. Her other three sons were unaffected as they did not receive the allele. One daughter had no children, her status as a carrier cannot be assessed. Two daughters had children, none of whom were haemophiliac, indicating that mothers probably were not carriers. Two other daughters were carriers giving birth to haemophiliac sons.

The possible historical influence of haemophilia is tantalising. Victoria's third child was princess Alice, whose daughter Alexandra married Czar Nicholas II of Russia. The Czarina, Alexandra, had four daughters before giving birth to the long awaited son Alexis - the heir to the Russian throne. Unfortunately, Alexis had the haemophilia allele, a legacy from his great grandmother – Queen Victoria. Distressed over their son's condition, the Czar and Czarina turned to the monk Rasputin. While affairs of the state deteriorated, culminating in Russian revolution, the Czar was preoccupied with the health of his son.

Among Victoria's descendants, eight of twenty five males in four generations were haemophiliacs. Queen Victoria almost certainly received the gene for haemophilia A, as a result of mutation on the X chromosome which she received from her father Edward, Duke of Kent. He was fifty-two years old at the time of Victoria's birth and such mutations may occur more frequently in the germ cells of older males.

In the recent years, a serious threat to victims of haemophilia has arisen due to their continuing dependence upon blood transfusions. Such transfusions are one means of transferring **Acquired Immune Deficiency Syndrome (AIDS)** and some haemophiliacs have in fact acquired AIDS in this way. Extensive surveillance of donor blood supplies is required to protect haemophiliacs and all others requiring transfusions.

Glucose 6-Phosphate Dehydrogenase (G-6PD) Deficiency: Another disease due to defective X-linked recessive allele is G-6PD. This is an important enzyme, for carbohydrate metabolism and maintaining stability of red blood cells.

Deficiency of enzyme G-6PD is a rare condition characterised by severe haemolytic anaemia (due to destruction of red blood cells) when exposed to environmental triggers such as fava beans, naphthalene and certain sulpha drugs.

Congenital Hyperuricemia – Lesch-Nyhan Syndrome: This disease is characterized by the excess production of uric acid. A mother contributes the X-chromosome with defective gene to a male zygote. Half of the male children of carrier mothers may be expected to inherit the disease. They are deficient for the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT). This enzyme is involved in nucleotide synthesis. Infants who receive the gene appear normal at birth. The initial symptom of the disease is the production of excessive uric acid in the urine as a result it appears as orange sand-coloured. By 10 months of age, the patients become abnormally irritable and lose motor control. Weak and flabby muscles prevent the child from sitting, walking and speaking normally. As the disease advances, there is deterioration of nervous system. Self-mutilation occurs, manifested by lip-biting, finger-chewing, teeth-grinding, and marked swinging of the arms. Eventually death occurs within a few years due to severe renal and neurological damage. Some of the patients live to their twenties.

Duchenne Muscular Dystrophy (DMD): It is another example of an X-linked recessive allele, that primarily affects young males. Half of the male progeny of a carrier female are expected to be affected. In the affected males deterioration begins between the ages of three and five years, but sometimes the affected individuals reach their teens. But they are confined to wheel chairs; and they die in their early twenties due to atrophy of their respiratory muscles. Only few affected males reproduce, so the condition transmitted mainly by female carriers. This disease occurs in about one in every four hundred newborn males; and is several times more frequent than haemophilia. In 1986, the defective gene that causes DMD was isolated and studied. It was found that the absence of a protein – *dystrophin* caused DMD.

Ichthyosis: So far you have seen the examples where the recessive X-linked are expressed in males. There are, however, instances where these are also expressed in females in certain situations. **Consanguineous matings** (see adjacent Margin Remark) can greatly increase the frequency of expression of X-linked traits in females. In consanguineous pedigrees containing X-linked recessive alleles, females have a high probability of carrying the X-linked allele, as they can receive the allele from either parent. In turn, matings of carrier females and affected males (Fig. 7.12) produce daughters and sons with an equal likelihood (i.e., 50%) of being affected.

Consanguineous means “of the same blood”. The term means sharing genes derived from a common ancestor, related by descent.

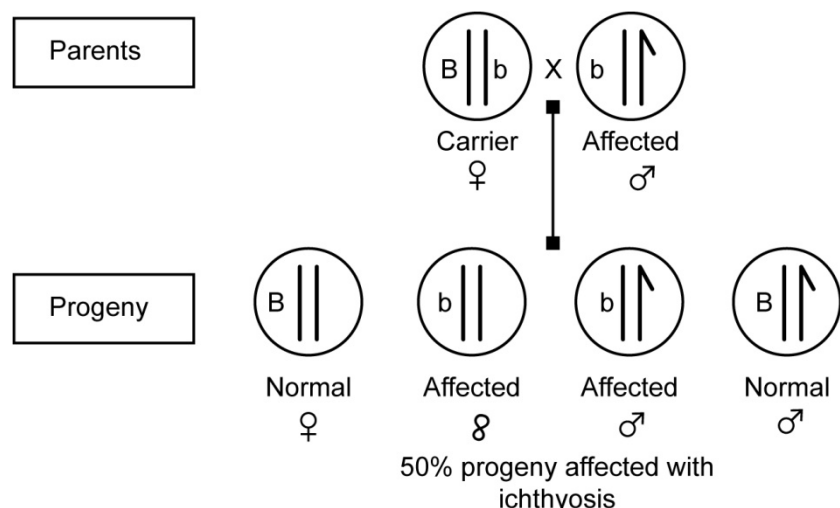


Fig. 7.12: Cross between ichthyosis carrier female and affected male showing 50% affected progeny.

Ichthyosis is a disorder characterised by extreme dryness, roughness and scalliness of the skin. The prefix 'ichthy' means fish-like. Children produced in situation as shown in Fig. 7.12 show ichthyosis at birth.

SAQ 2

A couple have a colour blind daughter and son with normal vision. What are the genotypes of the parent in this cross?

SAQ 3

Draw a three generation pedigree of a family starting from a couple, where male is a haemophiliac and the female is normal. They have 3 sons and a carrier daughter. The daughter marries a normal male and has 3 daughters and 2 sons. What is the probability of her children being carriers and haemophiliacs?

7.5.2 Y-Linked Traits in Humans

Any gene that occurs exclusively on the Y chromosome is said to be **holandric** and it is not expressed in females. Such a Y-linked gene normally occurs in males and is transmitted only from father to son – **holandric inheritance**. Only a few Y-linked genes have been identified up to now. One is the histocompatibility gene, known as the H-Y gene which is present on the short arm of the Y chromosome. Another important Y-linked gene is the TDF gene that codes for testis determining factor. This locus plays an important role in primary sex determination. The functional significance of TDF gene would be explained in Unit 5.

Another phenotype known to be associated with the Y chromosome is the condition **hypertrichosis**. The gene concerned with this condition leads to the development of hairy pinna (Fig. 7.13). This phenotype has been observed in the inhabitants of Australia, Ceylon, Israel, and India.



Fig. 7.13: Hen-feathering (top), and cock-feathering (bottom) in domestic fowl.

SAQ 4

A man has hypertrichosis of the ears, a condition due to a gene on the Y-chromosome. Show the types of male and female children he has.

SAQ 5

Is a Y-chromosome linked gene supposed to be dominant or recessive in order to be recognised? How is a Y-linked gene transmitted to grand children?

7.6 SEX-LIMITED AND SEX-ENFLUENCED TRAITS

Not all of the characters that differ in the two sexes are X-linked. There are certain traits that are determined by autosomal genes, but their expression is altered or influenced by the sex of the individual. Many a times these traits are confused with the sex-linked traits. Actually, they are entirely different in their mode of inheritance since their genetic determinants are not located on the sex chromosomes. There are two kinds of such traits: sex-limited, and sex-influenced traits.

7.6.1 Sex-Limited Traits

Sex-Limited Traits are traits expressed only in one sex, although the genes controlling them are present as well as transmitted to both the sexes. Therefore, males and females with the same genotype, with respect to a particular locus may have different phenotypes.

Sex-Limited Traits are determined by autosomal genes, whose phenotypic expression is determined by the presence or absence of one of the sex hormones. Since sex hormones are the limiting factors, the phenotypic expression of these genes is limited to one sex or the other. The most obvious examples are the secondary sex characteristics. Beard development in human beings is one such sex-limited character as men have beards, and women normally do not. Yet studies indicate no significant differences between the sexes in number of hairs per unit area of skin surface except in their development. This appears to depend on sex hormone production. Any disturbance in these hormones in women may result in the development of beard. Similarly, the full development of breasts in females, and presence of prostate glands in males are the examples of sex-limited traits seen in human beings. Traits like egg laying in chickens, oviposition behaviour in insects are some other such examples. Milk production in mammals is limited to females, but certain bulls are in great demand among dairy breeders and artificial insemination associations because their mothers and daughters have increased milk production records.



Fig. 7.14: Pattern-baldness in man.

Another classic example of a sex-limited trait is “cock feathering” in different birds. We consider here the example of domestic fowl, the males and females exhibit pronounced difference in their plumage. In the *leghorn* breed the males have long, pointed, curved, fringed feathers on tail and neck, but feathers on females are shorter, rounded, straighter, and without fringe (see Fig. 7.14). Thus males are cock-feathered and females are hen-feathered. In the breeds *Sebright bantam*, birds of both sexes are hen-feathered. However, in *Hamburg* and *Wyandotte*, both hen-, and cock-feathered males are seen, but all the females are hen-feathered. The feathering type depends on a single pair of alleles *H* and *h* in the following manner (Table 7.1).

Table 7.1: The Feathering Type in Domestic Fowl.

Genotype	Male	Female
HH	hen-feathered	hen-feathered
Hh	hen-feathered	hen-feathered
hh	hen-feathered	cock-feathered

Thus *Sebright bantams* are all HH . *Hamburgs* and *Wyandottes* may be $H-$, or hh , and *Leghorns* are all hh . Cock-feathering where it occurs is limited to the male sex.

7.6.2 Sex-Influenced Traits

Sex-Influenced or Sex-Controlled Traits appear in both sexes but occur in one sex more than the other.

The genes for **Sex-Influenced** Traits show differing patterns of expression in each sex—usually the trait behaves as dominant in one sex and a recessive in the other. Genes for **sex-influenced traits occur only on autosomes**.

The best documented example of sex-influenced inheritance is **pattern baldness** (Figs. 7.15 and 7.16). Individuals expressing pattern baldness begin to lose their hair on the front and the top of the head, relatively early in life, often in their twenties. Affected individuals are not totally bald: a distinct rim of hair surrounds their head in patterns varying from person to person. It has been proposed that a single pair of alleles is involved. The allele B_1 , which is responsible for pattern baldness, is dominant in males, and the heterozygous males therefore, express pattern baldness. In females, however, the gene is recessive. The allele for normal hair growth can be designated as B_2 . Individuals with the genotype $B_1 B_1$ show pattern baldness, regardless of sex. In such situations, in females there is marked thinning, rather than total loss of hair on the top of the head. Persons with $B_1 B_2$ genotype are bald if they are male but not bald if they are female. *The presence of male hormones, are strongly implicated in the expression of pattern baldness.*

Pattern baldness refers to a definite genetic pattern. In this condition hair usually thins on top ultimately leaving a fringe of hair low on the head (Fig. 4.13). Baldness may also arise due to various causes such as disease, radiation, thyroid defect.

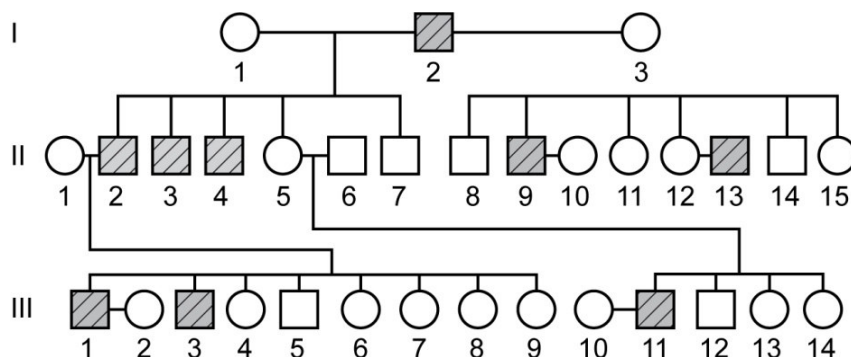


Fig. 7.15: Pedigree showing the incidence of pattern, baldness in a family. The men represented by the dark squares became bald before they reached the age of 35. Those represented by light squares are over 35 and have thick hair. No woman in this family pedigree expressed the trait (After Gardner et al. 1991, *Principle of Genetics*, John Wiley & Sons, Inc.)

Some human traits, such as certain types of white forelock, absence of upper lateral incisor teeth, a particular type of enlargement of the terminal joints of the fingers, and cleft-lip, exhibit a pattern of inheritance characteristic of sex-influenced genes.

A few well-known examples of sex-influenced genes in animals are: spotting in cattle (mahogany and white dominant in males, red and white dominant in females), horned versus hornless condition in sheep (Fig. 7.16) where the autosomal gene involved is dominant in males and recessive in females.

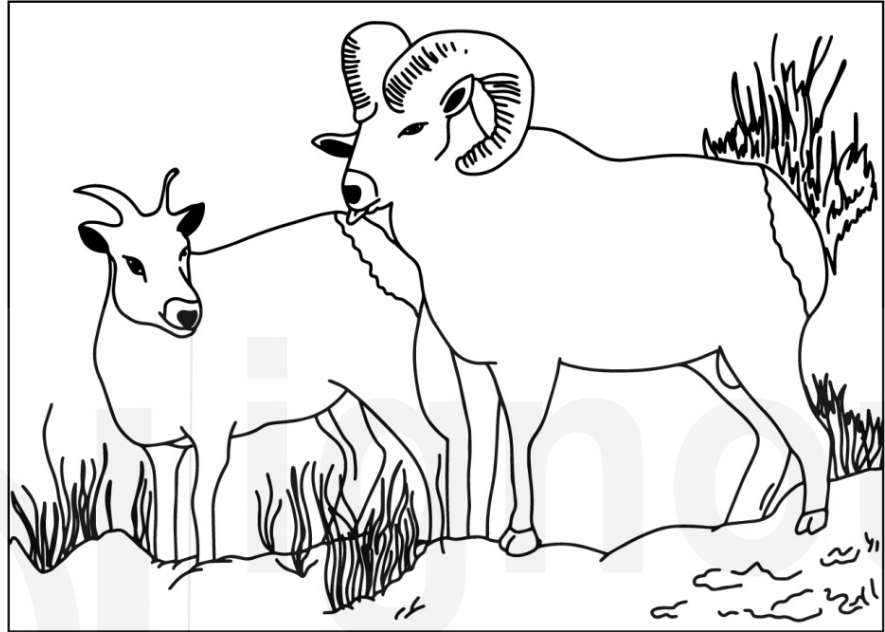


Fig. 7.16: Rocky mountain sheep showing sexual dimorphism in horn development. The male has large horns, whereas the female is devoid of them.

SAQ 6

Compare the inheritance pattern of the sex-limited traits with those of the sex-linked traits.

7.7 DOSAGE COMPENSATION

Recall that in the XX-XY chromosome system, males have only one X chromosome (hemizygous) while the females have two. Thus the males have half the number of X-linked genes as females. In other words, the males have only one dose of X chromosomes and the females have double dose of X-chromosomes. We know that the amount of gene product in cells is related to the number of gene copies present, it would be expected that females would have double the amount of X-linked gene products as compared to males. Now the question arises, is there any compensation for this dosage difference between sexes? The answer is 'Yes', **there is a mechanism which regulates the levels of gene products in such a way that both hemizygous and heterozygous/homozygous individuals that is, males and females, have the same amount of gene product. This mechanism is known as Dosage Compensation.**

7.7.1 In Man

In human and other mammals, the necessary dosage compensation is accompanied by **inactivation** or “**turning off**” of one **of the X chromosomes** in females so that both males and females have only one functional X chromosome per cell. The inactive X chromosome, in females becomes tightly coiled into ‘**heterochromatin**’ a condensed form of chromatin visible as a dark spot – **X-chromatin or Barr body** (after its discoverer M.L. Ban) in the nucleus of female cell (Fig. 7.17.). Thus Barr body is the inactivated X chromosome. One X chromosome is necessary for normal development in both sexes, but if an individual (or either sex) has more than one X-chromosomes, all but one are inactivated and are visible in stained somatic cells as Barr bodies. Thus somatic cell nuclei of normal males have no Barr body, and those of normal females have one (also see Box 7.1).



Barr body

Fig. 7.17: Barr Body in the nucleus of a cell of a normal female.

Box 7.1: Detection of Barr Body

A simple-way to demonstrate the Barr Body in humans is by scraping epithelial cells from the buccal mucosa of females, and staining them with a specific dye. The nuclei in many cells would show a small, diamond-shaped structure about 1 μm in diameter, more deeply stained than the surrounding chromatin and usually located at the periphery of the nucleus. This body stains positively in the **Feulgen reaction** for DNA.

The hypothesis that all but one *X chromosome(s) are inactivated* in each cell was proposed by the geneticist Mary F. Lyon in 1961 and is known as **Lyon hypothesis**. Crucial evidence for this hypothesis was provided by sexually aneuploid individuals. **Aneuploidy** (meaning not the true number) refers to the possession of an abnormal number of chromosomes. Aneuploid individuals have the normal diploid number, plus or minus one or more chromosomes. Females lacking one X chromosome exhibit **Turner's syndrome**, designated 45, XO (45 chromosomes, with one X missing). Males with an extra X-chromosome have **Klinefelter's syndrome** designated 47 XXY. Cells from 45, XO females and 46 XY males have no Barr bodies (Fig. 7.18 a); while those from 47, XXX females and 48 XXXY males have two (Fig. 7.18 b); 46, XX females and 47, **XXY** males have one Barr body (Fig. 7.18 c) and 48, XXXX females and 49, XXXXY males have three Barr bodies (Fig. 7.18 d). Examination of the number of Barr bodies can be done easily to screen for sex-chromosomes abnormalities. You have already seen in Box 7.1 how to make a preparation of the epithelial cells from the buccal cavity for examination. The mandatory "sex test" that have been required for Olympic athletes includes a count of Barr bodies. Males disguised as females can be identified as they have no Barr bodies.

The inactivation of X chromosomes during development occurs at random. Early in development, the maternally derived X is inactivated or **lyonised** in some, while the paternally-derived X is inactivated in others. Thereafter, descendants of a particular cell have the same X inactivated (Fig. 7.19). If a female is heterozygous for an X-linked gene, she is **mosaic** for that trait. One of her X-chromosomes is active in roughly half of her cells while the second X is active in other cells. That is to say that some cells express X-linked genes inherited from the father while others express those passed on by the mother.

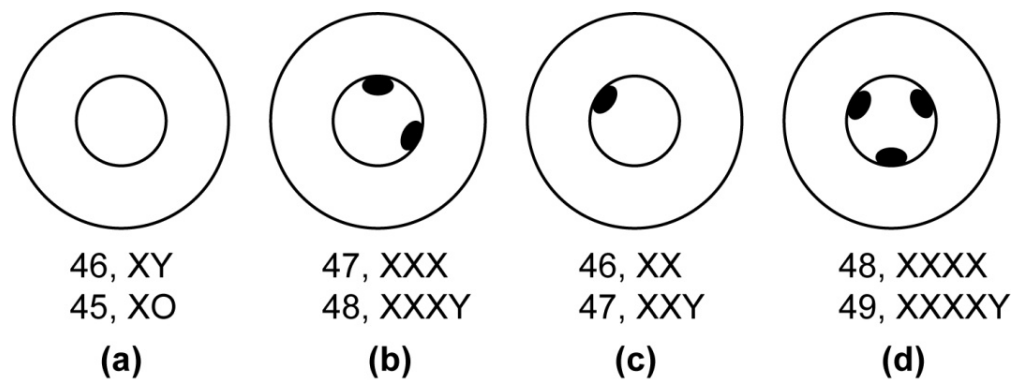


Fig. 7.18: Diagrammatic representation of varying number of Barr bodies in different genetic make ups.

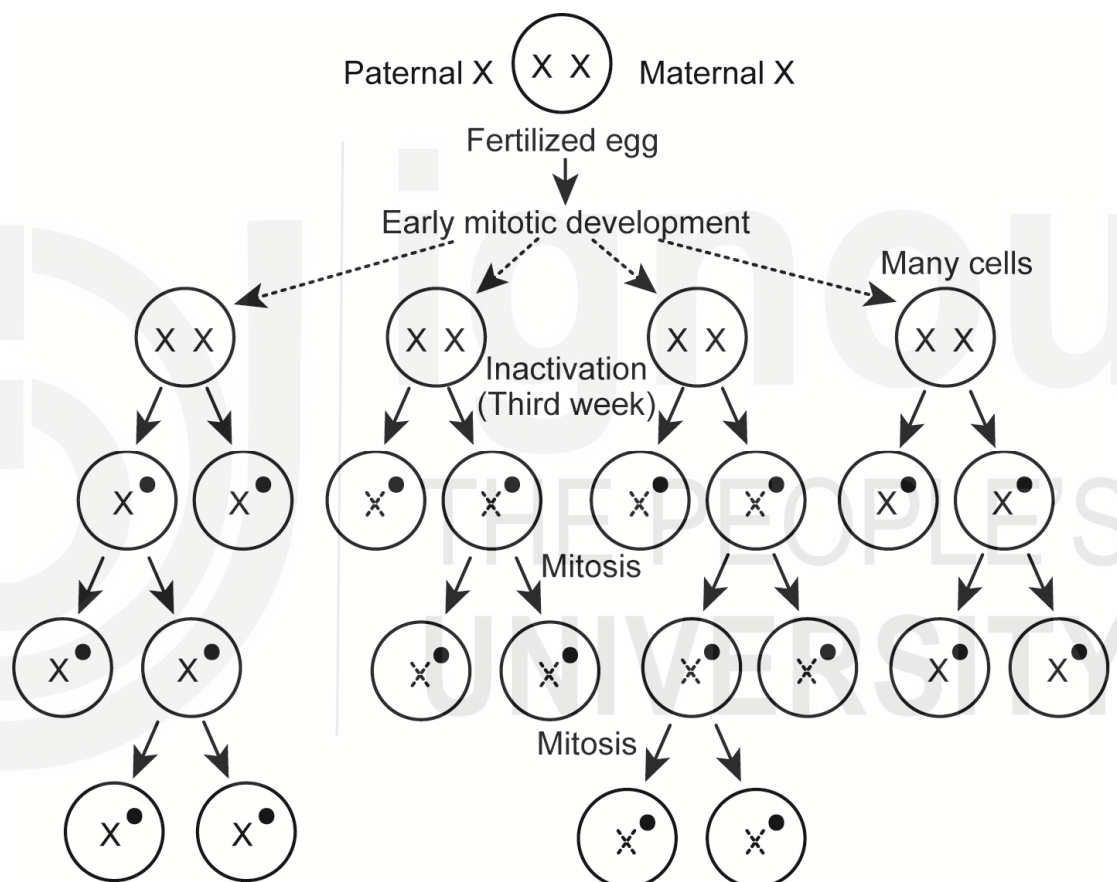


Fig. 7.19: Diagrammatic representation of the random inactivation of one of the two chromosomes in female cells, all progeny cells inactivate the same chromosome. All descendants of these cells have the same chromosome inactivated, so females are mosaics for their maternally-derived and paternally-derived X chromosomes.

Calico cats (Fig. 7.20) exhibit mosaicism, due to dosage compensation. Several loci control coat colour in cats, but only one X-linked locus is involved in producing calico individuals. Two alleles occur at that locus, *R* and *r*: In males (hemizygous), *R* produces rust coat colour and *r* black. In females *R* inactivation produces clones of *R*-bearing rust fur intermixed with *r*-bearing black fur – the calico cat. Thus almost all calico cats are females. Male calico cats, only result because of sex-chromosome aneuploidy. XXY males also undergo X inactivation, so an occasional calico male is seen.

When a female is heterozygous for a deleterious X-linked allele, the effects of the cell lines bearing the normal allele may compensate for the harmful effects of the cell lines bearing the deleterious allele. In females heterozygous for partial colour blindness, for examples, some cell clones in the retina are in fact colour blind, but the presence of other normal clones results in normal colour vision.

Another example that shows the genetic consequences of X-inactivation in females heterozygous for an X-linked gene is the enzyme G-6PD. Cell cultures from an individual heterozygous for the G-6PD gene, has alleles with two forms of the enzyme: G-6PD type A and G-6PD type B. In spite of the cells carrying both the alleles, half the cells express G-6PD type A enzyme, while the remaining express G-6PD type B enzyme. If one cell from this culture was made to grow in isolation then all cells arising from it express the same enzyme type as its parent cell. It confirms the hypothesis that X-inactivation is clonally transmitted, i.e., the same inactivated X-chromosome is passed to daughter cells throughout repeated cell divisions.

The inactivation of one of the two **X chromosomes** in females **must be reversible**, since females transmit both of their X chromosomes to their progeny in a functional state. This is especially clear in the case of hemizygous male progeny which receives either of the X chromosome of the mother with equal probability, because the single X chromosome that each son receives must be fully active given that the X chromosomes contains many genes that are vital to the growth and development, indeed to the survival. The **reactivation-“turning on”** of the inactive heterochromatic X chromosomes of mammalian females occurs in germ cell lineages prior to oogenesis. Both X chromosomes of a female are active in the oogonial cells.

The maintenance of the germ cells and ovarian structures requires the presence of two X-chromosomes. At this point you may wonder, whether normal reactivation ever fails to occur. There are considerable evidences that indicate abnormal reactivation of the heterochromatic X chromosomes. The most common form of inherited mental retardation in humans is its example.

7.7.2 In *Drosophila*

Dosage compensation occurs in fruit flies, but its mechanism is different from those of the mammals. No barr bodies are found in fruit flies. You have already learnt that in fruit flies, the X chromosome to autosome ratio is responsible for sex determination. Normal females have two X chromosomes and normal males have one X chromosome. *Dosage compensation in this case is achieved by increased transcriptional activity of genes on the single X chromosome in male cells relative to that of each of the X chromosome in female cells* The male has hyperactive X-chromosome, approaching the level of activity of both of the females. This hyperactivity of X-chromosome can be cytologically seen as “puffed” bands in the salivary gland chromosomes (Fig. 7.21). *This is in variance to the inactive X-chromosomes in mammals which appears condensed (sex chromatin body).*



Fig. 7.20: A calico cat with patches of colour resulting from random inactivation of X chromosomes bearing colour determining genes in cells giving rise to hair.

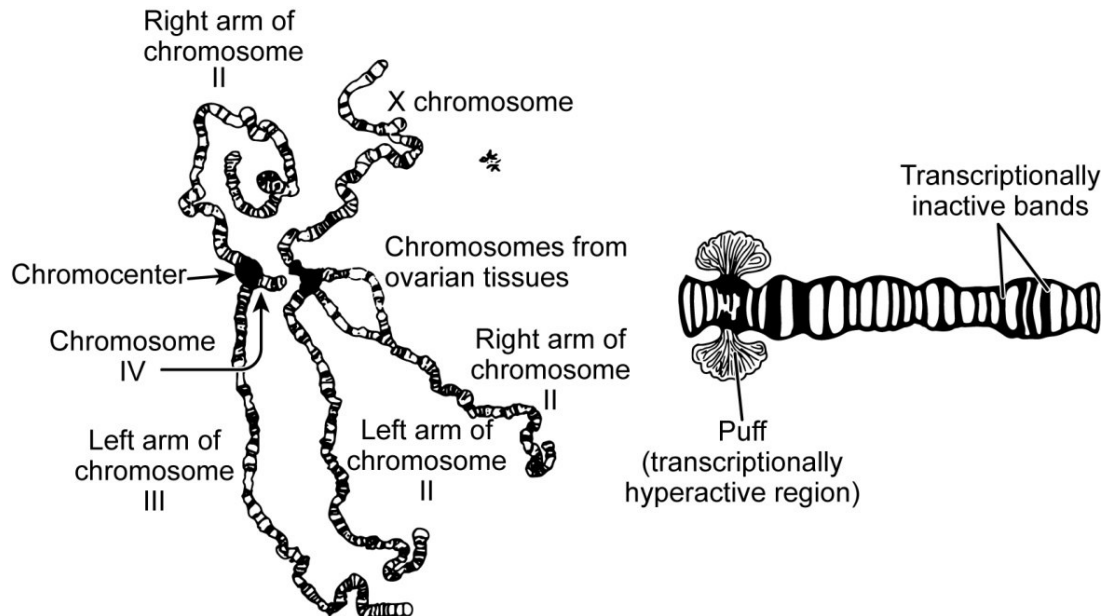


Fig. 7.21: a) The salivary gland chromosomes of *Drosophila melanogaster*. b) A portion of the puffed band enlarged.

SAQ 7

Indicate the expected number of Barr bodies in interphase cells of the following individuals: Klinefelter's syndrome; Turner's syndrome; and Karyotypes 47 XYY, 47 XXX, and 48 XXXX.

SAQ 8

Cat breeders are aware that kittens with the calico coat pattern are invariably females. Why?

7.8 SUMMARY

In this unit you have learnt that:

- The chromosomes are the carriers of genes and the transmission of chromosomes from one generation to the next closely parallels that of the genes.
- In species with an XX-XY mechanism, genes on the sex chromosomes may be X-linked or Y-linked.
- The mode of transmission of sex-linked traits is different from that of the autosomal ones.
- The dominant X-linked traits are always expressed in both the sexes.
- Recessive X-linked traits show a criss-cross pattern of inheritance. Female heterozygotes are carriers who pass the trait to 50% of their male offspring. Recessive X-linked traits are expressed far less commonly in females than in males.

- Only a few Y-linked genes have been identified and amongst them TDF (Testis determining factor) plays a role in male sex determination.
- Sex-limited and sex-influenced traits are the result of genes on the autosomes. The expression of these genes is sexually dimorphic.
- Dosage compensation regulates the level of gene products in such a way that both males and females have the same amount of gene products.
- In female mammals including man, dosage compensation occurs by the inactivation of all the X-chromosomes except one (forming Barr body or bodies) whereas in fruit flies it occurs by the hyperactivation of the single X-chromosome in males.

7.9 TERMINAL QUESTIONS

1. List at least **four** criteria for identifying X-linked recessive traits from pedigree studies.
2. Choose the correct answer.
 - i) Which one of the following statements does not apply to human sex chromosome?
 - a) carry allelic pairs
 - b) determine individual sex
 - c) are identical in women
 - d) are identical in man
 - e) both a and d
 - ii) Barr bodies result from:
 - a) inactivation of one X chromosome by the Y chromosome
 - b) a third X chromosome
 - c) inactivation of one X chromosome for dosage compensation
 - d) both b and c
 - iii) A man and a woman are both affected by vitamin D-resistant rickets which is a dominant sex-linked allele. All of the female offspring of these people are affected with rickets, but some of the males are not. What are the possible genotypes of the parents?
 - a) both are homozygous for the trait
 - b) the woman is heterozygous for the trait
 - c) the woman is homozygous and the man is heterozygous
 - d) this is not possible.

- iv) The fly *Drosophila melanogaster* has a gene that codes for white eyes as recessive and X-linked. Red eyes result from the wild type allele at the same locus. A cross between a heterozygous red-eyed female and white-eyed male would produce:
- all red-eyed progeny
 - all white-eyed males and all-eyed females
 - one red-eyed male and one white-eyed male
 - one red-eyed female and one white-eyed female
 - both c and d
- v) The gene for pattern baldness is dominant in men, but exhibits recessiveness in women. The difference in expression results from:
- the gene for baldness being X-linked
 - the gene for baldness being Y-linked
 - the expression of the gene depending upon the hormonal balance of the individual
 - both a and c
 - none of the above

3. Fill in the blanks:

- Men have ----- pairs of autosomes and one ----- pair of sex chromosomes.
- Women have ----- pairs of autosomes and one ----- pair of sex chromosomes.
- The fertilization of an egg by a Y sperm results in a ----- offspring.
- Genes which are Y-linked are called -----.
- Sex-limited genes are those whose phenotypic expression is determined by the presence or absence of sex -----.
- Beard development in humans is generally limited to one sex (male), yet studies indicate that there is no real difference in the number of hairs per unit area of skin between men and women. This indicates that beard development is a ----- trait.

4. Match the terms in column A with their appropriate descriptions in column B:

Column A	Column B
i) Dosage compensation	a) X-chromatin
ii) Turner's syndrome	b) inactivation of one X chromosome

iii) Klinefelter's syndrome	c) inactivation of one X chromosome so as to reduce to half the allele
iv) Barr body	d) inactivation of all but one X chromosomes
v) Lyon hypothesis	e) phenotypically female (XO) f) phenotypically male (XXY)

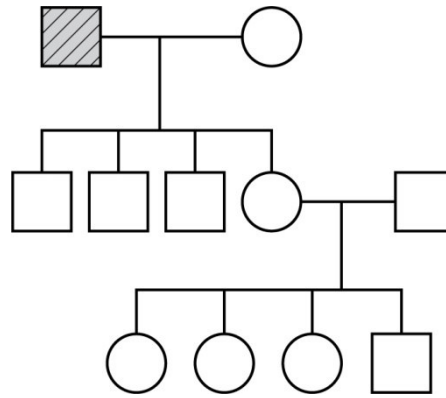
5. In sheep, the gene h^+ for the horned condition is dominant in males and recessive in females. If a hornless ram were mated to a horned ewe, what is the chance that:
- an F_2 male sheep will be horned or
 - an F_2 female sheep will be horned?
6. In chicken, the gene h which distinguishes hen-feathering from cock-feathering, is sex-limited. Males may be hen-feathered or cock-feathered, but female are always hen-feathered. If a cock-feathered male (hh) were mated to a homozygous (h^+h^+) hen-feathered female, what patterns of feathering might be expected among the (a) male F_2 and (b) female F_2 progeny?
7. In *Drosophila*, the gene for bobbed bristles (recessive allele bb , bobbed bristles; wild-type allele bb^+ , normal bristles) is located on the X chromosomes and on a homologous segment of the Y chromosome. Give the genotypes and phenotypes of the offspring from the following crosses: a) $X^{bb}X^{bb} \times X^{bb}Y^{bb+}$, b) $X^{bb}X^{bb} \times X^{bb}Y^{bb}$, c) $X^{bb+}Y^{bb}$, d) $X^{bb+}X^{bb} \times X^{bb}Y^{bb+}$.
8. Make a diagram for a cross between a normal woman (whose father was defective in green colour vision) and a green colour-defective man. Summarise the expected for sex and colour vision.

7.10 ANSWERS

Self Assessment Questions

- Since both the husband and wife have fathers with the X-linked trait, the husband will not carry the trait as it receives its X-chromosome from his mother, but the wife carries the trait as she gets one X-chromosome from her father. a) The probability of having a normal son is fifty per cent. b) The chances of having a normal daughter is hundred per cent as a female is not affected with X-linked recessive trait unless she receives two genes for the trait. c) There is fifty per cent probability of having affected son. d) There is no chance of the daughter being affected, but there is a fifty per cent probability that they may be carrier.
- Since the daughter is colour blind it can be assumed that both parents carry the genes for the trait. The father's genotype is therefore, hemizygous and that of the mother is heterozygous for the trait. The son must have received the normal X-chromosome from the mother and is therefore, normal.

3.



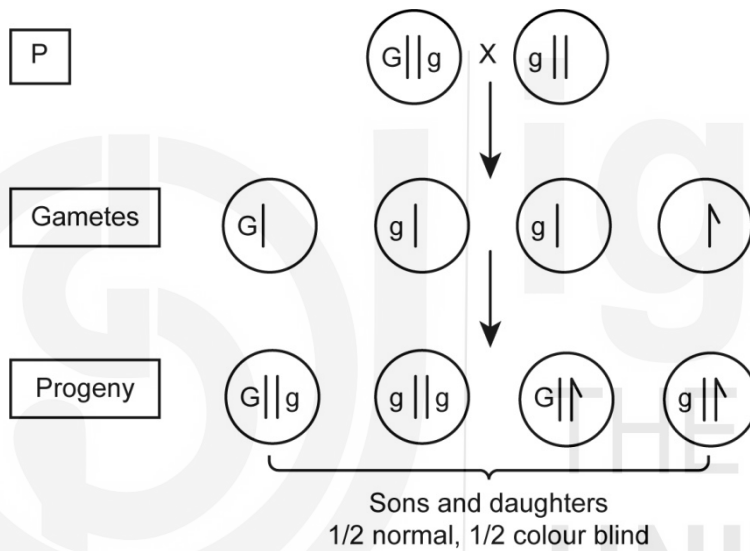
The probability is fifty per cent for the daughters being carriers and there is fifty per cent probability of the son being a haemophiliac.

4. Only the male children have hypertrichosis ear as the gene or it is Y-linked and the Y is passed from father to son. Females don't have the trait as they never possess a Y-chromosome.
5. Irrespective of whether a Y-linked gene is recessive or dominant it can be recognized as it is always present in hemizygous condition. The Y-linked gene is transmitted from grandfather to male grand-children through the father. The female grand-children are unaffected so are the daughters and the grand-children born from them.
6. Sex-linked inheritance patterns are quite different from those of sex-limited ones. The latter may be expressed in either sex, though with differential frequency. Genes for sex-limited traits express their effects in only one sex or the other and their action is clearly related to sex hormones. They are principally responsible for secondary sex characters.
7. Klinefelter-one; Turner-none; 47 XYY-none; 47 XXX-two; 48 XXXX three.
8. Because the mosaic coat pattern is due to the expression of sex-linked heterozygous alleles according to the Lyon hypothesis.

Terminal Questions

1.
 - i) The trait occurs more frequently in males than in females.
 - ii) Traits are transmitted from an affected man through his carrier daughters to half his grandsons.
 - iii) An X-linked allele is never transmitted directly from father to son.
 - iv) All affected females have an affected father and a carrier or affected mother.
2. i) e, ii) c, iii) b, iv) e, v) c.
3. i) 22, XY, ii) 22, XX, iii) male, iv) holandric, v) hormones, vi) sex-limited.

4. i) b, ii) e, iii) f, iv) a, v) d.
5. a) $3/4$, b) $1/4$.
6. a) 3 hen-feathered : 1 cock-feathered
b) All hen-feathered
7. a) $1/2 X^{bb}X^{bb}$ bobbed females, $1/2 X^{bb}Y^{bb+}$ wild males;
b) $1/2 X^{bb}X^{bb+}$ wild females, $1/2 X^{bb}Y^{bb}$ bobbed males;
c) $1/2 X^{bb}X^{bb+}$ and $X^{bb}X^{bb+}$ wild females, $1/4 X^{bb+}Y^{bb}$ wild males, $1/4 X^{bb}Y^{bb}$ bobbed males;
d) $1/4 X^{bb+}X^{bb}$ wild females, $1/4 X^{bb}X^{bb}$ bobbed females, $1/2 X^{bb+}Y^{bb+}$ and $X^{bb}Y^{bb+}$ wild males.
- 8.



THE NATURE AND STRUCTURE OF GENETIC MATERIAL

Structure

8.1	Introduction Objectives	8.4	The Physical Structure of DNA: The Double Helix The Double Helix DNA Denaturation and Renaturation
8.2	The Search of Genetic Material Proteins Nucleian Amounts of DNA and Protein DNA is the Genetic Material RNA as Genetic Material	8.5	Conformational Flexibility of DNA Molecules
8.3	The Chemical Composition of DNA and RNA	8.6	Summary
		8.7	Terminal Questions
		8.8	Answers

8.1 INTRODUCTION

In the preceding units, you have learnt that the systematic study of the science of genetics began through Mendel's investigations. You also studied the patterns of, transmission of traits from one generation to another, the origin of variations through recombination, and how the genes governing certain traits have been located on given chromosomes by establishing linkage and gene mapping.

You may recall that the involvement of discrete genes in determining phenotypic characters was known from Mendel's work. It also demonstrated that genes were transmitted unchanged between succeeding generations. But there was paucity of information regarding the nature and structure of the genetic material composing the gene. For decades after Mendel's work, many geneticists concentrated on establishing the chemical identity of the genetic material as well as describing its molecular structure.

We begin this **unit** by discussing the qualities that the genetic material possesses and also the evidences that DNA and RNA are the genetic materials. In addition, we shall also examine the molecular structure of the nucleic acids.

Objectives

After studying this unit you should be able to:

- ❖ describe the various investigations leading to the identification of the genetic material,
- ❖ draw diagrams of a nucleotide and a polynucleotide,
- ❖ distinguish between purines and pyrimidines,
- ❖ state the complementary bases found in nucleic acids,
- ❖ describe the Watson-Crick model of DNA,
- ❖ state the chemical and structural differences between DNA and RNA, and
- ❖ describe the conformation flexibility of DNA molecules.

8.2 THE SEARCH FOR GENETIC MATERIAL

You already know that the genetic or hereditary material for all species is nucleic acid. In most organisms, it occurs in the form of deoxyribonucleic acid – DNA; and in a few viruses it occurs as ribonucleic acid – RNA. Long before DNA and RNA were proved to carry genetic information, geneticists recognized that special molecules must fulfil that function. They postulated that the material responsible for inheritable traits should have the following three principal characteristics:

- i) It must contain **all the information** for an organism's cell structure, function, development and reproduction in a stable form.
- ii) It must **replicate accurately** so that the progeny cells have the same genetic information as the parental cells.
- iii) It must be capable of undergoing **variation**, and exist in an almost infinite variety of forms, as without variation (through mutation and recombination), organisms would be incapable of change, and adaptation and evolution would not occur.

In mid to late 1800's and early 1900's, scientists considered proteins rather than the nucleic acids as the prime candidates for hereditary material. But critical experiments that were performed subsequently, the details of which are discussed in the unit later on, went on to show that DNA is the genetic material. But let us first look into some salient features of proteins that made these likely candidates for hereditary material.

8.2.1 Proteins

Since proteins were known to be highly complex polymeric molecules, they were considered strong candidates for genetic substance. About 66% of the weight of the human body is water; 47% of the remainder (that is, 16% of the total weight) is protein. Protein fulfils a variety of crucial functions for the living organism. One category of proteins called enzymes are of particular importance, as they catalyse thousands of crucial biochemical reactions in the body.

Some proteins function to provide **physical support**, both for individual cells and for the entire organisms. Keratin is a structural protein that is the primary component of skin and hair. Tubulin forms part of the “**skeletal**” or supportive system of an individual cell. Muscle tissue consists of protein specialised for movement. **Antibodies** are proteins that protect the body from bacteria and other foreign substances. **Haemoglobin** is a protein specialised for the transport of gases in the blood. Some hormones are proteins that provide communication among some tissues of the body. We have seen the various roles that proteins play in the body. A brief introduction to the chemical structure of proteins will help to illustrate how proteins fulfil so many different functions, and why they seemed to be the most likely chemical to bear hereditary information. We shall now briefly consider some aspects of protein chemistry.

Many of the salient facts about protein chemistry that we still accept today were known in the 1930's. Proteins are composed of linear polymers of amino acids called polypeptides. Typically, amino acids have a basic amino ($-\text{NH}_2$) group and an acidic carboxyl ($-\text{COOH}$) group attached to a common carbon atom from which a variety of side chains extend (see Fig. 8.1). Twenty amino acids are commonly found in polypeptides. Amino acids are joined together in a linear polymer by bonds called peptide bonds, between the amino group of one amino acid and the carboxyl group of an adjacent amino acid (Fig. 8.2). As a result of these linkages, most polypeptides have a free, unbound amino group at one end (the amino terminal or N-terminal amino acid) and a free carboxyl group at the other end (the carboxyl terminal or the C-terminal amino acid) of the linear polymer.

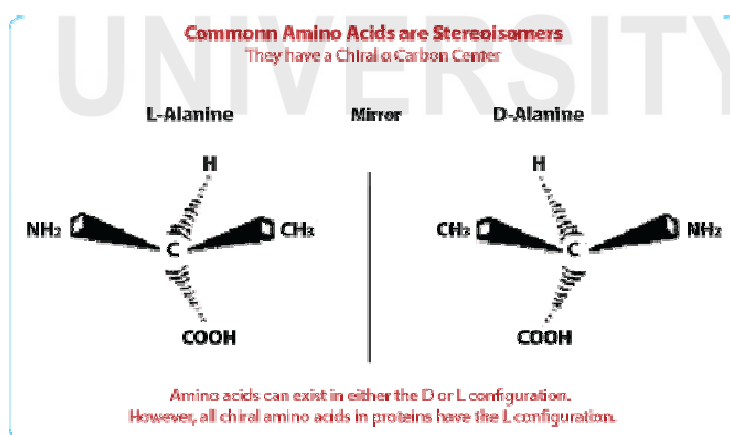
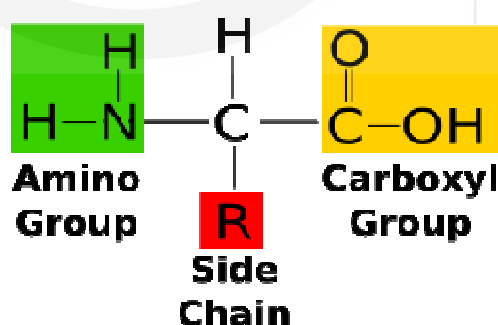


Fig. 8.1: Structure of amino acid (L and D forms with N and C terminus).

If two amino acids are bound by one polypeptide bond, the molecule thus formed is known as a **dipeptide**. And in the case of more than two amino acids, or if a chain of many amino acids is involved, it is known as a **polypeptide**. Proteins may contain only one polypeptide chain, several polypeptide chains, or polypeptide chains combined with other molecules. Haemoglobin, for example, is a protein consisting of four polypeptide chains, each bound to an iron-containing molecule (see Fig. 8.3).

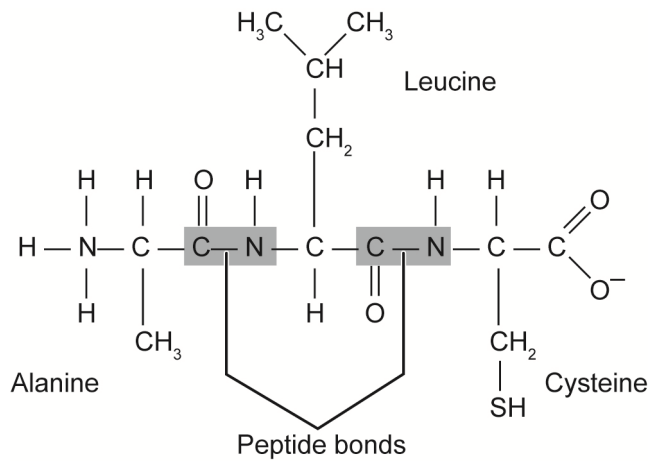


Fig. 8.2: A polypeptide formed from three amino acids. Note the two peptide bonds.

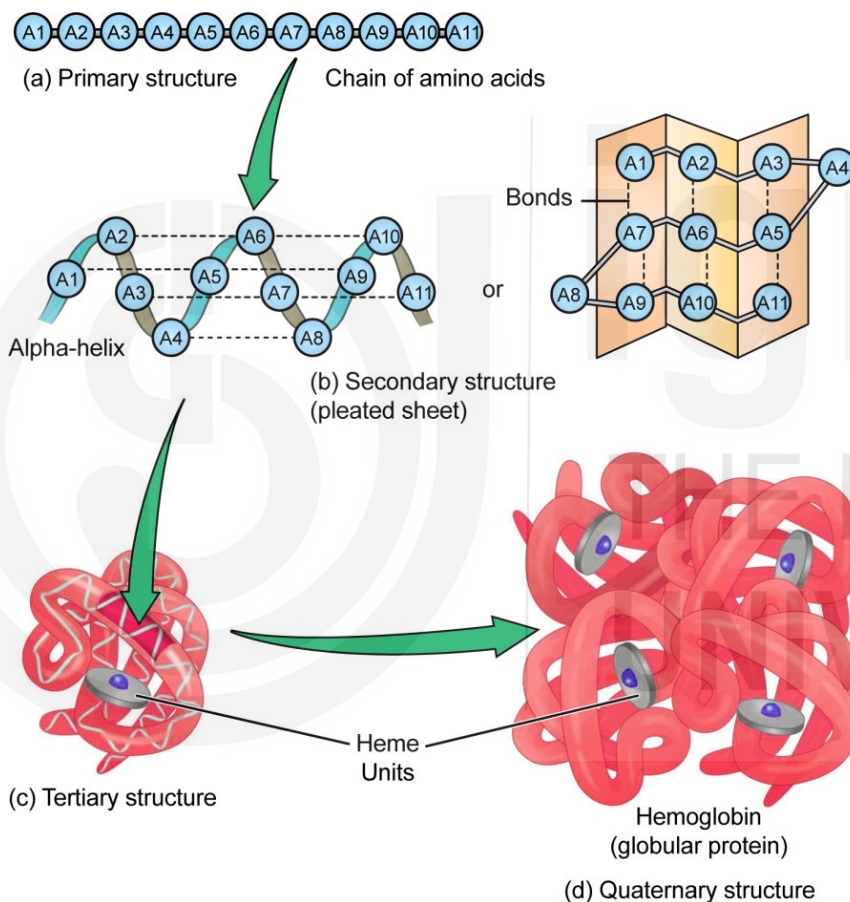


Fig. 8.3: The protein molecule haemoglobin showing four polypeptide chains.

Different proteins have different compositions and sequences of amino acids. The amino acid *composition* of a protein is the relative proportion of the different kinds of amino acids present in a protein. The amino acid *sequence* of a protein is the order in which the amino acids follow one after the other in the linear polypeptide chain. By convention, the amino acid sequence of a polypeptide is listed starting with the N-terminal amino acid or residue, and continues through the C-terminal amino acid. The smallest proteins or polypeptides have fewer than one hundred amino acids, for example, the hormone insulin has 51 amino acids, and some large proteins like myosin (involved in muscle contraction) have over 1,800 amino acids long polypeptide chain.

The number of different possible sequences of amino acids in a protein is 20^n , where n is the number of amino acids in the protein. For a polypeptide only four amino acids long, some 160,000 different combinations are possible. And then amino acids can produce 10,240,000,000,000 unique combinations. For a protein one hundred amino acids long, the number of combinations are unimaginable. The biochemists of the nineteenth century knew that a large molecule, as that of protein, manifests a great diversity of structure to fulfil the many functions as well as encode the immense amount of hereditary information needed to direct various biochemical reactions.

In addition to being of many different sizes, proteins also have widely varying physical properties because of varying amino acid content and the differing chemical and physical properties of the side chains of chains of the amino acids. Most of the side chains are neutral, but three (viz. lysine, histidine and arginine) are basic, and two (viz. aspartic acid and glutamic acid) are acidic. Furthermore, the various side chains have different affections for water. The basic and acidic amino acids are water loving, i.e., **hydrophilic** or water soluble. Other amino acids, such as leucine, isoleucine, phenylalanine, proline, valine and methionine are water-hating, i.e., **hydrophobic** or water-insoluble. But most amino acids, e.g., glycine, tyrosine and serine are **ambivalent**, they can take water or leave it.

The **primary** structure of protein refers to the sequence of amino acids in the polypeptide chain. Commonly, protein in a polypeptide a spiral or helix which is the **secondary structure** of proteins. The helix in turn is twisted or folded precisely into a complex three-dimensional conformation, crucial for the protein function, and is known as **tertiary structure**. Finally many proteins contain two or more polypeptides, forming the **quaternary structure**.

In the 1930's the biochemists knew that enzyme activities, the ability to catalyse chemical reactions in cells, were associated with proteins, implying that enzymes were proteins. What emerged in 1930's was the realisation that proteins are large, highly complex molecules, with a variety of physical properties and functional activities. This is still valid though greatly augmented by present knowledge.

8.2.2 Nuclein

In 1869, only a few years after the publication of Mendel's work, a Swiss biochemist, Johann Friedrich Miescher studied the nuclei of pus cells from waste surgical bandages. He found that these nuclei contained a novel phosphorus-bearing compound, which was a non-protein material that he named **nuclein**. He named it so based on the site of its origin. Nuclein is mostly **chromatin** – a complex of deoxyribonucleic acid (DNA) and chromosomal proteins.

The work by Miescher and other scientists, subsequently, demonstrated that the nuclei of the cells of all species contain nuclein and that nuclein was an important constituent of chromosomes. Quantitative analysis of chromosomes showed that they consisted of about 60% protein and 40% nuclein (DNA). This led the scientists of that time to infer that protein was the genetic material. DNA was not considered to be identical with the genetic material as it was

found to lack chemical diversity needed for the genetic substance. In contrast, proteins are diverse collection of molecules, so it was widely believed that protein was the genetic material and that DNA was merely the structural framework of chromosomes.

8.2.3 Amounts of DNA and Protein

Description of the meiotic behaviour of chromosomes supported their role in heredity. The diploid number was constant in the somatic cells of all members of a species, but was halved in the germinal cells and the diploid condition was resumed only after fertilisation.

Careful measurements of the amounts DNA and protein in somatic and sperm cells indicated the likely hereditary role of DNA, but not of proteins. Table 8.1 gives data on the DNA content of somatic and gametic cells in some organisms. Within a species, the amounts of protein in a sperm cell varied considerably. Furthermore, the amounts of protein in sperm and somatic cells bore no simple relationship to each other. On the other hand, the amount of DNA in sperm was relatively constant. Additionally, the amount of DNA in sperm cells was exactly one-half that in the somatic cells.

Table 8.1: DNA content in the somatic and genetic cells of some organisms. The unit for measuring DNA content is picograms 10^{-12} gram, or one trillionth of a gram. (After Maxson & Daugherty, *Human Genetics*, 1992, p.70).

Organism	Somatic Cell (2n)	Gametic Cell (n)
Chicken	2.6	1.3
Cow	6.6	3.3
Diatom	0.08	0.04
Fern	326.4	163.2
Frog	15.0	7.5
Fruit fly	0.4	0.2
Fungus	3.0	1.5
Man	6.4	3.2
Onion	36.7	18.4
Pine	138.7	69.4
Rat	6.0	3.0
Salamander	336.0	168.0

8.2.4 DNA is the Genetic Material

The identity between DNA and the genetic material was shown directly in the experiments that are described below:

Transformation in Pneumococcus

Frederick Griffith laid the foundation for the identification of DNA as the genetic material, in 1928, with his experiments on transformation in bacterium *Diplococcus pneumonia* or *pneumococcus*, now named *Streptococcus pneumonia*. In his experiment, he used two naturally occurring strains of *pneumococcus*. These two strains have markedly different properties. One strain possesses a smooth polysaccharide capsule (Fig.8.4), and thus produces smooth colonies. This strain was called strain S. It causes pneumonia in mice. Another strain, which lacks polysaccharide produces rough colonies. This strain was called strain R and did not have a pathological effect on mice. Bacteria of the rough strain are engulfed by the white blood cells of the mice; but the virulent smooth-strain bacteria survive because they are protected by their polysaccharide coating. Griffith found that neither the heat-killed S-type nor the live R-type cells, by themselves, caused pneumonia in mice. However, if a mixture of the live R-type and the heat-killed S-type cells was injected into the mice, they developed an infection identical to [hat caused by the living S-type cells (Fig. 8.5). Thus, something in the heat-killed S-cells transformed the R-type bacteria into the S-type cells. Griffith felt that some principle transferred from the killed S-type cells converted the R strain to virulence by enabling it to synthesise a new polysaccharide coat. In Griffith's terms, the transforming principle from the dead S cells served as a "pabulum" for the R cells.

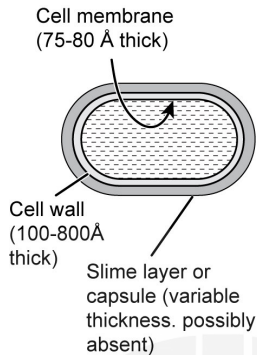


Fig. 8.4: A typical bacterial cell showing the surface layers including the capsule.

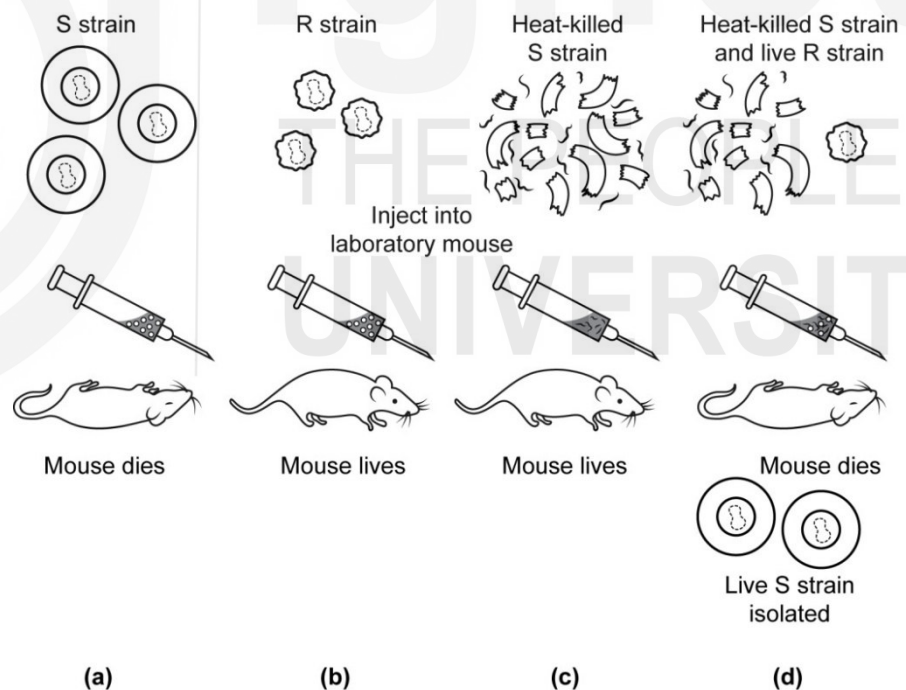


Fig. 8.5: Griffith's transformation experiment with smooth (S) and rough (R) strain or *Streptococcus pneumoniae*.

Proof that the Transforming Principle is DNA

Over the next decade and a half, a research team headed by Oswald T. Avery (Fig. 8.6) at the Rockefeller Institute earnestly pursued to identify the transforming principle. Between 1930 and 1933, they achieved transformation outside the body of a living mouse. Avery and his coworkers did most of their work under *in vitro* conditions, rather than the occurrence of pneumonia in the mice as evidence of transformation.

In 1944, sixteen years after Griffith's publication, Avery, MacLeod and McCarty published a remarkable paper in which they reported that they had purified the transforming factor. Analysis of molecular composition and weight indicated this factor to be DNA. Their strategy was to prepare a cell-free filtrate from the heat-killed S-cells (see Fig. 8.7, steps 1 to 4), containing the transforming principle and then to digest the individual components of this filtrate with specific degradative enzymes (see steps 5, 6, and 7, in Fig. 8.7). For instance, any of the three enzymes – protease, trypsin or chymotrypsin – could be used to degrade all the proteins in the filtrate (step 5). Similarly, a ribonuclease (step 6) can be employed to destroy the ribonucleic acid (RNA) – the second type of nucleic acid (step 7) present in living cells (though, unlike DNA, not a major constituent of chromosomes). The filtrate salvaged (step 4) was tested for its transforming ability (see step 8); that was found to be positive. Likewise after the respective enzymatic digestion the filtrate was also tested for retention of its transforming ability. Surprisingly (as genes were still thought to be made of protein), treatment with protease had no effect on the ability of the filtrate to transform the R cells into the S form (step 9). Similarly, ribonuclease treatment had no effect on the transforming activity (step 10). However, digestion of DNA with deoxyribonuclease (see step in Fig. 8.7) totally destroyed the transforming ability, so that the filtrate was, no longer able to convert one cell type into the other.

Gradually, it became clear to Avery and his colleagues that the transforming principle must be DNA. This idea was supported not only by the results of the enzymatic digestion experiments, but also by the analysis of purified transforming principle. The analytical tools that Avery et. al. used were: *ultracentrifugation, electrophoresis, ultraviolet absorption spectrophotometry, and elementary chemical analysis.*

On reading Avery et. al.'s paper even today, one has little doubt over the reliability of their results or the validity of their conclusions. Scientists, in 1944, were more sceptical. Doubts were raised about the efficiency of the enzymes used to digest the protein component of the S cell filtrate. It was suggested that as much as 2% residual protein in the 'pure' preparation of transforming principle would be undetected and might be responsible for the transformation of the R cell to S, if genes were made of proteins. These nagging doubts although unfounded prevented the results of Avery's group from being unanimously accepted by the scientific community. Therefore, a second experimental identification of genetic material was required.

Avery, born in Canada, studied medicine at Columbia University in New York City, obtaining his MD in 1904. He joined the Rockefeller Institute Hospital in 1933, remaining there until 1948, when he retired and moved to Nashville, Tennessee. Avery was a bachelor and a dedicated bacteriologist. He was actively engaged in research even towards the end of his career. He made several contributions to the study of pneumonia causing bacteria. His meticulous plan of work on the transforming principle though lasting several years, eventually culminated in 1944 with the clear identification that the active agent is DNA. Rene Dubos has written a biography of Avery: 'The Professor, the Institute and DNA', Rockefeller, University Press., New York, 1976.

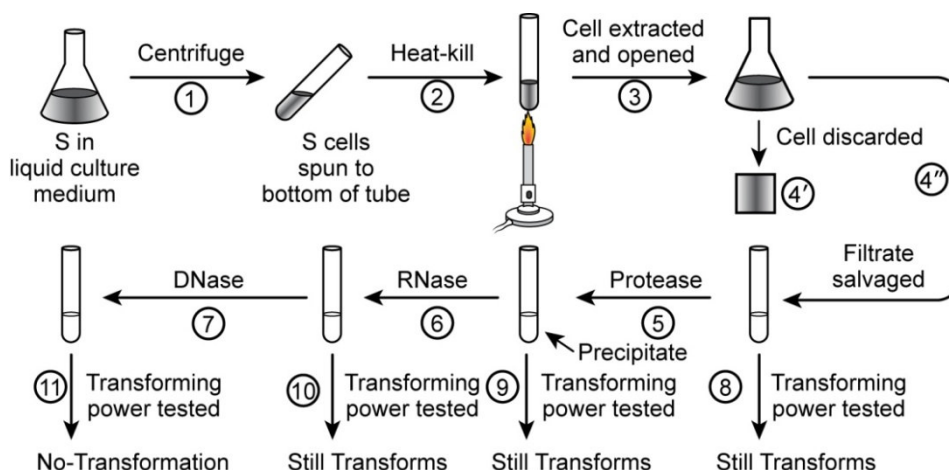


Fig. 8.6: An outline of Avery, MacLeod and McCarty's experiment demonstrating that DNA is the transforming principle.

SAQ 1

Insert the correct words in the blank space.

- i) Experiments on mice and showed that structural and physiological character can be from one bacterium to another (....., 1928). This transfer of characters appears to be associated with a transfer of (....., ... and, 19).

The Hershey Chase Experiment

The third experiment, that was directed at chemical characterisation of the genetic material, was carried out in 1951-53, seven years after Avery's work was published. By then experimental techniques had progressed, and new approaches to problem solving in genetics were available. In particular, the use of bacteriophages as experimental tools for studying molecular genetics had become fully established.

Bacteriophages or phages as they are commonly known, are viruses that specifically infect bacteria. Phage T2 (Fig. 8.7), for example, is one of several types of phages that are specific for the bacterium *Escherichia coli*. The phage consists of a protein coat which surrounds a core of DNA. Electron micrographs have revealed that the protein coat portion that contains the genetic material is hexagonal and this entire portion is called head. In addition, there is a tail also. The life cycle of bacteriophage T2 is shown in Fig. 8.8.

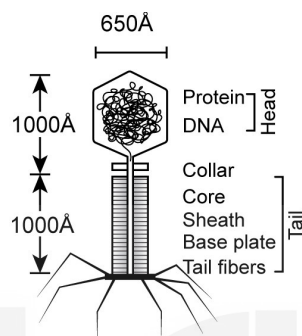


Fig. 8.7: Diagrammatic sketch of the *E. coli* phage T2 showing its various parts.

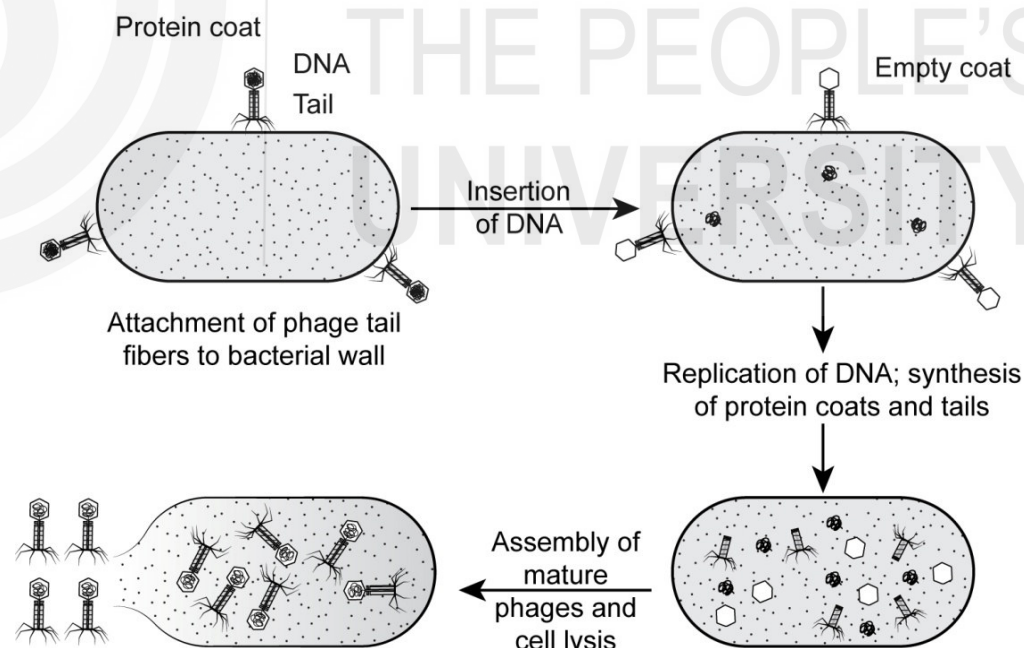


Fig. 8.8: The life cycle of bacteriophage T2.

Alfred Hershey and Martha Chase in 1952 published the results of experiments designed to clarify the events leading to phage reproduction. Several of the experiments clearly established the independent function of phage's coat protein and nucleic acid in the reproductive process associated with the bacterial cell. From the existing data Hershey and Chase knew that: (i) T2 phages consist of approximately 50 per cent protein and 50 per cent DNA; (ii) infection is initiated by adsorption of the phage through its tail fibers

to the bacterial cell; (iii) the reproduction of new viruses occurs within the bacterial cell.

While working with this phage, it appeared to Hershey and Chase that some molecular component of the phage DNA and/or protein enters the bacterial cell and directs viral reproduction. What could possibly this component be, was the main question arising in their minds. Both DNA and protein contain elements most characteristic of living organisms – carbon, hydrogen, oxygen and nitrogen. They differ in one major aspect, that is, **sulfur occurs only in proteins not in DNA while phosphorous occurs only in DNA and not proteins**. Based on the information that the two macromolecules are different, they designed their experiment. They differentially radiolabelled the macromolecules (DNA or protein) with radioisotopes ('marker') to follow the molecular components of phage during infection. Both P^{32} and S^{35} – radioactive isotopes of phosphorus and sulfur were used respectively.

Hershey and Chase began their experiment (Fig. 8.9) by growing cultures of phages T2 (Fig. 8.7) in suspensions of *E. coli* rich in one of two radioactive isotopes, sulfur – 35 (S^{35}) or phosphorus – 32 (P^{32}). They then proceeded to identify the material injected into the cell by phages attached to the bacterial wall.

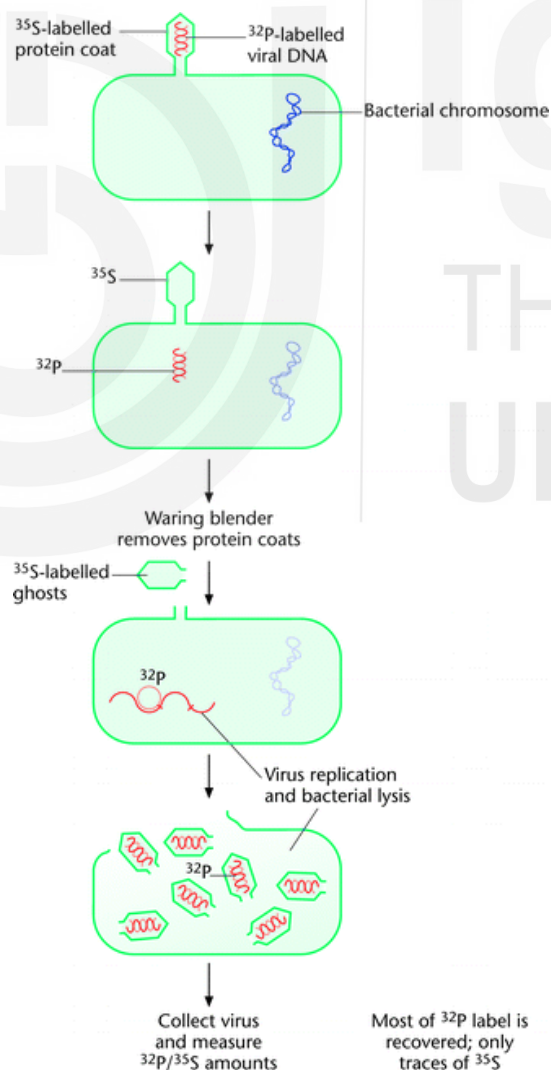


Fig. 8.9: Diagrammatic representation of Hershey and Chase's experiment, that demonstrated that DNA and not protein is responsible for directing the reproduction of phage T2 during the infection of *E. coli*. This is based on the fact that nucleic acid label (P^{32}) enters the bacterium during infection. The protein label (S^{35}) does not.

All elements exist in a number of forms called isotopes. Each isotope differs only in the numbers of neutrons in the atomic nucleus. Different isotopes of the same atomic number have different atomic masses.

Isotopes may be stable (e.g., C^{12}) or unstable (e.g., C^{14}). The unstable ones decay into a stable form (possibly not of the same element) with emission of radiation of characteristic energy. With the help of Scintillation Counter, we can measure the amount of radioactive disintegration and also identify the radioactive atom involved. The biological systems or cells cannot distinguish between isotopes, e.g., a bacterium cannot differentiate between C^{14} – glucose and C^{12} – glucose, and will use each equally efficiently as carbon source. Based on this Hershey and Chase prepared bacteriophages P^{32} and S^{35} .

Have you studied Figure 8.9 carefully? Then how about answering the following questions?

SAQ 2

- a) Which molecules of the phage in culture A will be radioactively labelled?
 - b) And which molecules of phage in B are radioactively labelled?
-

All the T2 derived from these cultures thus had their DNA labelled with P^{32} or the protein coats labelled with S^{35} . Next, the unlabelled cultures of *E. coli* were infected separately with S^{35} – labelled and P^{32} – labelled T2. In the case of the P^{32} labelled phages that were mixed with *E. coli* they found that the P^{32} – label entered the bacterial cells and the next generation of phages that burst from the injected cells carried a significant amount of P^{32} label. And in the S^{35} labelled phages mixed with unlabelled *E. coli*, the researchers found that the label stayed on the outside of the bacteria in the phage's coats. From these observations they concluded that the outer protein coat of a phage does not enter the bacterium it infects, whereas the phage's inner material consisting of DNA does enter the bacterial cell. Since the DNA is responsible for the production of new phages during the infection process, the DNA, not the protein must be the genetic material.

Transfection Experiments – During the eight years following the publication of the Hershey-Chase experiment, further research provided additional proof that DNA is the genetic material. These studies involved the same organisms as were used by Hershey and Chase.

In 1957, there were several reports showing that naked cells of *E. coli* could be obtained on treating the cells with the enzyme lysozyme. This enzyme dissolves the outer wall without destroying the bacterium. Such naked structures are called *protoplasts* or *spheroplasts*. John Spizizen and Dean Fraser independently reported that by using protoplasts, they were able to initiate phage multiplication with disrupted T2 particles. That is, provided protoplasts are used, it is not necessary for virus to be intact in order for infection to occur.

Later, in 1960, similar but refined experiments were reported by George Guthrie and Robert Sinsheimer. They purified DNA from the bacteriophage ϕ X-174. It is a small phage consisting of a single-stranded, circular DNA molecule of some 5500 nucleotides. When added to the *E. coli* protoplasts, the purified DNA resulted in the production of complete ϕ X-174 bacteriophages. This process of infection by only the viral nucleic acid, is called **transfection**, proves beyond doubt that the phage DNA alone contains all the necessary information for production of mature viruses. Thus, the evidence that DNA serves as the genetic material was further strengthened, even though all direct evidence had been obtained from bacterial and viral studies.

8.2.5 RNA as Genetic Material

All viruses are not limited to bacterial hosts. Other kinds of viruses infect and parasitise animal and plant cells. One plant virus, *tobacco mosaic virus* (TMV), that contains RNA, not DNA was an important tool for genetic experiments in the late 1940's. TMV infects tobacco, causing the infected regions on leaves to become discoloured and bristled. Different strains of TMV produce clearly different inherited lesions on the infected leaves. The common virus (TMV-*common*) produces a green mosaic disease, but a variant Holmes Ribgrass (TMV-*HR*), produces ringspot lesions. Moreover, the amino acid composition of the proteins of the two strains differ.

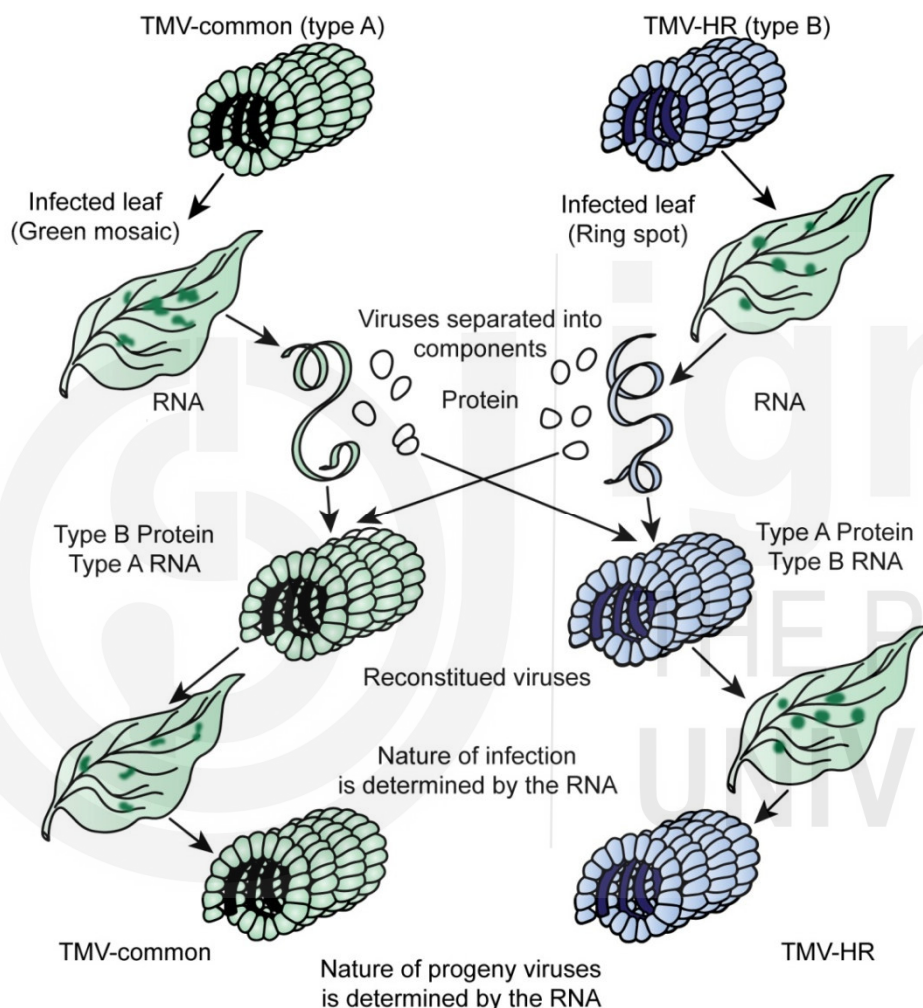


Fig. 8.10: Genetic effects of reconstituting tobacco mosaic viruses by using RNA and proteins from two different strains.

Heinz Fraenkel-Conrat and Bea Singer found that the virus could be broken into component parts and these could again be reassembled or reconstituted to form functional virus. From the two strains of TMV, they were able to reconstitute viruses with the RNA from TMV-*common* enclosed in TMV-*HR*-protein and TMV-*HR* RNA with TMV-*common* protein (Fig. 8.10). When these reassembled viruses were used to infect tobacco leaves, the progeny viruses produced were always found to be phenotypically and genotypically identical to the parent strain from which the RNA had been obtained. The reassembled viruses with TMV-*common* RNA and TMV-*HR* protein produced a green mosaic disease characteristic of TMV-*common*. Recovered progeny virus had protein characteristic of TMV-*common*. Similarly, the virus containing TMV-*HR*

RNA with TMV-*common* proteins produced ringspot lesions and the virus progeny had protein characteristic of TMV-*HR*. The source of the RNA determined the nature of the mosaic infection and of the proteins present in the progeny viruses indicated that nucleic acid in the progeny viruses indicated that nucleic acid in this case was RNA. Hence, RNA carries genetic information not proteins.

SAQ 3

Choose the correct answer:

- i) DNA is believed to be the genetic material because:
 - a) all the body cells of an individual seem to have identical amounts and composition of DNA, while reproductive cells have half the amount of DNA found in body cells.
 - b) the proteins are the same from cell to cell in an individual, but DNA differs, thus DNA must be the material that makes tissues different.
 - c) DNA is the largest type of macromolecule found in living organism.
 - d) DNA is found in the cell nucleus.
- ii) By differentially labelling the coat protein and the DNA of phage T2, Hershey and Chase demonstrated that:
 - a) only the proteins enter the infected cell
 - b) the entire virus enters the infected cell
 - c) the phage genetic material is most probably DNA
 - d) the phage coat protein directs synthesis of new progeny phage.

SAQ 4

What is the macromolecular composition of a bacterial virus or bacteriophage such as a phage T2?

SAQ 5

With respect to which particular component do the proteins and DNA differ from each other and that allowed researchers to specifically label one or the other of these macromolecules with radioactive isotope?

8.3 THE CHEMICAL COMPOSITION OF DNA AND RNA

The results of the experiments that we have described above, all point to nucleic acid, DNA or RNA as the carrier of genetic information. Now we need to examine the chemical structure of these molecules. Their structure will tell us a good deal about how they function.

Nucleic acids, both DNA and RNA are **macromolecules**, which means they have a molecular weight of at least a few thousand Daltons. One dalton is equivalent to a twelfth of the mass of the carbon¹² atom or 1.67×10^{-24} g. In this respect they differ from many other molecules important to cell function such as sugars and amino acids, which weigh from a hundred to a few hundred daltons.

Both the nucleic acids are polymeric molecules and are composed of repeating subunits called **nucleotides**. Each nucleotide is made up of three components: (1) a phosphate group, (2) a five-carbon sugar or pentose, and (3) a cyclic nitrogen containing compound called a nitrogenous base or simply base (Table 8.2; Fig. 8.11).

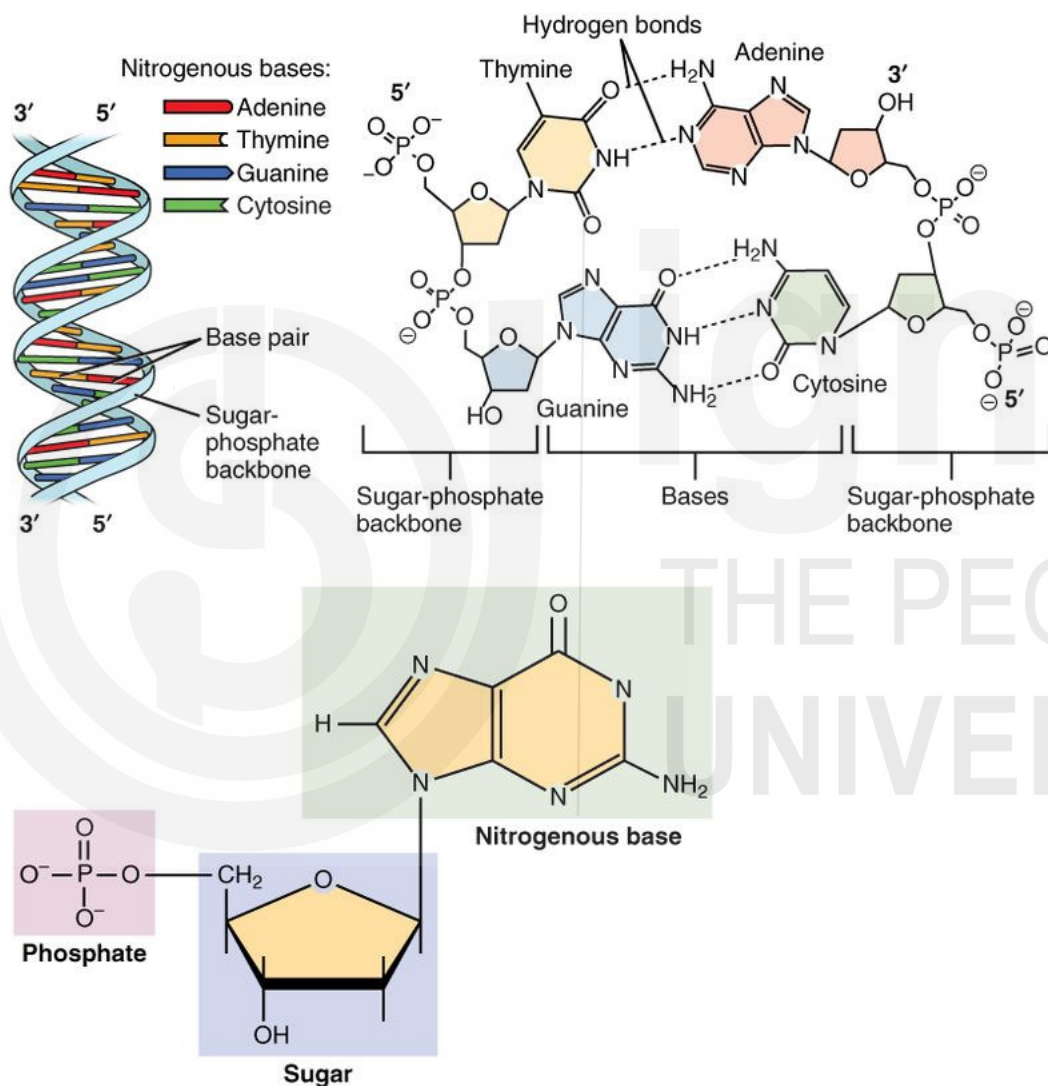


Fig. 8.11: Structural formulae of the components of nucleic acids; phosphate, sugars and bases. When the pentoses are present in nucleosides, nucleotides or nucleic acids, the five carbons are numbered.

When incorporated into a nucleic acid, a nucleotide contains one each of the three components. But, when free in the cell pool] nucleotides usually occur as triphosphates. The energy held in extra phosphates is used, among other purposes to synthesise the polymer. A **nucleoside** is a sugar-base compound. Nucleotides are therefore nucleoside phosphates (Fig. 8.12). Note that ATP, adenosine triphosphate, the energy currency of the cell, is a nucleoside triphosphate.

The sugars differ only in the presence (ribose in RNA) or absence (deoxysibose in DNA) of an oxygen in the 2' position. The carbons of the sugar are numbered 1' to 5'. The primes are used to avoid confusion with the numbering system of the bases (see Fig. 8.11). There are four different bases found in DNA: **adenine, guanine, thymine, and cytosine** (see Table 8.2 again). RNA also usually contains adenine, guanine, and cytosine, but has a different base, **uracil**, in place of thymine. Thus three of the nitrogenous bases are found in both DNA and RNA, whereas thymine is unique to DNA and uracil is unique to RNA. Adenine and guanine are double-ring bases called **purines**; cytosine, thymine and uracil are single-ring bases called **pyrimidines**.

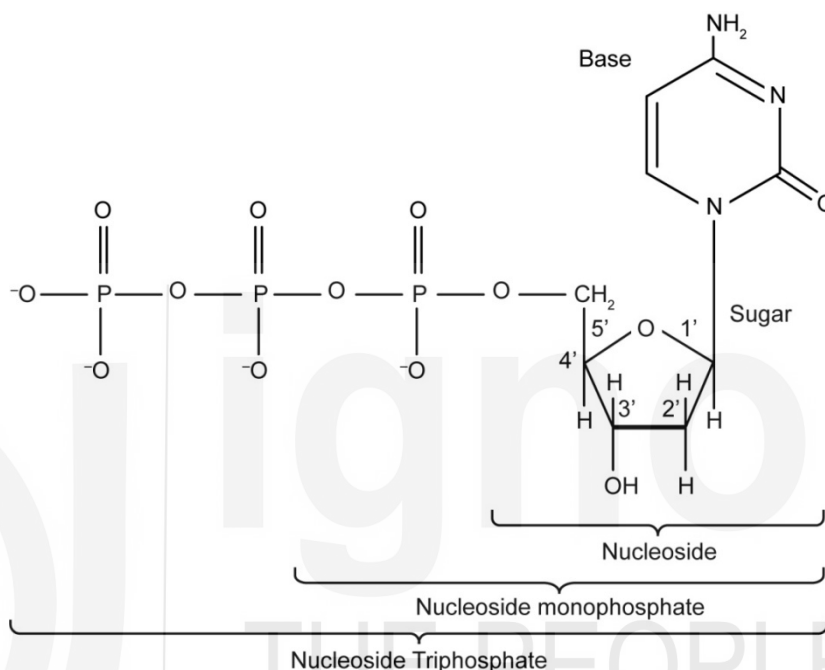


Fig. 8.12: The structure of a nucleoside and nucleotide: a nucleoside monophosphate and a nucleoside triphosphate.

Table 8.2: Components of Nucleic Acid.

			Base	
Nucleic acid	Phosphate	Sugar	Purine	Pyrimidine
DNA	Present	Deoxyribose	Guanine	Cytosine
			Adenine	Thymine
RNA	Present	Ribose	Guanine	Cytosine
			Adenine	Uracil

A nucleotide is formed, by the attachment of a base to the 1' carbon of the sugar and attachment of a phosphate to the 5' carbon of the same sugar (Fig. 8.13) – the first phosphate ester bond (see Fig. 8.14); it takes its name from the base (Table 8.3). Nucleotides are linked together or **polymerised** by the formation of a bond between the phosphate of one nucleotide and hydroxyl (OH) group at the 3' carbon of an adjacent molecule – the second phosphate ester bond. Very long strings of a nucleotides can be thus polymerised by the **phosphodiester bonding** (Fig. 8.14 and Box 8.1).

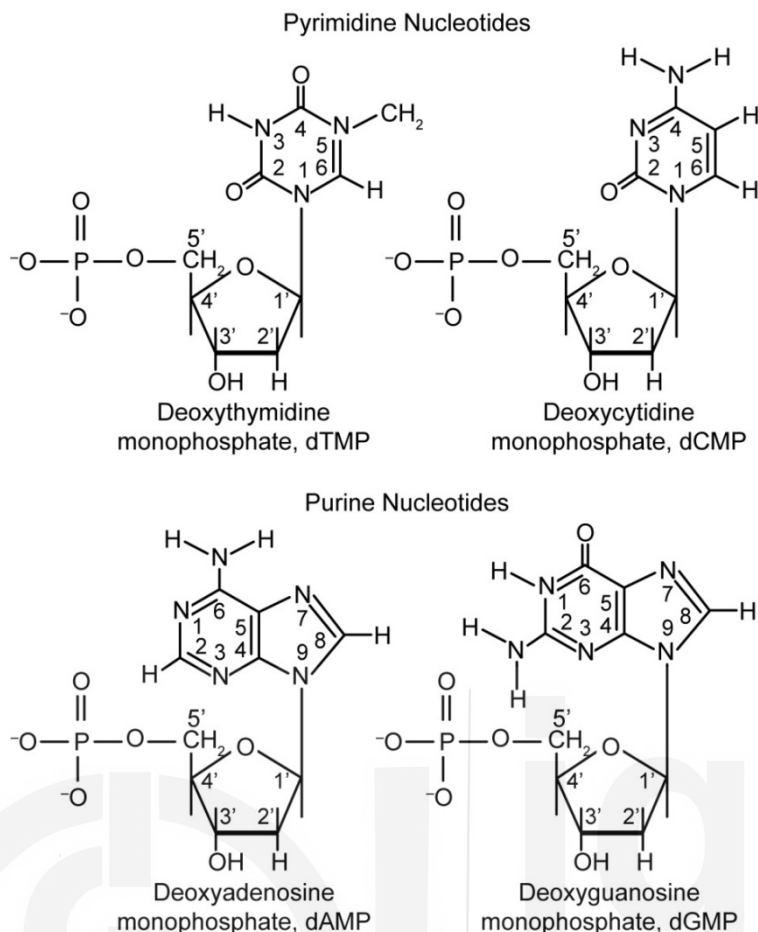


Fig. 8.13: Structure of the four common deoxyribonucleotides of DNA. RNA contains similar nucleotides, which contain the pyrimidines uracil and cytosine and the purines adenine and guanine.

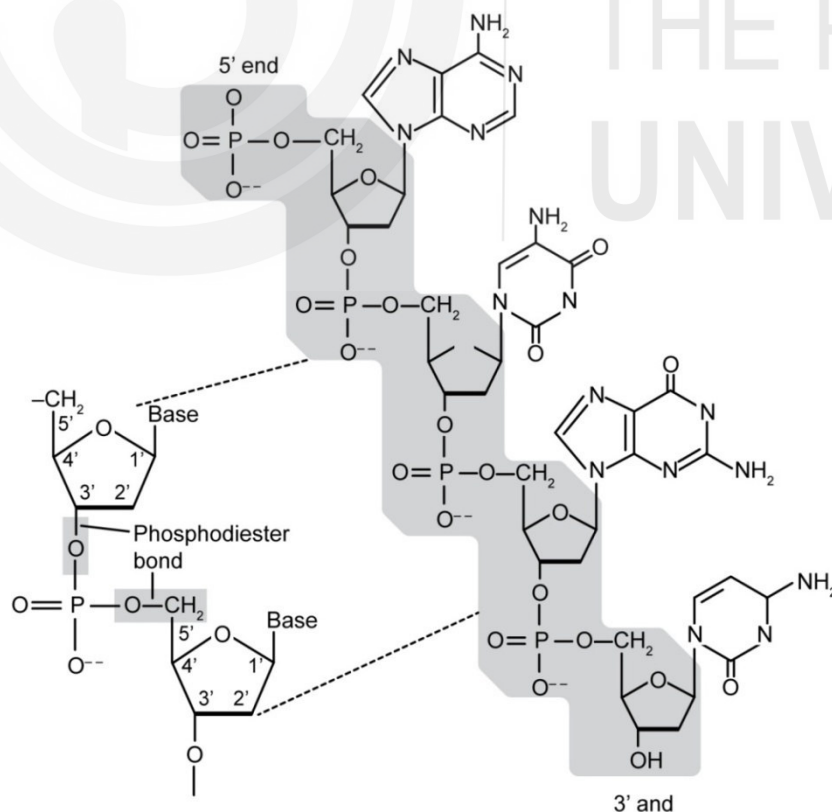


Fig. 8.14: Formation of a short polydeoxy ribonucleotide strand by polymerisation of adjacent nucleotides. Note the role of phosphodiester bonds. There is no limit to the length that the strand can be or the type of base attached to each nucleotide residue.

Table 8.3: Nucleotide Nomenclature.

Nucleotide (Nucleoside monophosphate)		Symbol					
		Monophosphate		Diphosphate		Triphosphate	
		Ribose	Deoxyribose	Ribose	Deoxyribose	Ribose	Deoxyribose
Guanine	Guanosine monophosphate	GMP		GDP		GTP	
	Deoxyguanosine monophosphate		dGMP		dGDP		dGTP
Adenine	Adenosine monophosphate	AMP		ADP		ATP	
	Deoxyadenosine monophosphate		dAMP		dADP		dATP
Cytosine	Cytidine monophosphate	CMP		CDP		CTP	
	Deoxycytidine monophosphate		dCMP		dCDP		dCTP
Thymine	Deoxythymidine monophosphate		dTMP		dTDP		dTTP
Uracil	Uridine monophosphate	UMP		UDP		UTP	

Both DNA and RNA, therefore, contain four different subunits or nucleotides, two purine nucleotides and two pyrimidine nucleotides (Fig. 8.13). RNA usually exists as a single-stranded polymer that is composed of a long sequence of nucleotides. DNA, however, has one very important additional level of organisation, it is usually a double stranded molecule.

After studying the chemical composition of DNA and RNA, how about attempting a couple of SAQs now?

SAQ 6

- Which are the four bases commonly found in DNA?
 - Which five-carbon sugar is found in DNA?
 - What is the difference between a nucleoside and a nucleotide?
 - Which base is unique to DNA and which base is unique to RNA?
-

SAQ 7

- How many phosphate groups are there per base in DNA, and how many phosphates are there in each precursor for DNA synthesis?
- Which chemical groups are at the ends of a single polynucleotide strand?
- Does the incorporation of nucleotides into a polynucleotide chain impose any limitations on the type of bases in the order in which these bases are arranged along the chain?

8.4 THE PHYSICAL STRUCTURE OF DNA: THE DOUBLE HELIX

The discovery of the double helix by James Watson and Francis Crick at Cambridge, in 1953, was one of the great deductive triumphs in the history of sciences, and it has influenced every aspect of molecular genetics, indeed of biology as a whole.

8.4.1 The Double Helix

Watson and Crick made use of the results of several lines of investigations concerning the structure of DNA. Based on these findings, they began to build models of various possible structures of DNA. Only the double helix was compatible with all the data. Their double helix model of DNA structure was based on two major kinds of evidence.

- Chargaff's Base Ratios** – E. Chargaff together with his colleagues E. Vischer and S. Zamenhof analysed the DNA of many different organisms. They used sensitive paper chromatographic techniques (see Box 8.2) to determine the exact amounts of each of the four nitrogenous bases in samples of DNA from different tissues of different organisms. Their results were quite startling. They observed that *the concentration of thymine was always equal to the concentration of adenine and the concentration of cytosine was always equal to concentration of guanine*. That is $A = T$ and $G = C$. This strongly suggested that thymine and adenine as well as cytosine and guanine were present in DNA with some fixed interrelationship. It implies that the total concentration of pyrimidines (thymine plus cytosine) always equals the total concentration of purines (adenine plus guanine). This rule is illustrated by Table 8.4.
- X-ray Diffraction Analysis** – The second piece of evidence utilised by Watson and Crick was the **X-ray diffraction pattern**. Maurice Wilkins, Rosalind Franklin and their colleagues in 1952 employed **X-ray crystallography** to analyse the structure of DNA. On analysing these pictures they found that DNA was a highly ordered, multiple stranded structure with repeating subunits placed every 3.4 Angstroms [$1 \text{ Angstrom } (\text{\AA}) = 10^{-8} \text{ cm}$] along the axis of the molecule.

Table 8.4: Chargaff's results in selected organisms indicating a consistent pattern of ratios of bases in DNA from different organisms. (Data from E. Chargaff, 1963, *Essays on nucleic acids*. Elsevier).

Organism	Ratio of bases in DNA sample	
	A:T	G:C
Cow	1.04	1.00
Man	1.00	1.00
Salmon	1.02	1.02
<i>E.coli</i>	1.09	0.99

Units of length

1 micrometer or	$= 10^{-6}$ meters
1 micron (μm)	= one- thousandth of a millimetre
1 nanometer (nm)	$= 10^{-9}$ meters = one- millionth of a millimetre
1 Angstrom unit (Å)	$= 10^{-10}$ meters

Watson and Crick considered all the evidence just described and began to build three-dimensional models for the structure of DNA. The model they devised, which fits all the known data on the composition of DNA molecule was the now famous double helix model for DNA. It is also known as the Watson-Crick model. According to this model two polynucleotide chains constitute the double helix. These two chains are coiled about one another in a spiral with the sugar phosphate backbones on the outside and bases on the inside (Fig. 8.15). Each polynucleotide chain is made up of nucleotides arranged in a sequence and linked together by **phosphodiester bonds**, joining adjacent deoxyribose moieties (see Fig. 8.15 and Box 8.1). The two polynucleotide strands are held together in their helical configuration by **hydrogen bonding** (Box 8.1) between the bases of the opposite strands, the resulting base pairs being stacked between the two chains perpendicular to the axis of the molecule like the steps of spiral staircase (Fig. 8.16). The **base pairing is specific**. That is, **adenine** is always **paired with thymine** and **guanine** is always **paired with cytosine** (see Fig. 8.16). Thus all base pairs consist of one purine and one pyrimidine. The specificity of base pairing results from the hydrogen-bonding capacities of bases in their normal configurations. Adenine and thymine form two hydrogen bonds and guanine and cytosine form three hydrogen bonds (Fig. 8.16).

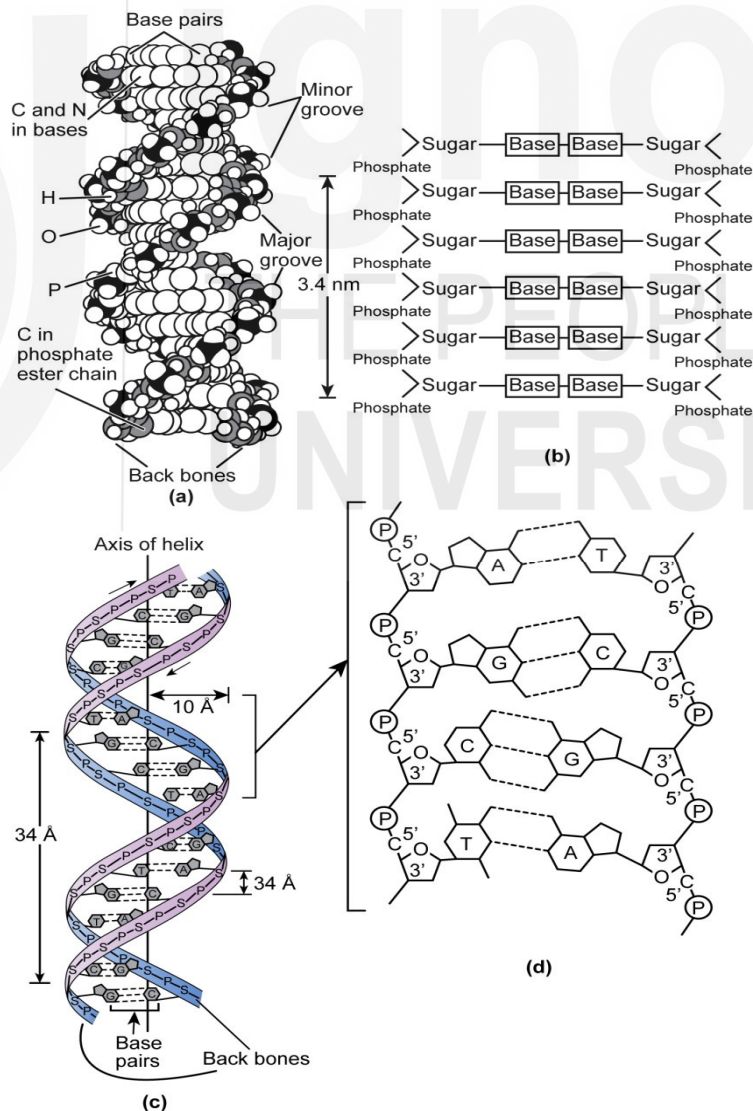


Fig. 8.15: Molecular structure of DNA, (a) space-filling model of the double helix. (b) diagrammatic representation of DNA double helix. (c) the arrangement of nucleotide components in the double helix, and (d) molecular structure of a fragment of DNA double helix.

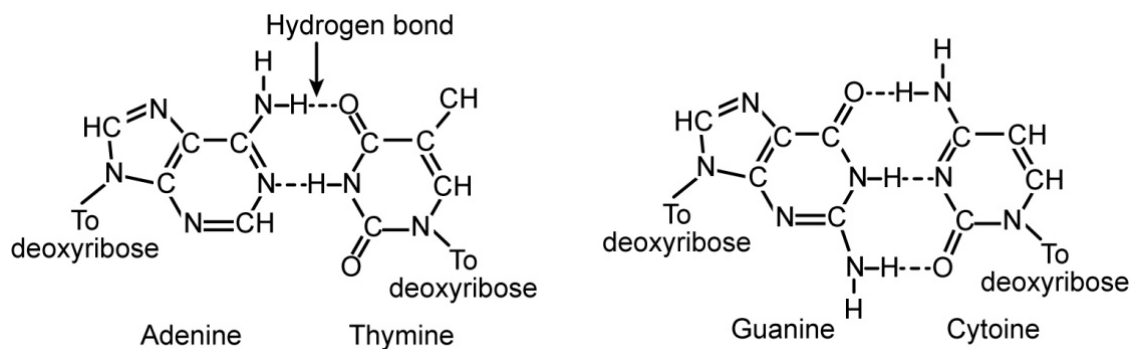


Fig. 8.16: Hydrogen bonding between nitrogenous bases in DNA.

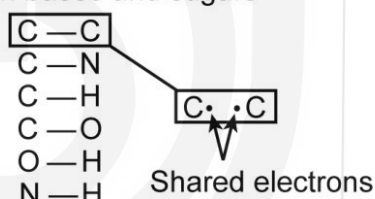
Box 8.1: Chemical Bonds Important in DNA Sequence.

1. Covalent Bond

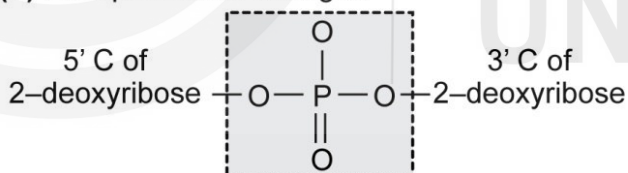
Strong bonds formed by sharing of electrons between atoms. Such bonds are about 1 Å long and generally have energies greater than 50 KCal mol⁻¹

2. Hydrogen Bond

(a) In bases and sugars

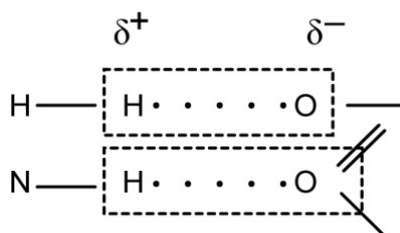


(b) Phosphodiester linkages



A hydrogen bond is a weak electrostatic attraction between an electronegative atom, such as oxygen and nitrogen and a hydrogen atom attached to a second electronegative atom. It arises because the electrons in a covalent bond such as O – H or N – H are displaced towards the electronegative atom so that the hydrogen atom possesses a partial positive charge and can therefore form a weak bond with a second electronegative atom. Actually, the hydrogen atom is shared between two electronegative atoms. Hydrogen bonds are longer than covalent bonds, about 2Å or more. A hydrogen bond usually has a bond energy between 3 and 10 KCal mol⁻¹ at 25°C. A large number of hydrogen bonds can cooperate to form a relatively strong attractive force. Hydrogen bonds play an important role in the three-dimensional structures of macromolecules such as nucleic acids and proteins.

3. Hydrophobic bonds



The association of nonpolar groups with each other when present in aqueous solutions is because of their insolubility in water. Water molecules are very polar (δ^- O and δ^+ Hs). Therefore, it acts as a good solvent for polar compounds but is a poor solvent for nonpolar compounds. Compounds which are polar are soluble in water ("hydrophilic"). Compounds which are non-polar (non longer charged groups) are insoluble in water ("hydrophobic"). The stacked base pairs provide hydrophobic core.



Describing the lengths of DNA molecules.

The basic monomeric unit of the double helix is the base pair with ten base pairs occurring per turn of helix. The length of a DNA molecule is usually described as so many base pairs abbreviated to bp (for example 100 bp, 250 bp). A kilobase pair, usually called a kilobase or kb is 1000 bp.

Once the sequence of bases in one strand of a DNA double helix is known, the sequence of bases in the other strand is also known because of the specific base pairing. The two strands of a DNA double helix are thus said to be **complementary (not identical)**. This property of complementarity of the two strands makes DNA uniquely suited to store and transmit genetic information.

The dimensions of the DNA double helix are indicated in Fig. 8.17. The distance between the backbones is $20\text{\AA}$ and each base pair is $3.4\text{\AA}$ apart. The orientation of the bases rotates with the rotation of the double helix, each base pair being offset by 36° from the adjacent ones. Thus a 360-degree rotation of the helix occurs every ten base pairs, or every $34\text{\AA}$. We can also identify two grooves, a **major groove** and a **minor groove**, bound by the sugar-phosphate backbones. The two grooves differ in size, because the two attachment points of the base pairs to the backbones are not opposite to each other (Fig. 8.17).

The sugar-phosphate backbones of the two complementary strands are antiparallel; that is, they have opposite chemical polarity (Fig. 8.18). As one moves unidirectionally along a DNA double helix, the phosphodiester bonds in one strand go from a 3' carbon of one nucleotide to a 5' carbon of the adjacent nucleotide, whereas those of complementary strand go from a 5' carbon to a 3' carbon. This opposite polarity of the complementary strands is very important in considering the mechanism of replication of DNA.

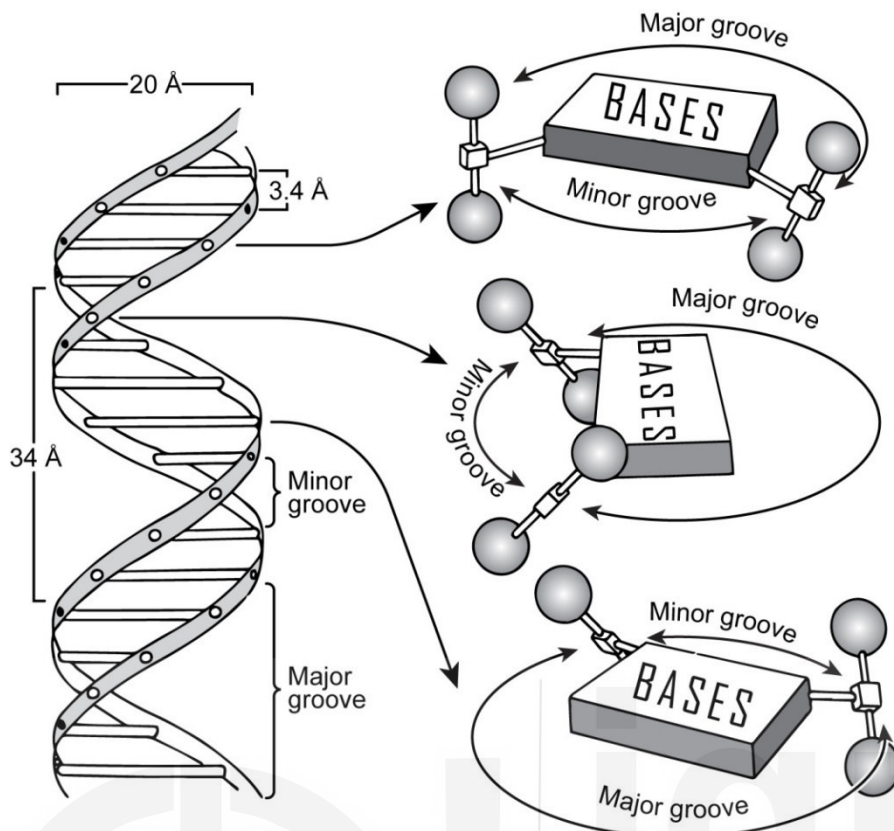


Fig. 8.17: Diagrammatic representation of the double helical structure of DNA (left). The horizontal rods represent base pair connecting the two ribbon-like sugar-phosphate backbones. On the right are shown the rotation of base pairs in double helix DNA. Cubes represent sugar molecules; spheres represent phosphate molecules.

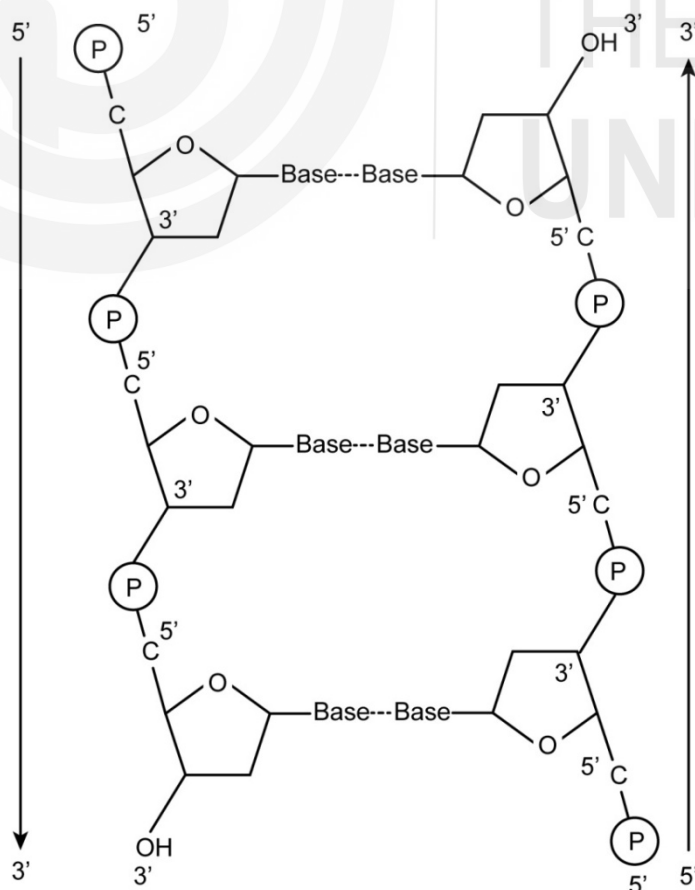


Fig. 8.18: Polarity of the DNA strands.

The high degree of structural stability of DNA double helices results in part from the large number of hydrogen bonds between the base-pairs (even though each hydrogen bond by itself is quite weak, much weaker than a covalent bond) and in part from the hydrophobic bonding (or 'stacking forces') between the stacked base pairs. The planar sides of the base pairs are relatively nonpolar and tend to be water insoluble ('hydrophobic'). This hydrophobic core base-pairs provide considerable stability to DNA molecules present in the aqueous protoplasm of living cells.

8.4.2 DNA Denaturation and Renaturation

When **denaturation** of double-stranded DNA occurs, the hydrogen bonds of the double helix break, the helix unwinds and the strands separate. However, the covalent bonds do not break. The strand separation can be induced by heat or chemical treatment. While heating a specimen of DNA, a point is reached when the thermal agitation overcomes hydrogen bonding and the molecule becomes denatured. It is also referred to as melting. It is logical that the more hydrogen bonds DNA contains, the higher the temperature needed to denature it. It follows that since a G – C (guanine-cytosine) base pair has three hydrogen bonds to every two in an A – T (adenine-thymine) base pair, the higher the G – C content in a given molecule of DNA, the higher the temperature required to denature that DNA. This relationship has been found to exist (Fig. 8.19).

Thermal denaturation of DNA is reversible by slow cooling. This renaturation process is due to the affinity between the long sequence of complementary A – T and G – C base pairs.

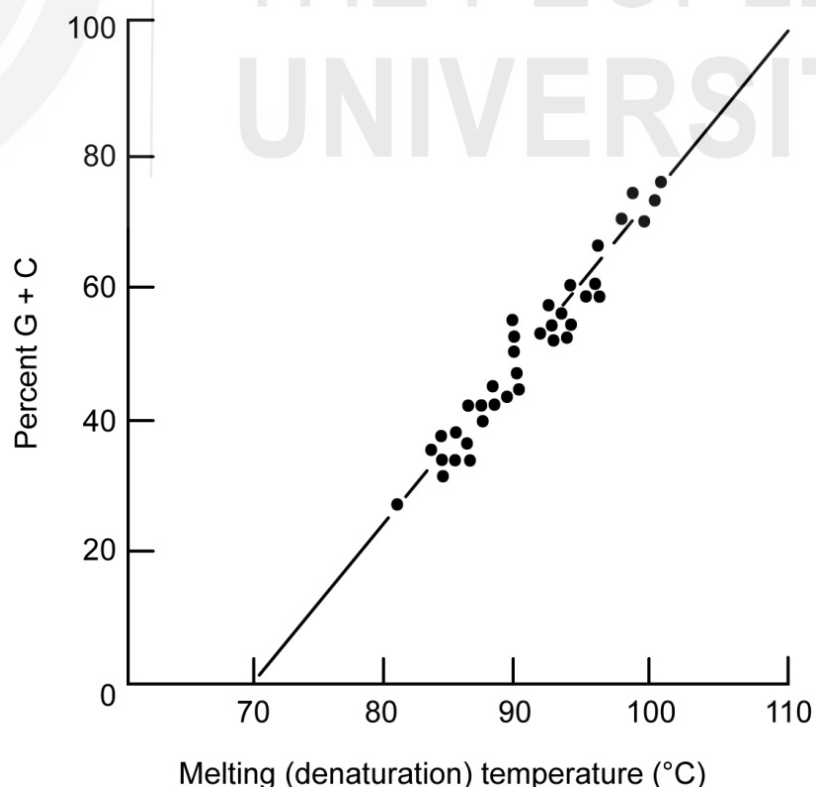


Fig. 8.19: Relationship of the number of hydrogen bonds (G-C content) and the thermal stability of DNA from different sources.

8.5 CONFORMATIONAL FLEXIBILITY OF DNA MOLECULES

The form of DNA we have described so far is called B-DNA. It is a right-handed helix. It turns in a clockwise manner when viewed down its axis. The bases are stacked almost exactly perpendicular to the main axis with about ten base pairs per turn. However, DNA can exist in other forms. DNA has also been described as molecule exhibiting a considerable amount of conformational flexibility.

The structures of DNA molecules changes as a function of their environment. The exact conformation of a given DNA molecule or segment of a DNA molecule will depend on the nature of the molecules with which it is interacting. When humidity is high DNA is in what is called the B-form (Watson-Crick's model). When humidity is relatively low or in dehydrated state, DNA exists in **A-form**, which has 11 nucleotides per turn. It is not yet clear to what extent A-DNA occurs under biological conditions. However, under certain conditions, short synthetic double-stranded fragments (e.g., CGG and GGTATACC along each strand) show a preference for assuming the A-configuration. A-T rich polymers show a preference for the B-form. Since A-DNA occurs under conditions of decreased hydration it has been suggested that it may be formed physiologically as a result of interaction with hydrophobic molecules or changing cellular conditions.

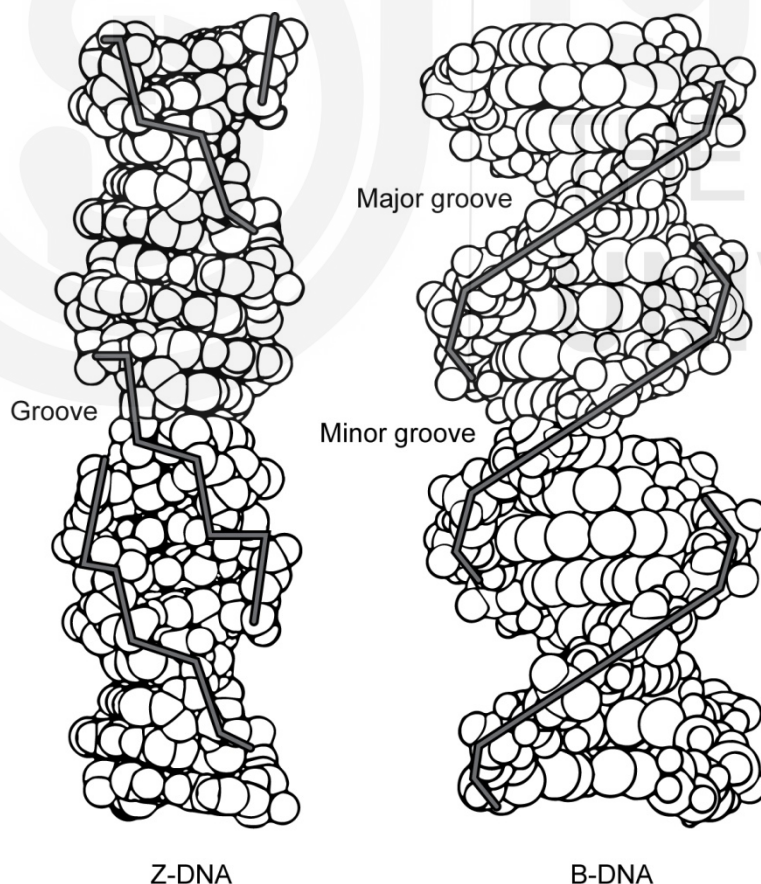


Fig. 8.20: Molecular models of Z-DNA and B-DNA. The irregularity of the Z-DNA backbone is illustrated by the dark lines that go from phosphate to phosphate residue along the chain. The groove in Z-DNA is quite deep and it extends to the axis of the double helix. The B-DNA on the other hand has a smooth line connecting the phosphate groups and two grooves.

Recently, certain DNA sequences have been discovered by Andrew Wang, Alexander Rich and their colleagues in 1979. They exist in a unique left-handed, double helical form called Z-DNA (Z for the zigzagged path of sugar-phosphate backbones of the structure, see Fig. 8.20). The Z-DNA helix contains 12 base pairs per turn. The helices of A- and B-form DNA are wound in a right handed manner. Moreover, specific segments of DNA molecules can undergo conformational shifts from the B-form to the Z-form and vice-versa. It has been found that certain regulatory proteins may bind only to the Z-form (or B-form) of a DNA sequence and cause it to shift to the B-form (or Z-form). There is some speculation that Z-DNA may be important in the regulatory aspects of gene expression.

SAQ 8

- a) If the nucleotide sequences of one polynucleotide of a DNA double helix is 5'-ATAGCAATGCAA-3', what will be the sequence of the complementary polynucleotide?
- b) DNA from the fungus *Neurospora crassa* has an AT content of 46%. What is the GC content?
- c) Is an adenine-thymine or guanine-cytosine base pair harder to break apart? Explain.
- d) For double-stranded DNA, which of the following base ratios always equals 1?
 - i) $(A + T) / (G + C)$
 - ii) $(A + G) / (C + T)$
 - iii) C/G
 - iv) $(G + T) / (A + C)$
 - v) A/G

SAQ 9

A double-stranded DNA molecule is 100,000 base pairs (100 kilobases) long.

- a) How many nucleotides does it contain?
- b) How many complete turns are there in the molecule?
- c) How long is the DNA molecule?

SAQ 10

- a) In what sense are the two strands of DNA antiparallel?
 - b) Compare and contrast between B-DNA and Z-DNA.
-

8.6 SUMMARY

In this unit you have learnt the following:

- Proteins fulfil many important functions for the living organism. In particular, proteins that are enzymes catalyse the biochemical reactions essential for life. Proteins consist of amino acids joined together by peptide bonds into polypeptides. Either one polypeptide chain or several chains bound together may form a protein. Proteins were once thought to be the hereditary molecules because they are long molecules of variable amino acids sequence, and are, therefore, capable of encoding immense amounts of information.
- The main feature that the geneticists were looking for in the genetic material was that it must be able to control the phenotype of a cell or organism (direct protein synthesis), replicate, and must be located in the chromosomes. Avery and his colleagues demonstrate that DNA was the genetic material when they showed that the transforming agent was DNA. Griffith had originally demonstrated the phenomenon of transformation of *Pneumococcus* in mice. Hershey and Chase demonstrated that it was the DNA of bacteriophage T2 that entered the bacterial cell. Fraenkel-Conrat and Singer showed that in viruses without DNA (RNA viruses) such as tobacco mosaic virus, the RNA acted as the genetic material. Thus by 1953, the evidence was strongly supportive of nucleic acids (DNA, or in its absence, RNA) as the genetic material.
- Chargaff showed a 1:1 relationship of adenine (A) to thymine (T) and cytosine (C) to guanine (G) in DNA. Wilkins, Franklin and their colleagues showed by X-ray crystallography, that DNA was a helix of specific dimensions. Following these lines of evidence, Watson and Crick in 1953 suggested the double-helical model of the structure of DNA. In their model, DNA is made up of two strands, running in opposite directions, with sugar-phosphate backbones and bases facing inward. Bases from the two strands form hydrogen bonds with each other with the restriction that only A and T and only G and C can pair. This explains the quantitative relationships that Chargaff found among the bases. Melting temperatures of DNA also support their structural hypothesis because DNAs with higher G-C contents have higher melting, or denaturation temperatures; G-C base pairs have three hydrogen bonds versus only two in an A-T base pair. The model of DNA presented by Watson and Crick is that of the D-form. DNA can exist in other forms, including the Z-form, a left handed double helix that may be important in gene expression.

8.7 TERMINAL QUESTIONS

1. Explain why biologists in early 1900s thought that genes must be made of protein?
2. Once possible explanation for Griffith's transformation result was that avirulent (R) bacteria somehow restored the dead virulent (S) cells to life. How did the experiments of Avery *et al.* eliminate this unlikely possibility?

3. Imagine that you are one of Avery's co-workers and helped conduct the transformation experiments demonstrating the hereditary function of DNA. How might you respond to the following criticisms of your conclusions?
 - a) DNA is not a hereditary molecule, but an agent, like ultraviolet light, that causes mutation.
 - b) Genes are not made up of DNA or proteins, but of DNA and proteins.
 - c) Like painting a house, transformation changes only the physical appearance of a bacterium.
4. Would C^{14} and H^3 have been suitable radioactive tracers for Hershey-Chase experiment?
5.
 - a) What was the objective of the experiment carried out by Hershey and Chase?
 - b) How was the objective accomplished?
 - c) What is the significance of this experiment?
6. How are the carbon and nitrogen atoms of the sugars, purines and pyrimidines numbered?
7. A double-stranded DNA molecule of a newly discovered virus was found by electron microscopy to have a length of 34 μm .
 - a) How many nucleotide pairs are present in one of these molecules?
 - b) How many complete turns of the two polynucleotide chains are present in such a double helix?
8.
 - a) Why was a double-helix chosen for the basic pattern of the DNA molecule?
 - b) Why were hydrogen bonds placed in the model to connect the bases?
9.
 - a) If a virus particle contains double-stranded DNA with 200,000 base pairs, how many nucleotides would be present?
 - b) How many complete spirals would occur on each strand?
 - c) How many atoms of phosphorus would be present?
 - d) What would be the length of the DNA configuration in the virus?
10. Indicate whether each of the following statements about the structure of DNA is true or false. Each letter is used to refer to the concentration of that base in DNA.
 - a) $A + T = G + C$

- b) $A = G ; C = T$
- c) $A/T = C/G$
- d) $T/A = C/G$
- e) $A + G = C + T$
- f) $G/C = 1$
- g) $A = T$ within each single strand.
- h) Hydrogen bonding provides stability to the double helix in aqueous solutions.
- i) Hydrophobic bonding provides stability to the double helix in aqueous cytoplasm.
- j) When separated, the two strands of a double helix are identical.
- k) Once the base sequence of one strand of a DNA double helix is known, the base sequence of the second strand can be deduced.
- l) Each nucleotide pair contains two phosphate groups, two deoxyribose molecules, and two bases.

8.8 ANSWERS

Self Assessment Questions

1. *Pneumococcus*; transformed; Griffith; transforming substance; Avery, McCleod, McCarty; 43.
2. a) Proteins, b) DNA.
3. i) a, b) c
4. About 1/2 protein, 1/2 DNA. A single long molecule of DNA is enclosed within a complete 'coat' that is composed of many proteins.
5. DNA contains phosphorus but no sulphur; it can be labelled with P^{32} . Proteins contain sulphur but usually no phosphorus; therefore, they can be labelled with S^{35} .
6. a) Adenine, guanine, thymine, cytosine.
b) The sugar is deoxyribose.
c) A nucleoside is a base attached to a sugar.
A nucleotide with one or more phosphate groups attached.
d) Thymine is unique to DNA and uracil is unique to RNA.
7. a) One phosphate group per base in DNA. Each precursor nucleotide contains three phosphates.
b) One end terminates with a 3'-OH group, that is, free hydroxyl on the 3' carbon of the sugar and the other terminates with a 5'-P group, that is, a free phosphate group on the 5' carbon of the sugar.

- c) No. Either of the four bases may be incorporated into a polynucleotide chain in any order.
8. a) 5-TTGCATTGCTAT-3'
- b) 54%
- c) The adenine-thymine base pair is held together by two hydrogen bonds but the guanine-cytosine base pair is held together by three hydrogen bonds. Thus, the guanine-cytosine base pair requires more energy to break it and is the harder pair to break apart.
- d) ii, iii, iv.
9. a) 200,000
- b) 10,000
- c) 3.4×10^4 nm
10. a) They are antiparallel in the sense that at the end of a double helix one strand terminates with a 3'-OH group and the other strand terminates with a 5'-P group.
- b) See Section 8.5.

Terminal Questions

1. See Subsections 8.2.1 to 8.2.3.
2. Avery *et al.* used cell-free extracts of virulent (S) cells as the transforming agent. Since no virulent cells were mixed with the avirulent ones, there was no possibility of resurrecting any virulent cells.
3. Write your view point. You may refer to Subsection 8.2.4.
4. No. Both protein and DNA contain C and H.
5. a) The objective was to demonstrate whether the genetic material was DNA or protein.
- b) By labelling phosphorus – a constituent of DNA, and sulphur – a constituent of protein, in a virus, it was possible to demonstrate that only the labelled phosphorus was introduced into the host cell during the viral reproductive cycle. This was enough to produce new phages.
- c) That DNA not protein is the genetic material.
6. See Fig. 8.16.
7. a) The distance between nucleotide pairs is $3.4 \text{ \AA}$ or 0.34 nm . The length of the molecule is 34 m or $34 \times 10^3 \text{ nm}$. Therefore the number of nucleotide pairs equals $(34 \times 10^3) / 0.34 = 10^5$.
- b) There are then nucleotide pairs per turn of the helix, and so the total number of turns equals $10^5 / 10 = 10^4$.

8. a) A multi-stranded, spiral structure was envisaged by the X-ray diffraction patterns. A double-stranded helix with specific base-pairing nicely fits the 1:1 ratio observed for A:T and G:C in DNA.
- b) Use of the known hydrogen-bonding potential of the bases provides a means of holding the two complementary strands in a stable configuration in such a double helix.
9. a) 400,000 nucleotides
- b) 20,000 complete spirals
- c) 400,000 phosphorous atoms
- d) 680,000 Å
10. a) False, b) False, c) True, d) True, e) True, f) True, g) False, h) True, i) True, j) False, k) True, l) True.



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MUTATIONS AND MUTAGENESIS

Structure

9.1	Introduction	Tautomerization
	Objectives	Deamination and Depurination
9.2	What is Mutation?	Base Analogs
	Somatic and Germinal Mutation	9.5 Transposable Genetic Elements (Jumping Genes)
	Spontaneous and Induced Mutations	9.6 Mutagenesis
	Reverse Mutations and Suppressor Mutations	Physical Mutagens
9.3	Detection of Mutations	Chemical Mutagens
	Detections in Humans	Environmental Mutagens
	Detection in <i>Drosophila</i>	9.7 DNA Repair Mechanism
	Detection in Haploid Organisms	9.8 Significance of Mutations
9.4	Molecular Basis of Mutation	9.9 Summary
	Base Pair Substitutions	9.10 Terminal Questions
	Mutations	9.11 Answers
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9.1 INTRODUCTION

In the previous unit you have learnt that the process of cell division, both meiosis or mitosis, transmit chromosomes that are morphologically intact and remain unchanged from one generation to another. At times, however, errors occur that result in the modification of one or more chromosomes within a cell resulting in cells and individuals with abnormal structures or numbers of chromosomes. Such visible chromosomal abnormalities have been found to occur in about six or seven of every 1,000 live births. A study of chromosomal abnormalities is important as these are associated with problems like infertility, miscarriages, several kinds of birth defects, delayed puberty, hermaphroditism and cancer.

You will study about chromosomal abnormalities that occur due to change in their structure or number. You should keep in mind the *two classes of abnormalities that are not mutually exclusive*. There are *some individuals who possess a numerical chromosomal change in conjunction with a structural change*. Such changes are also referred to as mutations.

Aberrations of structure refer to the changes in the parts of individual chromosomes rather than whole chromosomes or sets of chromosomes in a genome. You would study these basic types of mutations and their effects in this unit.

Objectives

After studying this unit you should be able to:

- ❖ describe the somatic and germinal mutations and their molecular basis,
- ❖ explain the mutagenesis and their types,
- ❖ discuss the DNA repair mechanism, and
- ❖ describe significance of mutations.

9.2 WHAT IS MUTATION?

Genome of a life form is subject to change from generation to generation. Mutation is a kind of variation wherein the organisms exhibit different phenotype. The altered organism is termed mutant. So, **the mutation can be defined as any heritable alteration in the genetic material of an organism or virus**. The genotypic variation may occur at gene or at chromosomal level leading to change in structure and number of chromosomes (Fig. 9.1).

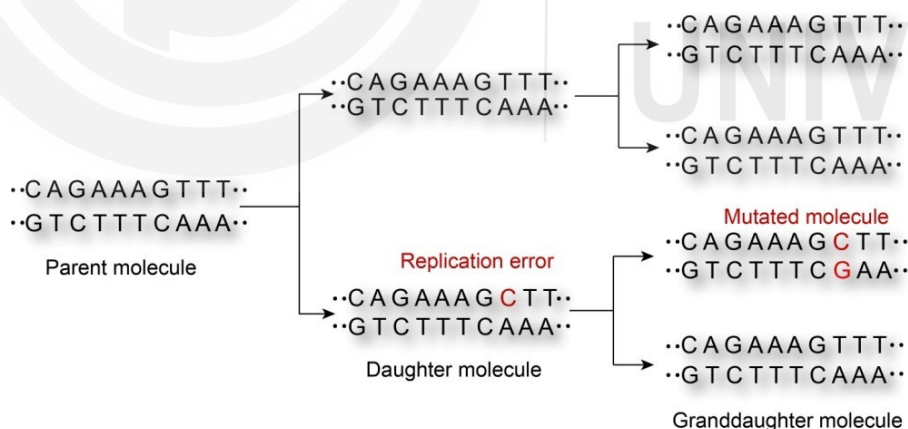


Fig. 9.1: Mutation showing an error in replication (Further replication leads to a double helix with a mutated sequence).

Historically, the earliest information on point mutation was reported in the year 1791, a New England farmer, Seth Wright, noticed a peculiar male lamb in his small flock of sheep. This lamb had unusually short legs which were somewhat, deformed. Mr. Wright recognized the unique advantage of sheep like this one, as they would not be able to jump the low stone fences and therefore, they would not damage the crops. He carefully bred his sheep and was able to raise a short legged flock. This breed was named ancon (Greek elbow) as the crooked legs resembled the human elbow.

Hugo deVries in 1901 used the term mutation as a result of his experiments on the plant evening primrose, *Oenothera lamarckiana*. He coined the term mutation to explain the variation he observed in crosses involving this plant. However, the variations he observed were results of chromosomal aberrations.

T.H. Morgan work in 1910 led to identify white eyed male flies in fruitfly. *Drosophila melanogaster*. Later on, several organisms were studied for mutations.

9.2.1 Somatic and Germinal Mutations

Mutations may occur in any type of cell and the changes in the genetic material lead to phenotypic changes in the cell in which it occurs.

Somatic Mutations: occur in somatic cells i.e. non-reproductive body cells. Somatic mutations phenotypically carry a chimeric tissue i.e. normal and mutant tissue. Somatic mutations will not be transmitted to the next progeny because reproductive cells are not affected and somatic cells die with the death of the organism.

However, mutations in somatic cells may sometimes cause damage including cancer, to the part of the body that arises from the mutated cells. The mutations in plants are kept alive to the next progeny by vegetative growth. Examples of some of the known beneficial somatic mutations are changes in colour of apples, i.e. golden delicious apples, and thornless sports of black berries.

Germinal Mutations: occur in the germ cells which form the gametes. These are heritable. The mutations may occur at chromosomal or gene level. The dominant mutation is expressed in the progeny. The effects of recessive mutants are suppressed in the diploid organisms. Genetic mutations may occur in autosomes or sex chromosomes. These are called autosomal and sex linked mutants respectively.

Mutation rate is the frequency of new mutation in a single gene or organism over time. The mutation rate does vary over the genome.

Mutant frequency is defined as the proportion of mutant cells in a population and is readily estimated.

The germinal mutations lead to variations in the progeny. As germinal mutations occur in gametes or germ cells, it may affect single individual gamete/progeny or several gametes/progenies because of the mutant gene.

9.2.2 Spontaneous and Induced Mutations

The mutations can originate spontaneously without a known cause or can be induced. Spontaneous mutations may result from metabolic error during DNA replication or movement of transposable genetic elements.

In humans, rate of the spontaneous mutations for individual genes may vary between or 1×10^{-4} to 4×10^{-6} per gene per generation. It is also known that many of the spontaneous errors are corrected by DNA repair system (see Table 9.1).

Table 9.1: Some examples of spontaneous mutation rates for various organisms.

Organism	Trait	Mutation Rate (per gene per generation)
<i>E.coil</i> (various sources)	Histidine auxotrophy	2×10^{-6}
	Streptomycin sensitivity	1×10^{-9}
	Phage T ₁ resistance	2.3×10^{-8}
<i>Drosophila</i> males	Brown eyes	3×10^{-5}
	Eyeless	6×10^{-5}
	Yellow body	12×10^{-5}
	Colourless kernel	2×10^{-6}
	Shrunken kernel	1.2×10^{-6}
Human	Achondroplasia	1×10^{-5}
	Aniridia	2.9×10^{-6}
	Retinoblastoma	6.7×10^{-6}
	Muscular dystrophy	9.2×10^{-5}
	(Duchenne type)	

Induced mutations arise when a mutagenic agent (Physical or Chemical agent) exposure reacts with DNA to cause mutations.

Mutagens: The agents which can cause mutations are called mutagens. All mutagenic agents induce mutations in either of the following ways.

- i) They may replace a base in the DNA.
- ii) They may alter the base in such a way which its specifically mis-pairs with another base.
- iii) They may damage the base so much that it can no longer pair with any base.
- iv) They may intercalate themselves in the DNA paving way for addition or deletion of base.

With the initial discovery of X-Rays as mutagenic agents a large numbers of other radiation and chemicals have been identified which have the capacity to induce mutations. In fact this list is growing as more and more agents are being studied and some of them are as follows.

A. Physical mutagens : Radiations (X-Rays, UV rays)

B. Chemical mutagens : (i) Mustard gas

(ii) Formaldehyde

(iii) Ethyl methane sulfonate (EMS) etc.

Induced mutations have a higher frequency rate than spontaneous mutations.

9.2.3 Reverse Mutations and Suppressor Mutations

Point mutations represent a mutational change from normal or wild type to a new genotype (dominant or recessive) and new phenotype. Such changes represent forward mutation.

Reverse mutations (Reverse or back mutations) represent the mutations that may restore the mutant to original wild type or partial wild type phenotype.

Reverse mutation occurs at the same locus as forward mutation while a suppressor mutation occurs at some other locus but it suppresses the original mutant genotype. **These two mutations can be identified by back crossing the reversal with wild type for reverse mutations.**

If mutant appears in the offspring of back cross, it represents suppressor mutation. **A suppressor mutation masks the effect of original mutation but it cannot reverse the original mutation.**

Suppressor mutation is called intragenic suppressor when it occurs at a different site of same gene carrying mutation.

Mechanism of two suppressor mutations for the production of functional /partial functional proteins are different.

SAQ 1

- i) Define mutation with an example and state who has coined this term.
- ii) Match the explanation given in Column II with the term given in Column I.

Column I	Column II
(A) Somatic mutation	(a) Mutation that masks the effect of original mutation
(B) Germinal mutation	(b) Mutations occur because of exposure to mutagenic agent
(C) Spontaneous mutation	(c) Mutations that restore the mutant to original wild type
(D) Induced mutation	(d) Mutations are naturally occurring
(E) Reverse mutation	(e) Mutations are heritable
(F) Suppressor mutation	(f) Mutations will not be transmitted to next generation

9.3 DETECTION OF MUTATIONS

Mutations are usually detected by a change in the phenotype. For different systems different methods of detection of mutations have been designed. The

detection methods are also based on the fact that most mutations are recessive in nature and their existence can be confirmed depending upon whether they are studied in diploid or haploid organisms or whether they are located on autosomes or sex chromosomes.

9.3.1 Detection in Humans

In human beings, the detection of mutation depends upon pedigree analysis. Pedigree analysis requires a family tree. Once any trait has been shown to be inherited, it is possible to predict whether the mutant allele is dominant or recessive and whether it is sex-linked or autosomal.

The detection of recessive mutations by this method may be difficult because it is not possible to predict whether a recessive trait has arisen because of the mating of the two heterozygous parents or through the mutation in one of them.

In case of recessive autosomal alleles, mutation is hidden in heterozygous condition. An affected individual and a homozygous normal individual will produce unaffected carrier children. Mating between two carriers will produce on an average, one-fourth affected offsprings. Because of the presence of single X-chromosome in human males, recessive sex-linked mutations can be easily detected by pedigree analysis. The woman, who is carrier of mutant trait, is not affected by the mutation because of the presence of dominant allele. However, she passes it to half of her male offsprings which will show up the affected trait. The most famous case of sex-linked mutation in humans is that of haemophilia. The pedigree analysis of seven generations in the royal family descendents of Queen Victoria has shown how the gene for this lethal genetic disease for which the Queen Victoria was the carrier has been transmitted to the offspring.

The dominant mutants are easy to be detected as they exert their effect immediately. If they are present on X-Chromosomes, affected fathers pass the phenotypic trait to all their daughters. If the mutation is located on one of the X-Chromosomes of female it will be passed on to 50% of her male offspring. If the condition is homozygous in female, all her male offsprings will be affected. If dominant mutations are autosomal, approximately 50 percent of the offspring of an affected heterozygous individual are expected to show the trait.

Genetic variations have also been detected in individuals (humans) on the basis of electrophoretic pattern, enzyme activity and DNA sequencing. The cells are grown *in vitro*. Cell culture technique is used for the biochemical estimation e.g. in sickle cell anaemia. Sickel cell erythrocytes (RBCs) contain a variant of normal haemoglobin. The above mentioned technique detects a mutation in one of the chain of globin.

Mutated haemoglobin gives sickle shape to erythrocyte due to which capacity for oxygen transportation is reduced and blood vessels are clogged. It may lead to death in severe cases (Fig. 9.2).

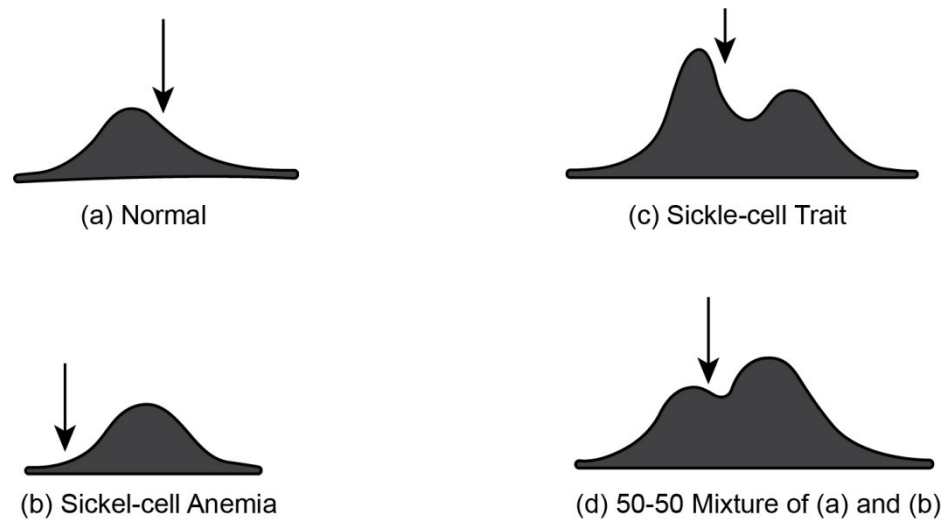


Fig. 9.2: Differences in the electrophoretic mobility of normal and sickle cell haemoglobin.

The adult haemoglobin (Hb A) has two identical α -globin chains and two identical β -globin chains. When Hb A (normal) was compared with Hb S (sickle cell), it was found that sixth amino acid from the amino ($-\text{NH}_2$) terminal end of the beta chain of Hb A is *glutamic acid* while Hb S contains *valine*. Hb A and HbS have identical α -chains. One amino acid change has led to the severe abnormality of the morphology of erythrocytes and is the cause of sickle cell anaemia.

9.3.2 Detection in *Drosophila*

The following four methods have been developed in *Drosophila* to detect mutations.

1. CIB method
2. Attached X-Chromosome method
3. Muller's – 5 method
4. Curly lobe – plum method

CIB method and Attached X-Chromosomes methods are used commonly and are described below.

CIB test system detects the rate of induction of sex-linked recessive mutations. The stock of CIB involves C – an inversion that suppresses the crossing over, I – a recessive lethal mutation and B – a dominant gene duplicate causing bar shape of the eye. Genes of all the traits are present on X-Chromosome.

In this technique, wild type (P) males are treated with a mutagenic agent (e.g. X-Rays) and are crossed with non-treated heterozygous female having one X-chromosome with CIB markers i.e. genes. F_1 generation consists of four types of offspring (Fig. 9.3). One type of offspring i.e. CIB male dies because it contains recessive lethal gene which is expressed in hemizygous conditions showing its total effect. The remaining type of male offspring is wild type. This wild type male is crossed with bar eyed female of F_1 -generation. These bar

eyed females receive CIB chromosome from their mother and irradiated X-chromosome from their father. F_2 generation, as a result of crossing over, will consist of bar eyed and wild type females and two types of males. One of which will be CIB types and will die because of reasons discussed above. In the other class of male offspring, a mutant may be found, if induced at P_1 level due to X-ray irradiation. A lethal mutation means no males will survive in F_2 generation. However, if morphological mutation is induced it will show up in this class of males. So, the sex-linked morphological or lethal mutation can be detected by this method.

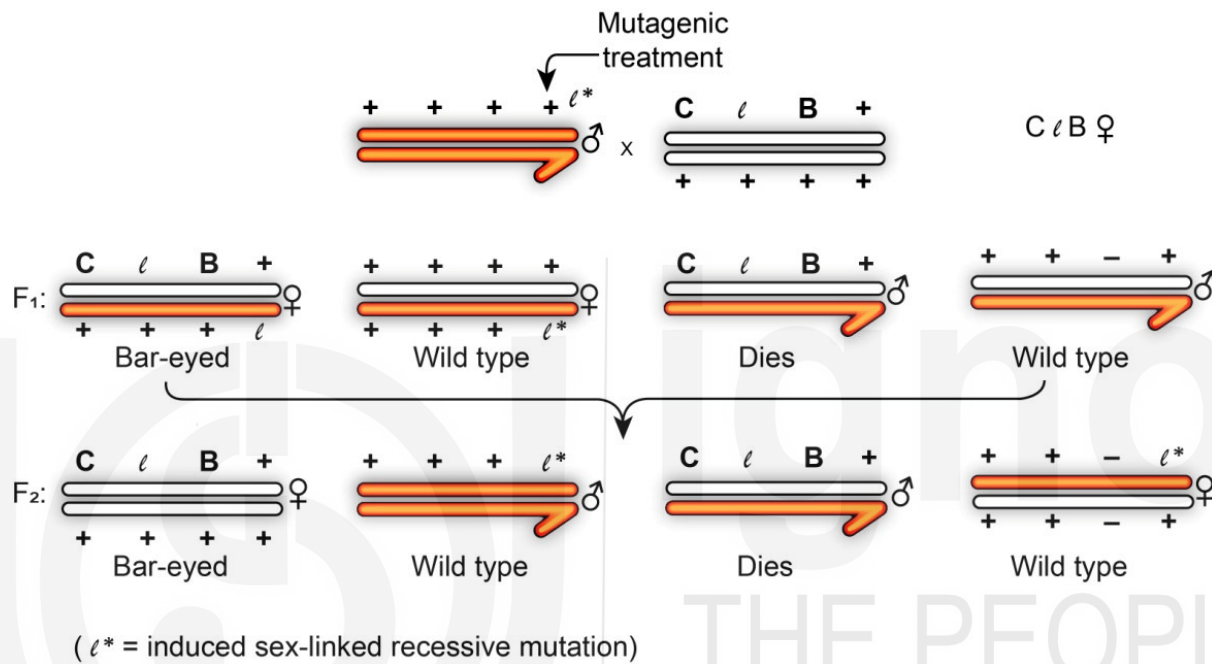


Fig. 9.3: Muller's CIB technique for detection of induced sex-linked mutation in *Drosophila*. In this case mutation is recessive and lethal.

Attached X-Chromosomes technique is comparatively a simpler technique as mutations can be detected in the first generation itself.

It is known that many times the two X-chromosomes fail to separate at anaphase and move to the same pole. This condition is known as attached-X. If this type of gamete is fertilized by Y-Sperm, a female fly of XXY-composition is produced. XXY or attached X female is crossed with a wild type or a normal XY male P_1 , which has been exposed to a mutagen, four types of progenies are produced in F_1 generation (Fig. 9.4).

- Triple-X (XXX) females which die.
- Attached – XY (XXY) females which are viable.
- YY males that also die.
- Normal XY males that are viable.

The induced sex-linked morphological mutations of P male will be expressed in both viable progenies. Lethal mutation will once again wipe out the males.

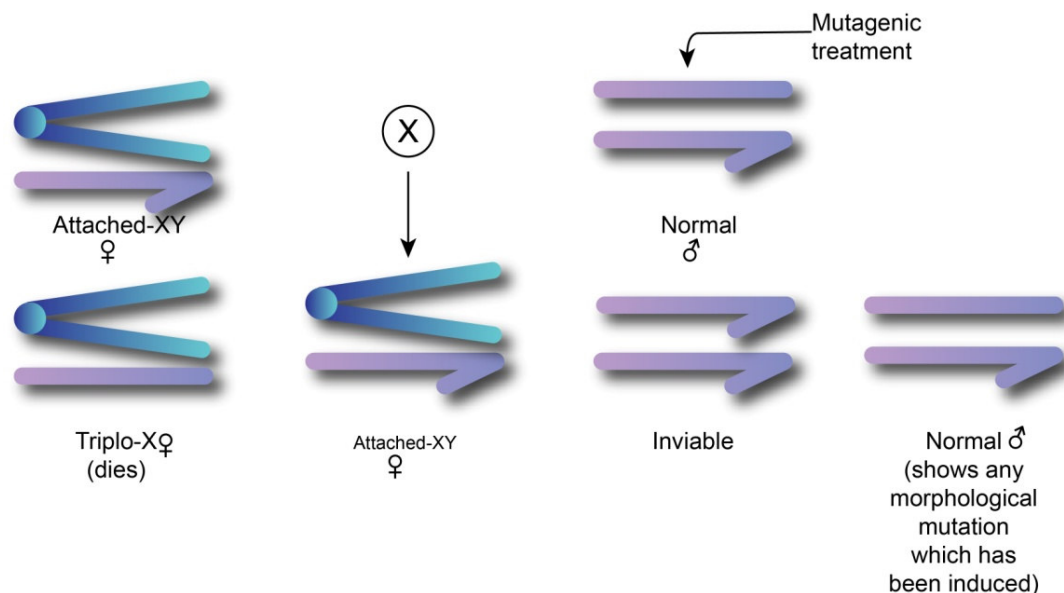


Fig. 9.4: The attached-X method for detection of induced mutations in *Drosophila*.

9.3.3 Detection in Haploid Organisms

The detection methods in haploid organisms are much simpler and straight forward because they contain only one chromosome (n) and also one set of allele. The mutations, if generated, whether recessive or dominant will express in the first generation. Detection depends on selection system where mutant cells are isolated easily from non-mutant cells e.g. the microorganisms that are nutritional wild type i.e. prototrophs will grow on minimal culture medium which consists of glucose, a few inorganic acids and salts, a nitrogen source such as ammonium nitrate and vitamin biotin. But the Induced nutritional mutants of some micro-organisms i.e. auxotrophs will not grow on minimal medium. They will grow on complete culture medium which apart from contents of minimal medium also contains amino acids, vitamins and nucleic acid derivatives. However, these mutants also grow on minimal medium supplemented with the compound, which as a result of mutations, are not able to synthesize e.g. **leu mutants** (Inability to synthesize leucine) will grow on minimal medium if supplemented with leucine. So, the process of identifying mutations in haploid organism is simple.

SAQ 2

Tick mark (✓) the correct option given in the parentheses in the following statements.

- Method for detection of mutation (*depends/does not depend*) upon whether it is located on autosomes or sex chromosome.
- Pedigree analysis is helpful in analysing mutations in (*humans/microorganisms*).
- It is (*necessary/not necessary*) to irradiate P_1 male with mutagenic agent to detect mutations in *Drosophila*.
- Mutations are detected in (F_1/F_2) generation in CIB technique.

- (v) Triple X females of F_1 generation in attached X-technique are (*viable/non-viable*).
- (vi) Mutation detection in microorganisms are simpler because they contain (*haploid/diploid*) set of chromosomes.

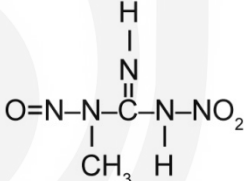
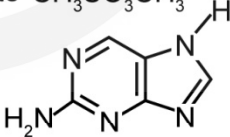
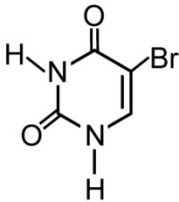
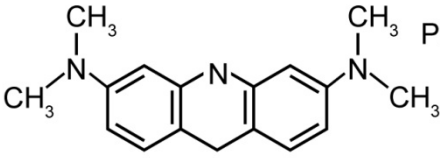
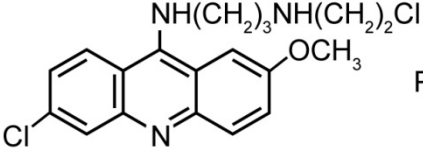
9.4 MOLECULAR BASIS OF MUTATION

Genes are made up of a segment of DNA at a specific locus on a chromosome having specific base sequence. The DNA molecule can be damaged and ultimately mutated by chemical mutagens, radiations, ultraviolet light and alkylation. Mutations involve alterations of the DNA sequence which include substitution or addition or deletions of one or more bases.

Mutagens that cause mutation may be present in the environment.

Carcinogens, Oncogens and teratogens fall in this category (see Table 9.2).

Table 9.2: Some common mutagens and their properties.

Mutagen	Structure	Mode of Action
X-rays	5 nm wavelength	Single and double-strand breaks
UV	254 nm wavelength	Pyrimidine dimers
Nitrous acid	HNO_2	Deamination of A, C, and G residues
Hydroxylamine	NH_2OH	Hydroxylation of cytosine
N-Methyl-N'-nitro-N-nitrosoguanidine		Production of 6-methyl guanine at replication fork, gives mainly transitions
Ethyl methane sulfonate	$\text{CH}_3\text{SO}_3\text{CH}_2\text{CH}_3$	Alkylation of purines, gives mainly transitions
Methyl methane sulfonate	$\text{CH}_3\text{SO}_3\text{CH}_3$	Alkylation of purines
2-Aminopurine		May replace adenine, may add hydrogen-bond to cytosine
5-Bromouracil		May replace thymine, may add hydrogen-bond to guanine
Acridine orange		Production of frameshifts
ICR 191 (a nitrogen mustard)		Production of frameshifts

Point mutations include changes that occur at specific sites in genes. These changes may be due to inaccurate replication of DNA caused by internal, natural or chemical factors.

Point mutations include the following:

- A. Base pair substitution mutations.
- B. Frame-shift mutations.

9.4.1 Base Pair Substitution Mutations

- A. Transition:** A mutation due to substitution of a purine by another purine or a pyrimidine in a polynucleotide chain is called **transition**.

Transition may be caused by

- (i) Tautomerization
- (ii) Deamination
- (iii) Base analogs

In other words transition is a change when a purine replaces a purine ($A \rightleftharpoons G$) or a pyrimidine replaces a pyrimidine ($T \rightleftharpoons C$).

- B. Transversion:** A mutation due to substitutions of a purine by a pyrimidine or pyrimidine for a purine in a polynucleotide chain is called **transversion**.

Transversion involves change where a purine base is replaced by a pyrimidine base or vice versa.



or



Transversion is caused by the following ways:

- (i) Depurination
- (ii) Alkylating agent

These types of mutations alter codon. The altered codon may code for a different amino acid and change property of protein. The effect may be seen in altered phenotype which in turn changes the property of the protein.

9.4.2 Frame Shift Mutation

A mutation by the addition or deletion of a single nucleotide or multiple nucleotides (other than three) which lead to shift in reading frame of DNA in a gene is called frame shift mutation.

Acridine dyes have been shown to cause deletion or insertion of a single base pair. Acridine dye (e.g. 5-amino acridine) intercalate between two adjacent purines which change the gap between the nucleotide from 3.4 Å to 6.8 Å. In this change, during DNA replication either a base is inserted or deleted which leads to frame shift mutations (see Tables 9.1 and 9.3).

Table 9.3: Effect of Important Chemical mutagens.

Mutagen	Mutation	Type of Mutation
1. Base Analogs		
(i) 5 – Bromouracil	$A = T \rightarrow G \equiv C$	Transition
(ii) 2 – Aminopurine	$G \equiv C \rightarrow A = T$	Transition
2. Deamination		
(i) Nitrous acid	$G \equiv C \rightarrow A = T$	Transition
	$A = T \rightarrow G \equiv C$	Transition
3. Alkylating Agent		
(i) Ethyl ethane sulfonate	$G \equiv C \rightarrow T = A$	Transition
4. Intercalating Agent		
Acridine	Deletion of bases, Frame shift Addition of Bases, Insertion	

9.4.3 Tautomerization

In a normal DNA structure in organisms, purine base adenine (A) is linked to pyrimidine base thymine (T) with two hydrogen bonds and purine base guanine (G) is linked to pyrimidine base cytosine (C) with three hydrogen bonds. Each nucleotide base can exist in alternate state. The shift from normal state to alternate state is called **tautomeric shift** and molecules are termed tautomers e.g. existence of two tautomers are termed the keto and eno forms. Keto form is a stable form and eno form may revert back to keto form. The existence of unstable form in tautomers may lead to error of base incorporation during DNA replication (Fig. 9.5).

The rare bases can introduce mutations e.g. enol-thymine form in the parent DNA strand can introduce guanine in the complementary DNA chain. Further replication of DNA will introduce cytosine in the complementary strand. It will lead to substitution of $T = A$ base pair by $G \equiv C$ base pair and new strand with mutated base will be synthesized.

The rare unstable (thymine) may revert back to common state and pairs with Adenine. This will undergo normal DNA replication. This change creates two types of DNA strands.

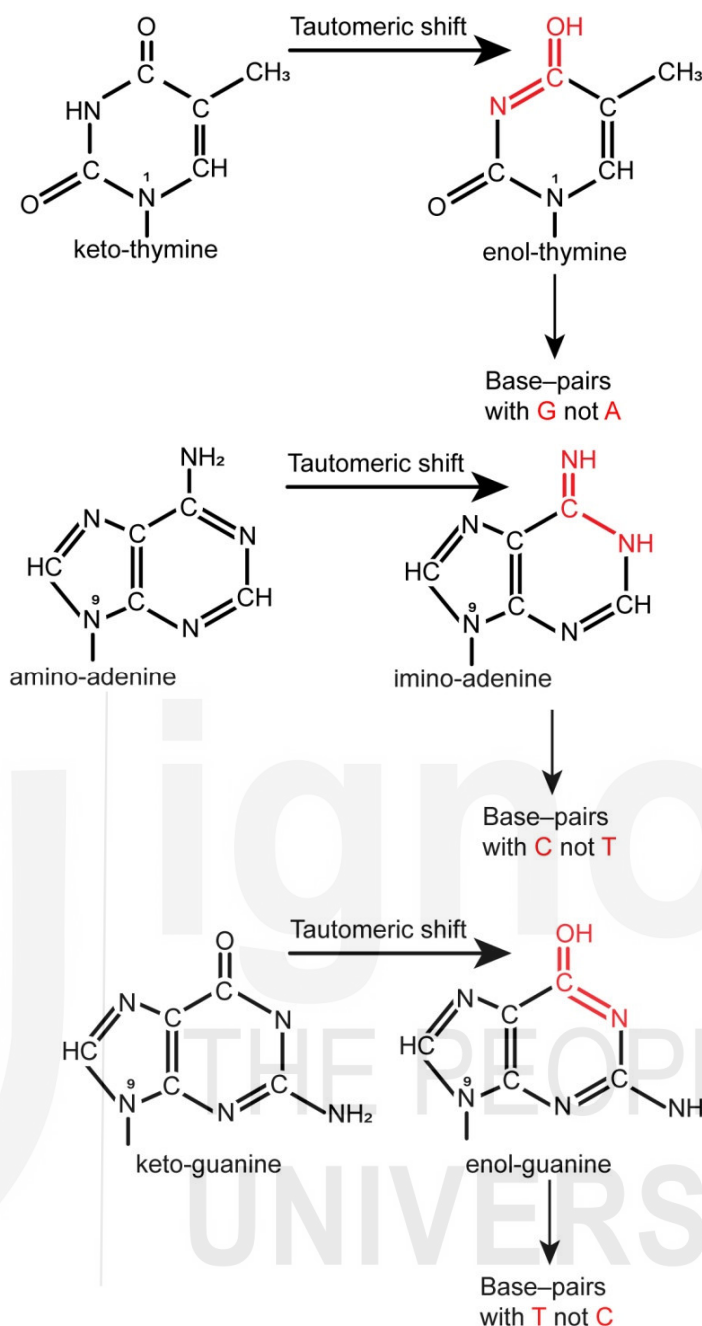


Fig. 9.5: The effects of tautomerism on base pairing.

9.4.4 Deamination and Depurination

Many chemicals [e.g. nitrous acid, hydroxylamine, ethyl methane sulfonate (EMS)] are known to change the base sequence in DNA. Nitrous acid (-HNO_2) and hydroxylamine replace amino group (-NH_2) by hydroxyl group (-OH) which leads to deamination of nitrogenous bases. For example, deamination of cytosine produces uracil (see Fig. 9.6). In case, uracil is not repaired back, it will direct the incorporation of adenine in the new DNA strand during replication. This ultimately results in conversion of CG base pair to a TA base pair i.e. a Transition mutation.

Depurination

Transversions as discussed earlier in this unit are caused by certain alkylating agents (e.g. ethyl methane sulfonate; EMS). The removal of purine from the strand of DNA is called depurination.

During DNA replication, any base can get inserted in the complementary strand if depurination occurs. Next cycle of DNA replication leads to transversion. This leads to genetic mutation which is irreversible (Fig. 9.6)

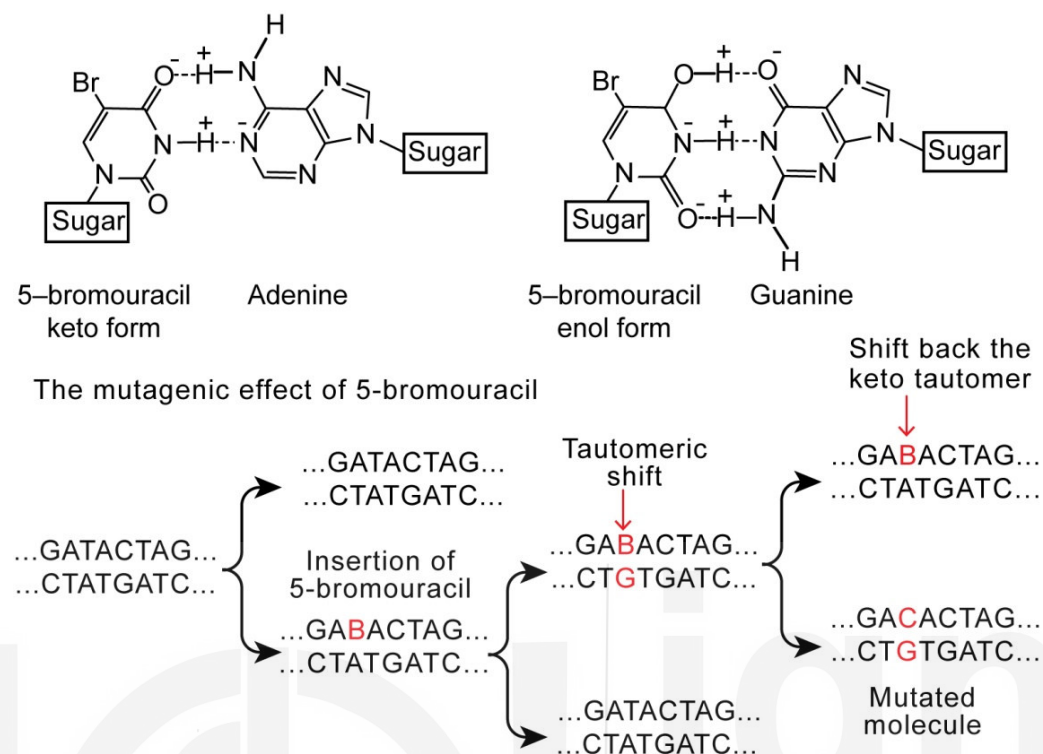


Fig. 9.6: 5-Bromouracil and its mutagenic effect:

A – The keto form of 5-bromouracil base pairs with adenine, and the enol form with guanine.

B – Three cycles of DNA replication are shown.

9.4.5 Base Analogues

Base analogues are the chemicals that have molecular structure that are extremely similar to bases of DNA. These chemicals act as mutagens and during DNA replication get incorporated so as to form base pairs with usual bases. One such chemical is 5- bromouracil (5 BU). 5 BU is a base analogue of thymine and usually pairs with adenine. The bromine atom in 5 BUdR so alters the charge distribution of the molecule that it may tautomerise to a 5 BUdR* form quite frequently. After tautomerisation it possesses the base pairing properties of cytosine, that is, it behaves like cytosine. You can see in Fig. 9.7 that the shift generates G → A transition.

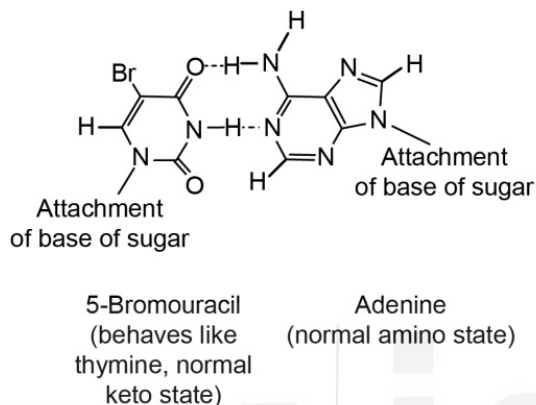
The changes we have discussed in the preceding subsections can bring out mutational effect in various ways. When a base pair change causes the change in mRNA codon resulting into a modified protein in place of one specified by wild-type, a **missense mutation** occurs. **Nonsense mutation** occurs when base pair changes result in the change in mRNA that generate a chain terminating codon which leads to premature termination of protein synthesis. **Neutral mutation** may go unnoticed as it is a base pair change that changes a codon in mRNA such that the resulting amino acid substitution does not alter the function of the protein. For example, change from codon AGG to AAG which substitutes amino acid lysine for arginine. Both amino acids

are similar in properties, so function of protein is not altered significantly.

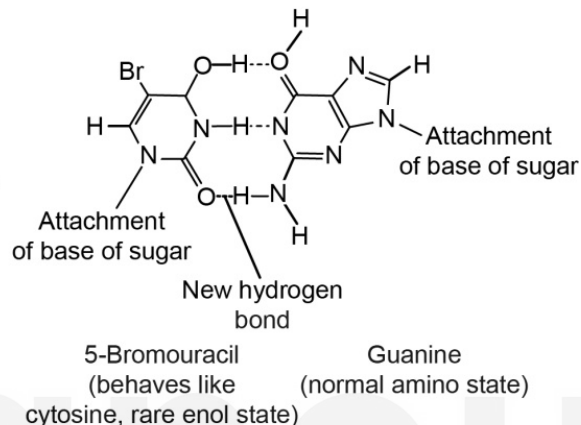
When base pair change alters a codon in mRNA which may still code for same amino acid, **silent mutation** occurs. For example, change from mRNA codon AGG to AGA both of which specify arginine.

It is time now that you should do another SAQ to see whether you have understood the molecular basis of mutations.

(a) Base pairing of 5-bromouracil in its normal state



(b) Base pairing of 5-bromouracil in its rare state



(c) Mutagenic action of 5 BU
AT to CG transition mutation

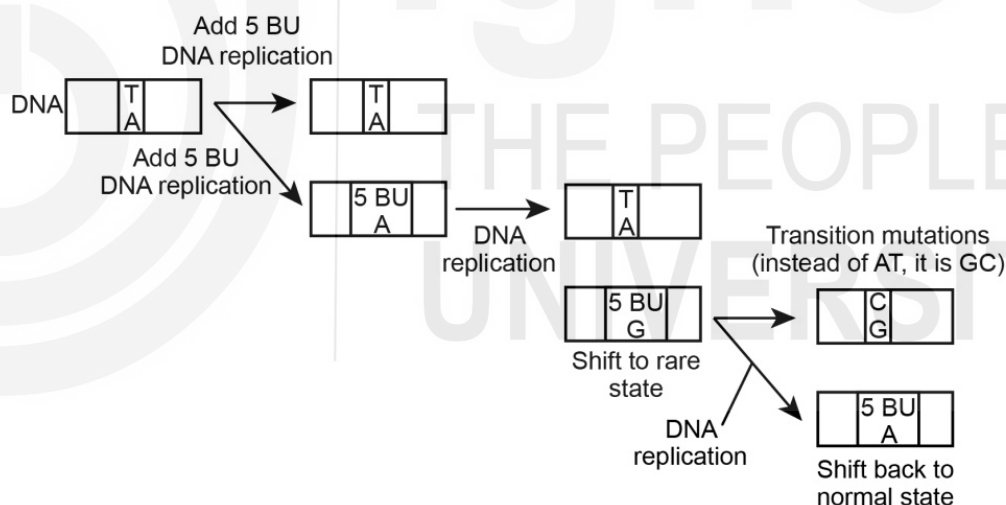


Fig 9.7: Mutagenic effects of base analogue 5-bromouracil (5BU). (a) In normal state 5 BU pairs with adenine; (b) in rare state it pairs with guanine; (c) mutations are induced when 5 BU gets incorporated into DNA in one form and then shifts to its alternate form during next replication stage.

SAQ 3

- Which of the base pair change represents for transition and which one for transversion?
 - AT→TA
 - AT→GC
 - GC→TA

2. Fill in the blanks:

- (a) Mutations caused by single base pair addition or deletion which change frame are called _____.
- (b) The existence of unstable form in tautomers may lead to _____ during DNA replication.
- (c) A mutagen that acts by removing _____ from nucleotide bases is called deaminating agent.
- (d) The removal of _____ from the DNA strand is called depurination.
- (e) _____ are chemical compounds having the structure similar to the naturally occurring nitrogen bases present in DNA.

9.5 TRANSPOSABLE GENETIC ELEMENTS (JUMPING GENES)

So far, in this unit we have discussed, how radiation, chemicals and base analogs can generate spontaneous and induced mutations. Genome has also a mutagen which has the capacity to generate mutations. These are termed as **mobile genetic elements** or **transposable elements**. These are from one part of genome to another within genome. These elements are also termed Jumping genes which were first discovered by Barbara McClintock in the 1940s. From her study of pigment patterns on kernels of the maize (*Zea mays*), McClintock inferred the existence of mobile genetic elements that influence gene expression in the corn. She has described these elements as “controlling elements”. McClintock was awarded Nobel Prize in 1983 for this work.

These elements when inserted into a gene can disrupt its expression and when they transpose, they produce an altered gene. Transposable elements are present in all organisms i.e. from bacteria to humans.

The transposable elements are categorized into two general classes on the basis of their movement.

Class I : encodes proteins that move the transposons (DNA) to new place or make a copy (replicate DNA) which gets integrated elsewhere in genome.

Examples : Found in prokaryotes and eukaryotes.

Class II : encodes a reverse transcriptase enzyme which can generate DNA molecule from RNA. The DNA copy can integrate at new site in genome. This class also includes mobile elements exclusively present in eukaryotes.

Example : (a) retroviruses which have RNA genome

(b) mobile elements found only in eukaryotes

Some example of eukaryotic transposons are **Ty elements in yeast**, **copia elements in *Drosophila*** and **retrovirus in birds and mammals**.

General features of Transposons (Fig. 9.8)

1. IS element is the simplest type of transposable element which consists of terminal inverted repeats on both sides of coding region.
2. Transposon (Tn) contains other genes and has genes for antibiotic resistance.
3. Eukaryotic transposable elements resemble bacterial transposons structure and transposition characteristics.
4. Junk DNA in eukaryotes has transposable elements.
5. Transposable element insertion in genes causes mutation. It can also cause chromosomal rearrangements and deletions.
6. Transposable elements may create new variations which may drive evolution.

Genetic elements are known by many names such as controlling elements, jumping genes, insertion sequences and transposons. However, these are generally called as transposable **genetic elements** (TGE), a widely accepted name. As you can see in Fig. 9.9 the copies of transposable elements can also be inserted at other points in the same chromosome causing disruption of the gene into which these are inserted. This often produces multiple physiological effects.

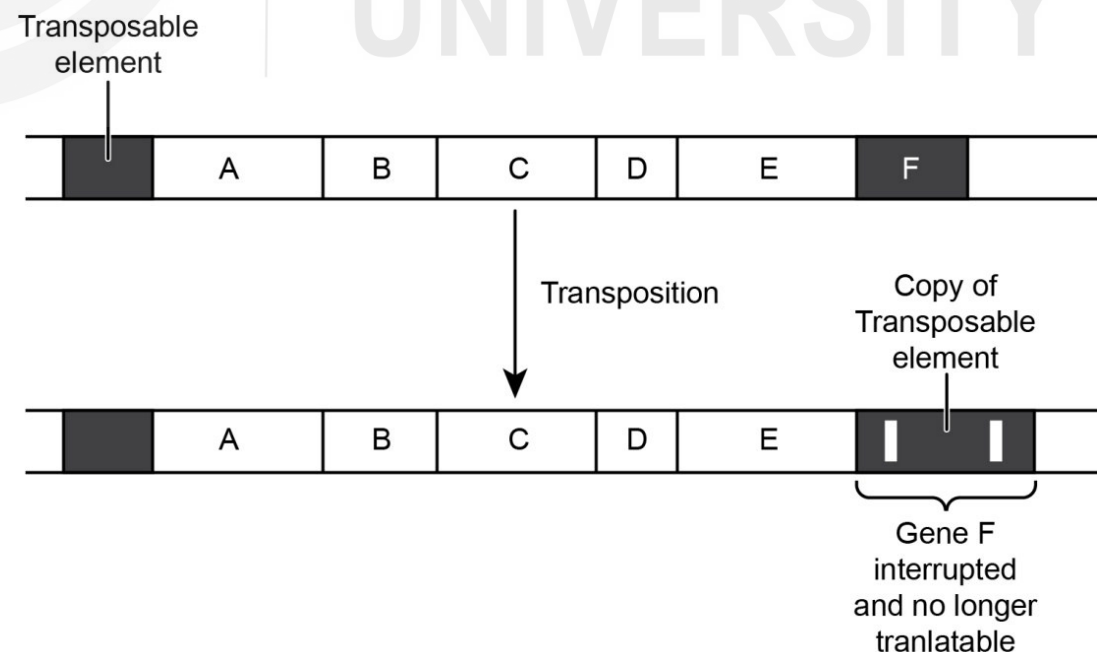


Fig. 9.8: When a transposable element is inserted in the middle of a gene, as in case of gene F in this figure the gene is disrupted. Such genes cannot be transcribed appropriately and thus do not function.

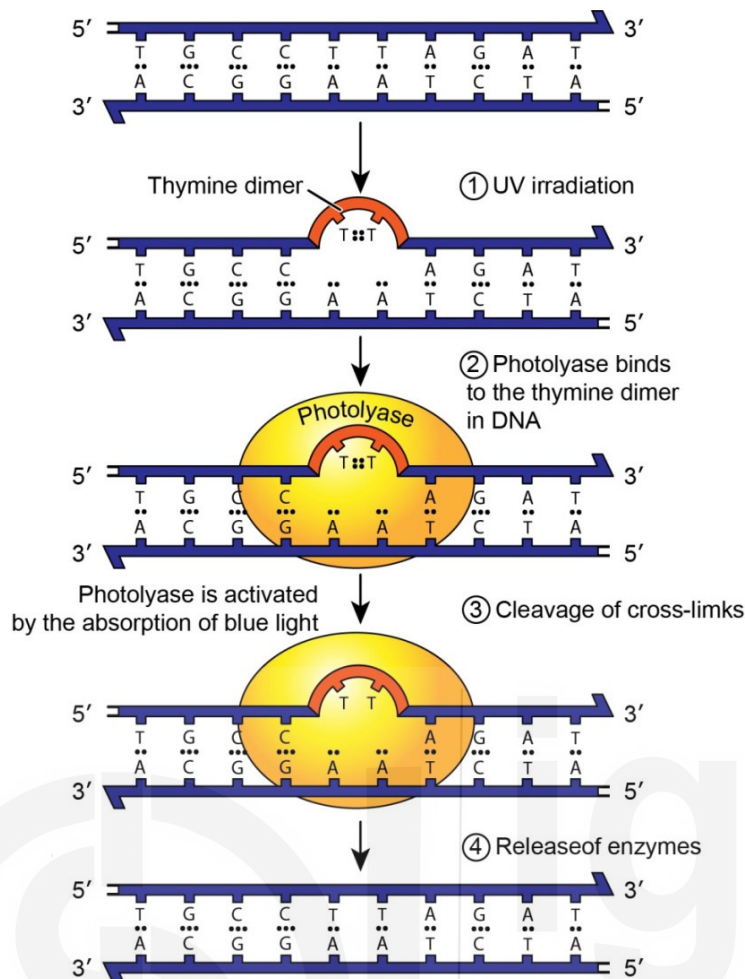


Fig. 9.9: Cleavage of thymine dimer cross-links by light-activated photolyase.

The arrows indicate the opposite polarity of the complementary strands of DNA.

9.6 MUTAGENESIS

Many agents (physical, chemical and environmental) have the mutagenic properties to cause mutations. They are known as **mutagens**. Mutagenic agents induce mutation in either of the following ways (Fig. 9.2).

- They may replace in the DNA.
- They may alter the base in such a way that it specifically mispairs with another base.
- They may damage the base so much that it can no longer pair with any base.
- They may intercalate themselves in the DNA paving way for addition or deletion of bases.

9.6.1 Physical Mutagens

These consist of high energy radiations which could penetrate living cells and affect the genetic material. The effect of radiations on living cells and tissues is directly proportional to the degree of penetration of the radiation. Radiations are of two types viz. electro-magnetic radiations and particulate radiations.

X-rays, gamma-rays and UV rays are short wavelength electromagnetic radiations which penetrate cells and tissues strongly. **Penetration power of electromagnetic radiation is inversely proportional to its wave length.**

Particulate radiations are in the form of subatomic particles emitted from atoms with high energy. Alpha particles, beta particles and neutrons fall in this category. Alpha particles and beta-particles are charged particles. However, beta particles being smaller in size are more penetrating than alpha particles. Neutrons ejected from radioactive isotopes do not carry any charge and hence are not deflected when they travel through living matter. Thus they are extremely penetrant and can cause severe damage to the living tissues as well as genetic material.

The physical mutagens are also divided as **high energy ionizing radiations** which include cosmic rays, X-rays, gamma-rays and particulate radiations and low energy **non-ionizing radiations** which include ultraviolet light.

The high energy radiations create ionization in the living cells. While passing through cells and tissues they collide with molecules such as water and cause the expulsion of electrons. This expulsion creates a positively charged ions. The ejected electron can not remain in the free state and therefore, is picked up by another ion creating a negative ion. The generation of free ions by radiations is the basis of extensive damage caused by them at the somatic and gametic level. These ions may combine with oxygen producing highly reactive chemical which may act on genes, chromosomes and other parts of the cells.

Peroxides which are mutagenic may be formed in the presence of oxygen following the splitting of water. Experiments have shown that sensitivity to a radiation and the rate of mutation are much lower in the organisms maintained in oxygen-free environment.

Non-ionizing radiations such as UV rays have major effect in the formation of dimers whereby adjacent pyrimidine bases become linked to one another by carbon to carbon bonds. Dimerization results in intra strand or inter strand cross linking which distorts the DNA **conformation**, thereby affecting the normal replication.

Many of these radiations are used in medicine, agriculture and warfare. So, working out dose effect relationship becomes important. Experiments have suggested, there is no dose which may be absolutely ineffective. Each doubling of dose results into the doubling of mutations induced.

9.6.2 Chemical Mutagens

Chemical mutagens are classified into four major groups on the basis of their specific reaction with DNA.

1. **Base Analogs:** These analogs have been discussed earlier in the Unit (Section 9.4.5).
2. **Deaminating Agents:** These agents have also been discussed in the Unit (Section 9.4.4).

The nitrogen atoms attached to the purines and pyrimidines are usually in the **amino** (NH_2) form (NH) configuration. Linkewise the oxygen atoms attached to the C 6 atoms of guanine and thymine normally have the **keto** ($\text{C}=\text{O}$) form and only rarely take up the **enol** (COOH) configuration.

3. **Alkylating Agents:** Such as ethyl methane sulfonate (EMS), ethyl ethane sulfonate (EES) and diethyl sulphate (DES) act on DNA by adding alkyl group (ethyl or methyl) to all four bases. However, these agents show a strong preference for base guanine. This results either in mispairing of affected base or its loss entirely, creating a gap thus causing mutations.
4. **Intercalating Agents:** This type of mutagen includes ethidium bromide and Acridine dyes (proflavin and acridine orange). Intercalating agents can mimic base pairs and slip between the base pairs in double helix and open the helix which leads to increase in distance between base pairs. This results in deletion or addition of base pairs during DNA replication.

9.6.3 Environmental Mutagens

These mutagens include air and water pollutants, agricultural chemicals, pesticides, cigarette and industrial smoke which includes chemicals like Benzidine, Vinyl Chloride etc. Mutagenic effects of urban Air pollutants like fine particulate matter have been shown. Some environmental mutagens are carcinogenic. Some of the biological agents may also act as mutagens which are listed below.

- a. Transposons.
- b. Virus – Inserted virus DNA into genome may change genetic function.
- c. Bacteria – Some bacteria like *Helicobacter pylori* cause inflammation and DNA damage which may lead to mutation.
- d. Parasitic Fungi of Field Crops .

Intercalation is a process in which mutagens such as acridines which are flat, aromatic molecules interact with DNA in such a way that they become wedged between the stacked bases of double helix.

SAQ 4

- (a) What are transposons? How do they cause mutation?
- (b) Fill in the blanks
 - (i) radiation induces dimerization of adjacent pyrimidine bases.
 - (ii) and are the examples of Intercalating agents.
 - (iii), ...are the examples of biological agents which may act as mutagens.

9.7 DNA REPAIR MECHANISM

Genome of Prokaryotes and Eukaryotes is under constant strain of damage every day due to various factors as described earlier. Errors may also occur when DNA replicates. To prevent the genome becoming dysfunctional, all

living organisms have DNA repair systems. Following five types of DNA repair system is known:

1. Direct repair system
2. Excision repair
3. Mismatch repair
4. Homologous recombination
5. Non-homologous and joining

Four common pathways are used in living organisms in repair mechanism.

- A. DNA damage is detected by enzymes.
- B. Nucleases remove mismatched or damaged nucleosides.
- C. Template strand is recognized by DNA polymerase to add correct nucleotide.
- D. Nicks are filled by DNA ligase.

Direct repair system and excision repair are described below.

Direct repair system

This system involves repairs of UV induced pyrimidine dimers. Kelner (1949) observed the UV damage to DNA of *E. Coli* could be reversed if UV irradiated cells are exposed to visible light in the blue range (blue light has wave length range of 320-370 nm). This process known as **photo reactivation** or **light repair** is found to be temperature sensitive.

Photoreactivation means that light induces an enzymatically controlled chemical reaction. The enzyme was isolated from *E. Coli* cells and was named as photoreactivation enzyme or photolyase. The enzyme cleaves the bond between thymine dimer, thereby, restoring the structure. The enzyme is also known to bind to the dimers in dark but is activated only when it absorbs a photon of light. Photolyases are apparently very effective since few thymine dimers and mutations are left after, photoreactivation (Fig. 9.7).

Another system of this kind corrects bases that have become alkylated by chemical mutagen such as EMS (Ethyl methane sulforate). An enzyme called alkyltransferase catalyzes the removal of the alkyl group from the base. So, in direct repair system the modified base is not removed from DNA but is corrected there itself.

Excision Repair: There are two types of Excision repair (a) base excision repair system which involves removal of damaged nucleotides or chemically modified bases from DNA. (b) nucleotide excision repair system which can repair intrastrand cross links, chemically modified DNA and thymidine dimers. Both mechanisms occur in prokaryotes and humans in dark.

A DNA glycosylase enzyme recognizes and removes modified base. There are different glycosylases. Each glycosylase recognizes and removes specific type of modified base like deaminated or oxidized base.

The glycosylase enzyme removes glycosylated bond between the changed base and sugar which creates apurinic or apyrimidinic sites (AP sites). AP endonuclease along with phosphodiesterases cleave sugar and create a nick in deoxyribose – phosphate group. Exonuclease removes altered nucleotide which is replaced by DNA polymerase based on complementary strand. DNA ligase seals the nick in the sugar – phosphate backbone. As for example, uracil glycosylase identifies and removes/uracil produced by deamination of cytosine (Fig. 9.8).

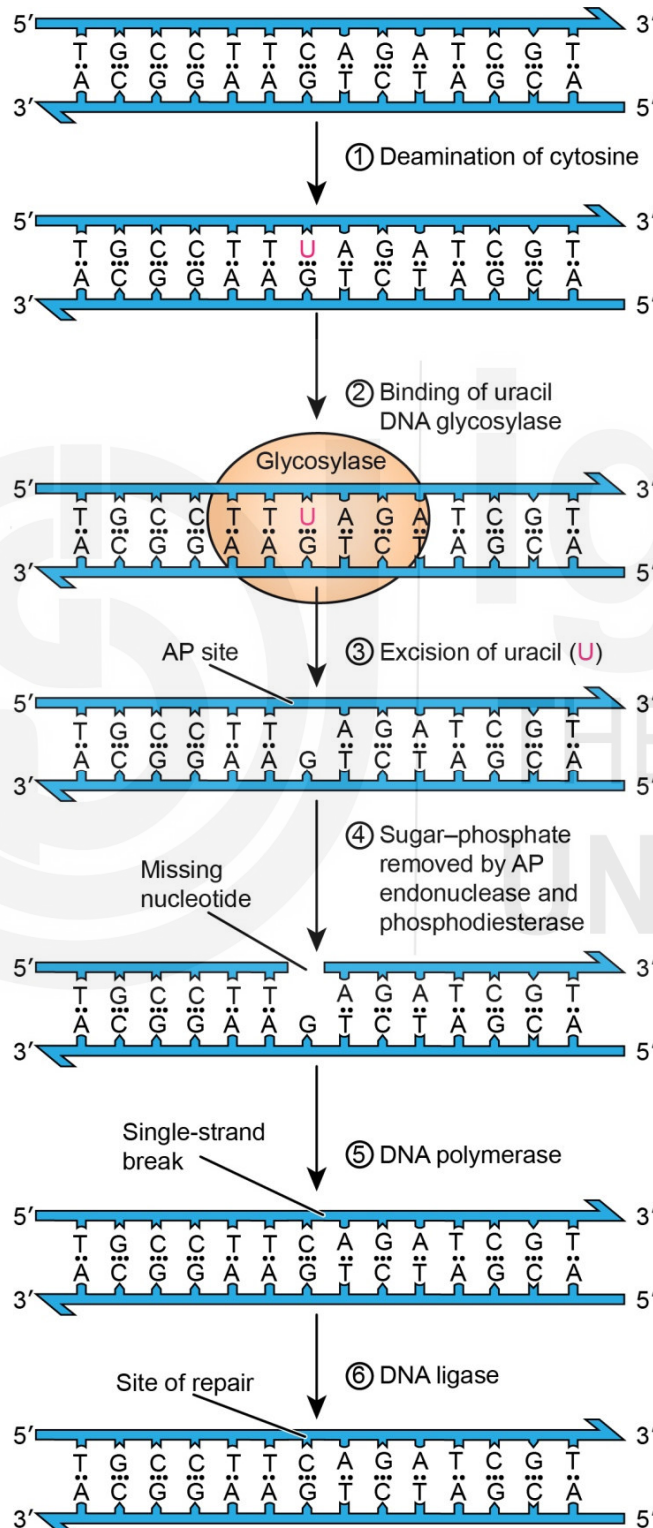


Fig. 9.10: Repair of DNA by the base excision pathway. Base excision repair may be initiated by any one of several different DNA glycosylases. In the example shown, uracil DNA glycosylase starts the repair process.

Nucleotide excision repair restricts thymidine dimer. Excinuclease enzyme excises either side of altered nucleotide(s) and cuts oligonucleotide containing altered nucleotide (see Fig. 9.9).

Defects in excision repair process leads to genetic diseases. In human, an inherited disease called xeroderma pigmentosum is caused by a recessive mutation that blocks this excision repair process. People with this disease are very sensitive to sunlight and particularly the UV wavelength in the sunlight. They demonstrate a very high incidence of skin cancer.

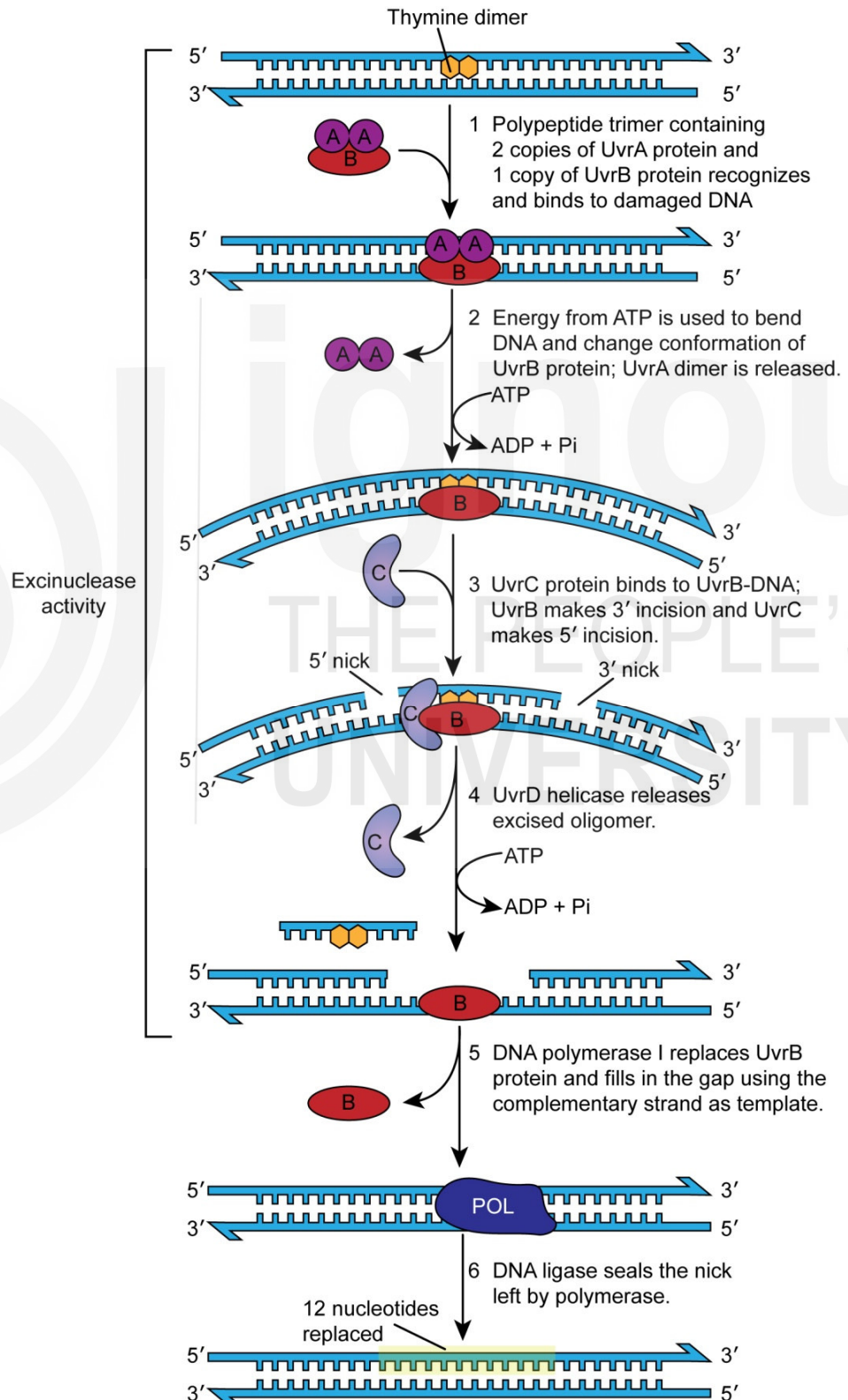


Fig. 9.11: Repair of DNA by the nucleotide excision pathway in *E. coli*.

SAQ5

- (a) Name the common pathways used by living organisms to repair damaged/altered DNA.
- (b) Fill in the blanks
- Base excision repair system removes or from DNA.
 - Nucleotide excision repair system repairs, ... and

9.8 SIGNIFICANCE OF MUTATIONS

In the previous sections, different type of mutations have been discussed. Mutations mostly produce detrimental effects. However, mutations can also be beneficial in the following ways:

- Mutations produce genetic variations within genome which help in the evolutionary process in the organisms.
- Mutant organisms help in understanding the metabolic pathways in organisms.
- Plant breeders rely on the genetic variability in crop plants for their improvement. (e.g. improved variety, high yield, disease and pest resistance etc.).
- In humans, a specific 32bp deletion (CCR5- delta 32), a genetic mutation, leads to HIV resistance to homozygotes and delays AIDS onset in heterozygotes.
- Mutagenic action of Ionizing radiations have been used to create sterilized female insects to control and eradicate pests.

Genetic recombination helps in the construction of an array that indicates the position of genes on a chromosome with respect to one another. When this is done by genetic technique, the array is called a genetic map.

9.9 SUMMARY

Let us summarise whatever you have learnt so far:

- A mutation is an alteration in genetic material including mitochondrial and chloroplast DNA.
- Mutation can occur spontaneously or induced experimentally by mutagens and makes the organisms different from the wild type.
- Mutations may occur in somatic cells or germinal cells. Somatic mutations are not heritable.
- Mutations can also be categorized as Reverse and Suppressor mutations.
- Mutations can be detected by a change in phenotype, pedigree analysis in humans, biochemical analysis or DNA sequencing and various other methods in different organisms.

- Mutations may be generated at DNA level by base-pair substitutions, addition or deletion of nucleotides (frame-shift mutations), tautomerization, deamination and depurination, base analogs, and transposable elements.
- Mutations are caused by various agents called mutagens. Mutagens can be physical (X-Rays, Y-Rays, UV rays etc.); Chemical (base analogs, deaminating agents, alkylating agents, intercalating agents) or environmental agents (biological agents, pollution, pesticides etc.)
- Cells of living organisms have different DNA repair mechanisms (e.g. Direct repair system, Excision repair system etc.) to correct the damaged DNA caused by mutations. This helps to prevent the genome becoming dysfunctional.
- Mutations are not always detrimental to genome but can also be beneficial. Mutations also produce genetic variability which advances the evolutionary process. They are helpful in advancement of studies in genetics and improvement of various useful characteristics of the crop plants.

9.10 TERMINAL QUESTIONS

1. Define mutation. Explain the following types of mutations briefly:
 - (a) Induced mutations
 - (b) Suppressor mutations
2. Explain briefly the CIB method of detection of mutation in *Drosophila*.
3. What is tautomerization?
4. What are transposable genetic elements? How they can cause mutations?
5. How the DNA damage is repaired by excision repair system?
6. How can the mutations be useful to human beings?

9.11 ANSWERS

Self Assessment Questions

1. i) Mutations can be defined as any heritable alteration in the genetic material of an organism or virus. **Hugo deVries** 1901 coined the term mutations. T.H. Morgan in 1910 identified white eyed male flies among red eyed male flies in *Drosophila melanogaster*.
ii) (A) f, (B) e, (C) d, (D) b, (E) c, (F) a.
2. i) depends, ii) humans, iii) necessary, iv) F₂, v) non-viable, vi) haploid.

3.
 - i). a – transversion; b-transition; c-transversion
 - ii). a – frameshift mutation;
b – error of base incorporation
c – amino group
d – purine
e – base analogs
4.
 - a) Transposons are genetic elements of long stretches of DNA that have the capacity to move from one location to another. They insert themselves at one or more sites of the genome. The mutational effect is caused when they change reading frame.
 - b) (i) UV; (ii) Ethidium bromide and Acridine; (iii) Transposon/Virus /Bacteria/Parasitic Fungi
5.
 - a) Refer to Section 9.7; Four common pathways used in living organism in repair mechanism.
 - b) (i) damaged nucleotides or chemically modified bases
(ii) Intra strand crosslinks, chemically modified DNA and thymidine dimers.

Terminal Questions

1. Mutation is any heritable alteration in the genetic material of an organism or virus. For Induced mutation and suppressor mutation – refer to Sections 9.2.1 and 9.2.2.
2. CIB method was devised by Muller for detecting mutations in *Drosophila*. This method can detect the rate of induction of sex linked recessive lethal mutation. For detail refer to Section 9.3.2.
3. Refer to Section 9.4.3.
4. These elements are able to move from one position to another in genome. They change the reading frame of the gene where they get inserted and thus cause the mutation.
5. Refer to Section 9.7 – Excision Repair.
6. Refer to Section 9.8 – use of mutations.

GLOSSARY

- Allele** : One of the two or more alternative forms of a gene, each possessing a unique nucleotide sequence. Different alleles of a gene are usually recognized by the phenotypes rather than by comparison of their nucleotide sequences.
- Autosomes** : Chromosomes other than the sex chromosomes.
- Back cross** : A mating between a heterozygote and an individual homozygous for either the dominant or the recessive allele; differs from test cross in that it involves the crossing of an offspring with one of the parents.
- Barr body** : Is the darkly stained condensed X-chromosome observed in the interphase of mammalian female cells.
- Centromere** : A chromosomal region that becomes associated with the spindle fibres during mitosis and meiosis.
- Chiasmata** : The places at which pairs of homologous chromatids are in contact (from late prophase of meiosis to the beginning of the first anaphase) and at which exchanges of homologous parts between nonsister chromatids have taken place by crossing-over (singular: chiasma).
- Chromatid** : In all duplicated chromosomes, each of the two longitudinal subunits that become visible during mitosis or meiosis.
- Chromosome** : A thread like structure found in the nuclei of cells, composed of chromatin that stores and transmits genetic information.
- Chromosome set or chromosome complement** : The normal gametic complement of chromosomes of a diploid individual.
- Chromosomal aberration** : Abnormal structure or number of chromosomes; includes deficiency, duplication, inversion, translocation, aneuploidy, polyploidy or any other change from the normal pattern.
- Cistron** : The region in a DNA molecule that specifies a polypeptide chain. Genetically defined as a region within which two mutations cannot complement each other.
- Codominance** : A situation in which the heterozygote exhibits the phenotypes of both homozygotes.

Colchicine	: A chemical that prevents formation of the spindle in nuclear division.
Complete dominance	: The case in which one allele is dominant to the other so that at the phenotypic level the heterozygote is essentially indistinguishable from the homozygous dominant.
Crossing-over	: The exchange of chromatid segments between homologous chromatids during meiosis; if different alleles are present on the chromatids, crossing-over can be detected by the formation of genetically recombinant chromatids.
Deficiency (deletion)	: Absence of a segment of a chromosome reducing the number of loci.
Denaturation	: Loss of native configuration of a macromolecule, usually accompanied by loss of biological activity.
Dexyribonucleic acid DNA	: A polynucleotide in which the sugar residue is deoxyribose and which is the primary genetic material of all cells.
Dihybrid cross	: A cross in which two pairs of alleles are being followed.
Diploid	: Having two complete haploid sets of chromosomes typical for the species or group.
DNA ligase	: An enzyme that creates a phosphodiester bond between the 5-PO ₄ end of one polynucleotide and the 3-OH end of another, thereby producing as single, larger polynucleotide.
DNA polymerase	: The enzyme responsible for synthesizing DNA from deoxyribonucleoside triphosphates under the direction of a template DNA strand.
Dominant	: An allele or phenotype, that is expressed both in the homozygous or heterozygous state.
Duplication	: The occurrence of a segment more than once in the same chromosome or genome.
Episome	: Extrachromosomal circular DNA molecule capable of self replication either independent of, or as in integral part of host chromosome.
Epistasis	: Interaction of alleles at different loci; the control or masking of the expression of alleles at one locus by alleles at a second locus.

Euchromatin	: Genetic material that is not stained so intensely by certain dyes during interphase and that comprises many different kinds of genes.
Exonuclease	: An enzyme that hydrolyses terminal phosphodiester bonds (at 3' or 5 ends) in a polynucleotide.
Expressivity	: The degree or range in which a phenotype for a given trait is expressed.
F-factor	: (fertility factor) bacteria have an independent circular DNA or episome which is responsible for fertility, and is also known as the sex factor.
First filial generation (F₁)	: The generation of individuals produced by the first parental generation (P ₁) or the first parents being considered.
Gene	: Classically a unit of inheritance occupying a specific site (locus) on a chromosome, that has one or more specific effects on an organism, and can both recombine with other such genetic units, and mutate independently to other allelic forms.
Gene expression	: The multistep process by which a gene is regulated and its product synthesised.
Genome	: The genetic content of cell or virus; in eukaryotes, it sometimes refers to only one complete haploid chromosomes set.
Genotype	: The genetic constitution of a cell or of an individual.
Haemophilia	: A disease in which there is a blood-clotting defect. Males are usually affected as the trait is a X-linked recessive one.
Haploid	: Having one complete set of chromosomes typical for that species.
Heterochromatin	: Chromatin staining darkly even during interphase, often containing repetitive DNA with few genes.
heterozygous	: Having alternative forms of a gene (alleles) at a given locus, one allelic form on each of the two homologues.
Homologous	: Of chromosomes or chromosome segments that are identical with respect to their constituent genetic loci and their visible structure.
Homozygous	: Having the same allele (gene form) present at a given locus on both homologous chromosomes.

Incomplete dominance	: With reference to two alleles at a locus the expression of a heterozygous phenotype which is distinct from, and often intermediate to, that of either parent.
Independent assortment	: The segregation at meiosis of a pair of alleles independently of other allelic pairs whose loci are found on different chromosomes.
Interference	: A measure of the degree to which one cross-over by a chromatid affects the probability of a second cross-over by the same chromatid.
Isogametes	: Morphologically identical gametes.
Karyotype	: The chromosome complement of a cell or an organism, characterized by the number, size and configuration of the chromosomes.
Lethal gene	: A gene whose expression results in death.
Linkage	: A measure of the degree to which alleles of two genes assort independently at meiosis or in genetic crosses.
Linkage group	: A set of gene loci that can be placed in linear order representing the different degrees of linkage between loci.
Miscarriage	: Spontaneous detachment and expulsion of embryo or foetus.
Monosomic	: A diploid organism lacking one chromosome of its proper complement ($2n-1$); an aneuploid. Monosome refers to the single chromosome, disome to two chromosomes of a kind, and trisome to three chromosomes of a kind.
Multiple alleles	: Three or more forms of a gene, any one of which can occur at a given locus on a specific chromosome.
Nondisjunction	: The failure of two sister chromatids or homologous chromosomes to separate during cell division, so that both go to the same pole, thus producing aneuploid nuclei.
Nucleic acid	: A macromolecule composed of phosphoric acid, pentose sugar, and organic bases; DNA and RNA.
Paracentric inversion	: An inversion that is entirely in one arm of a chromosome and does not include the centromere.
Parental type	: An association of genetic markers, found among the progeny of a cross that is identical to an association of markers present in a male gamete.
Parthenogenesis	: The production of an embryo from a female gamete without participation of a male gamete.

- Partial dominance** : See incomplete dominance.
- Pedigree** : A diagram showing the ancestral relationships among individuals of a family over two or more generations.
- Penetrance** : The frequency (expressed as a percentage) with which individuals of a given genotype manifest at least some degree of a specific (mutant) phenotype associated with a trait.
- Peptide bond** : A chemical bond holding amino acid subunits together in proteins.
- Pericentric inversion** : An inversion including the centromere, hence involving both arms of a chromosome.
- Phosphodiester bond** : In nucleic acids, the covalent bond formed between the phosphate group of one nucleotide and the 3'-OH group of the next nucleotide in line; these bonds form the backbone of nucleic acids.
- Plasmid** : Extrachromosomal circular DNA molecules capable of self-replication independent of host chromosomes.
- Pleiotropic** : The multiple phenotypic effects of a single allele.
- Polyploid** : An organism with more than two sets of chromosomes (2n diploid) or genomes (e.g. triploid – 3n, tetraploid – 4n, pentaploid – 5n) so on.
- Recessive** : Any gene form that is not expressed in the presence of the other allele in the heterozygote. Also, any trait that is expressed phenotypically only when the responsible gene form is present in double dose in a homozygote.
- Reciprocal crosses** : Crosses in which the dominant allele is contributed by one parent and the recessive allele by the other parent and vice versa. Those in which each of the two strains provides the males in one cross and the females in the other, as in ♂ A × ♀ B and ♂ B × ♀ A.
- Recombinant type** : An association of genetic markers, found among the progeny of a cross, that is different from any association of markers present in the parents (cf. parental type).
- Recombination** : The creation of a new association of DNA molecules (chromosomes) or parts of DNA molecules (chromosomes).
- Satellite DNA** : A component of the genome that can be isolated from the rest of the DNA by centrifugation, usually it consists of short, highly repetitive sequences.

- Sex chromosome** : A chromosome, such as X or Y in humans, which is involved in sex determination.
- Sex chromosomes** : Chromosomes that are connected with the determination of sex.
- Sex-linked** : A trait which is expressed in only one sex in spite of not being sex chromosome linked.
- Synaptonemal complex** : A structure found during meiosis, that mediates close pairing between homologous regions of chromatids.
- Test cross** : A mating between a heterozygote and an individual homozygous for the recessive allele.
- Transcription** : The process by which genetic information from DNA in the chromosome is transmitted to m-RNA and finally results in the formation of gene product.
- Trihybrid** : An individual heterozygous for three pairs of alleles.
- Wild-type** : The most commonly observed phenotype or genotype in a population, that is designated as the norm or standard.
- X chromosome** : A chromosome associated with sex determination. In most animals, the female has two and the male has one X chromosome.
- X-linkage** : Genes found on the sex chromosome which is present as a pair in the homogametic sex. There are numerous genes linked on X-chromosome.
- Y chromosome** : The partner of the X chromosome in the male of many animal species.
- Y-linkage** : Genes present on the sex chromosome responsible for the heterogametic sex. There are few known Y-linked genes.