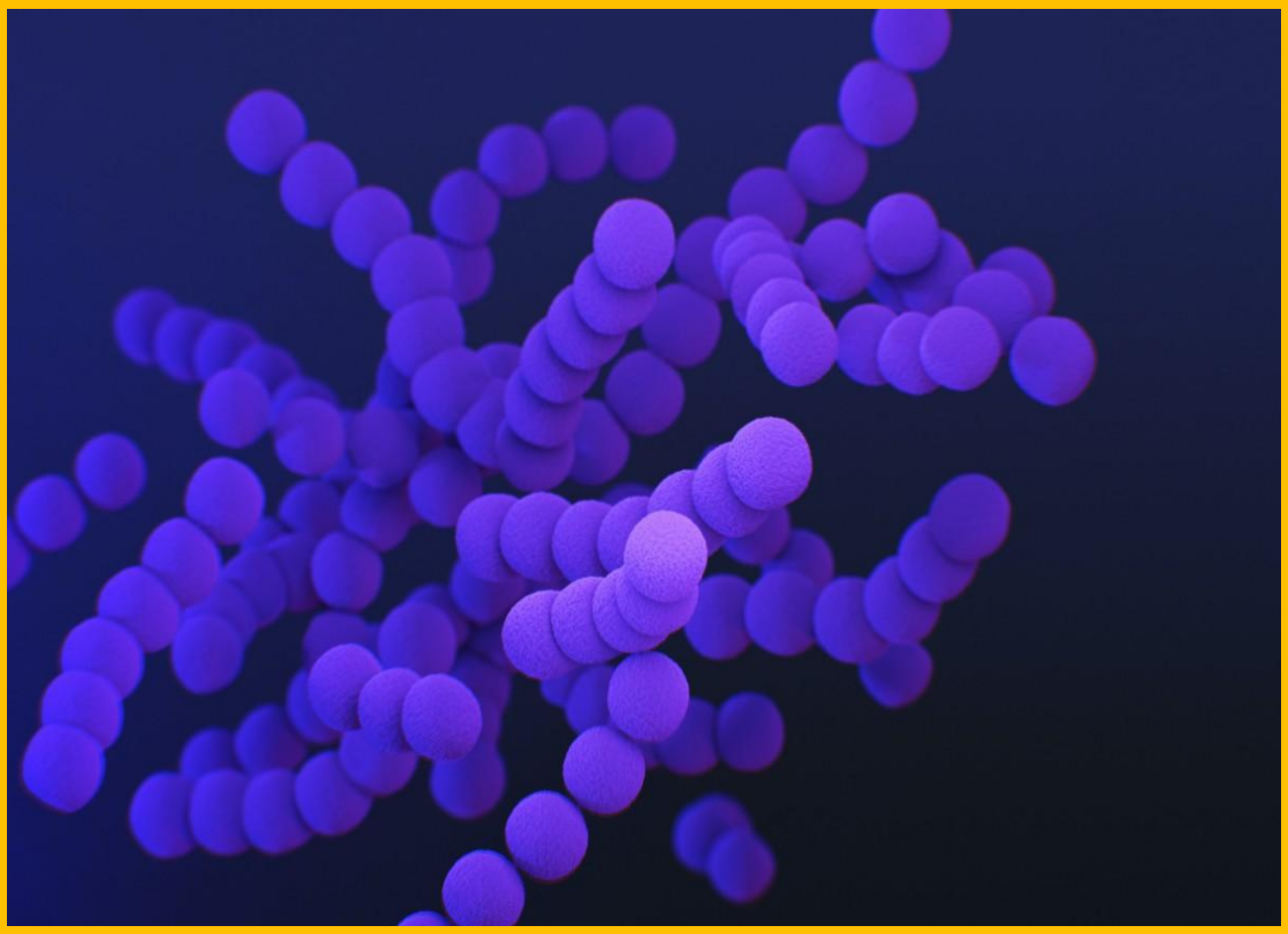
MSCZO-506

M. Sc. II Semester

CONCEPTS OF CELL BIOLOGY AND GENETICS



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Course 1: Concepts of Cell Biology and Genetics

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Unit 1 BIOLOGY OF CHROMOSOME

CONTENTS

1.1 OBJECTIVES

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

1.2 INTRODUCTION

Chromosomes are made up of a strand of DNA tightly wrapped around proteins called histones. This makes the DNA compact and able to fit inside the cell's nucleus. A gene is a segment of DNA that encodes for a trait. Therefore, genes are located on chromosomes. On each chromosome is a constriction point called a centromere, which divides the chromosome into different sections? An individual inherits 23 chromosomes from each parent.

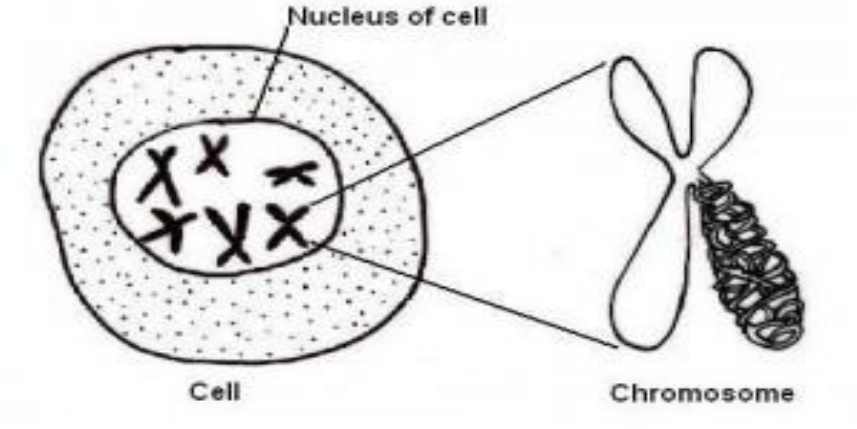

Fig.1.1 Structure of Chromosomes (online sources)

If there is a change in the number or structure of chromosomes, it can be problematic. For example, some cancers are the result of fragmented chromosomes pieced together. Additionally, Down syndrome, also known as trisomy 21, is the result of having an extra copy of the twenty-first chromosome. Individuals with trisomy 21 have 47 chromosomes.

1.2 CLASSES OF DNA

DNA is a group of molecules that is responsible for carrying and transmitting the hereditary materials or the genetic instructions from parents to offspring's. Nucleic acids are the organic materials present in all organisms in the form of DNA or RNA. These nucleic acids are formed by the combination of nitrogenous bases, sugar molecules and the phosphate groups that are linked by different bonds in a series of sequences. The DNA structure defines the basic genetic makeup of our body.

This is also true for viruses as most of these entities have either RNA. For instance, some viruses may have RNA as their genetic material, while others have DNA as the genetic material. The Human Immunodeficiency Virus (HIV) contains RNA, which is then converted into DNA after attaching itself to the host cell.

Apart from being responsible for the inheritance of genetic information in all living beings, DNA also plays a crucial role in the production of proteins. Nuclear DNA is the DNA contained within the nucleus of every cell in a eukaryotic organism. It codes for the majority of the organism's genomes while the mitochondrial DNA and plastid DNA handles the rest.

The DNA present in the mitochondria of the cell is termed as mitochondrial DNA. It is inherited from the mother to the child. In humans, there are approximately 16,000 base pairs of mitochondrial DNA. Similarly, plastids have their own DNA and they play an essential role in photosynthesis. DNA is known as Deoxyribonucleic Acid. It is an organic compound that has a unique molecular structure. It is found in all prokaryotic cells and eukaryotic cells**.**

DNA Types

There are three different DNA types:

- **A-DNA:** It is a right-handed double helix similar to the B-DNA form. Dehydrated DNA takes an A form that protects the DNA during extreme condition such as desiccation. Protein binding also removes the solvent from DNA and the DNA takes an A form.
- **B-DNA:** This is the most common DNA conformation and is a right-handed helix. Majority of DNA has a B type conformation under normal physiological conditions.
- **Z-DNA:** Z-DNA is a left-handed DNA where the double helix winds to the left in a zig-zag pattern. It was discovered by Andres Wang and Alexander Rich. It is found ahead of the start site of a gene and hence, is believed to play some role in gene regulation.

DNA Diagram

The following diagram explains the DNA structure representing the different parts of the DNA. DNA comprises a sugar-phosphate backbone and the nucleotide bases (guanine, cytosine, adenine and thymine).

DNA Structure

The DNA structure can be thought of like a twisted ladder. This structure is described as a double-helix, as illustrated in the figure above. It is a nucleic acid, and all nucleic acids are made up of nucleotides. The DNA molecule is composed of units called nucleotides, and each nucleotide is composed of three different components, such as sugar, phosphate groups and nitrogen bases.

The basic building blocks of DNA are nucleotides, which are composed of a sugar group, a phosphate group, and a nitrogen base. The sugar and phosphate groups link the nucleotides together to form each strand of DNA. Adenine (A), Thymine (T), Guanine (G) and Cytosine (C) are four types of nitrogen bases.

These 4 Nitrogenous bases pair together in the following way: **A** with **T,** and **C** with **G**. These base pairs are essential for the DNA's double helix structure, which resembles a twisted ladder.

The order of the nitrogenous bases determines the genetic code or the DNA's instructions.

Among the three components of DNA structure, sugar is the one which forms the backbone of the DNA molecule. It is also called deoxyribose. The nitrogenous bases of the opposite strands form hydrogen bonds, forming a ladder-like structure

The DNA molecule consists of 4 nitrogen bases, namely adenine (A), thymine (T), cytosine (C) and Guanine (G) which ultimately forms the structure of a nucleotide. The A and G are purines and the C and T are pyrimidines.

The two strands of DNA run in opposite directions. These strands are held together by the hydrogen bond that is present between the two complementary bases. The strands are helically twisted, where each strand forms a right-handed coil and ten nucleotides make up a single turn.

The pitch of each helix is 3.4 nm. Hence, the distance between two consecutive base pairs (i.e., hydrogen-bonded bases of the opposite strands) is 0.34. The DNA coils up, forming chromosome and each chromosome has a single molecule of DNA in it. Overall, human beings have around twenty-three pairs of chromosomes in the nucleus of cells. DNA also plays an essential role in the process of cell division.

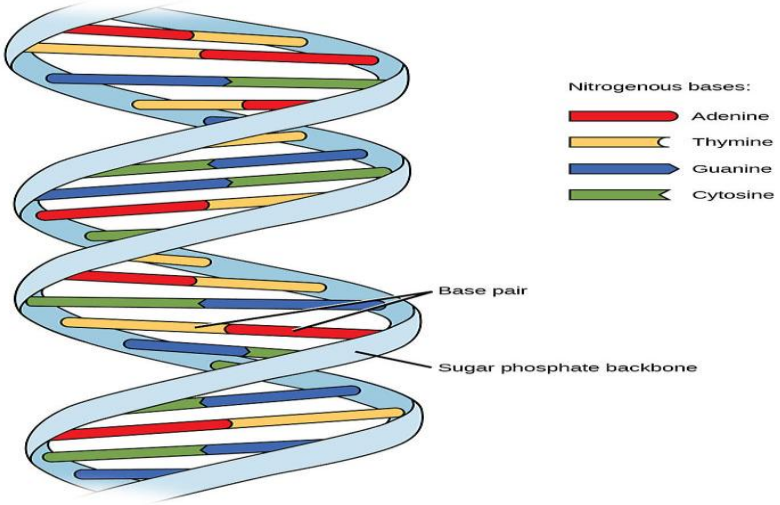

Fig 1.2 Structure of DNA(online source)

DNA Function

DNA is the genetic material which carries all the hereditary information. Genes are the small segments of DNA, consisting mostly of 250 – 2 million base pairs. A gene code for a polypeptide molecule, where three nitrogenous bases sequence stands for one amino acid.

Polypeptide chains are further folded in secondary, tertiary and quaternary structure to form different proteins. As every organism contains many genes in their DNA, different types of

proteins can be formed. Protein is the main functional and structural molecules in most of the organisms. Apart from storing genetic information, DNA is involved in:

- Replication process: Transferring the genetic information from one cell to its daughters and from one generation to the next and equal distribution of DNA during the cell division
- Mutations: The changes which occur in the DNA sequences
- Transcription
- Cellular Metabolism
- DNA Fingerprinting
- Gene Therapy

The following seven points will highlight the seven important criteria generally used for the classification of DNA.

The seven criteria which are generally used for the classification of DNA include: (1) Number of base pairs per turn (2) Coiling pattern (3) Location (4) Structure (5) Nucleotide sequences (6) Coding and Non-coding DNA and (7) Number of strands.

Number of Nucleotide Base per Turn: Depending upon the nucleotide base per turn of the helix, pitch of the helix, tilt of the base pair and humidity of the sample, the DNA can be observed in four different forms, namely A, B, C and D.

Type of DNA

B-DNA (Common form of DNA):

This form is the same as proposed by Watson and Crick and is the most common form. The B-form X-ray diffraction is observed when humidity is 92% and salt concentration is high. This is a right handed double helical structure. The pitch is 3. 4 nm and there are 10 base pairs per turn of the helix.

A-DNA: This form is found when humidity of the sample is 75%. This has pitch of 2.8nm and 11 base pairs per turn of helix. The base pairs are almost perpendicular in B-DNA, whereas base pairs

are titled in A-DNA. As a result, the depth of the deep grooves is increased and that of shallow grooves is reduced.

C-DNA:

The C-form of DNA is observed when humidity of DNA fiber is below 66%. This form of DNA has pitch of 3.1nm and 9.3 base pairs per turn of the helix. The base pair has negative tilt (-7.8).

D-DNA: This is the rare form. This form has 8 base pairs per turn of the helix and the tilt is negative but higher than C form (-16.7). The B-DNA is the most stable form. It can change to another form depending upon humidity and salt concentration of the sample.

Based on the location in the cell, DNA is of three types, viz., chromosomal DNA, cytoplasmic DNA and promiscuous DNA.

These are briefly described below:

Chromosomal DNA:

Mostly the DNA is found in the chromosomes. Such DNA is called chromosomal DNA or nuclear DNA in case of eukaryotes. The chromosomes are composed of DNA, histones and RNA. Thus, major amount of DNA is found in association with chromosomes.

Cytoplasmic DNA:

Some amount of DNA is also found in the cytoplasm especially in mitochondria and chloroplasts. Such DNA is referred to as cytoplasmic DNA, which plays an important role in the cytoplasmic inheritance. Cytoplasmic DNA has circular structure

Promiscuous DNA:

Some DNA segments with common base sequence are found in chloroplasts, mitochondria and nucleus. This suggests that some DNA sequences move from one organelle to other. Such DNA is referred to as promiscuous DNA. The first case of movement of DNA from chloroplasts to mitochondria was reported in maize. Later on, such movement of DNA was reported in yeast and sea urchin. Now movements of DNA have been reported in several crop plants such as spinach, mung bean, maize and peas.

1.3 CHROMOSOMAL PROTEINS: HISTONE AND THEIR MODIFICATIONS

Histones are proteins that condense and structure the DNA of eukaryotic cell nuclei into units called nucleosomes. Their main functions are to compact DNA and regulate chromatin, therefore impacting gene regulation. Histones H2A, H2B, H3 and H4 are known as the core histones, and they come together to form one nucleosome. The nucleosome core is formed of two H2A-H2B dimers and a H3-H4 tetramer. In general, eukaryotic histones repress gene transcription, but It is now known that histones can be both positive and negative regulators of gene expression. These interactions are the basis of the histone code.

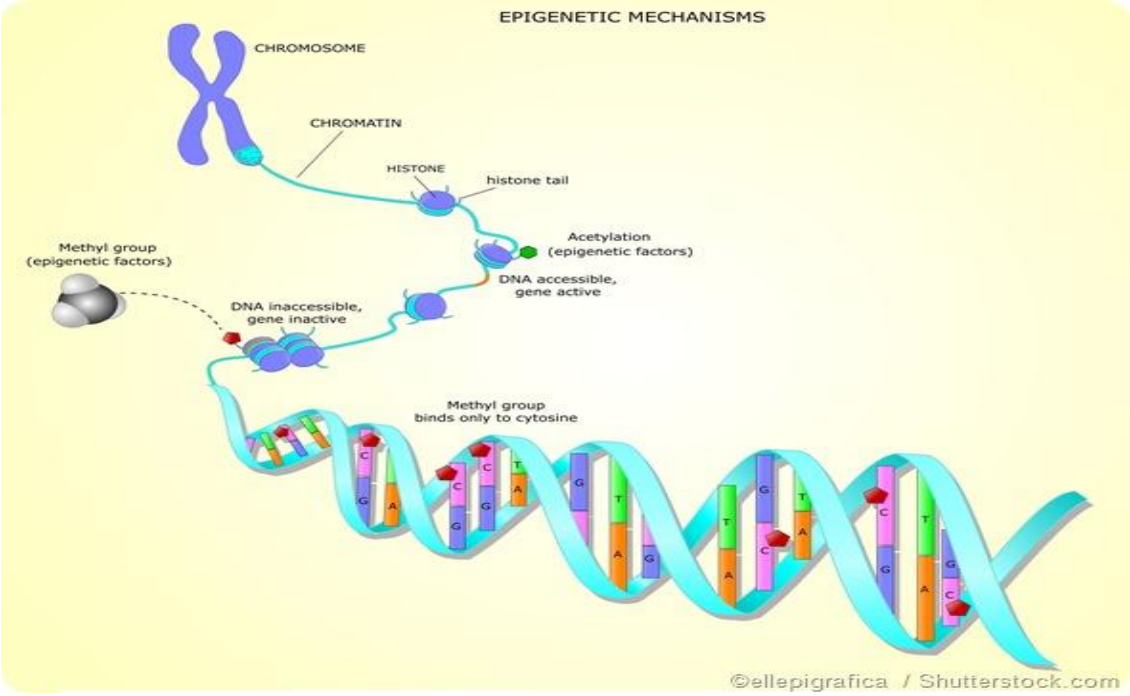

Fig 1.3 Chromosomal Histoneproteins (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3193420/)

Histone H2B H2B forms a (H2A-H2B)-2 tetramer. This tetramer and its component dimers are easily exchanged in and out of the nucleosome compared to H3 and H4, meaning that the modifications on H2A and H2B are less likely to be maintained in chromatin Histone H3K4 When you think H3K4, think activation. Whether it's methylated or acetylated, this site will turn genes fasters than you can say PRDM9. Acylation of all histone residues are activating, and H3K4 is no

Exception.

The real interest in H3K4 lies in its methylation. Histone H3K9 H3K9 does double duty. It can turn genes on by getting acetylated, but can silence them just as easily when methylated. H3K9ac is a particularly important acetylation: it is highly correlated with active promoters. H3K9ac also has a high co-occurrence with H3K14ac and

H3K4me3 which together are these three marks are the hallmark of active gene promoters Histone H3K27.
https://epigenie.com/key-epigenetic-players/histone-proteins-and-modifications/

Histone modifications in detail

Acetylation

Acetylation is one of the most widely studied histone modifications since it was one of the first discovered to influence transcriptional regulation. Unmodified lysine residues are positively charged but acetylation results in neutralization of the charge on histones, which reduces the interaction of histones and negatively charged DNA. The charge neutralization results in a weaker histone: DNA interaction allows transcription factor binding and significantly increases gene expression (Roth et al., 2001).

Histone acetylation is involved in cell cycle regulation, cell proliferation, and apoptosis and may play a vital role in regulating many other cellular processes, including cellular differentiation, DNA replication and repair, nuclear import and neuronal repression. An imbalance in the equilibrium of histone acetylation is associated with tumorigenesis and cancer progression.

Methylation

Methylation is added to the lysine or arginine residues of histones H3 and H4, with different impacts on transcription. Arginine methylation promotes transcriptional activation (Greer *et al*., 2012) while lysine methylation is implicated in both transcriptional activation and repression depending on the methylation site. This flexibility may be explained by the fact that that methylation does not alter histone charge or directly impact histone-DNA interactions, unlike acetylation.

Phosphorylation

Histone phosphorylation is a critical intermediate step in chromosome condensation during cell division, transcriptional regulation, and DNA damage repair (Rossetto et al., 2012, Kschonsak et al., 2015). Unlike acetylation and methylation, histone phosphorylation establishes interactions between other histone modifications and serves as a platform for effector proteins, which leads to a downstream cascade of events.

Phosphorylation occurs on all core histones, with differential effects on each. Phosphorylation of histone H3 at serine 10 and 28, and histone H2A on T120, are involved in chromatin compaction and the regulation of chromatin structure and function during mitosis. These are important markers of cell cycle and cell growth that are conserved throughout eukaryotes.

References: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3193420/

1.5 NUCLEOSOME MODEL

- The **chromosomes** are the nuclear components of the special organization, individuality, and function that are capable of self-reproduction and play a vital role in heredity, mutation, variation and evolutionary development of the species.
- Each chromosome is made up of **DNA** tightly coiled many times around proteins that support its structure.
- The proteins that bind to the DNA to form eukaryotic chromosomes are traditionally divided into two classes: the histones and the non-histone chromosomal proteins.
- The complex of both classes of protein with the nuclear DNA of eukaryotic cells is known as chromatin.
- Chromatin are a highly compacted structure consisting of packaged DNA and necessary so as to fit DNA into the nucleus.
- The assembly of DNA into chromatin involves a range of events, beginning with the formation of the basic unit, the nucleosome, and ultimately giving rise to a complex organization of specific domains within the nucleus.
- In the first step of this process, DNA is condensed into an 11 nm fiber that represents an approximate 6-fold level of compaction. This is achieved through nucleosome assembly.

- The nucleosome is the smallest structural component of chromatin and is produced through interactions between DNA and histone proteins.
- Each nucleosome consists of histone octamer core, assembled from the histones H2A, H2B, H3 and H4 (or other histone variants in some cases) and a segment of DNA that wraps around the histone core. Adjacent nucleosomes are connected via “linker DNA”.
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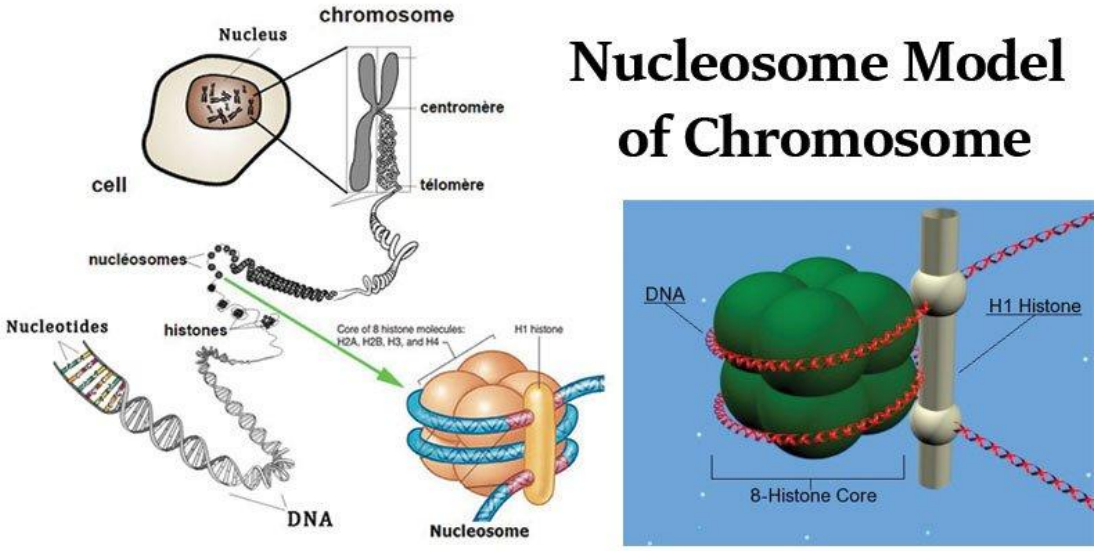


Fig 1.4 Nucleosome Model of Chromosome (online)

Introduction

1. Nucleosome model is a scientific model which explains the organization of DNA and associated proteins in the chromosome.
2. It also further explains the exact mechanism of the folding of the DNA in the nucleus.
3. The model was proposed by Roger Kornberg in 1974 and is the most accepted model of chromatin organization.
4. It was confirmed and christened by P. Oudet et al., (1975).

Features of the Nucleosome Model of Chromosomes

5. In eukaryotes, DNA is tightly bound to an equal mass of histones, which serve to form a repeating array of DNA-protein particles, called nucleosomes.
6. If it was stretched out, the DNA double-helix in each human chromosome would span the cell nucleus thousands of time.

7. Histones play a crucial role in packing this very long DNA molecule in an orderly way (i.e., nucleosome) into nucleus only a few micrometers in diameter.
8. Thus, nucleosomes are the fundamental packing unit particles of the chromatin and give chromatin a "beads-on-a-string" appearance in electron micrographs taken after treatments that unfold higher-order packing.
9. Each nucleosome is a disc-shaped particle with a diameter of about 11 nm and 5.7 nm in height containing 2 copies of each 4 nucleosome histones–H2A, H2B, H3, and H4.
10. This histone octamer forms a protein core [(i.e., a core of histone tetramer (H3, H4)2 and the apolar regions of 2(H2A and H2B)] around which the double-stranded DNA helix is wound 1¾ time containing 146 base pairs.
11. In chromatin, the DNA extends as a continuous thread from nucleosome to nucleosome.
12. Each nucleosome bead is separated from the next by a region of linker DNA which is generally 54 base pair long and contains single H1 histone protein molecule.
13. Generally, DNA makes two complete turns around the histone octamers and these two turns (200 bp long) are sealed off by H1 molecules.

The Folding of the DNA

14. The first step is the assembly of the DNA with a newly synthesized tetramer (H3-H4), are specifically modified (e.g. H4 is acetylated at Lys5 and Lysl2 (H3-H4)), to form a sub-nucleosomal particle, which is followed by the addition of two H2A-H2B dimers.
15. This produces a nucleosomal core particle consisting of 146 base pairs of DNA bind around the histone octamer. This core particle and the linker DNA together form the nucleosome.
16. The next step is the maturation step that requires ATP to establish regular spacing of the nucleosome cores to form the nucleo-filament.
17. During this step the newly incorporated histones are de-acetylated.
18. Next, the incorporation of linker histones is accompanied by folding of the nucleo-filament into the 30 nm fiber, the structure of which remains to be elucidated.
19. Two principal models exist- the solenoid model and the zig-zag.
20. Finally, further successive folding events lead to a high level of organization and specific domains in the nucleus.

References

1. https://www.easybiologyclass.com/nucleosome-model-of-chromosomes-in- eukaryotes-short-notes/

1.6 CENTROMERE, KINETOCHORE AND TELOMERE

Structure of Centromere*:*

The site of constriction in a chromosome under light microscope is generally taken as the position of centromere. It is generally believed that constitutive heterochromatin is present in the centromeric region. The component of centromere is mainly the kinetochore, and DNA associated proteins. Spindle fibers or microtubules are attached at this point which helps in moving the chromosomes or chromatids to the poles during cell division.

When microtubules of the spindle are attached at the centromere of metaphase chromosomes consisting of two chromatids, then sister chromatids separate and move to opposite poles of the spindle—and next step of division proceeds. Thus centromere has two functions, one is the attachment of sister chromatids, and second is the site for attachment of spindle fiber.

It has been observed under the electron microscope that a single spindle fibre is attached to the centromere of yeast, Saccharomyces cerevisiae, while multiple spindle fibres are attached to the centromere of other organisms.

The chromatin segment of the centromere in yeast has been analysed and found to contain a Protein-DNA complex of 220 to 250 base pairs. Four regions have been identified in the centromere of yeast as CDE 1, CDE 2, CDE 3 and CDE 4.

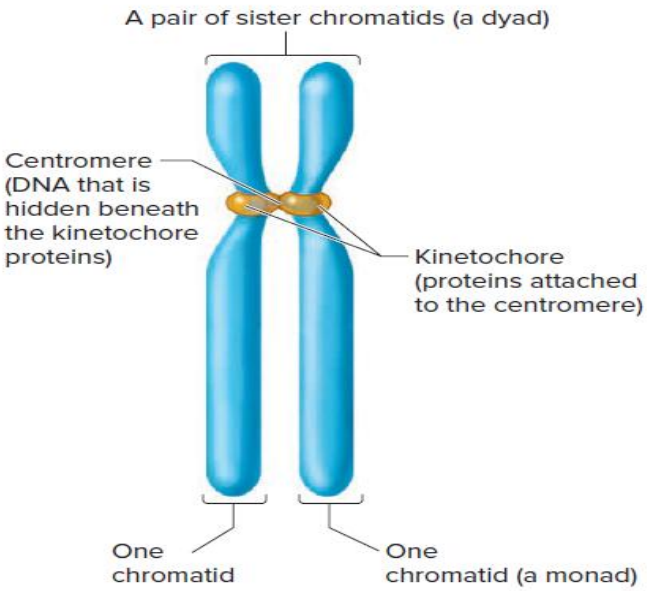


Fig 1.5 Structure of Centromere

The base sequences of first three regions are similar in all yeasts but the variation in base sequence is found in CDE 4.The centromeric DNA is protected from the digestion of nuclease by forming a structure called centromeric core particle. This particle contains more DNA than normal nucleosome core particle and associated proteins. The spindle fibre is attached to this particle that helps to separate chromosomes during cell division.

Types of Centromere:

(a) Structure of Telomeres:

All chromosomes have a special DNA-protein structure at the end called Telomeres. The telomeres have some important role in chromosome replication and stability. Microscopic observations show that chromosomes with broken ends become degraded leading sometimes to cell death. In an experiment, telomeres from Tetrahymena were transferred to the ends of linear plasmid DNA of yeast and these were then allowed to replicate in yeast. It has been noted that the addition of telomeric DNA helps the plasmid DNA to replicate as linear molecules showing thereby that telomeres are needed for replication. Telemore consists of repetitive DNA of large Kilo bases and are highly conserved containing clusters of G residues. The telomere sequences of mammals,

including human, axe AGGGTT. In Tetrahymena, the sequences are GGGGTT. Molecular studies show that the telomere sequences of a large number of eukaryotes are similar consisting of repeats of DNA sequences preferably clusters of G residues. The sequence of telomere repeats in human is AGGGTT, in Terahymean it is GGGGTT (Table 13.1). These telomeric sequences are repeated hundreds or telomeric replication thousands of times up to several kilo-bases.

(b) Telomeric Replication:

DNA polymerase has the capacity to synthesise a growing DNA chain in 5′ → 3′ direction but cannot synthesise up to telomeric ends. The replication process in telomere is unique and different. The problem of replication of telomeres has been done by a special mechanism with the help of an enzyme telomerase having reverse transcriptase activity. This enzyme (telomerase) is able to add telomeric repeats at 3′ end of the DNA strand forming a single-stranded overhang at the 3′ end of both template and new strand. Hence the 5′ end of each strand is shorter than 3′ end. Now the telomerase molecule incorporates an essential RNA molecule called Guide RNA at 5′ end which has specific sequences that are complementary to the telomere repeat. It then serves as a primer for telomere at 5′ end of the strand. When the elongation of the strand at 5′ end is complete—i.e., two ends of the strand are equal—then the splicing of RNA primer takes place and the gap is filled up by the polymerase.

1.7 METAPHASE CHROMOSOME BANDING

A gene is a functional unit of DNA, and your DNA is organized onto chromosomes. **Chromosome banding** is a little like tie-dying your chromosomes.

A **chromosome** is a unit of tightly-packed DNA. DNA has to wrap tightly around itself, because you have quite a lot of it. In fact, if you unrolled the entire DNA in a single one of your cells, it would be about three meters long. Humans have 46 chromosomes - 23 from Mom and 23 from Dad.

In **chromosome banding**, we treat chromosomes with chemicals to stain them and learn about a chromosome by how it stains. There are several different types of stains we can use.

There are several types of **chromosome banding**. Here, we will list a few of the most common types.

- **G-banding** uses a stain called Giemsa stain. G-banding gives you a series of light and dark stripes along the length of the chromosome. We will discuss G-banding in the most detail because you will likely see G-banding if you take genetics class.
- **Q-banding** uses a stain called quinacrine. Q-banding yields a fluorescent pattern. It is similar in pattern to G-banding but glows yellow.
- **C-banding** only stains the centromeres. Centromeres are little constricted portions of chromosomes. That's where sister chromatids (two copies of the same chromosome) will attach to each other when the cell is getting ready to divide.
- **R-banding** is the opposite of G-banding. R-banding stains complementary regions to those stained with G-banding and they are used together to determine chromosomal deletions.

Fig .1.6 Chromosome banding G binding

1.8 CHROMOSOME AND CHROMOSOMAL ABERRATIONS

Chromosomal aberrations, or abnormalities, are changes to the structure or number of chromosomes, which are strands of condensed genetic material. Humans typically have 23 pairs of chromosomes, of which 22 pairs are autosomal, numbered 1 through 22. The last pair of chromosomes is sex chromosomes, which determine an individual's sex assignment. At birth, most people with XY sex chromosomes are assigned male, and most individuals with XX are assigned female. In general, each parent contributes one set of chromosomes to their offspring, which collectively make up the 23 pairs of chromosomes. A change to any of the

chromosomes, in number or structure, creates a chromosomal aberration and may cause medical disorders.

What are the different types of chromosomal aberrations?

Chromosomal aberrations can be categorized as numerical or structural aberrations. Numerical aberrations, changes to the number of chromosomes present, are referred to as aneuploidies. The most common types of aneuploidy are monosomies, when only one chromosome of a pair is present, and trisomies, when there are three copies of a chromosome instead of a pair.

The four main types of structural chromosomal aberrations are deletion, duplication, inversion, and translocation. Deletions occur when a portion of the chromosome is deleted, or taken out, which can make that chromosome less functional. For example, when part of a short arm in chromosome 5 is deleted, this causes Cri-du-chat syndrome, common symptoms of which are reduced head size and high-pitched crying in infants. In duplication, part of the chromosome is duplicated, resulting in extra genetic material. Chromosomal aberrations are most often caused by errors during cell division. Cell division in humans occurs via mitosis or, only in sex chromosomes, meiosis. In mitosis, cells duplicate their chromosomes and produce daughter cells with an identical number of chromosomes as the original cell. In other words, a cell with 46 chromosomes will produce two cells, each with 46 identical chromosomes. Meanwhile, cell division by meiosis involves two rounds of cell division that allow for the recombination of genetic material, resulting in four sex cells with only half of the number of chromosomes. For example, a cell with 46 chromosomes undergoing meiosis will produce four unique daughter cells, each with 23 chromosomes.

Sometimes, chromosomal disorders are caused by mosaicism, when there are two or more different cell lines in one person. Mosaicism can occur after nondisjunction happens in a mitotic cell division during early embryonic development. This results in one line of cells with a chromosomal aberration while other lines may stay unchanged.Parents may reduce some risk of chromosomal aberrations in offspring by meeting their own nutritional needs, limiting exposure to problematic substances, and visiting a doctor before becoming pregnant. General risk reduction strategies include eating healthy, abstaining from smoking or drinking alcohol, and taking prenatal vitamins prior to pregnancy. Chromosomal aberrations are more likely to occur in pregnancies when the

pregnant individual is over the age of 35. If a chromosomal disorder has been identified in a family, a healthcare provider may recommend genetic counseling to discuss different options, including assisted reproduction techniques.

Resources: http:// www.nature

1.9 GIANT CHROMOSOMES: POLYTENE AND LAMPBRUSH CHROMOSOME

Giant chromosomes The following points highlights the five special types of chromosomes. The types are**:** 1. Polytene Chromosome or Giant Chromosome 2. B-Chromosome or Supernumerary Chromosome 3. Chimaera 4. SAT-Chromosome.

Special Types of Chromosomes: Chromosomes which significantly differ in structure and function from normal chromosomes are known as special chromosomes. Special chromosomes include lampbrush chromosome, polytene chromosome and B chromosome. i. Lampbrush Chromosome: These are special types of chromosomes in which large number of loops are projected out from the chromatin axis giving a lampbrush appearance. Such chromosomes are called lampbrush chromosomes. They are found in oocyte nuclei of both vertebrates and invertebrates and spermatocyte nuclei of Drosophila during diplotene stage. These chromosomes have three main features. a. Extra Ordinary Length: Lampbrush chromosomes have remarkable length. They are sometimes larger than polytene chromosomes. The length has been recorded up to 1 mm in urodele amphibian. b. Large Number of Loops: Lampbrush chromosomes have large number of loops. Loops are projected in pair from the chromomere .One to nine loops may arise from a single chromomere. The chromomere are connected by inter- chromomere fibres

Lamp-Brush Appearance: Projection of large number of pairs of loops from chromomeres leads to lampbrush appearance. The loops increase gradually in numbers, reach maximum in diplotene and gradually decline after diplotene and ultimately disappear. In diplotene stage, lampbrush chromosomes consist of two homologous chromosomes which are in contact only at certain points, called chiasmata. Each chromosome of the pair consists of two chromatids which lie together and

form the chromosome axis or main axis. The axis is differentiated into chromomeres (dark colour) and loops (light colour). Loops are formed on both sides of chromosomal axis. Each chromatid has one chromomere. The chromosomal axis, the chromomere and the loop axis all are made up of DNA and have hereditary function or are considered regions of genetic activity. ii. Polytene or Giant Chromosomes: The multiple replicates of the same chromosome holding together in a parallel fashion resulting in very thick chromosome are known as polytene chromosomes and such condition is referred to as polyteny. They were first reported by Balbiani (1881) in salivary glands of dipteran insects. Later on they were reported in salivary glands of Drosophila and several other insects. Since these chromosomes are generally found in salivary gland, they are also known as salivary gland chromosomes. These chromosomes have three main features as given below: a. Bands: The strips which are found in these chromosomes are known as bands. Some of the bands are visible in a swollen or expanded form which is known as puffs (Fig 4.4). When a puff becomes very much enlarged it is called a Balbiani ring.

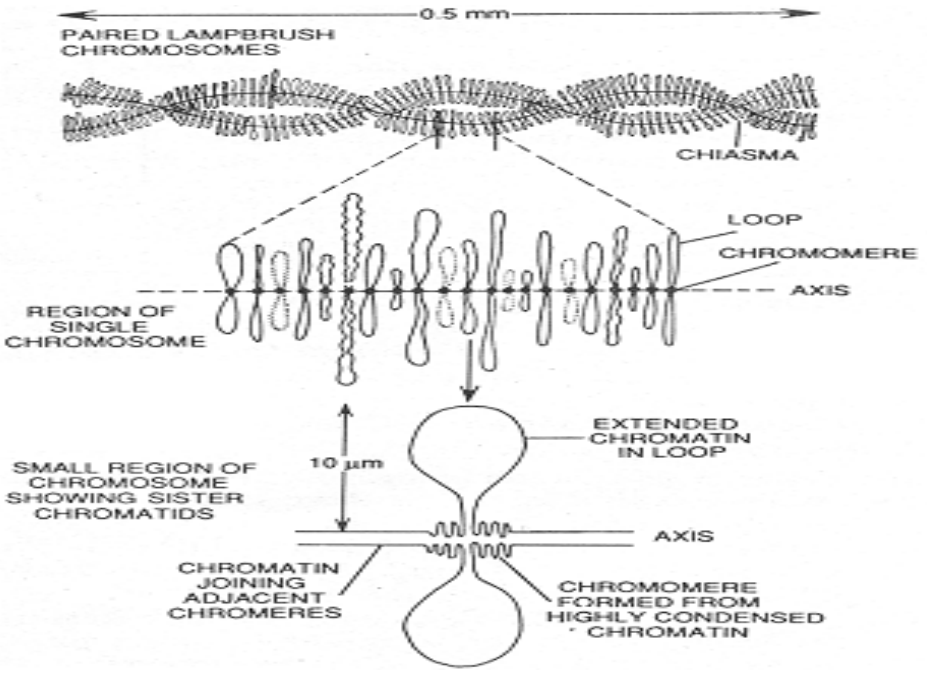


Fig 1.7 Lamp-Brush chromosome Source net

b. Puffs: The swollen regions are known as chromosome puffs or Balbiani rings. The puffs are reversible and considered as regions of genetic activity. Recently it has been found in Drosophila that each band contains the genetic material of a single gene. Origin of Puffs: Puffs originate from single band and are involved in RNA synthesis. The process of puff formation at different sites of polytene chromosomes is referred to as puffing. The first sign of puffing is the accumulation of an acidic protein at the pre-puff site followed by an increase in the rate of RNA synthesis at that site. There is a characteristic puffing in different tissues and at different time during larval development. The presence of a specific puff is related with the appearance of a specific protein, for example, salivary proteins were found to be associated with a particular puff. Significance of Puffs: The puffs represent sites of DNA synthesis, i.e., gene transcription. Transcription also occurs in the bands, but to a very small extent. The accumulation of ribonucleo protein has been demonstrated in the region of puff. Inhibitors of transcription such as actinomycin D and alpha aminitin prevent puff formation and lead to some amount of reduction of existing puffs. There is an increase in puffing during those stages of larval development when moulting hormone ecdysone is released from the prothorasic gland. This has also been shown experimentally by injection of ecdysone into fourth instar larvae which responds by increased formation of puffs. c. Giant Size: Polytene chromosomes have giant size. The size may be observed up to 200 times or more than the normal chromosomes. Because of their giant size, they are also referred to as giant chromosomes. These chromosomes are somatically paired and their number in the salivary gland cells always appears to be half of the normal somatic cells. Now these chromosomes have also been reported in malpighian tubes, larval fat bodies, debte gut epithelia, etc. These chromosomes can be easily studied in the salivary gland of Drosophila. For this purpose, the salivary glands are dissected out from third instar larvae and squashed inaceto-carmine. The slides can be viewed under light microscope. 1. Functions of Lampbrush chromosomes, (a) Synthesis of RNA: Functions of lampbrush chromosomes involve synthesis of RNA and protein by their loops. RNA is synthesized only at the thin insertion and then carried around the loops to the thick insertion. There it may be either destroyed or released into nucleus. (b) Formation of yolk material: There are some probabilities that lampbrush chromosomes help in the formation of certain amount of yolk material for the egg. II Polytene chromosomes: These are also giant chromosomes but relatively smaller than lampbrush chromosomes, found in the larvae of certain dipterans. Such banded chromosomes occur in the larval salivary glands, midgut epithelium, and rectum and Malpighian tubules of

various genera (Drosophila, Sciara, Rhynchosciara, and Chironomus). In these larvae the salivary glands contain salivary cells so large in size that they can easily be seen with the lens power of a dissecting microscope.

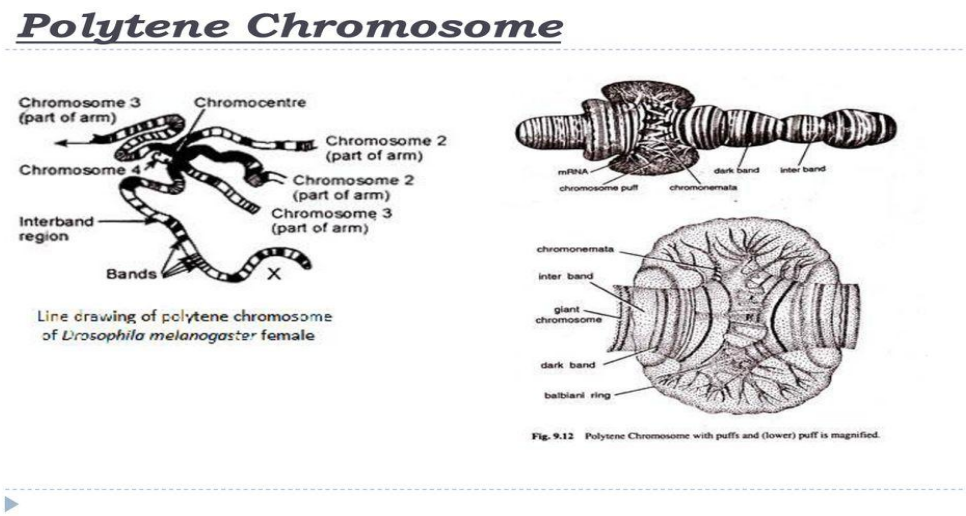


Fig 1.8 Polytene chromosome (source net)

Nuclei of these cells are much larger than those of ordinary cells being generally about 25μ in diameter, and chromosomes in nuclei are so large that they are 50 to 200 times as large as chromosomes in other body cells of the organism. They were first observed in 1881 by E.G. Balbiani in Chironomus and were studied by Korschelt (1884) and Corney (1884). Heitz and Bauer in 1933 studied these giant chromosomes in Bibio hortulanus larvae, while Painter (1933) described them in salivary glands of Drosophila. Because of their large size showing numerous strands these are named as polytene chromosomes (name suggested by Kollar) or commonly salivary gland chromosomes. The latter term is a misnomer as these chromosomes may occur in other somatic cells of body besides salivary gland cells. Polytene Function (1) Main function of the polytene chromosome is to carry genes which ultimately control physiology of an organism. These genes are formed of DNA molecules. (2) Shifting of heterochromatin in respect to euchromatin produces giant changes called position effects. These effects cause mutations in animals as well. (3) Heterochromatic regions contain fewer genes than euchromatic parts. Production of nucleolar material is entirely done by heterochromatin. (4) Chromosomes also help in protein synthesis

indirectly. Nucleolus contains RNA, and this RNA serves as a means of transmission of genetic information to the cytoplasm, leading to the formation of specific protein.

1.10 SUMMARY

In the nucleus of each cell, the DNA Molecule is packaged into thread-like structures called chromosomes. Each chromosome is made up of DNA tightly coiled many times around proteins called histones that support its structure. Chromosomes are not visible in the cell's nucleus—not even under a microscope—when the cell is not dividing. However, the DNA that makes up chromosomes becomes more tightly packed during cell division and is then visible under a microscope. Most of what researchers know about chromosomes was learned by observing chromosomes during cell division. Chromosomes are made up of a strand of DNA tightly wrapped around proteins called histones. This makes the DNA compact and able to fit inside the cell's nucleus. A gene is a segment of DNA that encodes for a trait. Therefore, genes are located on chromosomes. On each chromosome is a constriction point called a centromere, which divides the chromosome into different sections? Human cells normally contain 23 pairs of chromosomes, or 46 chromosomes total. An individual inherits 23 chromosomes from each parent.

DNA is a group of molecules that is responsible for carrying and transmitting the hereditary materials or the genetic instructions from parents to offspring's**.**Nucleic acids are the organic materials present in all organisms in the form of DNA or RNA. These nucleic acids are formed by the combination of nitrogenous bases, sugar molecules and the phosphate groups that are linked by different bonds in a series of sequences. The DNA structure defines the basic genetic makeup of our body. The chromosomes are the nuclear components of the special organization, individuality, and function that are capable of self-reproduction and play a vital role in heredity, mutation, variation and evolutionary development of the species. Chromosomal aberrations, or abnormalities, are changes to the structure or number of chromosomes, which are strands of condensed genetic material. Giant chromosomes the following points highlight the five special types of chromosomes. The types are: 1. Polytene Chromosome or Giant Chromosome 2 lamp brush chromosome.

1.11 TERMINAL QUESTIONS AND ANSWERS

1. Explain the chromosome structure and DNA material.
2. Describe the polyten chromosome and lamp brush chromosome.
3. What is Metaphase chromosome banding?
4. Explain the Nucleosome Model of Chromosomes.

1. DNA fingerprinting recognizes the differences in

(a) satellite DNA

(b) bulk DNA

(c) Repetitive DNA

(d) both (a) and (c)

Answer: (d)

2. This force can stabilize a DNA double-helix

(a) Hydrophilic sugar-phosphate groups are found on the exterior of the helix where interaction with water occurs

(b) Hydrophobic bases are present in the interior of the helix, each base-pair is stabilized by the same number of hydrogen bonds

(c) covalent base stacking interactions may take place between neighbouring bases within the same strand in the helix

(d) non-covalent N-glycosidic bonds may form between nitrogenous bases in opposite strands in the helix

Answer: (a)

3. In this type of DNA replication, of the two newly formed molecules, one is purely a new one and the other is an old one

(a) dispersive

(b) conservative

(c) semiconservative

(d) both (b) and (c)

Answer: (c)

4. The process of DNA replication is affected by an enzyme known as

(a) Mutase

(b) Ligase

(c) Polymerase I

(d) Ribonuclease

Answer: (c)

5. A DNA molecule in which both strands have radioactive thymidine is permitted to replicate in an environment that contains non-radioactive thymidine. What is the right number of DNA molecules which possess some radioactive thymidine post three duplications?

(a) one such molecule

(b) two such molecules

(c) four such molecules

(d) eight such molecules

Answer: (b)

6. If the DNA strand has nitrogenous base sequence ATTGCC, the mRNA will have?

(a) ATTGCA

(b) UGGACC

(c) UAACGG

(d) ATCGCC

Answer: (c)

7. The type of coiling in DNA is

(a) Zig-zag

(b) Left-handed

(c) Opposite

(d) Right-handed

Answer: (d)

8. In DNA, the enzyme which breaks the H2 bonds is

(a) Ligase

(b) Helicase

(c) Topoisomerase

(d) Polymerase

Answer: (b)

9. The total DNA comprises what amount of cytoplasmic DNA in cells?

(a) 95-99%

(b) 65-75%

(c) 45-50%

(d) 1-5%

Answer: (d)

10. The bases are held together in a DNA double helix by hydrogen bonds. These bonds are

(a) Ionic bonds

(b) Covalent bonds

(c) Non-covalent bonds

(d) Van der Waals forces

Answer: (c)

The term chromosome was coined by _____.

(a) Sutton

(b) Boveri

(c) Waldeyer

(d) Hoffmeister

Sol: (c) Waldeyer.

2. Chromosomes found in the salivary gland of Drosophila is_____.

(a) Polytene

(b) Lampbrush

(c) Supernumerary

(d) B-chromosomes

Sol: (a) Polytene.

3. Lampbrush chromosomes occur in_____.

(a) Oocytes

(b) Cancer cells

(c) Lymph glands

(d) Salivary glands

Sol: (a) Oocytes.

4. Which of the following is true about the Chromatids?

(a) It is a haploid chromosome

(b) It is a complete chromosome

(c) It is a duplicate chromosome

(d) It is one-half of the replicated chromosome

Sol: (d) It is one-half of the replicated chromosome.

5. The centromere is that part of the chromosome where_____.

(a) Nicking occurs

(b) Chromatids are attached

(c) Nucleoli are formed

(d) Crossing-over takes place

Sol: (b) Chromatids are attached.

6. The ends of the chromosome are called _____.

(a) Satellites

(b) Centromeres

(c) Telomeres

(d) Kinetochore

Sol: (c) Telomeres.

7. Chromosomes were first observed by_____.

(a) Fleming

(b) Waldeyer

(c) Strasburger

(d) Hoffmeister

Sol: (d) Hoffmeister.

8. A chromosome with sub-terminal centromere is_____.

(a) Acrocentric

(b) Acentric

(c) Metacentric

(d) Telocentric

Sol: (a) Acrocentric.

9. The giant chromosome with a number of chromonemeta is_____.

(a) Hetrochromosome

(b) Polytene chromosome

(c) Lampbrush chromosome

(d) Supernumerary chromosome

Sol: (b) Polytene chromosome.

10. A chromosome with centromere near the middle is called_____.

(a) Metacentric

(b) Acrocentric

(c) Telocentric

(d) Submetacentric

Sol: (d) Submetacentric.

11. Puffs or balbiani rings in the salivary gland chromosome are the sites of_____.

(a) Protein synthesis

(b) RNA synthesis

(c) DNA replication

(d) DNA duplication

Sol: (b) RNA synthesis.

12. Chromosomal theory of inheritance was proposed by_____.

(a) Sutton in 1902

(b) Boveri in 1902

(c) Correns in 1909

(d) Sutton and Boveri in 1902

Sol: (d) Sutton and Boveri in 1902.

13. More than 200 chromosomes occur in_____.

(a) Dog

(b) Amoeba

(c) Chicken

(d) Gorilla

Sol: (b) Amoeba.

14. A colour blind daughter may be born if the_____.

(a) Father is normal and the mother is a carrier

(b) Father is normal and the mother is colour blind

(c) Father is colour blind and mother is a carrier

(d) Father is colour blind and mother is normal

Sol: (c) Father is colour blind and mother is a carrier.

15. A somatic cell in a human male contains_____.

(a) No gene on the sex chromosome

(b) Genes on only on sex chromosomes

(c) Two genes for every sex-linked character

(d) Only one sex-linked gene for each character

Sol: (d) Only one sex-linked gene for each character.

16. The blue-green algae and bacteria contain _____.

(a) Three linkage groups

(b) Two linkage groups

(c) One linkage group

(d) None of the above

Sol: (c) One linkage group.

17. What is the number of linkage groups in the Drosophila?

(a) Two

(b) Four

(c) Eight

(d) None of the above

Sol: (b) Four.

18. Gene for colour blindness in man is located on _____.

(a) Both X and Y chromosome

(b) Y-chromosome only

(c) X-chromosome only

(d) Either X-chromosome or Y-chromosome

Sol: (c) X-chromosome only.

19. Chromosomal constitution in human females can be best written as_____.

(a) 46

(b) 44+2

(c) 44A+XY

(d) 44A+XX

Sol: (d) 44A+XX.

20. Which of the following disease is sex-linked?

(a) Hepatitis

(b) Leukaemia

(c) Malignancy

(d) Colour blindness

Sol: (d) Colour blindness.

UNIT 2: SEX CHROMOSOMES, SEX DETERMINATION AND DOSAGE COMPENSATION IN DROSOPHILA AND HUMAN BEINGS

CONTENTS

2.1 OBJECTIVES

In this unit you will learn

- About sex chromosomes.
- The theory of sex determination
- Dosage compensation in Drosophila and human beings.

2.2 INTRODUCTION

A chromosome is a long DNA molecule with part or all of the genetic material of an organism. They are thread-like structures located inside the nucleus of animal and plant cells. The chromosomes have been considered as the physical basis of heredity because they have a special organization, individuality, functions and are capable of self- reproduction. Each chromosome is made up of a single molecule of Deoxyribonucleic acid (DNA) and protein. They passed genetic informations from parents to offspring.Each chromosome is made up of DNA tightly coiled many times around proteins called histones that support its structure. DNA contains the specific instructions that make each type of living creature unique. Eukaryotic chromosomes mostly include packaging proteins called histones which, aided by chaperone proteins, bind to and condense the DNA molecule to maintain its integrity. These chromosomes display a complex three-dimensional structure, which plays a significant role in transcriptional regulation.

Chromosomes are not visible in the cell's nucleus—not even under a microscope—when the cell is not dividing. However, the DNA that makes up chromosomes becomes more tightly packed during cell division and is then visible under a microscope. Most of what researchers know about chromosomes was learned by observing chromosomes during cell division.

2.3 SEX CHROMOSOME

The sex chromosomes**,** either of a pair of chromosomes determine whether an individual is male or female. The sex chromosomes (X and Y) are of unequal size; shape and/ or staining quality (hence are heteromorphic). The X- chromosomes of man and *Drosophila* have been found to be straight,

rod –like and comparatively larger then Y chromosome. The Y chromosome of man and *Drosophila* is smaller than X chromosomes however, in Y chromosome of *Drosophila*, one end remains slightly curved or bent to one side. In one sex, there are two X chromosomes (XX) while in the other, there is only one X chromosome (X), whose partner is without gene and called the Y chromosome. A large number of genes carry the X chromosome in addition to those which determine the sex. The genes located in the X chromosomes and the characters controlled by them are called sex- linked as they are inherited in relation to the sex.

In humans the sex chromosomes contain of 23 pairs of chromosomes, a total of 46 chromosomes. The first 22 pairs of chromosomes are called autosomes. The chromosomes which have no relation with the sex and contain the gene which determine the somatic characters of the individuals are known as autosome (A). On genetical grounds, both X and Y chromosomes have many differences. Some portion of both types of chromosomes has homologous genes or identical, so called homologous region and remaining are called non-homologous or differential regions.

The genes which reside in differential or non-homologous regions X and Y chromosomes always inherit together according to differential regions of X and Y chromosome never undergo crossing over. Such genes are called completely sex – linked genes.

Types of sex chromosomes: In dioecious organisms, thus two types of chromosomes were recognized which are as follows:

(i) Autosomes: The chromosomes which have no relation with the sex and contain the gene which determine the somatic body characters of the individuals are known as autosomes (A).

(ii) Sexchromosomes: The chromosomes which are responsible for the determination of sex are known assex chromosome e.g., X and Y chromosomes.

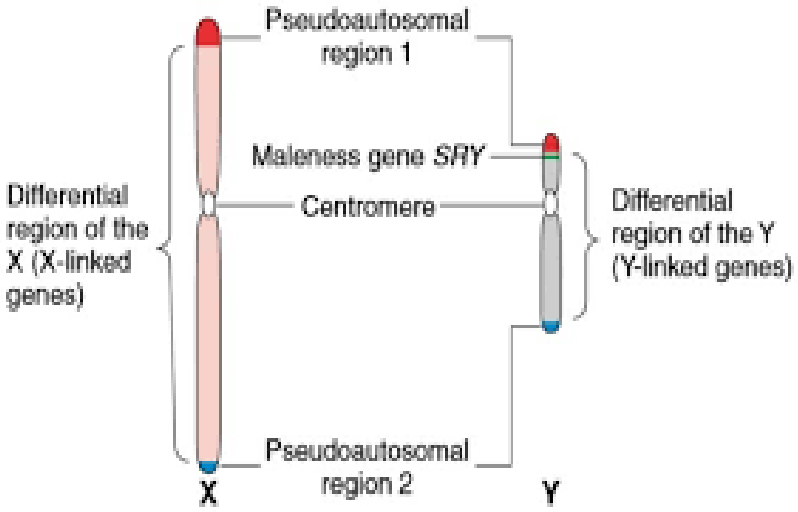


Fig.2.1 Sex chromosome (Source-https://www.google.com/search?q=Sex+chromosome+image&tbm=isch&ved=)

2.4 SEX DETERMINATION

The essential feature of sexual reproduction is the production of male and female gametes. This feature enables sexual reproduction to provide genetic variability. It is for this reason we believe that sexual reproduction has played an important role in evolution. The sex determination mechanisms were explained purely on the basis of sex chromosomes, the composition of which generally differed in male and female individuals.

Sex is the symbol of life which can be determined by several levels. It is the fundamental quality recognized in living organisms especially in higher forms of life. Primarily, sex determination relates to whether an individual develops testes or ovaries. Secondary sexual characters develop at certain periods of life, like the development of body hairs, spermatogenesis, oogenesis etc. which are also under the genetic control. All higher animals are present in two definite forms of life i.e., male and female which is an evolutionary outcome. Evidences are there which prove the sexual dimorphism has developed from a single origin.

The difficulty of sex determination remained a puzzle up to the year 1900. It was believed that nutrition of the developing embryo and the parents as well as internal and external environment

plays a role in determination of sex. There are three categories at the time of sex determination. Progamic: When the determination of sex takes place before the fertilization of the egg. Syngamic: determination takes place at the time of fertilization.

Epigamic: After the formation of zygote the sex is determined. Mostly determination of sex is progamic or syngamic.

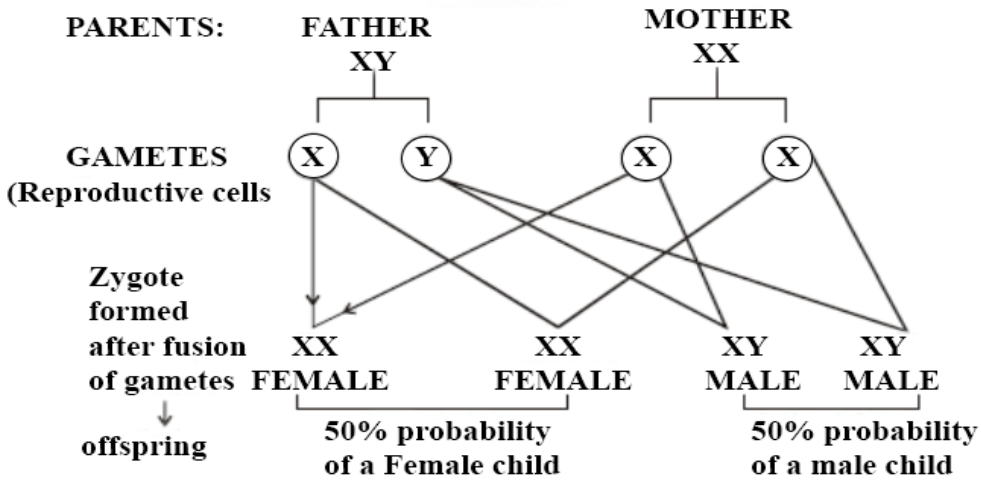


Fig.2.2 Sex determination in human

(Source:https://www.google.com/search?q=Sex+determination+in+human+image&client)

Modern geneticists have reported many different mechanisms of sex determination in living organisms. Some important and common mechanisms of sex determination are following:

A. Chromosomal theory of sex determination

According to the chromosome theory of sex determination, the male and female individuals would differ in their chromosome composition. There may be two types of chromosomes present in such individuals:i) Autosomes, that determines the phenotypic characters and ii) Sex chromosomes, that determines sex. In diploid individuals there are 2n-2 autosomes and two sex chromosomes. While in one sex, two sex chromosomes are homomorphic (XX), in the other sex these are heteromorphic (XY).

In (1891) Herman Henking observed dark stained chromatin element (namely X body) which was later found to be sex chromosome and named as X-chromosome. In (1905) Miss Stevens observed *Drosophila melanogaster* and found four pair of chromosomes. One pair of chromosomes was peculiar in male flies and one resembles with the X-chromosome of female while other is unequal in size. Later (1909), Wilson term this unusual chromosome as Y-chromosome. So, *Drosophila* could be described as XX female and XY male. The chromosome is responsible for sex determination was suggested by McClung (1902).

There are 23 pairs of chromosomes in human

- Female= (22 pairs of AA chromosomes + XX)
- Male = (22 pairs of AA chromosome + XY)

X and Y chromosomes differs from each other. In human, Y chromosome is shorter than X-chromosome while Y chromosome is larger than X-chromosome in *Drosophila.*

B. Genic balance theory of sex determination

In (1925) B. Bridge propose the genic balance theory of sex determination in which he declared that the sex of an individual is determined by a balance among the genes for maleness and femaleness present in an individual.

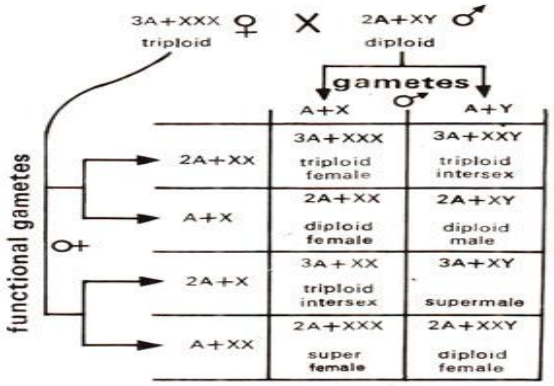


Fig.2.3Balance theory of sex determination X/A ratio in Drosophila

(Source:https://www.google.com/search?q=3Balance+theory+of+sex+determination+X/A+ratio+in+Drosophila)

Whereas in the study of *Drosophila,* it was established that Y chromosome is generally heterochromatin and play essential role in sex determination. The gene for maleness is present in autosomes whereas the gene for femaleness is present in X-chromosome. Therefore, all the individuals carry the gene for both male and female sex. However, it is actually the ratio among X-chromosome and the autosomes which governs the development of male or female sex. The ratio is recognized as sex index ratio.

Sex index ratio (X/A) = (X chromosome number/ Number of autosomes sets)

a. If the sex index ratio (X/A) is 0.5 or less, it gives male sex
b. If the sex index ratio (X/A) is 1 or more than 1 then it gives female sex
c. If the sex index ration (X/A) is between 0.5 and 1 then it gives intersex

Ploidy condition in *Drosophila*	**No. of X chromosome**	**No. of autosomal set (A)**	**Sex index ratio (X/A)**	**Phenotype character**
2n (XXX)	3	2	1.5	Super female (XXX)
3n	3	3	1	Female
4n	4	4	1	Female
2n	2	3	0.67	Intersex
2n	1	2	0.5	Male
3n	1	3	0.33	Super male
2n	2	4	0.5	Male

Table: 2.4 Shows sex expression in Drosophila according to sex index ratio

(Source-https://www.onlinebiologynotes.com/theories-of-sex-determination-in-organisms)

Genic balance theory representsthe sex determination in *Drosophila* on the basis of sex index , where Y-chromosome does not play role in sex determination. However, this genic balance theory of sex determination does not hold true for human or mammals and also in those individuals where Y chromosomes plays definite role in sex determination Fig. 2.3).

C. Haplo-diplomechanism of sex determination

Male haploidy or haplodiploidy parthenogenesis is particularly common in the hymenopterans insects such as ants, bees, sawflies and wasps. In these insects, since fertilized eggs develop into diploid females and unfertilized ones into haploid males, so **arrhenotoky (**a form of parthenogenesis in which the unfertilized eggs develop into males) is both a form of reproduction and a means of sex determination. Meiosis is normal in females, but crossing over and reduction in chromosomes number fail to occur during spermatogenesis in males due to their haploidy.

Thus arrhenotokous parthenogenesis determines the sex in hymenopterans and sex chromosomes have no identity here like the *Drosophila.* It seems that heterozygosity for specific genes induces femaleness;' that haploid can never be heterozygous.

D. Single gene effects sex determination

In certain organism such as *Chlamydomonas, Neurospora,Asparagus,*yeast, maize, *Drosophila*etc, sex determination is influenced by the differential action of single gene. The following cases are example for the determination of sex.

i) **Sex determination in *Neurospora*:** *Neurospora* has two sexes exactly identical and denoted as A and a. mating occurs only between gametes of unit sex (e.g.,AXa). The mating type A or a is determined by a pair of autosomal alleles and follows single Mendelian inheritance.

ii) **Sex determination in *Asparagus:*** *Asparagus*is adioeciousplant (a plant group that includes distinct male and female plants) however, sometimes the female flowers bear rudimentary anthers and the male flower with poorly developed pistil may set seeds. In one of the experiments when the seeds of such a rare male flower were raised into plants then the male and female plants were found to be present 3:1 ratio. When the

male plants raised thus were used to pollinate the female flowers on female plants, only two third of them showed segregation indicating that sex is controlled by a single gene. In this case, maleness should be dominant over femaleness and the male plants should ordinarily be heterozygous.

iii) **Monogenic sex determination in maize: maize is a monoecious plant (**a single plant that bears both male and female flowers) with male inflorescence (tassel) and the female inflorescence (silk) located on the same plant. A gene called tassel seeds converts the tassel into seeds bearing inflorescence, while another gene called silk less, (sk) is responsible for absence of silk. Therefore, a plant with genotype/ sk will be effectively a male plant and a plant with genotype ts/ts will be effectively a femaleplant. Thus, individual single gene (viz., sk or ts) can impose bisexually in maize.

iv) **Sex reversal gene (Sxr) in mammals:** Recently a sex reversal gene (Sxr) has been discovered in human being, so that in the presence of this gene XX female individuals may become males. Such cases of sex reversal are reported in goat and mice. Mice also contain two other gene Tdy and Tda-1 which interact, to cause sex reversal in XY male individuals to transform them into females.

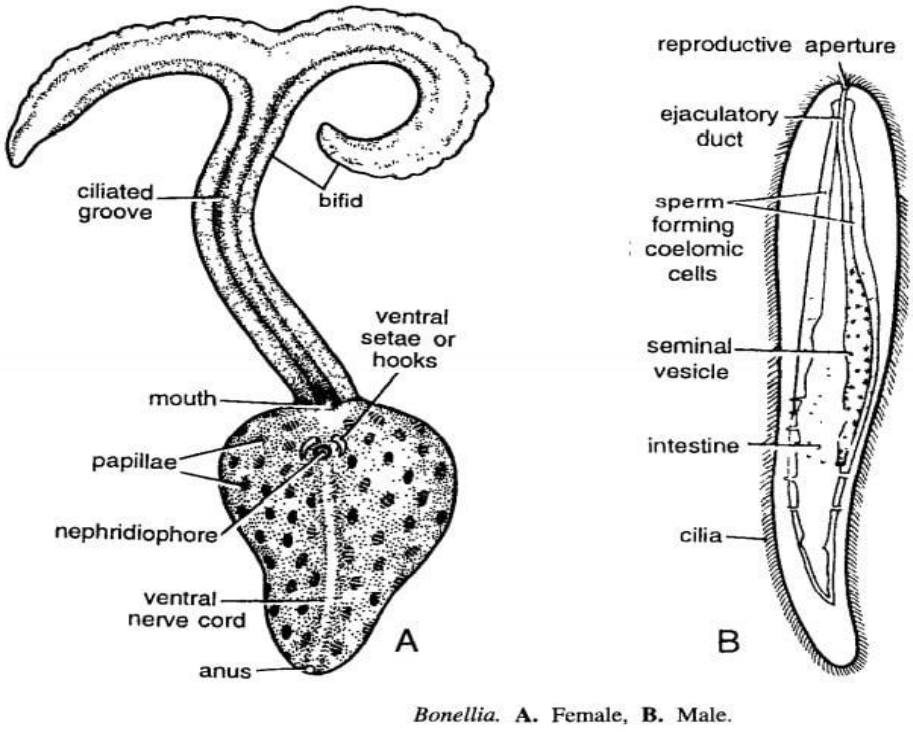
E. Metabolically controlled sex determining mechanism:

Certain workers have seen the possibility of sex determination in the phenomenon of metabolism. Crew suggested that sex is a physiological equitable division between anabolic and catabolic individuals. A. F. Shull and D. D. Whitney have shown that increasing metabolic rate in rotifers the occurrence of male individuals increase than the females. Likewise Riddle found that metabolism had some definite role in the determination of sex in pigeons and doves, because, increased rate of metabolism developed the male potency, while decreased rate of metabolism caused femaleness.

F. Hormonal control of sex determination:

A large number of cases are known where the sex is modified due to external environment or due to hormones secreted from the sex organs. Three examples of hormonal or environmental control of sex would be presented here.

a) Sex in *Bonellia:* In *Bonelliaviridis,* a marine worm, all the larvae are genetically and cytologically similar. If a particular larva settles near the proboscis of an adult female, it becomes a male individual. On the other, if it has to developed male is detached from the proboscis it become a female. Similarly, if a partly developed male is detached from the proboscis it becomes an intersex. Obviously, the proboscis secretes a substance suppressing femaleness.



Bonellia. **A.** Female, **B.** Male.

Fig. 2.3 The female and male of the echiuroid Bonellia

(Source:https://www.google.com/search?q=the+female+and+male+of+the+echiuroid+bonellia)

b) The Crew's hen: A case of complete sex reversal was reported in 1923 by Crew, where a fertile female fowl (hen), which had already produced offspring, changed over to a fully fertile male (cock) due to a damaged ovary in the female. It is believed that the ovary in the female secreted a male suppressing hormone. Therefore, in the absence of ovary, the testis could develop.

c) The freemartin: Another classical example of hormonal control of sex determination is found in cattle. In cattle, when twins calves of different sexes occur, the female one is

usually a sterile intersex called a freemartin. These are known as the freemartin after the name of the worker and would be produced only when there is a vascular connection between the two embryos. The male hormone perhaps suppresses the development of the ovary of the co-twin.

2.5 DOSAGE COMPENSATION

The method by which organisms adjust gene expression between individuals of different biological sexes is known as dosage compensation. Varying sexes are commonly distinguished by different types and numbers of sex chromosomes across animals. Diverse evolutionary branches have evolved various techniques to balance gene expression across the sexes in order to neutralise the substantial variation in gene dosage induced by differing numbers of sex chromosomes among the sexes. Different types of creatures have evolved differing strategies to cope with this discrepancy since sex chromosomes contain different quantities of genes. Because it is difficult to duplicate a gene, organisms instead equalise the expression of each gene. Females (XX) in humans, for example, silence the transcription of one of each pair's X chromosomes while transcribing all information from the other, expressed X chromosome. As a result, both females and men have the same number of expressed X-linked genes (XY), with both sexes having essentially one X chromosome per cell from which to transcribe and express genes.

Distinct lineages have evolved different ways to deal with the disparities in gene copy numbers observed on sex chromosomes between the sexes. Some lineages have evolved dosage compensation, an epigenetic mechanism that restores expression of X or Z-specific genes in the heterogametic sex to levels seen in the ancestor before the sex chromosome's development. Other lineages compensate for the expression of X- or Z-specific genes between sexes, but not at ancestral levels, implying that "dosage balancing" is insufficient. One example of this is X-inactivation in humans. The third reported type of gene dose regulatory mechanism is incomplete compensation without balance (sometimes referred to as incomplete or partial dosage compensation). In this system gene expression of sex-specific loci is reduced in the heterogametic sex i.e. the females in ZZ/ZW systems and males in XX/XY systems.

There are three essential mechanisms of attain dosage compensation which are widely documented in the literature and which are common to most species. These include random inactivation of one female X chromosome(as notice in *Mus musculus;* this is called X inactivation , a two-fold increasein the transcription of a single male X chromosome (as noticedin *Drosophila melanogaster*), and decreasetranscription by half in both of the X chromosomes of a hermaphroditic organism (as noticed in *Caenorhabditis elegans*). These mechanisms have been generally studied and manipulated in model organisms frequently used in the laboratory research setting. However, there are also other less common forms of dosage compensation, which are not as widely researched and are sometimes particular to only one species (as observed in certain bird and monotreme species).

2.5.1 DOSAGE COMPENSATION IN DROSOPHILA

In *Drosophila melanogaster*, dosage compensation takes place through the differential activity of some sex-linked (X-linked) genes. J. Tobler found about equal activity of some the enzyme tryptophanpyrolase in the two sexes, even though one X-linked gene for this eye pigmentation enzyme is present in male and two in females. Some regulating mechanism other than the loss of an X chromosome (Lyon hypothesis) is apparently responsible for this dosage compensation. It is assumed that in Drosophila, the dosage compensation in some cases results from the action of modifier in genes called dosage compensationgenes within the X chromosomes. These modifiers tend to cancel the effect of the different doses of a given gene by influencing genetic transcription, genetic translation or the biological activity of the protein products resulting from these processes.

- Dosage compensation is an epigenetic mechanism that balances gene expression from unequally distributed sex chromosomes between the sexes and in relation to the diploid autosomes. In *Drosophila melanogaster*, this is achieved by twofold up regulation of transcription from the single male X chromosome.
- The moderation of chromatin structure is a general principle of dosage compensation systems in numerous organisms. Concomitant with the evolution of sex chromosomes, pre-existing epigenetic regulators are often adapted for this novel task.

- In *D. melanogaster* males, the dosage compensation complex (DCC) uses the histone acetyltransferase MOF for global hyperacetylation of X-linked chromatin at histone H4 at lysine 16 (H4K16ac). The H4K16ac modification prevents chromatin compaction and is generally related with enhanced DNA availabilityand transcription.
- Identification of the X chromosome by the DCC involves the dynamic interplay between male sex lethal (MSL) proteins, male-specific RNAs on the X (roXs), and a limited number of X-specific DNA sequence elements. The DCC spreads from these high-affinity binding sites (HASs) to the transcribed regions of active genes, where it recognizes features of active chromatin such as transcription coupled histone marks.
- The dosage compensation complex (DCC) induces substantial alterations in the local and long-range structure of X-linked chromatin. The resulting permissive conditions within the X-chromosomal territory create a uniquely active compartment, leading to activation even of autosomal genes that get translocated in this environment.

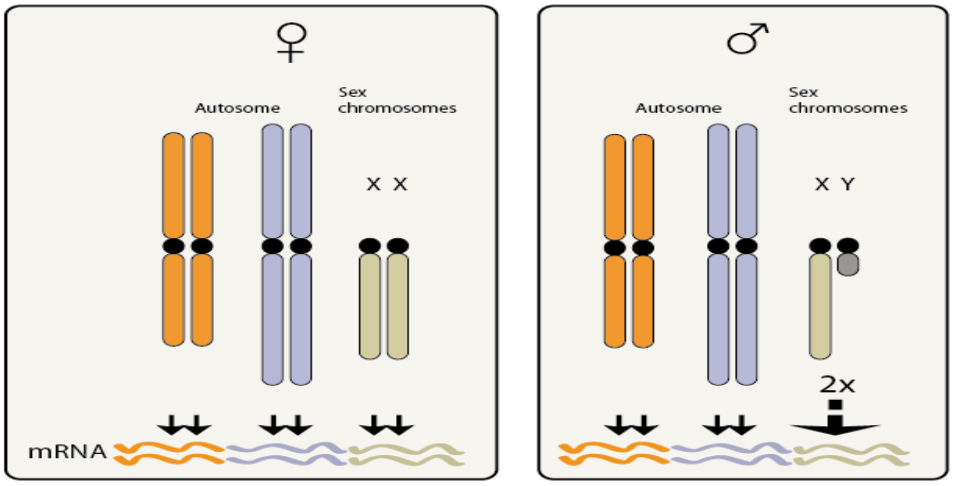


Fig. Dosage compensation in Drosophila melanogaster

(Source:https://www.google.com/search?q=Fig.+Dosage+compensation+in+Drosophila)

- The exact mechanism of transcriptional activation remains enigmatic to date. Traditionally, transcription elongation is thought to be enhanced by H4K16ac in the transcribed regions of genes, and recent evidence supports this idea. However, some data suggest that transcriptional initiation as well as the release of paused Pol II from gene promoters might also be targeted by the dosage compensate.

2.5.2 DOSAGE COMPENSATION IN HUMANS

In humans, as in several other organisms, there is a major chromosomal difference among the sexes. For example, humans have 22 pairs of chromosomes that are present in both males and females, known as autosome. In each pair, one member of inherited from the mother and one from the father. However there are two X and Y chromosomes,the sexchromosomes that differ between the sexes. The females have two Xs, while males have one X and one Y. This matter because even if the X is a medium-sized chromosome with more than 1000 genes, the Y is small and gene poor. A comparable condition exists in other mammals, as well as rodents and marsupials. Chromosomal variances are connected to the process by which sex is determined and seem to have evolved over several millions of years.

Sex chromosome difference presents the organism with a difficulty. The two sexes vary in the copy number of X-linked genes. This can lead to a difference in the amount of gene products (RNAs and proteins), which would, in turn, require variation in metabolic control and other cellular processes. To avoid this, dosage compensation mechanisms have evolved that balance the level of X-linked gene products between the sexes. There are three common methods by which this can be performed: first, a twofold up-regulation in the expression of X-linked gene in males; second, a twofold down-regulation of genes on each of the two X chromosomes in females; and lastly, the whole inactivation of one of the two X chromosomes in females. The first plan has been adopted in the fruit fly, *Drosophila* the second in the worm *Caenorhabditis elegans* and, it now seems, both the first and the last in mammals.

The studies of dosage compensation in mammals have obtainable crucial insights into essential epigenetic process and how patterns of equalized between the sexes and relative to the autosomes. In humans, females with two X gene expression are control through development. It can be surely predicted that they will pursue to do so.

X chromosome dosage requires being chromosomes undergo X inactivation of one chromosome; the remaining active X up-regulates its genes twofold. In flies, both female X chromosomes are active; male X-linked genes are up-regulated twofold. In worms, which utilize a

hermaphrodite/male sex determination pathway, hermaphrodite's express X-linked genes at half the rate of males, with both genotypes expressing two times the amount of X-linked genes.

2.6 SUMMARY

A chromosome is a long DNA molecule with part or all of the genetic material of an organism. They are thread-like structures located inside the nucleus of animal and plant cells. Each chromosome is made up of a single molecule of Deoxyribonucleic acid (DNA) and protein. They passed genetic informations from parents to offspring. Each chromosome is made up of DNA tightly coiled many times around proteins called histones that support its structure.

Chromosomes are not visible in the cell's nucleus—not even under a microscope—when the cell is not dividing. However, the DNA that makes up chromosomes becomes more tightly packed during cell division and is then visible under a microscope.

The sex chromosomes, either of a pair of chromosomes determine whether an individual is male or female. The sex chromosomes (X and Y) are of unequal size; shape and/ or staining quality (hence are heteromorphic). In one sex, there are two X chromosomes (XX) while in the other, there is only one X chromosome (X), whose partner is without gene and called the Y chromosome. A large number of genes carry the X chromosome in addition to those which determine the sex. The genes located in the X chromosomes and the characters controlled by them are called sex- linked as they are inherited in relation to the sex.

In humans the sex chromosomes contain of 23 pairs of chromosomes, a total of 46 chromosomes. The first 22 pairs of chromosomes are called autosomes.

The essential feature of sexual reproduction is the production of male and female gametes. This feature enables sexual reproduction to provide genetic variability. It is for this reason we believe that sexual reproduction has played an important role in evolution. The sex determination mechanisms were explained purely on the basis of sex chromosomes, the composition of which generally differed in male and female individuals.

The method by which organisms adjust gene expression between individuals of different biological sexes is known as dosage compensation. Varying sexes are commonly distinguished by different types and numbers of sex chromosomes across animals. Diverse evolutionary branches have evolved various techniques to balance gene expression across the sexes in order to neutralise the

substantial variation in gene dosage induced by differing numbers of sex chromosomes among the sexes.

In *Drosophila melanogaster*, dosage compensation takes place through the differential activity of some sex-linked (X-linked) genes.

In humans, as in several other organisms, there is a major chromosomal difference among the sexes. For example, humans have 22 pairs of chromosomes that are present in both males and females, known as autosome. In each pair, one member of inherited from the mother and one from the father. However there are two X and Y chromosomes, the sex chromosomes that differ between the sexes.

The studies of dosage compensation in mammals have obtainable crucial insights into essential epigenetic process and how patterns of gene expression are control through development. It can be surely predicted that they will pursue to do so.

2.7 TERMINAL QUESTION AND ANSWERS

1. Multiple Choice Questions:

1. _____ discovered XY sex chromosomes

A. M J D White

B. NettilStevans

C. R Brown

D. Mendel

2. The chromosomes accounted for sex determination are referred to as

A. Heterosis

B. Multiple alleles

C. Allosomes

D. Autosomes

3. Theory of linkage was put forward by

A. De Vries

B. Sutton

C. Bateson and Punnet

D. Morgan

4. Both Drosophila and mammals have XY system of sex determination. Dosage compensation y inactivation of one X chromosome is seen in _____

A. Mammals

B. Drosophila

C. Metaphase

D. Telophase

5. Which one of the following is true for Drosophila?

A. They don't have X chromosome

B. One of the female X chromosome is inactivated

C. The male Y chromosome is inactivated

D. The male X chromosome is hyper active

Answers: 1 B, 2 C, 3 D, 4 A, 5 D.

2. Short Answer Question:

1. What is sex determination?

2. What is dosage compensation?

3. Explain genic balance theory of sex determination?

4. In short describe the chromosomal theory of sex determination.

3. Long Answer Question:

1. Describe the different theories of sex determination.

2. What is sex determination? Describe various examples of sex chromosomal mechanism of sex determination.

3. Explain dosage compensation in *Drosophila.*

4. Describe the dosage compensation in human beings.

2.8 GLOSSARY

Autosome: an autosome is one of the numbered chromosomes, as opposed to the sex chromosomes.

Chromosome: a long, stringy aggregate of genes that carries heredity information (DNA) and is formed from condensed chromatin.

Deoxyribonucleic acid (DNA): The molecule that carries the genetic information for most living systems. DNA molecule consists of four bases (adenine, cytosine, guanine, and thymine) and a sugar-phosphate backbone, arranged in two connected strands to form a double helix.

Dioecious: the animals in which male and female reproductive organs are present in separate individuals are called dioecious animals.

Gene: a segment of chromosome that encodes the necessary regulatory and sequence information to direct the synthesis of a protein or RNA product.

Gene Expression: The process through which a gene is activated at particular time and place so the functional product is produced.

Heteromorphic: differing from the normal form in size, shape, and function

Oogenisis: is the process of formation of female gametes.

Spermatogenesis: is the process by which haploid spermatozoa develop from germ cells in the seminiferous tubules of the testis.

Transcription: the enzymatic process, involving base pairing, by which the genetic information contained in DNA is used to specify a complementary sequence of base in an RNA molecule.

Sex- chromosome: a sex chromosome is a type of chromosome involved in sex determination. Humans and most other mammals have two sex chromosomes, X and Y that in combination determine the sex of an individual. Females have two X chromosomes in their cells, while males have one X and one Y.

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Unit 3: CELL DIVISION

CONTENTS

3.1 *OBJECTIVES*

After studying this unit, students will learn

- Mechanism of Mitosis and Meiosis
- Cell cycle regulation

3.2 *INTRODUCTION*

The organism size depends generally on its total cell mass, which depends on both the total number of cells and cell size. Cell number depends on the balance between cell division and cell death. Unicellular organisms grow and divide as fast as they can and nutrients availability determines their rate of proliferation. In multicellular organism cells divide only when more cells are needed by the organism. Three fundamental processes determine the size of an organism: cell growth, cell division, and cell death. These processes are independently regulated by intracellular programs and extracellular signal molecules. The extracellular signal molecules mainly are soluble secreted proteins, proteins bound to the surface of cells, or components of the extracellular matrix.

The factors that promote organism growth are of three major classes:

1) Mitogens: - Stimulate cell division by relieving intracellular negative controls that otherwise block progress through the cell cycle. First discovered mitogen is platelet-derived growth factor (PDGF).
2) Growth factors: - Stimulate cell growth by promoting the synthesis of proteins and other macromolecules and by inhibiting their degradation.
3) Survival factors: - Promote cell survival by suppressing apoptosis.

Formation of a new cell required division of the paternal cell into two daughter cells. Cell division encompasses a controlled process of activation of a number of molecular events encoded in the DNA and executed by proteins. Cell division process involves a period of cell growth, during which proteins are made and DNA is replicated, followed by cell division that results in division of a cell into two daughter cells. Cell division is a highly regulated process and only happens when an organism replaces worn out cells or makes more cells in response to a new need (growth of muscle

in response to exercise or damage). If the regulation of cell cycle gets disturbed it results in formation of tumor (cancerous or noncancerous).

3.3 *MITOSIS*

The cell cycle is generally governed by the four coordinated processes: cell growth, DNA replication, distribution of the duplicated chromosomes to daughter cells, and cell division. In bacteria, cell growth and DNA replication take place throughout most of the cell cycle, and duplicated chromosomes are distributed to daughter cells in association with the plasma membrane. Eukaryotic cell cycle is characterized by four discrete phases. DNA synthesis occurs during a particular phase of cell cycle and the replicated chromosomes are then distributed to daughter nuclei by a complex series of molecular mechanisms leading to cell division. All these stages are well regulated by a particular regulatory system and progression between stages of the cell cycle is controlled by a regulatory system. Mitosis is the method in eukaryotes for partitioning the genome equally during cell division. In Plant and animal cells it is done with the help of mitotic apparatus (captures the chromosomes and then pushes and pulls them to opposite sides of the dividing cell) and distributes the DNA equally.

Phases of the Cell Cycle

The cell cycle comprises two phases: Interphase and Mitosis. Interphase is marked by the decondensed chromosomes which are distributed throughout the nucleus, so the nucleus appears morphologically uniform. At Interphase cell growth and DNA replication occur in an orderly manner to prepare cells for division. Mitosis is separation of daughter chromosomes and usually ends with cell division (cytokinesis). However, because mitosis and cytokinesis last only about an hour, interphase—the time between mitoses—takes up around 95 percent of the cell cycle. The chromosomes are decondensed and disseminated throughout the nucleus during interphase, resulting in a morphologically homogeneous nucleus. Interphase, on the other hand, is the time when both cell growth and DNA replication proceed in an orderly manner in preparation for cell division at the molecular level. Some cells in adult animals stop cell division altogether (e.g. nerve cells) and many other cells divide only occasionally, when injured and dead cells are needed to

replace (skin fibroblasts and internal organs, such as the liver, kidney, and lung). These cells exit G_1 to enter a quiescent stage of the cycle called G_0, where they remain metabolically active until cell division is initiated by appropriate extracellular signals.

The G_1 phase of the cell cycle denotes the time between mitosis and the start of DNA replication (gap). The cell is metabolically active and grows at a constant rate during G_1, but it does not replicate its DNA. Following G_1, the S phase (synthesis) occurs, during which DNA replication occurs. The G2 phase (gap 2) follows the end of DNA synthesis, during which cell growth continues and proteins are generated in preparation for mitosis.

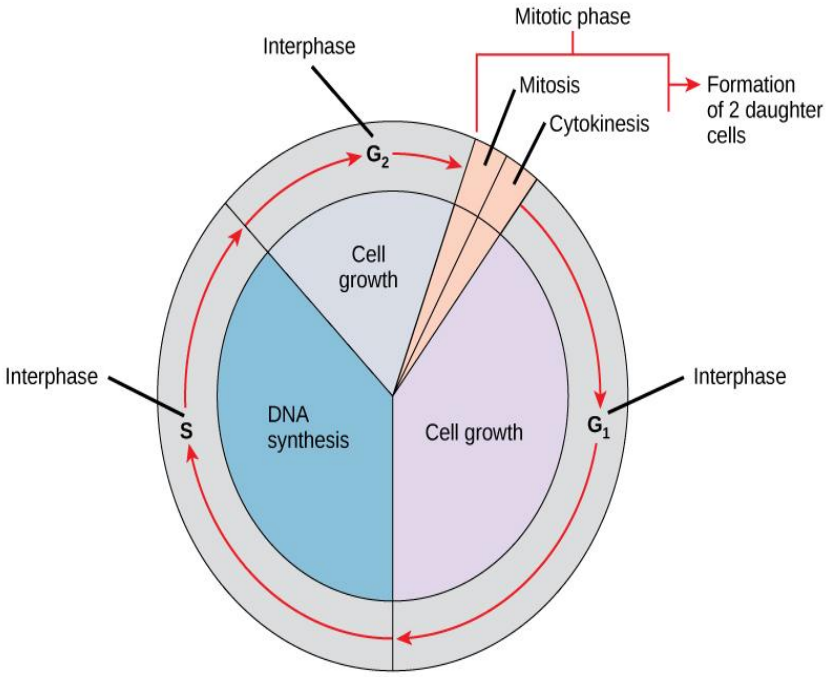

Fig.3.1 Phases of cell cycle (Credit: https://commons.wikimedia.org/wiki/File:Figure_10_02_01.jpg)

Cell cycle duration varies from organism to organism and between cell types. Duration of cell cycle in human cells is 24 hours (G_1 phase 11 hours, S phase 8 hours, G2 phase 4 hours, and M phase 1 hour). Budding yeast's cell cycle is about 90 minutes. Early embryo cells have an even shorter cell cycle of 30 minutes or less shortly after fertilization of the egg. In this case, though, cell growth does not occur. Instead, these early embryonic cell cycles rapidly divide the egg

cytoplasm into smaller cells. There is no G_1 or G_2 phase, and DNA replication occurs very fast leading to very short S phases alternating with M phases.

Regulation of the Cell Cycle

The progression of cell cycle is highly regulated by extracellular signals from the environment, and internal signals which monitor and coordinate the various processes of cell cycle. In yeast (*Saccharomyces cerevisiae)* cell cycle regulatory point occurs late in G_1 and controls progression from G_1 to S, where it is known as START. Once cells have passed START, they are committed to entering the S phase and undergoing one cell division cycle. The passage through START is tightly regulated by external signals, such as the availability of nutrients, as well as by cell size. During nutrient shortage yeasts cells arrest their cell cycle at START and enter a resting state rather than proceeding to S phase. Polypeptide factors that signal yeast mating also arrest the cell cycle at START, allowing haploid yeast cells to fuse with one another instead of progressing to S

In most animal cells division is similarly regulated in the G_1 phase of the cell cycle. Regulatory point is called the restriction point in animal cells, and functions analogously to START in yeasts. The animal cell cycle is regulated by extracellular growth factors that signal cell proliferation. When appropriate growth factors are present cells pass the restriction point and enter the S phase. After passing the restriction point the cell is committed to proceed through S phase and the rest of the cell cycle, even in the absence of further growth factor stimulation. In absence of appropriate growth factors during G_1 phase, progression through the cell cycle stops at the restriction point. Such arrested cells then enter a quiescent stage of the cell cycle called G0.

Generally the cell cycle is regulated primarily in G_1 but in some cases cell cycle is instead controlled principally at G_2. In fission yeast *Schizosaccharomycespombe* cell cycle is regulated primarily by control of the transition from G_2 to M, which is the principal point at which cell size and nutrient availability are monitored. Vertebrate oocytes can remain arrested in G_2 for long periods of time (several decades in humans) until their progression to M phase is triggered by hormonal stimulation.

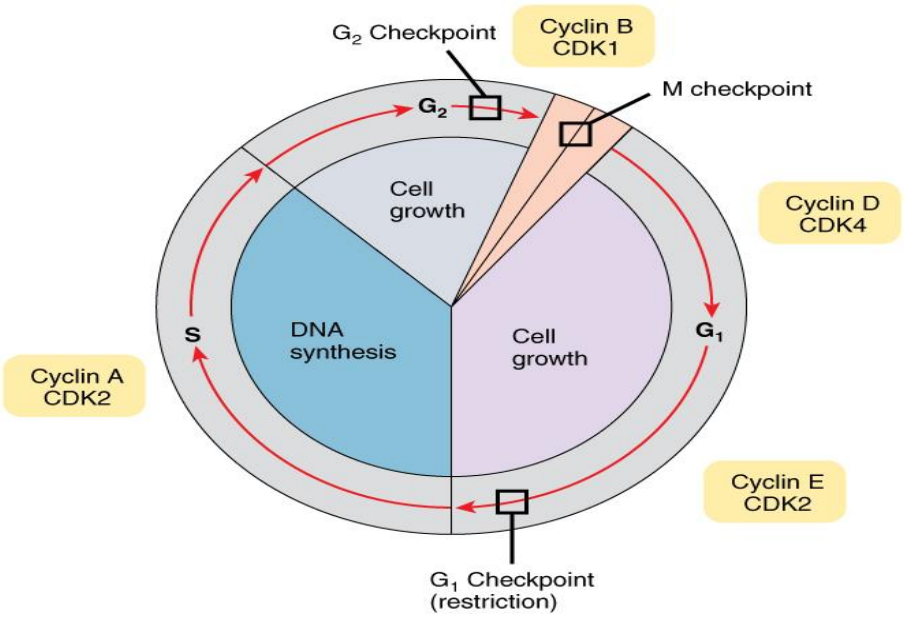

Fig.3.2 Cell cycle Regulation (Credit: https://commons.wikimedia.org/wiki/File:0332_Cell_Cycle_With_Cyclins_and_Checkpoints.jpg)

Cell Cycle Checkpoints

The cell cycle events are highly coordinated with one another because it is very important that DNA replication is complete before mitosis. If division occurs without complete DNA replication the daughter cell will fail to inherit complete copies of the genome. Generally different phases of the cell cycle coordinate with one another via a system of checkpoints and feedback controls that prevent entry into the next phase of the cell cycle until the events of the preceding phase have been completed.

At G_2 phase various checkpoints ensure that incomplete or damaged chromosomes are not replicated and passed on to daughter cells. They prevent the initiation of mitosis until DNA replication is completed by sensing unreplicated DNA, which generates a signal that leads to cell cycle arrest. G_2 checkpoint prevents the progression to M phase before complete DNA replication. G_2 checkpoint also ensures that DNA is not damaged and if it is damaged, they arrest the cell cycle in G_2 state. Cell cycle will only proceed when the damage is repaired.

DNA damage not only arrests the cell cycle in G_2, but also arrests cell cycle progression at a checkpoint in G_1. This checkpoint arrest may allow repairing of the damaged DNA before the cell cycle enters S phase. In mammalian cells, arrest at the G_1 checkpoint is mediated by the action of a protein known as p53, which is rapidly induced in response to damaged DNA (mutation in p53 causes cancer). The p53 protein is a transcriptional regulator which stimulates expression of the Cdk inhibitor p21.

Checkpoint towards the end of mitosis maintains the integrity of the genome that occurs toward the end of mitosis. It ensure proper alignment of chromosomes on the mitotic spindle, thus ensuring that a complete set of chromosomes is distributed accurately to the daughter cells

The DNA should only replicate once per cell cycle, to ensure this, control mechanisms prevent initiation of a new S phase prior to mitosis. These control mechanisms regulate cells in G_2 from reentering S phase and block the initiation of another round of DNA replication until after mitosis, to the point when the cell has entered the G_1 phase of the next cell cycle.

Family of proteins (called MCM proteins) is involved in restriction of DNA replication once per cell cycle. They bind to replication origins together with the origin replication complex (ORC) proteins. MCM protein binding to DNA is regulated and it can only bind to DNA (replication origin during G_1stage allowing DNA replication to initiate when the cell enters S phase. Once initiation has occurred, the MCM proteins are displaced from the origin, so that replication cannot initiate again until the cell passes through mitosis and enters G_1 phase of the next cell cycle.

Eukaryotic cell cycle is under the control of a conserved set of protein kinases, which control the transition of the cell cycle from one stage to another stage. They are cdks (cyclin dependent kinases and cyclin protein. They are first discovered in oocytes and named as MPF (Maturation promoting factor). The MPF not only restricted to the entry of oocytes into meiosis but it also present in somatic cells, where it induces transition from G_2 to M mitotic cycle. MPFis a dimer of Cdc2 and CyclinB. Cyclin B is a regulatory subunit which regulates the catalytic property of the Cdc2 protein kinase by phosphorylation and dephosphorylation of Cdc2.

In mammalian cells, cyclin B production start at S phase. Cyclin B then accumulates and forms complexes with Cdc2 throughout S and G_2. This complex remain inactive throughout S and G_2 because Cdc2 is phosphorylated at two critical regulatory positions by Cdc2 kinase activity and protein kinase called Wee1 that eventually inhibits Cdc2 activity and leads to the accumulation of inactive Cdc2/cyclin B complexes throughout S and G_2.

The Progression from G_2 to M occurs by activation of the Cdc2/cyclin B complex as a result of dephosphorylation by an enzyme protein phosphatase called Cdc25. The activated Cdc2 protein kinase phosphorylates a variety of target proteins that results in initiation of M phase events. Cdc2 activity also leads to ubiquitin-mediated proteolysis of cyclin B, which results in inactivation of Cdc2. Inactivation of Cdc2 cause cell to exit mitosis, undergo cytokinesis and return to interphase.

Various researches then shows that Cdc2 and cyclin B are members of large families of related proteins and members of these families regulate different phase of cell cycle. In Yeast, Cdc2 when associated with mitotic B-type cyclins (Clb1, Clb2, Clb3, and Clb4) regulate transition from G_2 to M. Passage through START is regulated by Cdc2 and G_1cyclins or Cln's. Progression through S phase is regulated by Cdc2 and different B-type cyclins (Clb5 and Clb6), these different associations cause Cdc2 to phosphorylate different substrate proteins for the transition of cell cycle through a particular phase,

Eukaryotic cell cycle is regulated by multiple cyclinsand Cdc2-related protein kinases known as Cdk's (cyclin-dependent kinases). Cdc2 is also known as Cdk1 and other members are named from Cdk2 through Cdk8. Cdk in association with specific cyclins, control the progression of cell cycle through different stages. As, G_1 to S progression is regulated by Cdk2 and Cdk4 (and in some cells Cdk6) with cyclins D and E. Progression through restriction point in G_1 is regulated by Cdk4 and Cdk6 with the D-type cyclins (cyclin D1, D2, and D3). Cyclin E which is expressed later in G_1 with Cdk2 control transition from G_1 to S transition and initiation of DNA synthesis. Cdk2/cyclin A control progression of cells through S phase. G_2 to M transition is control by Cdc2 with cyclin B.

Mitosis is divided into four sub stages

Prophase is marked by breakdown of nuclear membrane and releasing of replicated chromosomes, (each comprising two identical chromatids and condensed into compact packets) into cytoplasm. The newly synthesized DNA molecules remain intertwined throughout S and G_2 but during the process of condensation become untangled. The condensed sister chromatids are then held together at the centromere. Centromere is a sequence of DNA to which proteins bind to form the kinetochore. Kinetochore is the site of attachment of the spindle microtubules. During prophase cytoplasmic changes lead to the development of the mitotic spindle. The centrosomes (which had duplicated during interphase) separate and move to opposite sides of the nucleus. During late prophase centrosomes serve as the two poles of the mitotic spindle.

As the prophase completes the cell enters prometaphase (transition period between prophase and metaphase). Prometaphase is marked by attachment of microtubules of the mitotic spindle to the kinetochores of condensed chromosomes. The kinetochores of sister chromatids are oriented on opposite sides of the chromosome, so they attach to microtubules emanating from opposite poles of the spindle. At Metaphase chromosomes align on the metaphase plate in the center of the spindle and spindle fibers attach to the centromere of each pair of sister chromatids.

Anaphase is marked by sorting of chromosomes, and movement of each chromatid of a pair to opposite sides of the cell. Most cells spend a brief period in metaphase before proceeding to anaphase. The transition from metaphase to anaphase is triggered by breakage of the link between sister chromatids leading to their separation and movement to opposite poles of the spindle.

Telophase leads to re-formation of a membrane around each set of chromosomes. Chromosome decondensation also occurs during telophase. Cytokinesis usually begins during late anaphase and is almost complete by the end of telophase, causing division of the cytoplasm to yield two daughter cells

3.3.1 *ROLE OF MATURATION PROMOTING FACTORS (MPF)*

MPF and Progression to Metaphase

Condensins, a protein complex, help in driving chromosome condensation by wrapping DNA around itself, compacting chromosomes into the condensed mitotic structure. Cdc2 protein kinase phosphorylate condensins leading to chromatin condensation by activating condensins as cells enter mitosis. Nuclear envelope breakdown, one of the most dramatic steps of mitosis, is initiated by maturation promoting factors (MPF). Phosphorylation of lamins caused by Cdc2 leading directly to depolymerization of the nuclear lamina. The nuclear membrane is then fragmented into minute vesicles, which eventually fuse to generate new daughter nuclei during telophase. Golgi and ER membrane similarly fragment into small vesicles by MPF. Golgi and ER membrane breakdown is also induced by MPF, and may in part be mediated by Cdc2 phosphorylation of the Golgi matrix protein GM130.

Increase in the activity of MPF leads to dramatic change in the dynamics of microtubules. First, increase in the rate of microtubule disassembly, causing depolymerization and shrinkage of the interphase microtubules. The disassembly of microtubule is as result of phosphorylation of microtubule-associated proteins, either by MPF itself or by other MPF-activated protein kinases. Furthermore, the quantity of microtubules originating from the centrosomes rises, replacing the interphase microtubules with a significant number of short microtubules emerging from the centrosomes.

3.3.2 *CHROMOSOMAL MOVEMENT*

Proteolysis and the Inactivation of MPF: Anaphase and Telophase

Cell cycle progression from metaphase to anaphase is initiated by ubiquitin-mediated proteolysis of key regulatory proteins, triggered by activation of a ubiquitin ligase called the anaphase-promoting complex. MPF causes the anaphase-promoting complex to activate at the start of mitosis, hence MPF eventually causes its own destruction. The anaphase-promoting complex remains inactive until the cell passes the metaphase checkpoint. After passing metaphase

checkpoint, activation of the ubiquitin degradation system leads to transition from metaphase to anaphase and progression through the rest of mitosis.

The sister chromatids are attached to mitotic spindle originated from different poles and the attached mitotic spindle exert force on the two sister chromatids to pull them towards opposite poles. But this pulling forces are initially resisted due to tight attachment between two sister chromatids. The sister-chromatids are attach to each other due to a complex of proteins called the cohesin complex. Anaphase starts with breaking of chromosome attachment and their movement to opposite poles of the spindle. This process involves the activation of the APC enzyme complex. APC cause degradation of a protein called securin. Before anaphase, securin binds to and inhibits the activity of a protease called separase. So, at the end of the metaphase the destruction of securin releases separase, which in turn cleave one of the subunits of the cohesin complex leading to the degradation of the cohesin complex, and the separation of sister chromatids. This separation of sister chromatids allow them to segregate by moving to opposite poles of the spindle.

3.3.3 *EXIT FROM MITOSIS*

Degradation of cyclin B by anaphase-promoting complex leads to inactivation of MPF, which is required for the cell to exit mitosis and return to interphase. Inactivation of MPF leads to reassembly of the nuclear envelope, chromatin decondensation, and the return of microtubules to an interphase state.

3.3.4 *CYTOKINESIS*

At the end of the cell cycle the cell is divided into two daughter cells by the process of cytokinesis. This process is initiated in late anaphase by the inactivation of MPF. In animal cells cytokinesis is mediated by a contractile ring of actin and myosin II filaments that forms beneath the plasma membrane. The position of this ring is determined by the location of the mitotic spindle. Cell cleavage occurs from the plane that passes through the metaphase plate perpendicular to the spindle. Contraction of the actin-myosin filaments pulls the plasma membrane inward and causes the pinching of the cell in half.

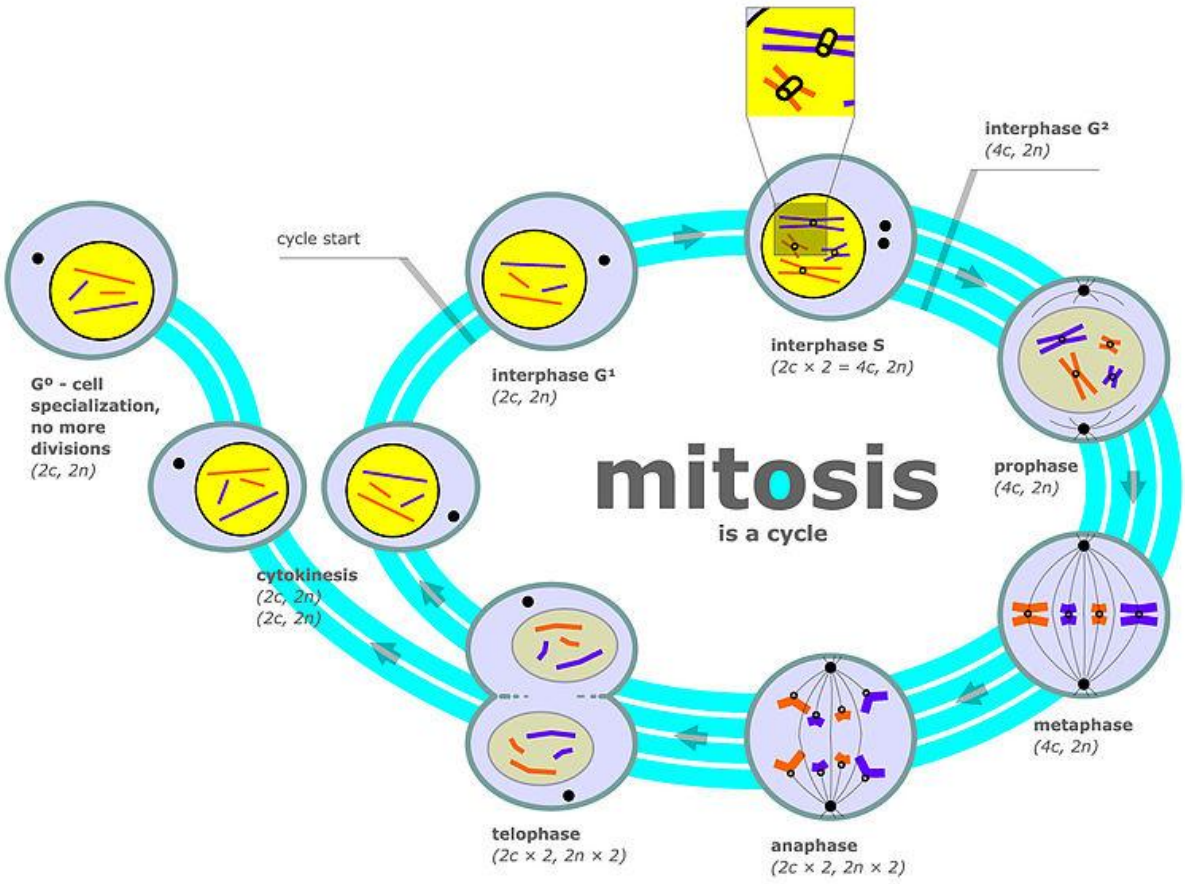

Fig.3.3 Mitosis (Credit: https://commons.wikimedia.org/wiki/File:Mitosis_diagram.jpg)

3.4 *MEIOSIS*

Mitosis involves division of a cell into diploid daughter cells with identical genetic complements. In contrast meiosis results in production of haploid daughter cells (reduces the chromosome number by half). In animal, meiosis occurs only in germ cells to produce haploid gametes (the sperm and the egg). During fertilization fusion of two haploid gametes results in production of diploid zygote.

Meiosis is a reductional division resulting in reduction of chromosome number, which is accomplished by two sequential rounds of nuclear and cell division (called meiosis I and meiosis II), though DNA replication occurs only once.

After completion of S phase (DNA replication) meiosis I is initiated. Chromosome segregation pattern in meiosis is different from mitosis, here homologous chromosomes first pair with one another and then segregate to different daughter cells. Meiosis I result in the formation of daughter cells containing a single member of each chromosome pair (consisting of two sister chromatids).

Meiosis I is followed by meiosis II, which is similar to mitosis in that the sister chromatids separate and segregate to different daughter cells. Meiosis II thus results in the production of four haploid daughter cells.

3.4.1 *CHROMOSOME PAIRING AND RECOMBINATION*

Recombination is a very significant process that occurs during meiosis. Pairing of homologous chromosome occur during prophase of meiosis I. Prophase of meiosis I is divided into five stages (leptotene, zygotene, pachytene, diplotene, and diakinesis) on the basis of chromosome morphology. Leptotene is marked by initial association of homologous chromosomes by base pairing between complementary DNA strands before the chromatin becomes highly condensed. During the zygotene stage synapsis takes place with the help of zipperlike protein structure, called the synaptonemal complex, which forms along the length of the paired chromosomes. Synaptonemal complexes maintain homologous chromosomes close association and alignment with one another through the pachytene stage. Recombination occurs during pachytene stage, leaving the chromosomes linked at the sites of crossing over (chiasmata; singular, chiasma).

Diplotene stage is marked by synaptonemal complex disappearances and separation of homologous chromosomes. However each chromosome pair (bivalent) consists of four chromatids remains associated at the chiasmata, which is critical for their correct alignment at metaphase. Finally, in Diakinesis chromosomes are fully condensed and the cell cycle progresses to metaphase.

During metaphase I the bivalent chromosomes align on the spindle and the kinetochores of sister chromatids are adjacent to each other and oriented in the same direction, while the kinetochores of homologous chromosomes are pointed toward opposite spindle poles. So, microtubules from the

same pole of the spindle attach to sister chromatids, while microtubules from opposite poles attaches to homologous chromosomes.

Anaphase I is marked by chiasmata disruption at which homologous chromosomes are joined. The homologous chromosomes get separated, while sister chromatids remain associated at their centromeres. Cytokinesis results in each daughter cell acquiring one member of each homologous pair, consisting of two sister chromatids.

Meiosis II starts immediately after cytokinesis. Meiosis II resembles a normal mitosis. During meiosis II the chromosomes align on the spindle with microtubules from opposite poles of the spindle attached to the kinetochores of sister chromatids during metaphase II. During anaphase II the link between the centromeres of sister chromatids is broken leading to segregation of sister chromatids to opposite poles. Cytokinesis leads to formation of haploid daughter cells.

3.4.2 *GENETIC REGULATION OF MEIOSIS*

Maturation promoting factor like mitosis, here also induces chromosome condensation, nuclear envelope breakdown and formation of the spindle. Anaphase promoting complex B activation results in progression from metaphase to anaphase during meiosis I with decreased activity of MPF. MPF activity increases again after cytokinesis and high activity of MPF arrest the cell in metaphase II. In oocytes the activity of MPF during metaphase is maintained by a cytostatic factor (CSF). Mos(protein-serine/threonine kinase) is an essential component of CSF. During meiosis the Mos is synthesized by the cell to increase the activity of MPF during meiosis II and to maintain its activity during metaphase II arrest. So, activation of Mos is done by ERK MAP kinase, which in turn is activated by another protein kinase called Rsk, which inhibits action of the anaphase-promoting complex and arrests meiosis at metaphase II.

The egg is surrounded by very large numbers of sperm at one time, so as to ensure that one sperm fuse to give rise to normal diploid embryos a regulatory system works. Binding of sperm to the plasma membrane receptor of egg increases the level of Ca^{2+} in the egg cytoplasm because of hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2). Increases in intracellular Ca^{2+} level result in alterations that prevent additional sperm from entering the egg. The surface alterations are

due to Ca^{2+}-induced exocytosis of secretory vesicles present in large numbers beneath the plasma membrane of the egg. They release their contents which alters the egg's extracellular coat resulting in further sperm entry. Increase in cytosolic Ca^{2+} due to fertilization also initiates completion of meiosis. The progression of metaphase to anaphase during metaphase II arrest is also initiated by Ca^{2+}-dependent activation of the anaphase-promoting complex. MPF inactivation causes completion of the second meiotic division, with asymmetric cytokinesis (as in meiosis I) giving rise to a second small polar body.

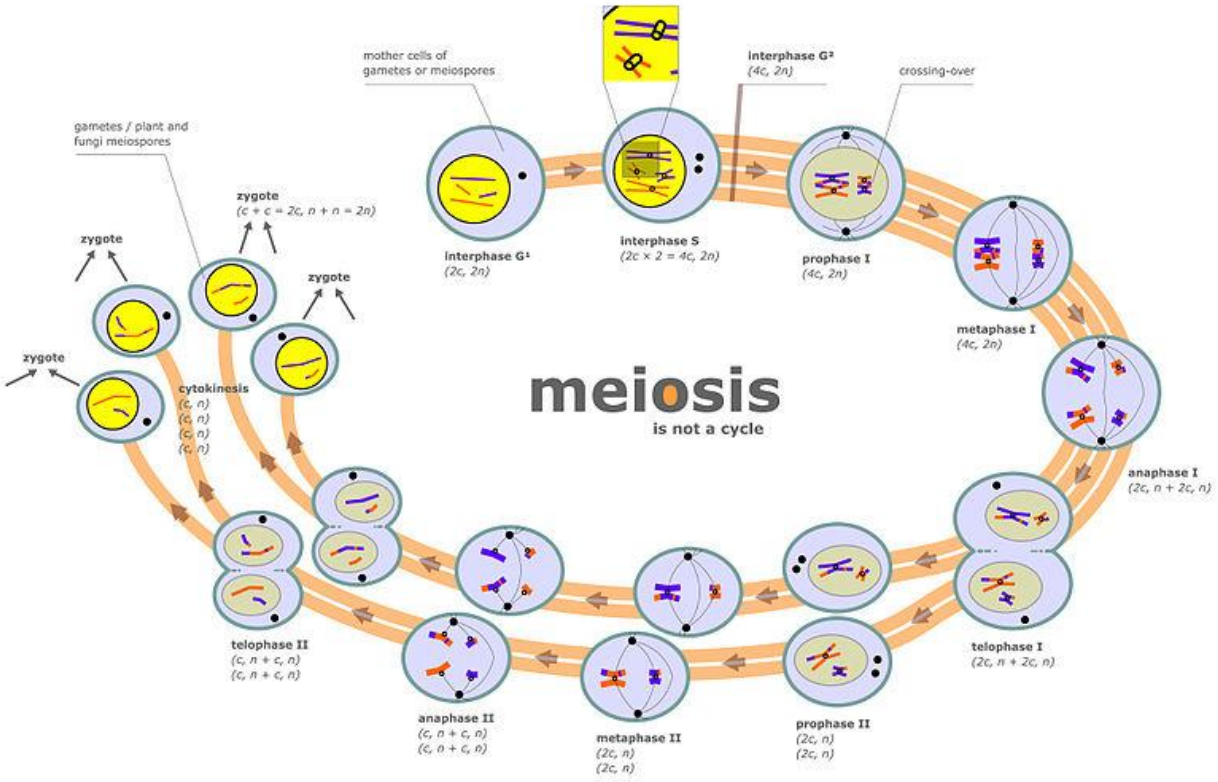

Fig 3.4 Meiosis (Credit: https://commons.wikimedia.org/wiki/File:Meiosis_diagram.jpg)

3.5 *SUMMARY*

Cell number depends on the amounts of cell division and cell death. Unicellular organisms grow and divide as fast as they can and nutrients availability determines their rate of proliferation. In multicellular organism cells divide only when more cells are needed by the organism. Three

fundamental processes determine the size of an organism: cell growth, cell division, and cell death. Some cells in adult animals stop cell division altogether (e.g. nerve cells) and many other cells divide only occasionally, when injured and dead cells are needed to replace (skin fibroblasts and internal organs, such as the liver, kidney, and lung). These cells exit G_1 to enter a quiescent stage of the cycle called G_0, where they remain metabolically active until cell division is initiated by appropriate extracellular signals. Cell cycle duration varies from organism to organism and between cell types. Duration of cell cycle in human cells is 24 hours (G_1 phase 11 hours, S phase 8 hours, G_2 phase 4 hours, and M phase 1 hour). Cell cycle is highly regulated and controlled by the checkpoints. Maturation promoting factor induces chromosome condensation, nuclear envelope breakdown, and formation of the spindle. Mitosis is the method in eukaryotes for partitioning the genome equally during cell division. Plant and animal cells with the help of mitotic apparatus (captures the chromosomes and then pushes and pulls them to opposite sides of the dividing cell) distribute the DNA equally. Mitosis involves division of a cell into diploid daughter cells with identical genetic complements. In contrast, meiosis results in production of haploid daughter cells (reduces the chromosome number by half). Meiosis is a reductional division resulting in reduction of chromosome number, which is accomplished by two sequential rounds of nuclear and cell division (called meiosis I and meiosis II), though DNA replication occurs only once.

3.*6 TERMINAL QUESTIONS& ANSWERS*

1. Cells which no longer divide remain in which phase of the cell cycle?

A. S phase

B. G0 phase

C. M phase

D. Prophase

2. When pair of sister chromatids align at the center of the cell is called

A. Metaphase

B. Interphase

C. Prophase

D. Anaphase

3. CDK is a protein

A. That bind to RNA
B. That bind to DNA
C. That bind to Cyclin
D. None of the above

4. Which of the following is the primary growth phase of the cell cycle?

A. G0
B. G2
C. M
D. G1

5. DNA replication occur in which stage of the cell cycle

A. M
B. S
C. G1
D. G2

6. In which phase of the cell cycle nuclear envelope formation occur

A. Metaphase
B. Anaphase
C. Telophase
D. Prophase

7. Condensation of chromosome occur in which phase of cell cycle

A. Prophase
B. Metaphase
C. G2
D. Anaphase

8. P53 is

A. Involve in cytokinesis
B. Involve in cell cycle regulation
C. Involve in protein structure
D. All of the above

9. Sequences that correctly describes the cell cycle is

A. G1-S-M-G2-cytokinesis
B. S-M-G2-G1-cytokinesis
C. G1-S-G2-M-cytokinesis
D. M-G1-G2-S-cytokinesis

10. Which event does not occur during interphase,

A. Chromatin condenses
B. Protein synthesis
C. Organelles replication
D. DNA replication

Answers 1(B), 2(A), 3(C), 4(D), 5(B), 6(C), 7(A), 8(B), 9(C), 10(A)

Q11. Describe the process of cell cycle.

Q12. Discuss various cell cycle checkpoints.

Q13. Write a short note

a) Maturation Promoting Factor
b) Genetic Regulation of meiosis
c) Chromosome pairing and recombination

Q14. Differentiate between Mitosis and Meiosis.

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UNIT 4 SOMATIC CELL GENETICS

CONTENTS

4.1 *OBJECTIVES*

In this unit you will learn

- What is cell fusion?
- How does Fusion take place?
- Heterokaryon-selecting hybrid and chromosome segregation
- Application of somatic cell genetics

4.2 *INTRODUCTION*

To understand various physical, physiological and biochemical processes, biochemical analysis is required. Biochemical procedures generally require isolation of large quantities of cells and then their physical disruption to obtain their components. If tissue samples are taken, a heterogeneous population of cells is obtained. To study a single type of cell in this heterogeneous population of cells, biologists have developed techniques to isolate cells from tissues and separating the various types. Homogeneous population of cells can then be analyzed—either directly or after increasing their number by cell culture. The good quantity of viable dissociated cells is typically obtained from fetal or neonatal tissues. The cells can be isolated from tissue by digesting their extracellular matrix using proteolytic enzymes (such as trypsin and collagenase) and adding agents (such as ethylenediaminetetraacetic acid, or EDTA) that bind, or chelate, the Ca^{2+} on which cell-cell adhesion depends. After this by gentle agitation tissue can be teased apart. To separate different cell types from the mixture of cell suspension various techniques are used. Centrifugation is one of those techniques used to separate large cells from the small cells and also different components of the cells. Another method employed is based on the ability of some cell types to adhere strongly to glass or plastic, which permits them to be separated from cells that adhere less strongly. Use of antibodies can increase the specificity of this technique. They specifically bind with the surface molecule of only one type of the cell in a tissue and can be coupled to a particular matrix (collagen, polysaccharide beads, or plastic) to create an affinity surface which allows only cells recognized by the antibodies to adhere. The cell then can be unbound by gentle shaking or by treating with enzymes (trypsin to digest the proteins that mediate the adhesion and collagenase in the case of a digestible matrix collagen). Antibodies can also be coupled with fluorescent dye to

label particular cells. The labeled cells can then be separated by an electronic fluorescence-activated cell sorter. Microdissection process can also be utilized for separation of cells. In this procedure cells can be acquired by dissecting them from thin tissue slices that have been prepared for microscopic examination. The Homogeneous population of the cells can be used for biochemical analysis and as a starting material for cell culture. Cell culture increases the number of a particular type of cell and this helps in understanding the complex nature of the cell under particular conditions of a culture dish. Under a proper environment, cells can survive, multiply and can express differentiated properties in a tissue-culture dish. These cells are observed continuously under the microscope and their biochemical analysis is also done. Effects of various factors (e.g. Hormone or growth factors) can be studied. The interaction between two different cell types can be studied by mixing them together.

Experimental design to settle a controversy in the field of neurobiology led to the beginning of tissue culture in 1907. To culture tissue cells require the surface to grow and divide (surface of a culture dish). The culture dish is usually coated to specific extracellular matrix components, such as collagen or laminin. Primary cultures are the cells prepared directly from the tissue sample without their proliferation in the culture dish. These primary cultured cells are then used for the proliferation of a large population of cells called secondary culture. These cells can be subculture for weeks or months. Initially fluid clots were used as a medium for cell culture but now were replaced by liquid media (containing salts, glucose, amino acids, and vitamins). Horse or fetal calf serum or a crude extract of chick embryos is also used in most media. But use of these media made it difficult for researchers to know which particular macromolecule a specific type of cell requires to thrive and to function normally. So, this problem is solved by various serum-free, chemically defined media. These media also contain specific proteins (growth factors and transferrin) required for cell survival and proliferation. Normal eukaryotic cells stop dividing after a finite number of cell divisions in culture, a process called cell senescence. This limited proliferation capacity is because of progressive shortening of the cell's telomeres (repetitive DNA sequences at the ends of each chromosome). Enzyme telomerase maintains telomeres but it is turned off in human somatic cells. Some human cells have long telomeres but still they stop dividing in culture because of activation of cell cycle control that arrests the cell cycle. So, to immortalize these cells, telomerase is added as well as activation of the checkpoint is done using certain cancer-promoting oncogenes

derived from tumor viruses or chemicals. Cancer cells easily generate cell lines and they grow without attaching to a surface. Both transformed and immortal cell lines can be stored in liquid nitrogen at -196°C and used for a long period of time. Human embryonic stem (ES) cell lines are commonly used in cell culture. These cells are harvested from the inner cell mass of the early embryo. ES have the ability to proliferate indefinitely while retaining their ability to produce any part of the body. Cells of a cell line are almost similar but not identical. To produce genetically similars cell cloning is done, in which a single cell is isolated and allowed to proliferate.

4.3 *CELL FUSION AND HYBRIDS*

When mixture of different parental cells are treated with either polyethylene glycol (PEG) or inactivated Sendai virus there plasma membranes fuses with adjacent cells, mixing the cytoplasm and incorporating the different nuclei into a single large cell called a heterokaryon (*hetero,* "different" and *karyon,* "chromosome"). When these heterokaryon undergoes cell division, the fusion of different nuclei occur, results in combining the different sets of chromosomes in one large nucleus to form a somatic cell hybrid. During the fusion process not only, different types of cells fused to form heterokaryons but same types of cells also fused to form homokaryons. For example, fusion between two parental cells A and B, results in formation of five different types of cells in various proportions: unfused A cells, unfused B cells, A-A homokaryons, B–B homokaryons, and the desired A–B heterokaryons. The desired hybrid cells are occur in small amount, because the total percentage of cells that undergo fusion in a treated mixed cell culture is quite low and they are also concealed among hybrids derived from two (or more) cells of the same type.

Now we have to select desired hybrid cells from the mixture. Let's suppose only A type of parental cell can survive under certain selection conditions, and B type of parental cell can survive under a different set of selection conditions. When the mixture of hybrid cells is cultured under combined selection pressures, only A–B hybrids that have acquired the capability to survive under both sets of conditions will be recovered. This process is called complementation, and its use for selection of desired hybrid is called somatic hybrid selection.

Somatic cell hybridization helps in study of gene interactions between somatic cells by formation of hybrids in intraspecific or interspecific crosses. The phenotypic changes in cell hybrids (permanent or transient) are monitored quantitatively by conditional genetic markers. The interaction between two cells can be studied by heterokaryons. Example if Chicken red blood cells fuse with tissue culture cells then their nucleus interacts with cytoplasm of a growing cell leading to reactivation of RNA synthesis and replication of DNA. When it is exposed to the cytoplasm of a growing tissue-culture cell by fusion.

4.3.1 *AGENTS AND MECHANISM OF FUSION*

One cell fuse with another cell to form a heterokaryon, a combined cell with two separate nuclei. To fuse two different cells various methods are used like treatment of a suspension of cells with inactivated viruses or with polyethylene glycol, each of which alters the plasma membranes of cells in a way that induces them to fuse. Numbers of methods are used for the fusion of the cells; Sendai virus is one of those. First the virus was inactivated by using UV irradiation without affecting its fusion efficiency. Then sendai virus will be mixed with the cells leading to fusion of their membranes and mixing of their cytoplasm. The fusion of similar or different cells results in formation of multinucleated homo- and heterokaryons respectively. It was therefore possible to remove the difficulty of virus replication in fused cells. Fusion of a variety of cell types, including differentiated and nondividing cells is possible because many cells carry receptors for Sendai virus, including those of different species. B-propiolactone is also used for the inactivation of the virus, most of it is subsequently degraded by phagocytic vacuoles of the cell. Other viruses like Newcastle disease viruses are less used for cell hybridization. A measure of control of fusion efficiency is possible by encouraging cell contact, either in suspension or on a surface, and by changing the proportion of each cell type used. The susceptibility of fusion varies between different cells and it depends on the presence of virus receptors and composition of the plasma membrane of the cell.

Chemical fusion methods have advantages over virus fusion methods because they prevent fuse cells refractory to virus. Lysolecithin and other lipids can be used for fusion but their use is limited because they cause extensive cell lysis. Another promising alternative is microsurgery. In which

pre-selected cells, synchronized in telophase, can be aligned and induced to fuse by micromanipulation and hybrid cells can then be isolated without the use of selection media. This method is inconvenient then mass fusion methods but it provides a method to control and directly observe early clone evolution. In addition to viruses and lipids, other techniques like high temperature, Ca at pH 10.5, or phospholipase C can initiate fusion. These systems have the advantage of synchronized, massive fusion which can be easily monitored by phase-contrast microscopy. Polyethylene glycol (PEG) is also used for the fusion process. PEG has advantage over Sendai virus as it is easily commercially available and consistency of quality was recognized quickly.

4.3.2 *VARIOUS STEPS ARE INVOLVED IN THE FUSION PROCESS.*

Firstly, the virus binds with the cell surface receptor which is independent of ions and occurs readily in the low temperature. Fusion process is temperature dependent. Then the two closely adjacent membranes fuse and membrane continuity is established. At this stage the cells appear more spherical in suspension. Membrane disruption leads to a repair process and there is not significant turnover of phospholipids or other membrane components. Virus-induced fusion is optimal at 7.6-8.0 pH. Ca^{++} and Mn^{++} cations are critical for the fusion process in most of the cases and may play a role in membrane stabilization or in a repair process since cell lysis results in their absence.

Fusion is an energy dependent process and uses oxidative phosphorylation or glycolysis as an energy source. Human erythrocytes and their ghosts can fuse without the use of ATP. Dibucaine (Local anesthetics), drugs of the phenothiazine class and cytochalasin B inhibit fusion. They act either directly on the plasma membrane or by an indirect mechanism.

4.3.3 *LIPID-INDUCED FUSION*

Small-chain saturated fatty acids, unsaturated fatty acids (oleic acid) and their esters and retinol can fuse with avian erythrocytes, particularly in presence of dextran to control cell lysis. The fusion induced by lipid requires presence of Ca^{++} ions but it occurs at pH of 5-6. The fusion of animal cells can also be induced by Liposomes made up of phospholipids.

4.4 *HETEROKARYON-SELECTING HYBRID AND CHROMOSOME SEGREGATION*

In the cell culture different types of cells are present and from this mixture, the hybrid cells are isolated. Heterokaryons cells contribute only a small portion in the complex fusion mixture of cells and only 10% of these cells can develop into a permanently hybrid cell line. Several selection techniques based on variations of cell morphology, growth characteristics, and genetic markers which screen for "mutants," or "variants," in somatic cells are there. Various methods of hybrid selection are fluorescent probes, the flow cytometer or biochemical inhibitors. One method of hybrid selection is the exclusion of parents with different genetic defects by a combination of mutant selection techniques that permits hybrids with complementary functional genes to grow. In 1964 Littlefield introduced this concept for eukaryotic cells. Initially it was used to select hybrids produced from two drug-resistant mouse strains, resistant to 8-azaguanine (8 AG) and resistant to 5-bromodeoxyuridine (BrdU). The medium used for the selection contains hypoxanthine, aminopterin, and thymidine and is commonly known as "HAT medium". Now this method has become the most commonly used method and also leads to development of other selective media for other drug-resistant mutants, auxotrophic mutants, and temperature-sensitive mutants. The selection of hybrids is done in vitro on the basis of ability to grow but it is also possible to perform selection in vivo to select cancer cells from heterogeneous cell populations. This method is based on the fact that cancer cells show differential adherence or growth e.g. Human peripheral blood lymphocytes can grow in suspension and do not proliferate continuously in culture except after transformation with Epstein-Barr virus and that’s why they can be easily eliminated during serial passage.

The most commonly used selection system to isolate somatic cell hybrids is called *HAT* selection. This technique is based on the fact that normal mammalian cells utilize two pathways to synthesize the nucleotides. The de novo pathway which produces new nucleotides using amino acids and the salvage pathway which produces produce new nucleotides from the routine degradation of used up nucleic acids. So, when the de novo pathway is blocked, using inhibitor aminopterin, the cells can survive by utilizing the salvage pathway to synthesize nucleotides. The salvage pathways involve two enzymes, one is hypoxanthine–guanine phosphoribosyl transferase (HGPRT), which

initiates the production of guanosine monophosphate (GMP) and adenosine monophosphate (AMP) from hypoxanthine, and other enzyme is thymidine kinase (TK), which utilizes thymidine to produce thymidylate. Cells which are deficient in salvage pathway enzymes hypoxanthine guanine phosphoribosyl transferase (HGPRT), an X-linked marker, or thymidine kinase (TK), on chromosome 17, are not able to survive aminopterin containing medium. Aminopterin blocks endogenous synthesis of purine and pyrimidine. These deficiencies can be due to an inborn error as in the Lesch-Nyhan syndrome (HGPRT), or can be introduced by mutagenesis and selection with nucleoside analogues ex. azaguanine (HGPRT) or bromodeoxyuridine (TK). Aminopterin or amethopterin (methotrexate) are folic acid analogs and they are stoichiometric inhibitors of folic acid reductase which can block the endogenous synthesis of purines and certain amino acids production. They are also able to inhibit the conversion of deoxyuridylic acid to thymidylic acid in pyrimidine biosynthesis. Normal cells in the presence of aminopterin or amethopterin can utilize preformed hypoxanthine and thymidine to produce the nucleic acid precursors through the purine and pyrimidine salvage pathways.

When hybrid cells are cultured in HAT medium (having aminopterin and supplemented with hypoxanthine and thymidine), only those cells can survive which are capable of expressing both the enzymes HGPRT and TK. If one parental cell type is deficient for the enzyme thymidine kinase (TK) and another parental cell type is deficient for enzyme hypoxanthine phosphoribosyl transferase (HPRT), the parental cell type will not survive but hybrid cells having both parental cell characteristics can survive. Thus, HAT medium selects only hybrid cells which have both parental cells characteristics. So, cells deficient in either of these enzymes will die in the presence of aminopterin, since neither the de novo nor salvage pathways is functional for the synthesis of nucleotides.

The desired hybrids are not “normal” cell because they contain extra sets of chromosomes acquired during fusion. This extra chromosomes set makes the hybrid cell unstable, and it loses chromosomes randomly during following cell divisions until normal diploid state of one set of paired chromosomes is reached . The preservation of the hybrids under the proper selection conditions ensures that only the desired ones survive. So, mitosis of heterokaryon leads to production of hybrid cells in which the two separate nuclear members disintegrate so all the

chromosomes are brought together in a single large nucleus. These hybrid cells can be cloned to produce hybrid cell lines but they tend to lose chromosomes therefore genetically unstable. For instance, mouse-human hybrid cells predominantly lose human chromosomes randomly leading to generation of variety of mouse-human hybrid cell lines, each of which contains only one or a few human chromosomes. These type of hybrid cells can be used in mapping the locations of genes in the human genome (hybrid cells with only human chromosome 11 synthesize human insulin indicating that the gene encoding insulin is located on chromosome 11). These hybrid cell can be used for creating chromosome-specific human DNA libraries.

4.5 *APPLICATION OF SOMATIC CELL GENETICS*

1. Various biological processes like endocytosis and secretion involve fusion of membranes So, cell fusion method could provide a useful model system for studying the biochemical and ultrastructural aspects of the fusion process.
2. The cell fusion technique is applied to study many problems of the genetics and cellular biology of somatic cells. Example - linkage analysis and chromosome assignment, virus rescue by cell hybridization, and the application of cell hybridization to the study of malignancy.
3. These hybrid cell can be used for creating chromosome-specific human DNA libraries.
4. Hybrid cells can be used for chromosome and gene mapping.
5. B lymphocyte cell clones are used for the production of antibodies but limited life span in culture is the basic drawback of this procedure. To overcome this limitation, B lymphocytes from an immunized mouse or rat are fused with B lymphocyte tumor cells (immortal) to produce a hybrid cell. These hybrid cells have the ability to produce a particular antibody and to multiply indefinitely in culture are selected. These hybrid cells can propagate as individual clones and provide a permanent source of monoclonal antibodies.

4.6 *SUMMARY*

Biochemical procedures generally require isolation of large quantities of cells and then their physical disruption to obtain their components. Homogeneous population of cells can then be analyzed either directly or after increasing their number by cell culture. The good quantity of viable dissociated cells is typically obtained from fetal or neonatal tissues. The Homogeneous population of the cells can be used for biochemical analysis and as a starting material for cell culture. Cell culture increases the number of a particular type of cell and this helps in understanding the complex nature of the cell under particular conditions of a culture dish. Under a proper environment, cells can survive, multiply and can express differentiated properties in a tissue-culture dish. Experiment design to settle a controversy in the field of neurobiology led to the beginning of tissue culture in 1907. To culture tissue cells require the surface to grow and divide (surface of a culture dish).When mixture of different parental cells are treated with either polyethylene glycol (PEG) or inactivated Sendai virus there plasma membranes fuses with adjacent cells, mixing the cytoplasm and incorporating the different nuclei into a single large cell called a heterokaryon (*hetero,* "different" and *karyon,* "chromosome"). When these heterokaryon undergoes cell division, the fusion of different nuclei occur, results in combining the different sets of chromosomes in one large nucleus to form a somatic cell hybrid. One cell fuse with another cell to form a heterokaryon, a combined cell with two separate nuclei. To fuse two different cells various methods are used like treatment of a suspension of cells with inactivated viruses or with polyethylene glycol, each of which alters the plasma membranes of cells in a way that induces them to fuse. Various methods of hybrid selection are fluorescent probes, the flow cytometer or biochemical inhibitors. One method of hybrid selection is the exclusion of parents with different genetic defects by a combination of mutant selection techniques that permits hybrids with complementary functional genes to grow. In 1964 Littlefield introduced this concept for eukaryotic cells. The most commonly used selection system to isolate somatic cell hybrids is called HAT selection. This technique is based on the fact that normal mammalian cells utilize two pathways to synthesize the nucleotides.

4.7 *TERMINAL QUESTIONS & ANSWERS*

1. Polyethylene glycol is-

A. Used for cell Fusion

B. Stimulate cell division

C. Stimulate DNA synthesis

D. Differentiation stimulant

2. Somatic hybrids are produced by

A. Protoplast fusion

B. Tissue culture

C. Pollen culture Polyethylene

D. Hybridoma process

3. HAT medium Contains

A. Hydrogen, amino acid and thymidine

B. Hypoxanthine, aminopterin, and thymidine

C. Hypoxanthine, amino acid and thymidine

D. Halogens, aminopterin and thymidine

4. The salvage pathways involves two enzymes,

A. Dehydrogenase and thymidine kinase

B. Kinase and phosphorylase

C. Glucose transferase and protein kinase

D. Hypoxanthine–guanine phosphoribosyl transferase and thymidine kinase

5. Sendai virus is used for

A. Cancer treatment

B. Transformation

C. Fusion of cells

D. None of the above

6. Heterokaryon are

A. Contain two set of chromosome from two parietal cells
B. Contain different proteins
C. Contain different RNA
D. All of the above

Answers 1(A), 2(A), 3(B), 4(D), 5(C), 6(A)

7. What are somatic hybrids?

8. Write applications of somatic hybridization technology.

9. How somatic hybrids are produced?

10. Write a short note on the mechanism of cell fusion.

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UNIT-5: HUMAN CYTOGENETICS

CONTENTS

5.1 *OBJECTIVES*

In this unit you will learn

- What is Karyotype and types of chromosomes?
- Common syndromes caused by Aneuploidy, mosaicism, deletion and duplication
- What are Chromosomal anomalies in malignancy?
- About Human genome

5.2 *INTRODUCTION*

Human cytogenetics is a field of science that focuses on studying the number, structure, function and origin of chromosomal abnormalities. Chromosomal abnormalities are responsible for a considerable proportion of human morbidity and mortality. The field of human cytogenetics has important contributions to explain the etiology of many congenital malformation/mental retardation syndromes. With the advancement in molecular biological techniques, cytogenetics continues to explore the knowledge of clinical genetics, chromosomal structure and prenatal diagnosis. A karyotype is an individual's collection of chromosomes. The karyotype is used to detect abnormal numbers or structures of chromosomes. Karyotyping is the method of pairing and ordering all the chromosomes of an organism. Standardized staining techniques are used in the preparation of karyotypes that show characteristic structural features for each chromosome. Human karyotype analysis is done to detect gross genetic changes like changes in chromosome number and to reveal more subtle structural changes (deletions, duplications, translocations, or inversions). Information from karyotypes is used for diagnosing specific birth defects, genetic disorders and even cancers.

5.3 *KARYOTYPE AND NOMENCLATURE OF METAPHASE CHROMOSOME BANDS*

The chromosomes of a cell at metaphase and prometaphase of the cell cycle have the most condensed conformation. So, mitotic cells whose cycle is arrested in metaphase or prometaphase

are used in the preparation of karyotypes. The sample (cells) for karyotype can be obtain from variety of tissue ex. tumor biopsies or bone marrow samples are used for cancer diagnosis, amniotic fluid or chorionic villus specimens are used for prenatal diagnosis and peripheral blood specimens or a skin biopsy use for various other diagnosis.

First the cells from sample are cultured and after a period of multiplication, cells are arrested in metaphase by colchicine (inhibit spindle formation). The cells are then placed in hypotonic solution resulting in swelling of their nuclei and bursting of the cells. The nuclei are then treated with a chemical fixative, dropped on a glass slide, and stained with various stains that reveal structural features of the chromosomes. Initially chromosomes are grouped according to their size and position of their centromeres. But advancement in the staining technique help in generating characteristic banding patterns for different chromosomes. In 1970 TorbjornCaspersson and his colleagues described the first banding technique, known as Q-banding. Q-banding involves the use of fluorescent dye quinacrine that alkylates DNA and is subject to quenching over time. Caspersson*et al*. demonstrated that quinacrine produced characteristic and reproducible banding patterns for individual chromosomes. Now a days Giemsa dye is used in staining for better resolution of individual bands and to produces a more stable preparation. These chromosomes can be observed under ordinary bright-field microscopy.

A-T (gene poor) rich heterochromatic regions of DNA stain darkly in G banding in comparison with less condensed GC rich (more transcriptionally active) regions of DNA which appear as light bands in G-banding.R- banding also involves use of Giemsa stain but this generates the reverse pattern from G-banding. In this procedure the chromosomes are heated before staining. The heat results in preferential melting of A-T base pairs of DNA which bind with Giemsa stain strongly; this leads to only the G-C region to bind with stain.C-banding is used for staining specifically heterochromatin or genetically inactive DNA. In this technique chromosomes are stained at centromeres region (A-T rich satellite DNA) using Giemsa stain.

The image of each chromosome is arranged in a standardized format known as a karyotype or a karyogram to obtain maximum diagnostic information. As per international conventions, human autosomes are arranged in descending order by size (except 21 and 22) and sex chromosomes are generally arranged at the end of a karyogram. Individual chromosomes has a short p arms—p for

"petite," at the top, and their long q arms—q for "queue"—at the bottom separated by centromere. The ends of the linear chromosome are known as telomeres.

The chromosome arm is marked into regions, or cytogenetic bands, which can be seen under microscope and using special stains. The cytogenetic bands are marked as p1, p2, p3, q1, q2, q3, etc., counting from the centromere out toward the telomeres. Under higher resolutions, sub-bands can be seen within the bands. The sub-bands are also numbered from the centromere out toward the telomere. For example, the cytogenetic map location of the CFTR gene is 7q31.2, which specifies it is on chromosome 7, q arm, band 3, sub-band 1, and sub-sub-band 2.

The chromosome ends are termed as ptel and qtel. For example, the notation 7qtel refers to the end of the long arm of chromosome 7.

5.4 *TYPES OF CHROMOSOMES THEIR ANOMALIES AND DISEASE*

Classification of chromosomes:

Human cell contain 46 chromosome and they are classified as

1) Autosome: - 1-23 chromosome are considered as autosome

2) Sex chromosome: Chromosome X and Y are classed as Sex chromosome. Normal human female contains 46,XX and normal human male 46,XY.

Centromere position can also be utilized to identify the gross morphology, or shape, of chromosomes. Type of chromosome on the basis of centromere position

1) Metacentric chromosomes have p and q arms of nearly equal lengths because the centromere is situated exactly in the center of p and q arms. For e.g. 1, 3 and 16 chromosomes

2) Submetacentric chromosomes have centromere slightly displaced from the center. For e.g.2, 6 and 10 chromosomes

3) Acrocentric chromosomes have centromere located near their ends. For e.g.14, 15 and 21 chromosomes.

4) Telocentric chromosomes have centromere located very close to the p arm and thus the p arm is barely visible or absent.

Based on the location of the centromere and size the 23 pairs of human chromosomes can be divided into 7 groups

Group	**Size**	**Position of centromere**	**Number of chromosomes**
Group A	Large	Metacentric	1, 2 and 3
Group B	Large	Submetacentric	4 and 5
Group C	Medium	Submetacentric	6, 7, 8, 9, 10, 11,12 and X
Group D	Medium	Acrocentric	13, 14 and 15
Group E	Relatively short	Submetacentric	16, 17 and 18
Group F	Short	Meta or submeta	19 and 20
Group G	Short	Acrocentric	21, 22 and Y

Karyograms help in identification of abnormalities in chromosomes because banding patterns between homologous chromosomes are nearly identical. Karyotyping can detect chromosomal change of a few megabases and can help in diagnoses of certain abnormalities.

Chromosomal anomalies and disease

1) **Aneuploidy:** Chromosomal disorder due to an incorrect chromosome number. Aneuploidy is caused by nondisjunction during meiosis, which involves movement of both

members of a homologous pair to the same daughter cell. So, the fertilized egg either has one or three copies of the chromosome instead of the usual two. This type of abnormalities involves disturbance of number of genes and are embryonic lethal especially if loss of chromosome occurs. Abnormalities which are not lethal usually cause sterility, because they prevent meiosis from proceeding normally. For instance, Down syndrome generally occurs due to trisomy of chromosome 21sometime due to a duplication or translocation of a specific region of chromosome 21. Trisomies of chromosome 13 or 18 also occur but are much less common in live born infants than is Down syndrome. Turner syndrome in women is due to receiving only a single X chromosome, Klinefelter syndrome in men is due to receiving two X chromosomes in addition to the Y chromosome.

2) **Genetic mosaicism:** Genetic mosaicism is characterized by the presence of two or more cell lineages with different genotypes arising from a single zygote in a single individual. It is due to postzygotic mutation. Mosaicism arises due to various genetic mutations (Single nucleotide variants, chromosomal aberrations, copy number variants, etc.); in either germline or somatic cells. Mosaicism's effect on tissues is determined by the amount and proportion of the cells affected by it. It may be restricted to gonadal tissue, non-gonadal tissue, or may affect all tissues of the body. Mosaic aneuploidies are produced by two processes: post-zygotic mitotic non-disjunction or post-zygotic mitotic trisomy rescue after a meiotic non-disjunction. Phenotypic expression of mosaicism depends on the number of cells affected by it and phenotypic expression can occur during intrauterine life, after birth, or even in late-life on the basis of the trigger factors. Mosaics sometimes do not affect the function, this is due to nonexpressive mutation, recessive mutation, or low number of cells affected. Conditions arises as a result of mosaicism are following

- Mosaic Klinefelter syndrome (46, XY/47, XXY) results in small size of testes and low secretion of testosterone by the gonads.
- Warkany syndrome is due to mosaic trisomies of chromosomes 8.
- Pallister–Killian syndrome due to isochromosome 12p, which is an abnormal chromosome 12.
- Rett's Syndrome is X- Chromosome associated disorder which is due to mutation of MECp2 gene. It is fatal for male because male have only one X chromosome. The

male can survive if he is mosaic for the specific gene that means more cells are with normal X-chromosome.

- Sporadic retinoblastoma severity and the onset of the retinoblastoma is due to mosaic.
- Cornelia de Lange syndrome (CdLS) patients have a mosaic variant on SMC1A in the buccal mucosa cells.
- Mosaic loss of Y chromosome in blood cells enhance morbidity and mortality in old age.
- Hereditary tyrosinemia type 1 is a condition in which the liver cells of the patient are mosaic. It is due to mutation in fumarylacetoacetate hydrolase (*FAH*) gene.
- Bloom syndrome is due to mutation in BLM gene which encode DNA helicase enzyme. It is characterized by predisposition of malignancy, growth disorder, and immunodeficiency.
- Duchene muscular dystrophy is associated with Dystrophin gene mosaicism.

3) **Deletions:** Chromosomal abnormalities due to deletion of parts of chromosomes. For e.g.DiGeorge syndrome, (T cell immunodeficiency and cardiac anomalies)is due to microdeletion of chromosome 22and Prader-Willi syndrome, (mental retardation, infantile hypotonia, and a compulsive eating disorder) due to a microdeletion of chromosome 15. Cri du chat syndrome, is due to deletion on the short arm of chromosome 5. It is characterized by malformations of the larynx, mental retardation, as well as a number of physical defects.

4) **Duplication:** In this process the extra copies of a chromosomal region are formed, resulting in different copy numbers of genes within that area of the chromosome. If the duplicated sections are adjacent to the original, the process is known as tandem duplication, whereas if they are separated by nonduplicated regions, the duplication is said to be displaced. Duplications may affect phenotype by altering gene dosage. For example, the amount of protein synthesized is often proportional to the number of gene copies present, so extra genes can lead to excess proteins.

   a. MECP2 duplication syndrome affects only men and causes mild to severe intellectual impairment. Individuals suffer from this shows weak muscular tone,

feeding difficulties, poor or missing speech, muscle stiffness (rigidity), and delayed development of motor abilities like sitting and walking during infancy. About half of patients experience seizures of tonic-clonic type (loss of consciousness, muscle rigidity, and convulsions). Some people who are afflicted also lose previously acquired talents (developmental regression). Recurrent respiratory tract infections are a leading cause of death in people with this disease.

b. 7q11.23 duplication syndrome is characterized by various neurological and behavioral problems and other abnormalities. This syndrome results in delayed development of speech and motor skills such as crawling and walking. Patients with 7q11.23 duplication syndrome may also have weak muscle tone (hypotonia) and show abnormal movements like involuntary movements of one side of the body that mirror intentional movements of the other side. About one-fifth of patients also experience seizures.

5) **Inversions:** In this process part of the chromosome has broken off, turned upside down and reattached. Chromosome 9 inversion is most common structural balanced chromosomal variants. In few cases, it results in infertility, congenital anomalies, growth retardation, recurrent pregnancy loss and cancer.

6) **Rings:** In this process a part of a chromosome has broken off and formed a circle or ring. This process can take place with or without loss of genetic material. Its example is ring chromosome 14 syndrome. This condition is characterized by seizures and intellectual disability. During infancy or early childhood recurrent seizures develop and these seizures are resistant to the treatment by anti-epileptic drugs in most of the cases. It may also characterize by some degree of intellectual disability or learning problems. Development may be delayed, mainly the development of speech and of motor skills such as walking, sitting and standing.

5.5 *COMMON SYNDROMES CAUSED BY ANEUPLOIDY, MOSAICISM, DELETION AND DUPLICATION*

Aneuploidy	Down syndrome, Turner syndrome Klinefelter syndrome
Mosaicism	Mosaic Klinefelter syndrome Warkany syndrome Pallister–Killian syndrome Rett's Syndrome Cornelia de Lange syndrome (CdLS) Hereditary tyrosinemia type 1 Bloom syndrome Duchene muscular dystrophy
Deletion	DiGeorge syndrome Prader-Willi Cri du chat
Duplication	*MECP2* duplication syndrome 7q11.23 duplication syndrome

5.6 *CHROMOSOMAL ANOMALIES IN MALIGNANCY*

Cancer is a progressive disease which means multiple mutations are required during tumor progression. These changes result in disruption of cellular control mechanisms involving disrupting of proto-oncogenes and tumor suppressor genes. Peter Nowell and David Hungerford in 1960 discovered the first chromosomal abnormality associated with cancer using cytogenetics.

They discover an abnormal minute chromosome (Philadelphia chromosome) in chronic myeloid leukemia (CML) patients. CML is a type of cancer that results in unrestricted growth of myeloid cells in the bone marrow.

Chromosomal deletions, inversions and translocations are commonly detected in chromosome region 9p21 in gliomas, non-small-cell lung cancers, leukemias and melanomas. These chromosomal changes inactivate a tumor suppressor called cyclin-dependent kinase inhibitor 2A. Wilms' tumor and retinoblastoma are due to gene deletions or inactivations. In various types of cancer inactivation of tumor suppressor genes occur because chromosomal regions associated with tumor suppressors are commonly deleted or mutated. Gene duplications and increases in gene copy numbers is also associated with cancer. Chromosomal region 12q13-q14 which encodes for MDM2 a binding protein of tumor suppressor p53 is strikingly amplified in many sarcomas. Amplification of MDM2 prevents p53 from regulating cell growth resulting in tumor formation. The overexpression and increases in copy number of the *ERBB2* gene result in certain types of breast cancer. This gene codes for human epidermal growth factor receptor 2. Increases in chromosomal number, such as chromosomes 1q and 3q, also result in cancer risk.

Disturbance in the gene involved in control of the cell cycle can lead to cancer progression. For example, if gene controlling chromosomal segregation is mutated then it results in duplications and deletions. Likewise, mutation in the gene controlling accurate sorting and segregation of chromosomes during mitosis result in chromosome instability and abnormalities in the number of chromosomes (polyploidy and aneuploidy) which can cause cancer. Abnormal centromeres during the mitotic cycle result in the abnormal loss of chromosomes leading to cancer progression.

5.6.1 *CHRONIC MYELOID LEUKEMIA*

Peter Nowell and David Hungerford discovered that Philadelphia chromosome is associated with chronic myeloid leukemia (CML). Then in 1973 using quinacrine fluorescence and G-banding cytogenetic techniques Janet Rowley examine CML patient's karyotypes and discover that Philadelphia chromosome formed due to translocation of DNA between chromosomes 9 and 22. This translocation cause shortening of chromosome 22 to form the Philadelphia chromosome (Ph^1), while simultaneously result in lengthening of chromosome 9 ($9q^+$) (Rowley, 1973). This

translocation result in formation of an abnormal, fused gene called *bcr-abl*, that leads to production of an aberrant, new protein (kinase molecule). The expression of this protein is constitutive and it in turn, activates cell cycle controlling proteins and enzymes. This disturb the cell cycle regulation and leads to uncontrolled cell growth which in turn cause cancer. The translocation involves the *ABL* proto-oncogene on chromosome 9 and *BCR* for breakpoint cluster region gene on chromosome 22. Under normal physiological conditions Abl protein is highly regulated and involved in cell cycle regulation. The Bcr protein is ubiquitously expressed. Its N-terminal is a serine–threonine kinase. The substrate for kinase is Bap-1 and Bcr itself. The center of the molecule involved in stimulation of the exchange of guanidine triphosphate (GTP) for guanidine diphosphate (GDP) on Rho guanidine exchange factors, which in turn may activate transcription factors such as NF-κB. The C-terminus has GTPase activity for Rac, a small GTPase of the Ras superfamily that regulates actin polymerization and the activity of an NADPH oxidase in phagocytic cells.

5.7 *HUMAN GENOME*

The genetic information of humans is stored in its DNA and passes from generation to generation. The DNA is a polymer of nucleotides guanine [G], adenine [A], thymine [T] and cytosine [C]. A gene is all the DNA that encodes the primary sequence of some final gene product, which can be either a polypeptide or an RNA with a structural or catalytic function. Other than gene, DNA also contains regulatory sequences which either control the transcription or act as an initiation point for replication or recombination. The human genome is made up of approximately 3.1 billion base pairs with include almost 29,000 genes on 24 different chromosomes (22 pairs and X and Y sex chromosomes). Each human chromosome is a single, very large, duplex DNA molecule. Each human chromosome has a particular set of genes. The coding regions of DNA (gene) consist of one or more intervening segments that do not code for the amino acid and these are called introns. The coding sequence is called exons. Human genes typically contain more intron sequences than exons sequences. Half of the human genome is in the form of moderately repeated sequences generated from the transposable elements. Transposons are DNA segments of a few hundred to several thousand base pairs which can move from one location to another in the genome. In the human genome some transposons are active and moving in a low frequency but most are

evolutionarily altered via mutation inactive relics. These are noncoding sequences but play an important role in evolution by redistributing genomic sequences. Approximately 3% of the human genome contain highly repetitive sequences (10bp long), also known as simple-sequence DNA or simple sequence repeats (SSR). They are sometimes repeated millions of times per cell and also called satellite DNA. This highly repetitive DNA is very important because it plays a role in the functional process of the cell as it is associated with centromeres and telomeres. The centromere is a sequence of DNA which serves as an attachment point of the mitotic spindle during cell cycle. The centromeric sequences are simple-sequence DNA, which consists of thousands of tandem copies of one or a few short sequences of 5 to 10 bp, in the same orientation. The end sequences of linear chromosomes are called telomeres which help in stabilizing chromosomes. Telomeres are multiple repeated sequences of (5) (TxGy)n (3)(AxCy)n where x and y are generally between 1 and 4. The number of telomere repeats, n, is generally more than 1,500 in mammals. Telomeric sequences cannot be routinely replicated by replication enzymes. Enzyme telomerase helps in replication of telomeric sequences in eukaryotic.

Chromatin is made up of fibers which contain DNA and protein in approximately equal proportions in terms of mass, along with small proportion of RNA. In chromatin the DNA is tightly associated with histone protein (small, basic proteins), which helps in packing and ordering the DNA into nucleosomes. Chromatin also contains nonhistonic protein in which some help in maintain chromosome structure and other help in regulation of expression of particular genes. There are 5 major classes of Histones which differ in molecular weight and amino acid composition. The compaction of DNA into chromosome involves various levels of highly organized folding. Histones are like beads of protein space at a regular interval to which DNA is wrapped. Compaction of DNA leads to supercoiling which is corrected by enzyme eukaryotic topoisomerases. The structure of condensed chromosome is maintained by SMC proteins (structural maintenance of chromosomes). Human genome project helps in sequences the entire genome of human. No two human have same genomic sequence except identical twins. Human genome is not static but keeps on changing. Changes may be neutral, advantageous or lethal. Comparison of human genome with other organism provide evolution relationship between them. Migration of human to different places leads to variation in genome that are became differentially

fixed in different populations. Human genome (gene) knowledge helps in presymptomatic genetic diagnosis of hereditary associated diseases like breast cancer and colon cancer.

5.8 *SUMMARY*

Karyograms help in identification of abnormalities in chromosomes because banding patterns between homologous chromosomes are nearly identical. Karyotyping can detect chromosomal change of few megabases and can help in diagnoses of certain abnormalities. Chromosome banding revealed by different staining techniques. a) Giemsa banding b) Q- banding c) R-banding and d) C-banding. Canceris a progressive disease which means multiple mutations are required during tumor progression. These change result in disruption of cellular control mechanism involving disrupting of proto-oncogenes and tumor suppressor genes. Peter Nowell and David Hungerford in 1960 discovered the first chromosomal abnormality associated with cancer using cytogenetics. They discovered that Philadelphia chromosome is associated with chronic myeloid leukemia (CML). Philadelphia chromosome formed due to translocation of DNA between chromosomes 9 and 22. This translocation causes shortening of chromosome 22 to form the Philadelphia chromosome (Ph1), while simultaneously resulting in lengthening of chromosome 9 (9q+) (Rowley, 1973). This translocation result in formation of an abnormal, fused gene called bcr-abl, that leads to production of an aberrant, new protein (kinase molecule) The human genome is made up of approximately 3.1 billion base pairs with include almost 29,000 genes on 24 different chromosomes (22 pairs and X and Y sex chromosomes). Each human chromosome is a single, very large, duplex DNA molecule. Each human chromosome has a particular set of genes.

5.9 *TERMINAL QUESTION & ANSWERS*

1. What is cytogenetics?

a. Study of protein
b. Study of nucleus
c. Study of cell
d. Study of Chromosomes

2. Aneuploidy represents

a. Increase or decrease number of chromosome
b. Increase in multiple sets of chromosome
c. Deletion of some parts of chromosome
d. Addition of some parts of chromosome

3. Patau's syndrome is caused due to which of the following

a. Trisomy of 13th chromosome
b. Tetrasomy of 13th chromosome
c. Tetrasomy of 14th chromosome
d. Trisomy of 14th chromosome

4. Trisomy 21 is better known as:

a. Edwards syndrome
b. Patau syndrome
c. Down syndrome
d. Warkany syndrome 2

5. The human genome is:

a. All of our genes
b. All of our DNA
c. All of the DNA and RNA in our cells
d. Responsible for all our physical characteristics

6. How many chromosomes do humans have?

a. 46
b. 48
c. 54
d. 56

7. Genes are made up of:

a. DNA
b. RNA
c. Proteins
d. Enzymes

8. Scientists now think humans have how many protein-encoding genes:

a. 20 - 25,000
b. 30 - 40,000
c. 65 - 75,000
d. more than 100,000

Answers: 1(d), 2(a), 3(a), 4(c), 5(b), 6(a), 7(a), 8(a)

Q 9 Write a short note on the human genome.

Q10 Describe chromosomal abnormalities in malignancy.

5.10 *REFERENCES*

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Unit 6: Microbial Cytogenetics

Content

6.1 OBJECTIVE

In this unit you will learn

- Bacterial transformation
- Transduction
- Transformation
- Bacteriophage: Types, Structure, Morphology and Morphogenesis.

6.2 INTRODUCTION

Cytogenetics is a branch of genetics that studies chromosomes, which are long strands of DNA and protein that store genetic information in cells. It covers chromosome structure and composition, chromosome analysis methods, chromosome abnormalities linked to disease, chromosome functions in sex determination, and chromosome changes over time. The field of cytogenetics emerged in the early twentieth century, when scientists realized that chromosomes are the physical carriers of genes.

Microbial cytogenetic studies microorganisms for different purposes. The microorganisms that are observed are bacteria, and Archaea. Some fungi and protozoa are also subjects used to study in this field. The studies of microorganisms involve studies of genotype and expression systems. Genotypes are the inherited compositions of an organism. Genetic Engineering is a field of work and study within microbial cytogenetics. The process involves creating recombinant DNA molecules through manipulating a DNA sequence that DNA created is then in contact with a host organism.

6.3 BACTERIAL TRANSFORMATION, TRANSDUCTION, CONJUGATION, BACTERIAL CHROMOSOME

This section includes the various means of gene transfer in bacteria and their use in bacterial genetics. The ways of genetic recombination in bacteria include transformation, transduction, and conjugation.

Bacterial transformation

It was first reported in *Streptococcus pneumoniae* by Griffith in 1928. It is a process of horizontal gene transfer in bacteria in which bacteria take up foreign naked DNA from the environment. Transformation is a key step in DNA cloning. It occurs after restriction and digestion and transfers newly made plasmids to bacteria. After transformation, bacteria are **selected** on antibiotic plates. Bacteria with a plasmid are antibiotic-resistant, and each one will form a **colony.**

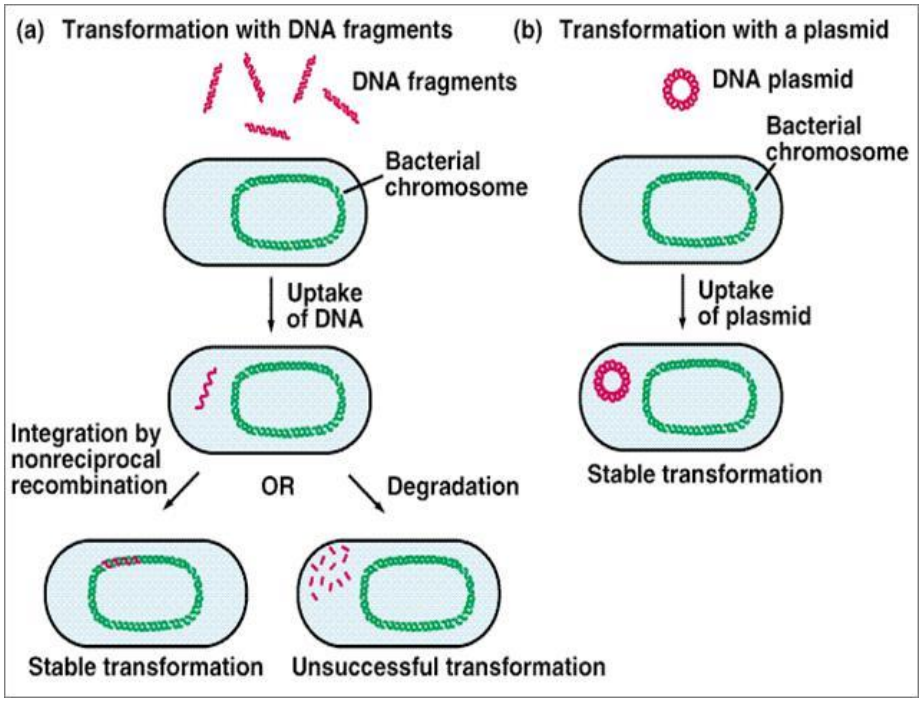

Figure 6.1: Gene transfer in Bacteria by transformation (Source: medbullets.com)

The process of gene transfer by transformation does not require a living donor cell but only requires the presence of persistent DNA in the environment. The prerequisite for bacteria to undergo transformation is its ability to take up free, extracellular genetic material. Such bacteria are termed as competent cells.

Once the transforming factor (DNA) enters the cytoplasm, it may be degraded by nucleases if it is different from the bacterial DNA. If the exogenous genetic material is similar to bacterial DNA, it may integrate into the chromosome. Sometimes the exogenous genetic material may co-exist as a plasmid with chromosomal DNA.

Transduction:

Transduction was discovered by Zinder and Lederberg in *Salmonella*. Transduction is a process by which foreign DNA is introduced in a cell by a virus vector. Transduction is commonly used in genetic engineering for inserting foreign DNA into the host cell. Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA (which occurs in conjugation), and it is DNAase resistant.

Transduction happens through either the lytic cycle or the lysogenic cycle. Transduction is especially important because it explains one mechanism by which antibiotic drugs become ineffective due to the transfer of antibiotic-resistance genes between bacteria.

Steps of Transduction in Bacteria:

1. Infection of the bacterial cell by bacteriophage.
2. The virus uses the host machinery to make multiple copies either directly by the lytic cycle or first gets incorporated into the bacterial genome by the lysogenic cycle and followed by the lytic stage.
3. During assembly of bacteriophages, the bacterial genome also gets packed by mistake in the viral head alongside the viral genome. In the lysogenic cycle, during excision of prophage, some parts of the bacterial genome that flank the prophage are also excised and go inside the assembled viral head together with the viral genome.
4. When these viruses infect another bacterial cell, they inject the viral DNA as well as donor DNA into the host cell.
5. The bacterial DNA either forms plasmids or gets inserted into the recipient DNA if it is homologous to the recipient genome. Most of the time it remains as an extra chromosomal DNA. It can also get inserted with the prophage if it is a temperate phage. So the fate depends on the portion of bacterial DNA and also on the nature of bacteriophages.

Transduction is common in both virulent and temperate phages, i.e. by lytic or lysogenic cycle. Transduction is of two types:

- Generalized Transduction – In this, the phage can carry any part of DNA.
- Specialized Transduction – In this, the phage carries only the specific part of DNA.

Generalized Transduction

Generalized transduction can occur by both lytic and lysogenic cycle. Here, any random part of DNA gets packed in bacteriophages by mistake along with the viral genome. It occurs at the lytic stage of the phage life cycle. When the virus-containing bacterial DNA infects another cell, it can get inserted into the host genome or if it was a plasmid, then it can reform the plasmid. Generalized transduction is used to study linkage information, gene mapping, comparing genomes of two different bacteria, mutagenesis, etc.

Example of generalized transduction includes *E. coli* transduction by P1 phage.

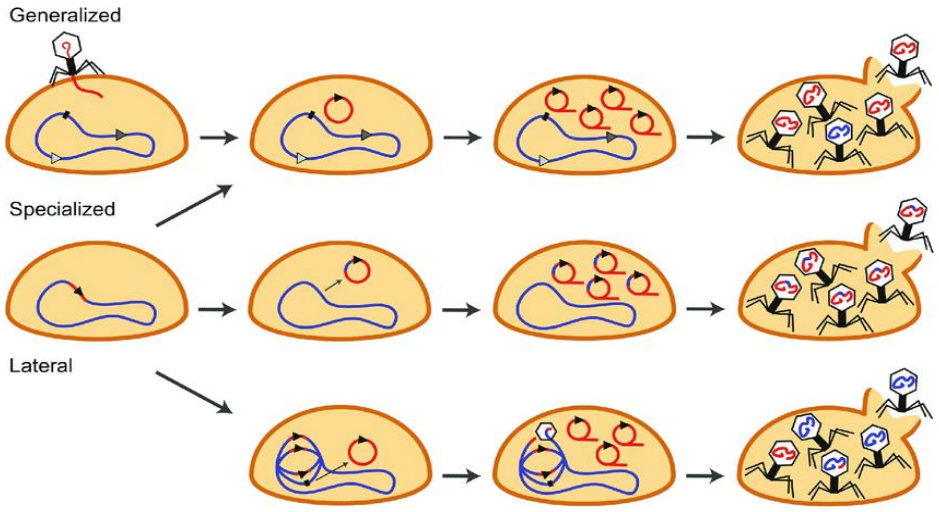

Figure 6.2: Generalised and specialized transduction (Source: researchgate.net)

Specialized Transduction

Specialized transduction can occur only through the lysogenic cycle, i.e. by temperate phage. Here, only the specific part of the bacterial DNA is packed into the virus. It occurs when the prophage, i.e. viral DNA, gets inserted into the bacterial genome in the lysogenic cycle. When prophage

excises from bacterial DNA, some parts of bacterial DNA, which are flanked on both sides of the prophage are also excised. Here, the newly packed phage genome consists of both bacterial and viral genome. Later, when the virus with the recombinant genome infects a new bacterial cell, the bacterial gene also gets inserted into the host genome with the viral genome through lysogeny. The recipient cell now shows the newly acquired characteristics. Specialized transduction is commonly used for isolation and insertion of genes of choice.

Example of specialized transduction includes *E. coli* transduction by λ phage.

Conjugation

Conjugation is a mechanism of DNA transfer during which plasmid DNA is transferred from one bacterium (the donor) of a mating pair into another (the recipient) via a pilus. The F factor present on chromosome is responsible for this conjugation. It requires cell-to-cell contact. Bacterial conjugation was first described by Lederberg and Tatum in 1946 in *Escherichia coli*.

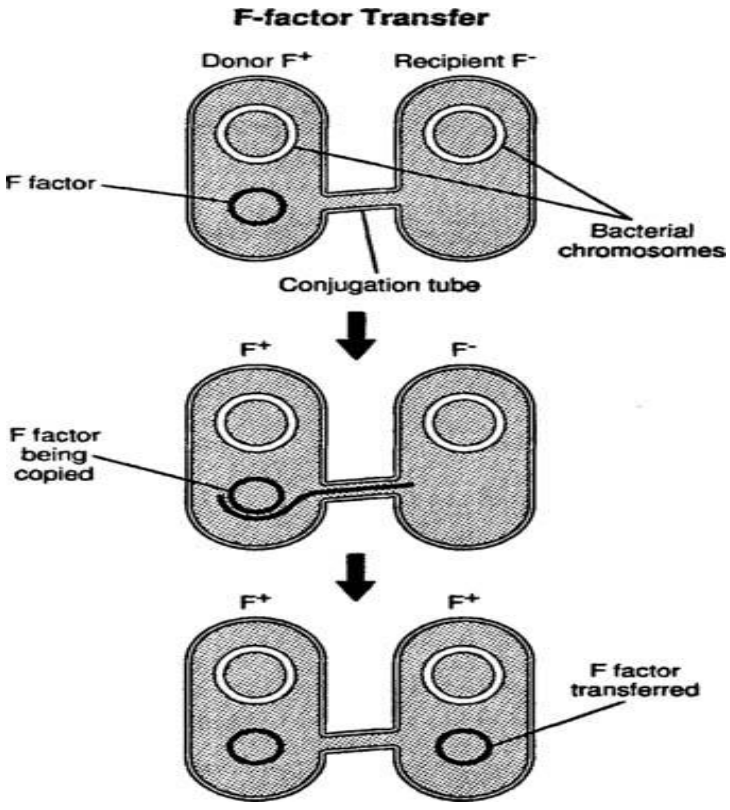

Figure 6.3: Bacterial Conjugation (Source: ccelms.ap.gov.in)

Conjugation is dependent on tra genes found in conjugative plasmid, which among other things encodes instruction for the bacterial cell to produce a sex pilus. Sex pilus is a tube like appendage which allows cell to cell interaction to ensure the protected transfer of a plasmid DNA copy from donor cell to a recipient.

Transduction is one of the most important tools for genetic engineering.

- Transduction is used to insert the genes of choices in animals and plant cells to modify the genetic constituents and get the desired characteristics.
- It can be used for gene therapy. It has huge potential to cure genetic diseases.
- It is an important tool in genetics and molecular biology research.

Bacterial chromosome

The bacterial genome is composed of a single molecule of chromosomal deoxyribonucleic acid or DNA and is located in a region of the bacterial cytoplasm visible when viewed with an electron microscope called the nucleoid. Unlike the eukaryotic nucleus, the bacterial nucleoid has no nuclear membrane or nucleoli. The bacterial chromosome is one long, single molecule of double stranded, helical, supercoiled DNA. In most bacteria, the two ends of the double-stranded DNA covalently bind together to form both a physical and genetic circle. The chromosome is generally around 1000 µm long and as many as 3500 genes *E. coli*, a bacterium that is 2-3 µm in length, has a chromosome approximately 1400 µm long. Since bacteria are haploid, that is they have only one chromosome and only reproduce asexually, there is also no meiosis in bacteria.

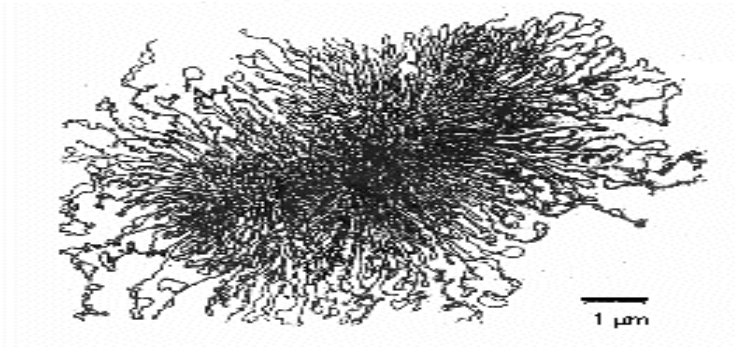

Figure 6.4: Electron Micrograph of a Bacterial Chromosome (Source: biolibretext.org)

6.4 BACTERIOPHAGE

The term *bacteriophage* is coined by bacteriophage which means bacteria eater, to describe the bactericidal ability of virus. Bacteriophages, also known as phages, are viruses that infect and replicate only in bacterial cells. Phage genome consists of either DNA or RNA, and can contain as few as four genes or as many as several hundred. Bacteriophages were discovered independently by Frederick W. Twort in Great Britain (1915) and D'Hérelle in France (1917). Like all viruses, phages are simple organisms that consist of a core of genetic material (nucleic acid) surrounded by a protein capsid. The nucleic acid may be either DNA or RNA and may be double-stranded or single-stranded. There are three basic structural forms of phage: an icosahedral (20-sided) head with a tail, an icosahedral head without a tail, and a filamentous form.

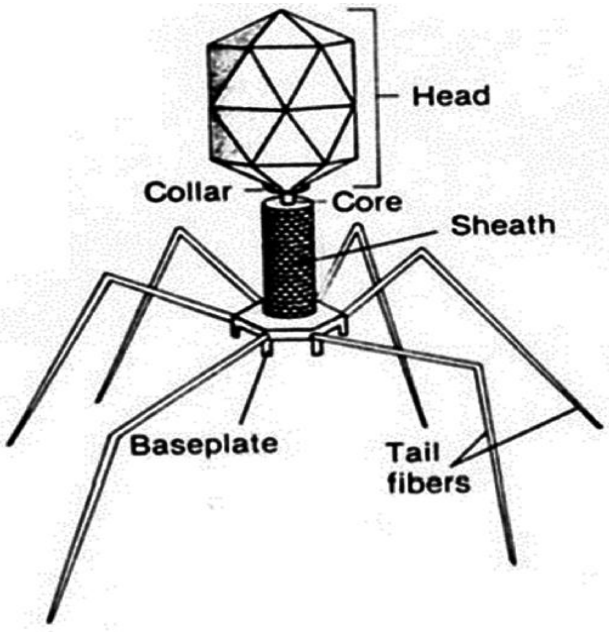

Figure 6.5: Structure of Bacteriophage (Source: Researchgate.com)

Structure of Bacteriophage:

Phages have a very simple structure. Their genetic material is contained in a prism shaped head, surrounded by a protein capsid. This is connected to the elongated sheath (sometimes called the tail) by a neck or collar region. The sheath forms a hollow tube through which the viral DNA/RNA

is injected into the host cell and is surrounded by protective sheath proteins. At the bottom of the sheath is the base plate to which the tail fibers (normally six) that facilitate attachment to the host cell are attached. In order to reproduce, phage must first enter the host cell. They bind to specific receptors on the bacterial cell surface with their tail fibres (adsorption) and create a hole, a process which, along with attachment, is coordinated by the base plate. A rigid tube is propelled out of the sheath, puncturing a hole in the bacterial cell membrane through which they inject their genetic material (DNA or RNA, double or single stranded). During infection a phage attaches to a bacterium and inserts its genetic material into the cell. After that a phage usually follows one of two life cycles, lytic (virulent) or lysogenic (temperate).

The lytic cycle: The phage infects a bacterium, hijacks the bacterium to make lots of phages, and then kills the cell by making it explode (*lyses*). Lytic phages take over the machinery of the cell to make phage components. They then destroy, or lyse, the cell, releasing new phage particles.

The stages of the lytic cycle are:

1. **Attachment**: Proteins in the "tail" of the phage bind to a specific receptor on the surface of the bacterial cell.
2. **Entry**: The phage injects its double-stranded DNA genome into the cytoplasm of the bacterium.
3. **DNA copying and protein synthesis**: Phage DNA is copied, and phage genes are expressed to make proteins, such as capsid proteins.
4. **Assembly of new phage**: Capsid assembles from the capsid proteins and is stuffed with DNA to make lots of new phage particles.
5. **Lysis**: Late in the lytic cycle, the phage expresses genes for proteins that poke holes in the plasma membrane and cell wall. The holes let water flow in, making the cell expand and burst like an overfilled water balloon.

The **lysogenic cycle** allows a phage to reproduce without killing its host. Some phages can only use the lytic cycle, but the phage we are following, lambda (λ), can switch between the two cycles. In the lysogenic cycle, the first two steps (attachment and DNA injection) occur just as they do for the lytic cycle. However, once the phage DNA is inside the cell, it is not immediately copied or

expressed to make proteins. Instead, it recombines with a particular region of the bacterial chromosome. This causes the phage DNA to be integrated into the chromosome. Lysogenic phages incorporate their nucleic acid into the chromosome of the host cell and replicate with it as a unit without destroying the cell. Under certain conditions lysogenic phages can be induced to follow a lytic cycle.

The integrated phage DNA, called a **prophage**, is not active: its genes aren't expressed, and it doesn't drive production of new phages. However, each time a host cell divides, the prophage is copied along with the host DNA, getting a free ride. The lysogenic cycle is less flashy (and less gory) than the lytic cycle, but at the end of the day, it's just another way for the phage to reproduce.

Under the right conditions, the prophage can become active and come back out of the bacterial chromosome, triggering the remaining steps of the lytic cycle (DNA copying and protein synthesis, phage assembly, and lysis).

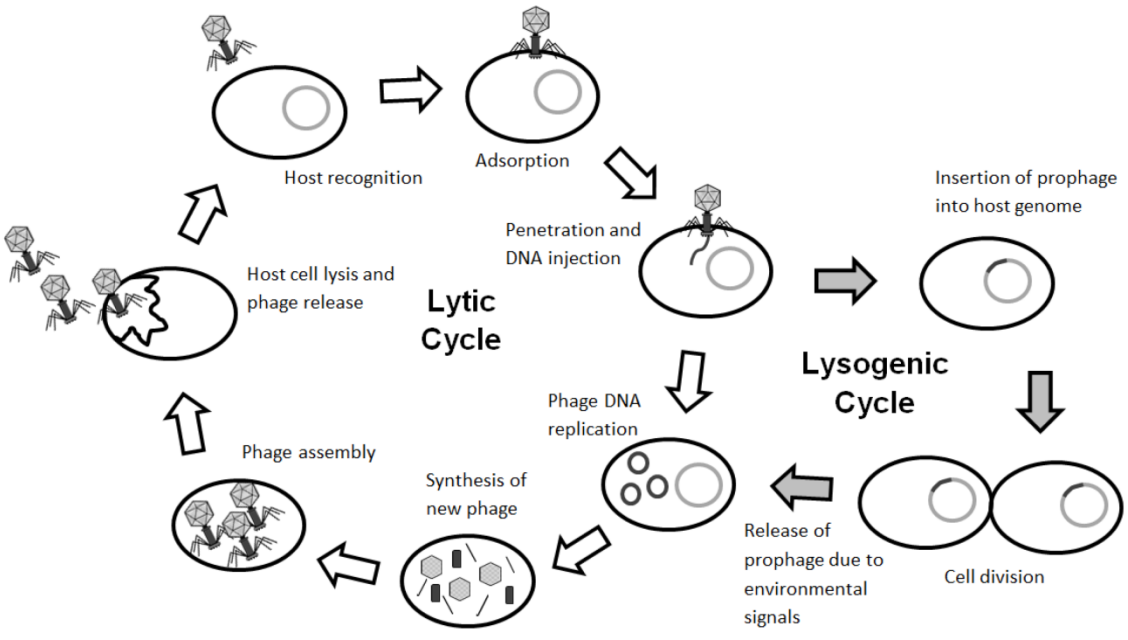

Figure 6.6: Lytic and lysogenic cycle of Bacteriophage

6.4.1 TYPES:

Bacteriophages occur abundantly in the universe, with different genomes, and lifestyles. Phages are classified by the International Committee on Taxonomy of Viruses (ICTV) according to morphology and nucleic acid.

Order	Family	Morphology	Nucleic acid	Examples
Belfryvirales	*Turriviridae*	Enveloped, isometric	Linear dsDNA	
	Ackermannviridae	Nonenveloped, contractile tail	Linear dsDNA	
	Autographiviridae	Nonenveloped, noncontractile tail (short)	Linear dsDNA	
	Chaseviridae		Linear dsDNA	
	Demerecviridae		Linear dsDNA	
	Drexlerviridae		Linear dsDNA	
	Guenliviridae		Linear dsDNA	
	Herelleviridae	Nonenveloped, contractile tail	Linear dsDNA	
	Myoviridae	Nonenveloped, contractile tail	Linear dsDNA	T4, Mu, P1, P2
Caudovirales	*Rountreeviridae*		Linear dsDNA	
	Salasmaviridae		Linear dsDNA	
	Schitoviridae		Linear dsDNA	
	Zobellviridae		Linear dsDNA	
Halopanivirales	*Sphaerolipoviridae*	Enveloped, isometric	Linear dsDNA	
	Simuloviridae	Enveloped, isometric	Linear dsDNA	
	Matshushitaviridae	Enveloped, isometric	Linear dsDNA	

6.4.2 STRUCTURE AND MORPHOLOGY OF T4 PHAGE

T4 phage structure is divided into three parts: the head, the tail and the long tail fibres. The prolate head encapsulates a 172 kbp concatemeric dsDNA genome. The 925 Å-long tails is surrounded by the contractile sheath and ends with a hexagonal baseplate. Six long tail fibres are attached to the baseplate's periphery and are the host cell's recognition sensors. The sheath and the baseplate undergo large conformational changes during infection. X-ray crystallography and cryo-electron microscopy have provided structural information on protein–protein and protein–nucleic acid interactions that regulate conformational changes during assembly and infection of *Escherichia coli* cells.

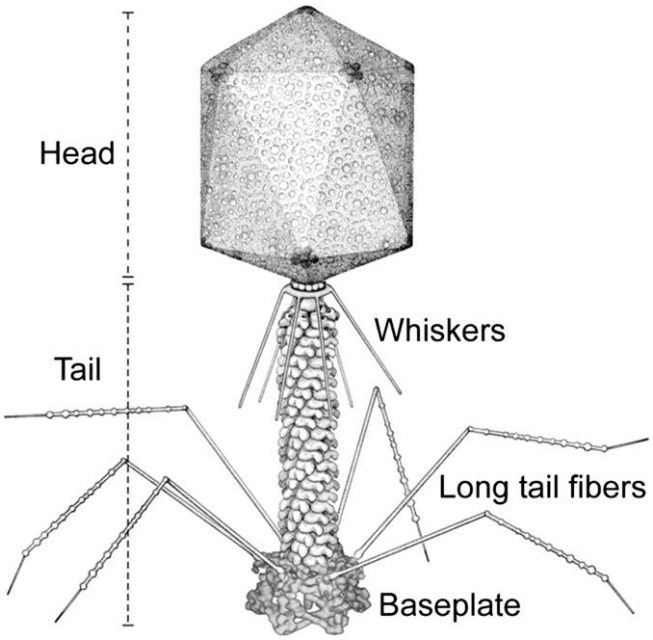

Figure 6.7: Structure of T4 bacteriophage (Source: semanticscholar.org)

6.4.3 MORPHOGENESIS

Bacteriophage T4 is one of the most complex viruses. More than 40 different proteins form the mature virion, which consists of a protein shell encapsulating a 172-kbp double-stranded genomic DNA, a tail, and fibres, attached to the distal end of the tail. The fibres and the tail carry the host

cell recognition sensors and are required for attachment of the phage to the cell surface. The tail also serves as a channel for delivery of the phage DNA from the head into the host cell cytoplasm. The tail is attached to the unique 'portal' vertex of the head through which the phage DNA is packaged during head assembly.

Head morphogenesis:

There are several stages in the assembly of the head:

1. Prohead formation,
2. Prohead proteolysis,
3. DNA packaging,
4. Expansion of the prolate head and
5. Binding of the Hoc and Soc accessory proteins.

The initiation complex first attaches to the inner side of the host cytoplasmic membrane followed by binding of the prohead core (scaffold) proteins gp21, gp22, gp67, gp68, the initiation proteins IPI, IPII, IPIII and gpalt. Subsequently, the capsid proteins gp23 and gp24 start to form a shell around the core proteins to assemble into a prohead. The T4 prohead starts to assemble with the formation of the membrane-bound initiation complex, which comprises the portal protein gp20 mediated by the chaperone protein gp40.

The genome-packaging machine consists of three components: the dodecameric portal protein gp20, the pentameric large terminase gp17 and the small terminase gp16. The external shape of the dodecameric portal protein assembly has a cylindrically shaped structure with the wider end inside the capsid, whereas the narrower end protrudes out of the capsid creating an attachment platform for the packaging motor. These portal proteins have less than 20% sequence identity, but are similar in their overall shape and structure. The large terminase gp17 consists of two functional domains, the N-terminal ATPase domain and the C-terminal nuclease domain, connected by a flexible linker.

There are two DNA-binding grooves on opposite sides of the C-terminal nuclease domain. One of these sites functions to cleave the concatenated DNA molecule to create an end for initiating

packaging and again after packaging has been completed. The other binding sites are used to bind the DNA during DNA translocation into the head.

A peptide linker between the N terminal ATPase domain and C terminal nuclease is essential for DNA translocation. A flexible peptide linker between the N- terminal ATPs domain and the C-terminal nuclease domain is essential for DNA translocation.

Five copies of gp17 assemble into packaging motor on the protruding 'stalk' of the dodecameric portal, thus creating a symmetry mismatch between the portal and the motor.

The small terminase, gp16, is involved in initiating genome packaging and regulating the gp17 functions. In phages such as lambda and SPP1, the small terminase binds to a specific sequence and brings it to the large terminase for initial cleavage to start the packaging mechanism. Gp16 may bind weakly to nonspecific DNA sequences to initiate DNA packaging. The functional oligomeric state of T4 gp16 is uncertain as the crystal structures of the small terminases vary from eight to 12 among three different phages. The domain organization of the small terminases is conserved and consists of an N-terminal DNA-binding domain, a central oligomerization domain, and a C-terminal large terminase-binding domain. The central oligomerization domain forms a ring-shaped structure. The N-terminal domains fold into a helix-turn-helix structure located around the periphery of the ring, whereas the C-terminal domains form a crown over the end of the ring assembly. Crystal structures, biochemical analyses and mutational studies suggest that the DNA wraps around the small terminase assembly.

Tail morphogenesis: The tail consists of the tail tube surrounded by a helical sheath. Both tube and sheath are attached to the dome-shaped base plate at the end away from the head.

The fully assembled base plate is a prerequisite for the assembly of the tail tube and the sheath both of which polymerize into the extended structure using the base plate as the assembly nucleus.

The structure of the baseplate:

During the infection process, the baseplate undergoes a dramatic conformational change that relaxes the high-energy dome-shaped structure to a low-energy star-shaped structure. The

transition between these structures has been studied by analyzing the structures of the component proteins. Crystal structures of nine baseplate proteins. Proteins at the periphery of the baseplate (gp11, gp10, gp9) have threefold symmetry. These proteins are the adaptors to connect the LTFs to the baseplate and transmit a signal to the baseplate on recognizing a host cell surface lip polysaccharide molecule.

The tail tube and tail sheath terminator:

The polymerized tail tube and sheath are capped by the terminator proteins gp3 and gp15, respectively, to prevent depolymerisation before the tail attaches to the head.

6.5 SUMMARY

Cytogenetics is the study of the structure and function of chromosomes in relation to phenotypic expression. Chromosomal abnormalities underline the development of a wide variety of diseases and disorders ranging from Down syndrome to cancer. The studies of microorganisms involve studies of genotype and expression systems. Microbial cytogenetics involves use of genetic engineering. The various means of gene transfer in bacteria and their use in bacterial genetics. The ways of genetic recombination in bacteria include transformation, transduction, and conjugation. . Transformation is a process of horizontal gene transfer in bacteria in which bacteria take up foreign naked DNA from the environment.

Bacteriophage is obligate intracellular viruses that specifically infect bacteria. Phage has a very simple structure. Cell bursting, or lysis, releases hundreds of new phages, which can find and infect other host cells nearby. Bacteriophages, also known as phages, are viruses that infect and replicate only in bacterial cells. Their genetic material is contained in a prism shaped head, surrounded by a protein capsid. In the **lytic cycle**, a phage acts like a typical virus: it hijacks its host cell and uses the cell's resources to make lots of new phages, causing the cell to **lyse** (burst) and die in the process. The mature virion consists of a protein shell encapsulating a 172 kbp double stranded genomic DNA, a tail, and fibres, attached to the distal end of the tail. The fibres and the tail carry the host cell recognition sensors and are required for attachment of the phage to the cell

surface. The fibres and the tail carry the host cell recognition sensors and are required for attachment of the phage to the cell surface. The tail also serves as a channel for delivery of the phage DNA from the head into the host cell cytoplasm. The genome-packaging machine consists of three components: the dodecameric portal protein gp20, the pentameric large terminase gp17 and the small terminase gp16. There are two DNA-binding grooves on opposite sides of the C-terminal nuclease domain.

6.6 TERMINAL QUESTIONS AND ANSWERS

Long answer type questions:

1. Differentiate between specialized and generalized transduction.

2. Explain the process of bacterial conjugation in detail.

3. Write a note on bacterial chromosome.

4. Draw a well labelled diagram of Bacteriophage.

5. Write about T4 bacteriophage structure and morphology.

6. Explain the lytic and lysogenic cycle in bacteriophage.

7. Write a note on morphogenesis in Bacteriophage.

8. What are the types of bacteriophage?

Multiple choice questions

Q.1 The first demonstration of recombination in bacteria was achieved by _____.

a) Lederberg and Tatum

b) Luria and Delbruck

c) Joshua and Lederberg

d) Luria and Tatum

Q.2 which type of *E. coli* strain was chosen to prove the experiment of conjugation?

a) Prototrophs

b) Auxotrophs

c) Polyauxotrophs

d) Autotrophs

Q.3 what is the shape of DNA in the male cells of *E. coli*?

a) Linear

b) Supercoiled

c) circular

d) Relaxed

Q.4 which of the following cells of *E. coli* are referred to as F⁻

a) Male cells

b) Female cells

c) Both male and female cells

d) Neither male nor female cells

Q.5 which of the following is true for an Hfr X F— cross?

a) Frequency of recombination high, transfer of F factor low

b) Frequency of recombination high, transfer of F factor high

c) Frequency of recombination low, transfer of F factor high

d) Frequency of recombination low, transfer of F factor low

Answer: 1(a), 2(c), 3(c), 4(b), 5(a)

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UNIT 7 MOLECULAR CYTOGENETIC TECHNIQUE

CONTENTS

7.1 *OBJECTIVES*

In this unit we will be familiar with some of the important molecular cytogenetic techniques such as:

- FISH
- GISH
- DNA Fingerprinting
- Flow cytometry

7.2 *INTRODUCTION*

Molecular cytogenetics is the combination of two areas of study molecular biology and cytogenetics. Molecular biology is the branch of biology in which we learn about the molecular basis of biological activities between cells; such as DNA synthesis, structure of DNA, transcription, translation, DNA repair process, DNA recombination etc. Cytogenetics is the branch of genetics which deals with study of chromosome structure, chromosome organization in the genome, chromosome behaviors during mitosis and meiosis. Molecular cytogenetics techniques involve the assessment of chromosome structure which help to distinguish normal and diseased cells. In modern times molecular cytogenetic techniques used in diagnosis and treatment of various types of malignancies. These techniques focused on studying the evolution of chromosomes, their number, structure, function and origin of chromosomes abnormalities.

7.3 *FISH& GISH*

Fluorescence in situ hybridization (FISH) is remarkable technique for identifying specific DNA sequences, diagnosing inheritable diseases, mapping genes, and uncovering novel oncogenes or chromosomal defects that contribute too many types of cancers. It is a version of hybridization analysis in which an intact chromosome is examined by probing it with a fluorescent labeled DNA molecule. It has now become the preferred method for localization of specific nucleic acids sequences in native context as it allows very precise spatial resolution of morphological and genomic structures. The technique is rapid, simple to implement, and offers great probe stability.

The genome of a particular species, entire chromosomes, chromosomal- specific regions, or single copy unique sequences can be identified, depending on the probe used.

History: In the early versions of in situ hybridization the probe was radioactively labeled but this procedure was unsatisfactory because it is difficult to achieve both sensitivity and resolution with a radioactive label. These problems were solved in the late 1980s by the development of non-radioactive fluorescent DNA labels. These labels combine high sensitivity with high resolution and are ideal for in situ hybridization.

Principle:

A cell with intact DNA in its nucleus is treated to denature the DNA, forming single stranded regions. The fluorescently labeled DNA probe is added, and the single stranded probe can anneal with the corresponding sequence inside the nucleus. The hybrid molecules will fluoresce when the light from a fluorescent microscope excites the tag on the probe. This technique can localize the gene of interest to different areas of the nucleus or to individual chromosomes.

FISH in action:

FISH was first used to study metaphase chromosomes. These chromosomes, which are made from dividing nuclei, are extremely compact. A fluorescent signal produced by FISH on metaphase chromosomes is mapped by measuring its position relative to the end of the short arm of the chromosome. Because of the very compressed form of the metaphase chromosome, only low-resolution mapping is achievable. To be resolved as a separate hybridization signal, two markers must be at least 1Mb apart. For the creation of usable chromosomal maps, this level of resolution is insufficient. As a result, metaphase FISH has primarily been used to determine the chromosome on which a novel marker is placed and to estimate its map position. Interphase chromosomes are more useful because this stage of the cell cycle (between nuclear division) is when the chromosomes are most condensed. Resolution down to 25 kb is possible, but chromosome morphology is lost so there are no external reference points against which to map the position of the probe. This technique is therefore used after preliminary map information has been obtained.

Types of probes for FISH:

Generally, researchers use three different types of FISH probes, each of which has a different application:

- **Locus specific probes:** bind to a particular region of a chromosome. This type of probe is useful when researcher have isolated a small portion of a gene and want to determine on which chromosome the gene is located.
- **Alphoid or Centromeri**c repeat probes are generated from repetitive sequences found in the centromere of each chromosome.
- **Whole chromosome probes** are actually collection of smaller probes, each of which binds to a different sequence along the length of a given chromosome. Using multiple probes labeled with a mixture of different fluorescent dyes, scientists are able to label each chromosome in its own unique color. The resulting full color maps of the chromosome probes are particularly useful for examining chromosomal abnormalities, for example, when a piece of one chromosome is attached to the end of another chromosome.

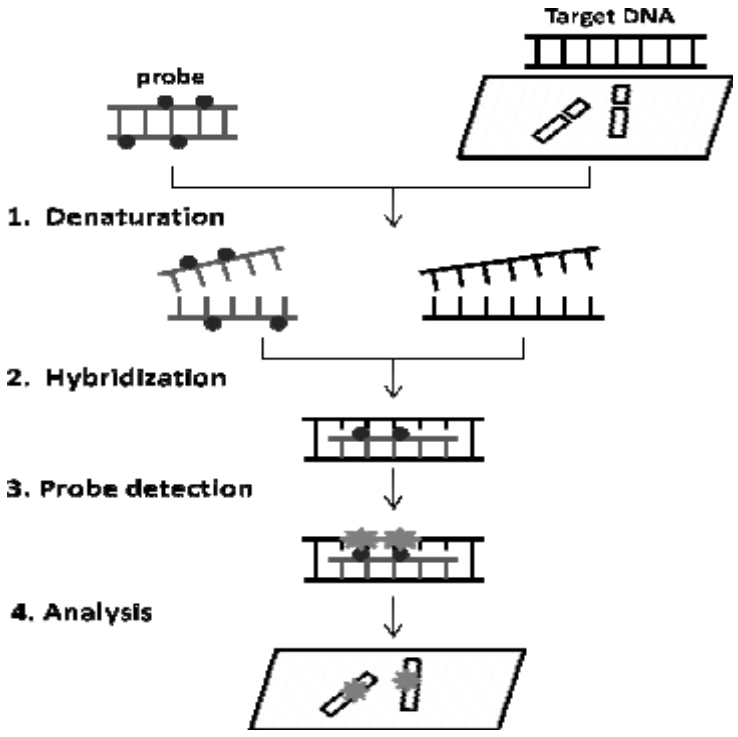

Figure 7.1: Process of FISH (copyright: researchgate.net)

Process of FISH:

1. First, a probe is constructed. The probe must be large enough to hybridize specifically with its target but not too large to impede the hybridization process. The probe is tagged directly with fluorophores.
2. Then, an interphase or metaphase chromosome preparation is produced. The chromosomes are firmly attached to a substrate, usually glass.
3. The probe is then applied to the chromosome DNA and incubated for approximately 12 hours while hybridizing. Several wash steps remove all unhybridized and partially hybridized probes.
4. The result is then visualized and quantified using a microscope that is capable of exciting the dye and recording images.

GISH: GISH (genome in situ hybridization) is a cytogenetic technique for radio labeling portions of the genome within cells. GISH was created for plant breeding and animal hybrid cell lines. The GISH approach, which is an improvement on FISH, is commonly used to examine plants. GISH approaches aid in the differentiation of a cell's genomes. Because this strong technique can discriminate genomes in hybrids, GISH is now being utilized to research hybrid cell lineages, genetic improvement programs, and polyploid evolution. GISH is a tool for analyzing meiotic behavior in hybrids and polyploids, which provides information about species relationships.

The Main steps involved in the genomic in situ hybridization are:

1. Direct or indirect labeling of probe.

2. Blocking DNA fragmentation

3. Preparation of slide.

4. Denaturation of Probe and blocking DNA in a hybridization mixture.

5. Addition of the probe and the blocking DNA with the hybridization mixture.

6. Chromosome DNA denaturation.

7. Hybridization of blocking DNA and probe in the target sequence of the chromosome.

8. Detection of the probe in the chromosome of one parent.

9. Chromosome DNA molecule of the second parent related to the unlabeled blocking DNA.

10. Visualization of hybridization signals in a fluorescence microscope. Unlabeled chromosomes are visualized with a counter-stain (blue).

The major application of GISH technique are as follows:

1. Meiotic studies of chromosome.

2. Determination of phylogenetic relationship.

3. Determine the positions of translocation breakpoints in chromosome

4. Comparative genomic studies of malignant and normal cells of an individual

5. Identification of unknown genome

6. To identify the hybridized genome of crop varieties

7.4 *DNA FINGERPRINTING*

DNA Fingerprinting is a molecular biology technique which was first developed in 1984 by British geneticist Alec Jeffreys. He noticed that certain sequences of highly variable DNA (i.e., VNTRs), which do not contribute to the functions of genes, are repeated within genes are known as minisatellites. Jeffreys recognized that each individual has a unique pattern of minisatellites. Therefore, this technique can assist in the identification of individuals or samples by their respective DNA profiles. This was the main idea behind this marvelous invention.

More than 99.1% of the genome is same throughout the human population, the remaining 0.9% of human DNA shows variations between individuals. These variable DNA sequences, termed molecular markers, can be used to both differentiate and correlate individuals.

The procedure for creating a DNA fingerprint involve following steps:

1. Obtain a sample of cells, such as skin, hair, or blood cells, which contain DNA.
2. The DNA is extracted from the cells and purified.
3. The DNA was then cut at specific points along the strand with restriction endonuclease enzymes which are known as molecular scissors.
4. This enzyme produces fragments of varying lengths.
5. These fragments are then separated by gel electrophoresis technique.
6. The shorter the fragment, the more quickly it moved toward the positive pole (anode).

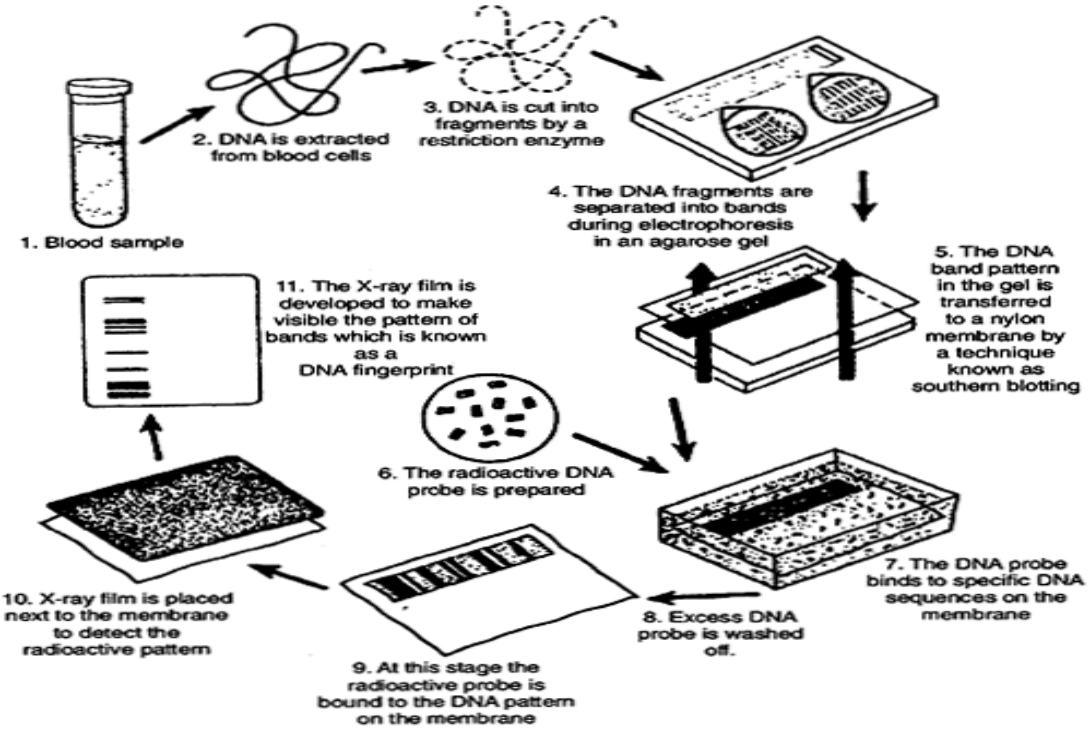

Figure 7.2: Steps involved in DNA fingerprinting (Source: pinterest.com)

7. The sorted double-stranded DNA fragments were then subjected to a blotting in which they were split into single strands and transferred to a nylon sheet.
8. The fragments underwent autoradiography in which they were exposed to DNA probes (i.e., pieces of synthetic DNA) that were made radioactive and that bound to the minisatellites.
9. A piece of X-ray film was then exposed to the fragments, and a dark mark was produced at any point where a radioactive probe had become attached. The resultant pattern of marks could then be analyzed.

Applications of DNA fingerprinting:

1. It is used in forensics to identify the culprit of a crime after comparing the DNA sequence of the potential suspects.
2. It is used to establish paternity and family relationships.
3. It is used to identify and protect the commercial varieties of crops and livestock.
4. It is used to find out the evolutionary history of an organism and trace out the linkages between groups of various organisms.

7.5 *FLOW CYTOMETRY*

Flow cytometry is a strong tool for determining morphology, cellular characteristics, and cell cycle stage of cells. Flow cytometry (etymologically derived from the Greek words cyto=cell and metry=measure) is a technique for determining the biological and physical properties of cells and other particles suspended in a high-velocity fluid stream while passing under a laser beam. Flow cytometry is used to evaluate living cells that have been stained with specific fluorescently tagged antibodies. Light scattering and fluorescence characteristics of cells are used in this technique. It's a common molecular biology approach for determining the expression of cell surface and intracellular chemicals, as well as identifying and characterizing various cell types in a heterogeneous cell population. This method can be used to interpret data on viability, apoptosis, cell cycle, cell physiology, immunophenotyping, and protein synthesis.

FACS: Fluorescence activated cell sorting is a specialized type of flow cytometry. It is a method which helps in sorting of heterogenous mixture of biological cells into two or more containers, one cell at a time, based on the specific light scattering and fluorescent characteristics of each cell.

Multicolor flow cytometry: Multicolor flow cytometry is a useful technique when examining mixed populations of cells, such as blood and tissue cells in human and animal samples. A specific cell type is marked with fluorescent dye like fluorophore or propidium iodide. The ability to use multiple fluorescent markers at a time allows identification of multiple cell types.

Working of Flow Cytometer: Main principle of using flow cytometry is the ability to analyze the complete cell cycle and analyze DNA content in different phases. Monitoring the natural events of the cell cycle can provide information for disease diagnosis and therapy prognosis. The different phases of the cell cycle can reveal altered DNA content and other anomalies indicated tumor presence or signs of advanced cell death. The data expressions are stored in a computer via specialized flow cytometry software associated with chosen instrument use during the time of analysis. Flow cytometry data is typically documented in two distinct ways: 1) Histogram and 2) A dot plot

The flow cytometer consists of three parts:

1. The fluidics system of a flow cytometer is responsible for transporting samples from the sample tube to the flow cell, past the laser, sorted and/or discarded.
2. The components of the optical system include excitation light sources, lenses, and optical filters used to collect and move wavelengths of light around the instrument and the detection system that generates the photocurrent. The difference of wavelength response in the data helps analyze cell type. Fluorochrome used for the detection of target proteins emit light when excited by laser with the corresponding excitation wavelength. These fluorescent stained cells or particles can be detected individually.
3. The electronics or flow cytometer instrumentation.

Sheath fluid focuses the cell suspension, causing cells to pass through a laser beam one cell at a time. Forward and side scattered light is detected, as well as fluorescence emitted from stained

cells. Light scattered from the cells or particles is detected as they go through laser beam. Detector measure the forward scatter (FS) and side scatter (SS). Cells passing through the beam scatter light which is detected as FS and SS. FS proportional to the cell size and Side scatter (SS) to the granularity of the cells. By this method cells can be distinguished based on differences in their size and granularity alone.

- ❖ Larger and more granular cells produce a scatter plot which shows high SS and FS.
- ❖ Monocytes are large agranular cells will produce a scatter plot with high FS but lower SS.
- ❖ Smaller agranular lymphocytes produce will produce a scatter plot with low FS and low SS.

Applications of Flow Cytometry:

1. Cell cycle status- It is a powerful tool to assess cells in G0/G1 phase versus S phase, G2 or polyploidy, including analysis of cell proliferation and activation.
2. Identification of cell types within a heterogenous sample for example distinguishing central effector memory cells from exhausted T cells or regulatory T cells.
3. Flow cytometry studies are used to identify and quantify immune cells and characterize hematological malignancies.
4. It helps to analyze protein expression throughout the entire cell, even in the nucleus.

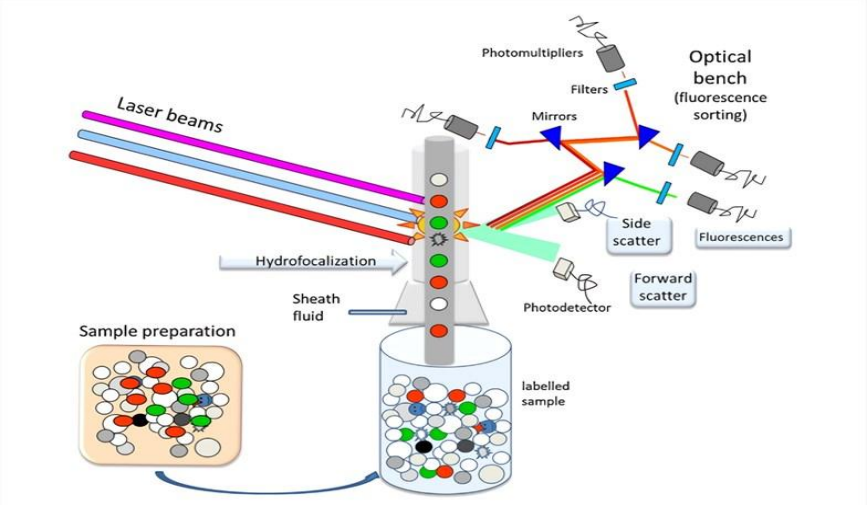

Figure 7.3: Flow cytometer (Source: creative biolabs)

7.6 SUMMARY

Molecular cytogenetic techniques play an important role in examinations of a variety of human disorders. Over the years Molecular cytogenetic analysis emerged as a successful diagnostic tool. These techniques involve fluorescence in situ hybridization, or FISH, GISH, DNA fingerprinting and Flow cytometry. FISH is based on the use of chromosome region specific and fluorescent-labeled DNA probe. In FISH, DNA probes are labeled with colorful tag to visualize specific area of the genome. Fluorescence activated cell sorting is a specialized type of flow cytometry. FISH was first used to study metaphase chromosomes. These chromosomes, which are made from dividing nuclei, are extremely compact. A fluorescent signal produced by FISH on metaphase chromosomes is mapped by measuring its position relative to the end of the short arm of the chromosome. It is a method which helps in sorting of heterogenous mixture of biological cells into two or more containers, one cell at a time, based on the specific light scattering and fluorescent characteristics of each cell. GISH is advancement in the FISH. DNA fingerprinting is the technique employed by forensic scientists that helps in the identification of DNA sequence of potential crime suspect. A flow cytometer utilizes laser as light sources to produce both scattered and fluorescent light signal that are read by detectors.

7.7 TERMINAL QUESTIONS AND ANSWERS

Question 1: Write a note on the following:

a) FISH
b) GISH
c) VNTR
d) PROBE
e) FACS

Question 2: Describe the principle and working of following technique:

a) DNA Fingerprinting
b) Flow cytometry

c) FISH

Question 3: Make a list of the applications of FISH, Flow cytometer, and DNA fingerprinting techniques.

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UNIT 8: GENOME ANALYSIS AND GENETICS OF CELL CYCLE

CONTENTS

8.1 OBJECTIVES

We understand the in this topic about the Genome and Genomic Analysis and read about the C-Value and C-value Paradox, we also read about the Molecular analysis of Genomic DNA, Gene Mapping & Genetic regulation of Cell Division.

8.2 INTRODUCTION

Genomic analysis is the identification, measurement or comparison of genomic features such as DNA sequence, structural variation, gene expression, or regulatory and functional element annotation at a genomic scale. Genome projects typically involve three main phases: DNA sequencing, assembly of DNA to represent original chromosome, and analysis of the representation. DNA sequencing is the process to determine the nucleotide order in a specific DNA molecule, which is useful when attempting to understand its function and consequent effects in the organism it resides in. DNA sequence assembly involves the alignment and merging of DNA fragments to reconstruct the DNA so that smaller sections of the genome can be analyzed.The analysis of DNA phase is the final step in genome analysis. It brings together the discoveries from the previous phases of the project to form conclusions, which can offer true value to further our knowledge of the genome and be applied in relevant situations.

8.3 C- VALUE PARADOX

C-value is the amount, in picograms, of DNA contained within a haploid nucleus (e.g. a gamete) or one half the amount in a diploid somatic cell of a eukaryotic organism. In some cases (notably among diploid organisms), the terms C-value and genome size are used interchangeably; however, in polyploids the C-value may represent two or more genomes contained within the same nucleus. Greilhuber et al. have suggested some new layers of terminology and associated abbreviations to clarify this issue, but these somewhat complex additions are yet to be used by other authors.

The **C-value enigma** or **C-value paradox** is the complex puzzle surrounding the extensive variation in nuclear genome size among eukaryotic species. At the center of the C-value enigma is the observation that genome size does not correlate with organismal complexity; for example,

some single-celled protists have genomes much larger than that of humans. Some prefer the term C-value enigma because it explicitly includes all of the questions that will need to be answered if a complete understanding of genome size evolution is to be achieved (Gregory 2005). Moreover, the term paradox implies a lack of understanding of one of the most basic features of eukaryotic genomes: namely that they are composed primarily of non-coding DNA.

In 1948, Roger and Colette Vendrely reported a "remarkable constancy in the nuclear DNA content of all the cells in all the individuals within a given animal species", which they took as evidence that DNA, rather than protein, was the substance of which genes are composed. The term C-value reflects this observed constancy. However, it was soon found that C-values (genome sizes) vary enormously among species and that this bears no relationship to the *presumed* number of genes (*as reflected by* the complexity of the organism).For example, the cells of some salamanders may contain 40 times more DNA than those of humans. Given that C-values were assumed to be constant because genetic information is encoded by DNA, and yet bore no relationship to presumed gene number, this was understandably considered paradoxical; the term "C-value paradox" was used to describe this situation by C.A. Thomas Jr. in 1971.

The discovery of non-coding DNA in the early 1970s resolved the main question of the C-value paradox: genome size does not reflect gene number in eukaryotes since most of their DNA is non-coding and therefore does not consist of genes. The human genome, for example, comprises less than 2% protein-coding regions, with the remainder being various types of non-coding DNA.

C-value enigma

The term "C-value enigma" represents an update of the more common but outdated term "C-value paradox" (Thomas 1971), being ultimately derived from the term "C-value" (Swift 1950) in reference to haploid nuclear DNA contents. The term was coined by Canadian biologist Dr. T. Ryan Gregory of the University of Guelph in 2000/2001. In general terms, the C-value enigma relates to the issue of variation in the amount of non-coding DNA found within the genomes of different eukaryotes.

8.3.1 Detailed account of various models of Prokaryotic Genomes

Human colon bacterium, E. coli has been used extensively for the study of prokaryotic genome. Studies in 1950's and 60's made use of phase-contrast microscopy and autoradiography techniques to reveal the presence of single circular chromosome having the essential genes of E. coli and packed within the cell nucleoid (Mason & Powelson, 1956; Cairns, 1963). The bacterial chromosome is tightly packed so that it can fit into the small volume of the bacterial cell. It involves supercoiling of DNA and further twisting the twisted chromosome. Scaffold of RNA and protein contributes 50% to the chromosome and DNA domains are held together them. The entire nucleoid is attached to the cell membrane. It helps in the segregation of the chromosomes during cell division. Histone proteins are absent in bacteria which are present in eukaryotes. However, it is believed that polyamines (organic molecules with multiple NH2 amine groups) such as spermidine, as well as some basic proteins are present in prokaryotes and help in reducing the size of bacterial chromosome. Chromosomes are circular in prokaryotes, replication starts at single point, called Ori C and proceeds in both directions till the two replication forks meet. The parameters which best convey bacterial genomes are the shape, size, replicon number, geometry and the G-C content. In prokaryotes gene expression is co-transcribed with the help of operons.

Earlier the complexity of the genome was related to the genome size, now referred to as the C-value paradox.

a) Chromosomal: The chromosome is highly varied and ranges from a single circular to double circular or even a linear molecule of DNA e.g., Agro bacterium tumefaciens has two chromosome a) linear chromosome of 2.1 Mb and b) circular chromosome of 3.0Mb. The chromosome can be easily distinguished from the plasmid due to its large size, E. coli has a super coiled chromosome (Fig: 1). the region to which the DNA remains confined is known as the nucleotide. Changes in the genes are much more prone than the change in entire chromosome due to the continuous process of selection operating within the genes. Base composition patterns of the prokaryotic genomes reveal that there is a direct correlation between the genome size and GC content of the genome. GC content is higher in the genomes of soil dwelling prokaryotes and gets reduced in genomes of host obligates and tend to have more of AT content. Free living bacteria have shown to contain about

49% of GC content and the figure is 38% in the case of host obligates. Another reason for this shift of nucleotides is due to the unchecked point mutations. The most common one being a change from C to T (Uracil formed due to deanimation of cytosine followed by subsequent replication to thymidine) or G to A. The mean GC content within the bacterial genome varies from 25-75% (for e.g.: Wigglesworth sp. has 22.4 % GC content and Streptomycin coelicolor has 72.1% GC content, thus we get an average of 46.8% GC content). Different regions of chromosome show different concentrations of AT and GC nucleotides. It has been found that AT is in abundance in the intergenic, non coding sequences and the wobble position in coding sequences for reduced sized genomes. Upstream promoter regions are AT rich to facilitate a curved rigid conformation which unwinds more easily. The distribution of GC does not follow a set pattern in the genome. However, it has been observed that a preference for GC is near the origin and AT is preferred near the origin of replication. The pattern of GC nucleotide distribution is also dependent on a whole host of functional and environmental circumstances.

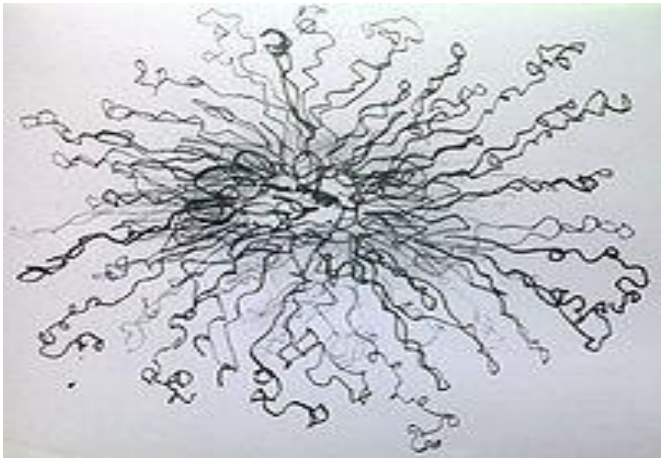

Figure 8.1. Supercoiled E.coli chromosome

b) Plasmid: Plasmid is the extra chromosomal DNA in a prokaryotic cell, capable of independent replication, stably inherited. The prokaryotic organism can survive even in the absence of plasmid. The number and shape (circular or linear) of the plasmid varies in different organisms. Plasmid are different from the chromosome; they are found adjacent to the chromosome smaller in size having non essential genes (genes not involved to carry the housekeeping function instead they give resistance from antibiotic and helps to grow in specific adverse conditions). Thus, they help in

providing an additional selective advantage to the host. e.g.; R factors (such plasmids are known as resistance or R-factor). Additional beneficial traits include heavy metal resistance, degradation of complex molecules, disease causing factors necessary for infection of plant or animal cell which are passed on to other hosts/bacterium through the process of conjugation. Three major types of bacterial plasmids have been extensively studied.

(i) F and F' plasmids: These are conjugation fertility factors.

(ii) R Plasmids: These plasmids have genes that confer resistance to antibiotics or other antimicrobial drugs.

(iii) Col plasmids: These are the plasmids that have genes encoding colicins proteins that kill sensitive E. coli cells.

Other plasmids are also present that code for bacteriocins and vibriocins. Vibriocins have the capability to kill sensitive Vibrio cholerae Plasmids may be divided into two groups (i) Conjugative or transmissible plasmids: These plasmids mediate the transfer of DNA by conjugation. Examples: F and F' plasmids, many R plasmids and some Col plasmids (ii) No conjugative or no transmissible plasmids: These plasmids do not mediate transfer of DNA by conjugation.

c) Episome: Episomes are distinguished from other extra chromosomal DNA like plasmids on the basis of their size; usually they have a molecular weight of 62kb. It is a form of plasmid which can replicate on its own in addition to the ability of integrating into a bacterial chromosome. It does not form part of the genome and is a non essential element which originates outside the host. It exits in various forms a) F+ form (autonomous extra chromosomal form) which is attached to the bacterial cell membrane. We can call it an independent form i.e. without integrating into the host genome, it is able to destroy the host by making use of all the host machinery for its own survival; b)Hfr form(high frequency recombination form) which forms a part of the bacterial chromosome by integrating into it. We'll call it the dependent form i.e. when it integrates into the host genome and makes its own copies by replicating along the host chromosomes. They are passed on to the daughter cells as they multiply with the host's cell division. Both the forms i.e., F+ and Hfr forms are capable to donate genetic material during conjugation in various bacteria e.g; Salmonella, E.

coli, Serratia, Pseudomonas. Conjugation results in converting a F- cell (cell lacking episome) into a F+ cell or Hfr cell depending upon the conjugating partner type. Another type known as F or F prime found outside the chromosome, but with a part of chromosomal DNA attached to it.

8.3.2 VIRAL GENOME AND EUKARYOTIC GENOMES

The viral genome is packed inside a symmetric protein capsid, composed of either a single or multiple proteins; each of them is encoding a single viral gene. Due to this symmetric structure, viruses could encode all the necessary information for constructing a large capsid using a small set of genes.

Viral diseases have an enormous impact on human health worldwide. Genomic technologies are providing infectious disease researchers an unprecedented capability to study at a genetic level the viruses that cause disease and their interactions with infected hosts. An enormous variety of genomic structures can be seen among viral species; as a group, they contain more structural genomic diversity than plants, animals, archaea, or bacteria. There are millions of different types of viruses, although only about 5,000 of them have been described in detail.

Eukaryotic genome has two sets of chromosome one from each parent. It differs from the prokaryotic genome in bulkiness as it has a lot of non-coding DNA also referred to as the junk DNA. Junk DNA acts as a sink to absorb a lot of mutations. The entire eukaryotic DNA is confined in the nucleus and remains associated with proteins. This association varies depending upon the requirements of the cell at various stages of the cell division. It's a multi-level system of DNA packing. Chromatin is formed when the double stranded DNA is wound around histone proteins to pack and condense the bulky DNA (Fig: 8.2). The word chromatin was first used by Walter Flemming, he explored cytology (study of chromosomes) and studied the process of mitosis in detail, In 1879 he stained salamander embryos using aniline dyes (byproduct of coal tar) and was able to identify and visualize the chromosome movements during cell division which he described in his book Zell-substanz, Kern und Zelltheilung (Cell-Substance, Nucleus, and Cell-Division), Chromatin is present almost in all cells with an exception of erythrocytes. During interphase chromatin is distinguishable as two different forms i.e., euchromatin and

heterochromatin.Histones are the most abundant proteins in chromatin. They are mostly made of lysine and arginine amino acids, which are responsible for contributing a net positive charge to histone proteins. Positive charge is helpful in binding with the negative charge of the phosphate molecule in DNA; this complementarily of the charges helps in holding the DNA in close contact with the histones. Five types of histone proteins associated with the chromatin are H1, H2A, H2B, H3 and H4.

a) Chromatin structural organization: It consists of a multi-level system of packing the DNA. Histones are the first level to initiate DNA packing. Uncoiled chromatin appears like a string with beads of nucleosomes (made of histone proteins having DNA coiled on it). Nucleosome forms the basic unit of chromatin, joined together by short stretches of DNA itself. Changes in the chromatin organization help in the process of replication and transcription. During the process of replication histones leave the DNA only transiently whereas they stay with the DNA during transcription. The changing shape and positional variation of the histones provide the movement of the RNA polymerase along the DNA molecule. Core histones consist of H2A, H2B, H3 and H4. There are two molecules of each type of core histone protein which result in the formation of the "Histone Octamer" complex. H1 is referred to as a "Linker histone" which seals the entry/exit position of the DNA strand on the nucleosome. H1 histone and the nucleosome bead together is known as chromatosome. Binding of the DNA and nucleosmes is highly non-specific depending upon the requirements of the cell and their function in general DNA packing.

A) Functions of chromatin:

1) Packing of DNA to fit within the cell's nucleus

2) To allow flexibility to DNA for carrying out the process of mitosis

3) To protect DNA from damage;

4) To regulate and control the genetic expression through DNA replication and transcription.

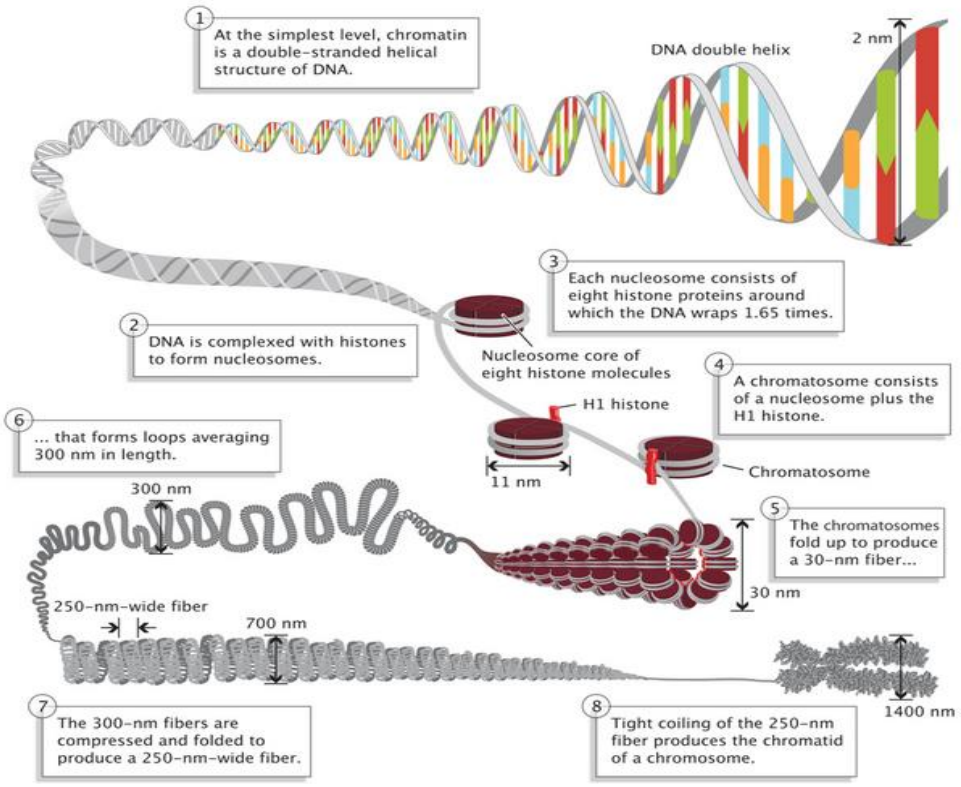

Fig.8.2. Chromatin structural organisation

a)Euchromatin and Heterochromatin: Chromatin is easily distinguishable into two phases during interphase: 1) Euchromatin, which consists of active DNA, i.e., region which code for specific genes e.g., being expressed as protein and repetitive sequences are absent. It shows the normal cycle of condensation and de-condensation in the cell cycle. Consequently, shows change in intensity of staining from darkest in the metaphase stage to lightest in the S phase. 2) Heterochromatin, which consists of mostly inactive DNA or the DNA which is not expressed as genes or proteins. It remains condensed and shows dark staining throughout the cell cycle as compared to euchromatin. It seems to serve structural purposes during the chromosomal stages. Heterochromatin can be further distinguished into two types: (i) Constitutive heterochromatin, located around the centromere, telomere and adjacent to the nucleolus mainly having certain repetitive sequences which is never expressed. It is present in all cells at identical positions of both homologous chromosomes of pair. (ii) Facultative heterochromatin, which is sometimes expressed. It varies in state in different cell types and developmental stages. The Barr body, inactivated X chromosome in somatic cells of XX mammalian females is an example of facultative

heterochromatin. Which X chromosome i.e paternal or maternal would be inactivated is a matter of chance, but once one of the X chromosomes inactivated, all the cells arising from that cell will keep the same inactive chromosome. Therefore, mammalian females are mosaic with some parts having the alternate allele expressed. Chromosomal studies early in this century revealed that certain regions remained condensed during the interphase and prophase while the other chromosomes were still as long and thin threads. These were called as hetero chromosomes by the earlier cytologists and considered to be the sex chromosomes. However, Heitz (1929) showed that differential condensation was not limited to the sex chromosome; and was part of an otherwise normal chromosome. Thus, two names heterochromatin and euchromatin were coined to describe the unusually condensed (which remains condensed throughout the events of cell cycle) and typical segments (which condense and decondense during the cell cycle), respectively. Chromatin is a feature of eukaryotic cells whose structure depends on several factors e.g.: stage of the cell cycle. During interphase, the chromatin opens up to allow the access of DNA and RNA polymerases that replicate and transcribe the DNA. The unwinding of the chromatin regions depends on the coding and non-coding regions of the DNA. The coding genes which need to be actively transcribed are loosely packed to facilitate the binding of the RNA polymerase whereas the DNA coding inactive genes (non-coding regions of DNA/junk DNA) remain associated with the structural proteins and are more tightly packed. Chromatin structure is altered by epigenetic chemical modifications which include particularly methylation, acetylation, and phosphorylation of histone proteins. When cell enters into cell cycle, there is tight packaging of chromatin to facilitate segregation of the chromosomes during anaphase. In general terms, there are three levels of chromatin organization. DNA wraps around histone proteins forming nucleosomes; the "beads on a string" structure. Multiple histones wrap into a 30 nm fibre and finally higher-level DNA packaging of the 30 nm fibres into the metaphase chromosome. There are certain exceptions to this organization. For example, spermatozoa and avian red blood cells have more tightly packed chromatin than most eukaryotic cells, and trypanosomatid protozoa do not condense their chromatin into visible chromosomes for mitosis.

8.4 MOLECULAR ANALYSIS OF GENOMIC DNA IN YEAST

S. cerevisiae contains a haploid set of 16 well-characterized chromosomes, ranging in size from 200 to 2,200 kb. The total sequence of chromosomal DNA, constituting 12,052 kb, was released in April, 1996. A total of 6,183 open-reading-frames (ORF) of over 100 amino acids long were reported, and approximately 5,800 of them were predicated to correspond to actual protein-coding genes. A larger number of ORFs were predicted by considering shorter proteins. In contrast to the genomes of multicellular organisms, the yeast genome is highly compact, with genes representing 72% of the total sequence (<2% in the human genome!). The average size of yeast genes is 1.45 kb, or 483 codons, with a range from 40 to 4,910 codons. A total of 3.8% of the ORF contain introns. Approximately 30% of the genes already have been characterized experimentally. Of the remaining 70% with unknown function, approximately one half either contain a motif of a characterized class of proteins or correspond to genes encoding proteins that are structurally related to functionally characterized gene products from yeast or from other organisms.

Ribosomal RNA is coded by approximately 120 copies of a single tandem array on chromosome XII. The DNA sequence revealed that yeast contains 262 t-RNA genes, of which 80 have introns. In addition, chromosomes contain movable DNA elements, retrotransposons (Ty elements), that vary in number and position in different strains of S. cerevisiae, with most laboratory strains having approximately 30.

Other nucleic acid entities also can be considered part of the yeast genome. Mitochondrial DNA encodes components of the mitochondrial translational machinery and approximately 15% of the mitochondrial proteins. P^0 mutants completely lack mitochondrial DNA and are deficient in the respiratory polypeptides synthesized on mitochondrial ribosomes, i.e., cytochrome b and subunits of cytochrome oxidase and ATPase complexes. Even though ρo mutants are respiratory deficient, they are viable and still retain mitochondria, although morphologically abnormal.

The 2-μm circle plasmids, present in most strains of S. cerevisiae, apparently function solely for their own replication. Generally ciro strains, which lack 2-μm DNA, have no observable

phenotype. However, a certain chromosomal mutation, nib1, causes a reduction in growth of cir+ strains, due to an abnormally high copy number 2-μm DNA.

Similarly, almost all S. cerevisiae strains contain dsRNA viruses that constitute approximately 0.1% of total nucleic acid. RNA viruses include three families with dsRNA genomes, L-A, L-BC, and M. Two other families of dsRNA, T and W, replicate in yeast but so far have not been shown to be viral. M dsRNA encodes a toxin, and L-A encodes the major coat protein and components required for the viral replication and maintenance of M. The two dsRNA, M and L-A, are packaged separately with the common capsid protein encoded by L-A, resulting in virus-like particles that are transmitted cytoplasmically during vegetative growth and conjugation. L-B and L-C (collectively denoted L-BC), similar to L-A, have a RNA- dependent RNA polymerase and are present in intracellular particles. KIL-o mutants, lacking M dsRNA and consequently the killer toxin, are readily induced by growth at elevated temperatures, and chemical and physical agents.
Yeast also contains a 20S circular single-stranded RNA (not shown in Figure 2.1) that appears to encode an RNA-dependent RNA polymerase, that acts as an independent replicon, and that is inherited as a non-Mendelian genetic element. Only mutations of chromosomal genes exhibit Mendelian 2:2 segregation in tetrads after sporulation of heterozygous diploids; this property is dependent on the disjunction of chromosomal centromeres. In contrast, non-Mendelian inheritance is observed for the phenotypes associated with the absence or alteration of other nucleic acids.

8.5 TRANSPOSABLE ELEMENTS IN PROKARYOTES AND EUKARYOTES

Two classes of transposable elements/mechanisms of movement: 1. Encode proteins that (1) move DNA directly to a new position or (2) replicate DNA and integrate replicated DNA elsewhere in the genome (prokaryotes and eukaryotes).

Transposable elements are mobile genetic elements which can insert themselves into many different DNA sites without showing much target site specificity. Studies in molecular biology, genetics, and genomics have revealed structure of various transposable elements and mechanisms of transposition. Many new transposable elements have been identified, broadening their

taxonomic distribution. Genomic studies are revealing relationship between TEs and viruses as well as their role in evolution of eukaryotic genome. Most transposons do not show very frequent movement, yet given the period on evolutionary time scale, their accumulation in the genome has been rather tremendous. They are divided broadly into three classes based on the transposition intermediate and mechanism of movement. Retroviral-like retro-transposons (Class 1), which move using RNA intermediate. They are called retroviral like as they resemble retroviruses not only in structure but also in their method of integration. Both possess gag, and pol genes; retroviruses in addition have env gene coding for the envelope. It is the absence of functional env in retrotransposons that they are no longer capable of forming infectious particle and hence are called endogenous retroviruses.

DNA of the transposon is transcribed into RNA by RNA polymerase. This RNA then serves as a template for enzyme reverse transcriptase, transcribing this RNA into DNA and thus generating a new copy of the transposon. This DNA is then inserted into a new site in the genome.

Transposase-like enzyme catalyzing this insertion reaction is called integrase rather than transposase (for historical reasons). These transposons have long terminal repeats (LTR) and hence are also called LTR retrotransposons. Human LTR elements account for approximately 8% of the genome. Having accumulated a number of mutations they are not currently showing much activity. But certainly these elements have played important role in the past in the genesis of some of the evolutionary novelties as discussed below. Nonretroviral retrotransposons, (Class2), also move by a nearly similar method with one difference.

LINEs code for enzyme catalyzing their movement and are hence autonomous. ORF1 e ncodes an RNA-binding protein and ORF2 encodes a protein with endonuclease and reverse- transcriptase activities.

Transposon DNA is transcribed into RNA, which leaves the nucleus, enters cytoplasm,

translating into two proteins, coded by ORF1 and ORF2, which combine with the RNA that coded it, making it a ribonucleoprotein complex. This complex moves back to nucleus, and endonuclease cleaves at 5'-TTTT/AA-33' consensus site of the target DNA. This frees the 3'hydroxyl group at the nick where reverse transcription of RNA starts. Second strand of the target DNA is cleaved and into this is inserted the complementary copy of the DNA strand which was reverse- transcribed

from the RNA. In this case RNA molecule selects the target sequence in the DNA, hence it is called target primed reverse transcription (TPRT). These elements have internal promoter for RNA polymerase II and do not have terminal inverted repeats and hence are also called Non-LTR retrotransposons, e.g., LINE-1, SINE (e.g.Alu) elements. They constitute nearly one third of the genome, and are currently

active in humans. This allows for the retrotransposition process to be target-primed reverse transcription. Out of the 500,000 L1 elements inserted in human genome, less than 100 copies are intact. A typical full-length Alu element is ~300base pairs, formed by fusion of two monomers derived fromthe 7SL RNA gene. Its 5' region contains an internal promoter for RNA polymerase III and 3' region ends with an oligo dA-rich tail of variable length. SINEs do not encode any enzyme and are therefore non-autonomous, and use the transposition machinery of LINEs.

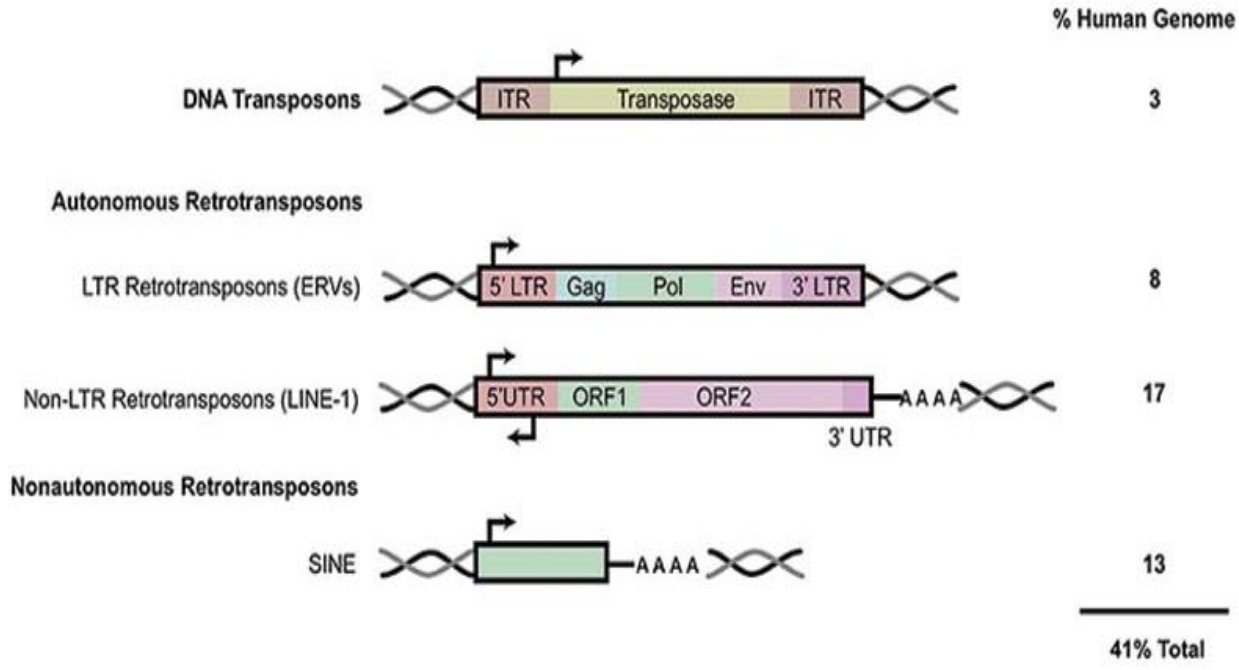

Fig.8.3 Different Transposable Elements

DNA transposons (Class3) exist as DNA through-out their life cycle. Eukaryotic DNA transposons have been divided into three major sub-classes;

i. Classic cut-and-paste transposons have terminal inverted repeats (TIR) and are cut as double-stranded DNA. First, flanking DNA is cut, transposon DNA removed and inserted into a new target DNA site which need not share homology with the ends of the element. The entire process uses the specific enzyme, transposase, coded by DNA transposon.

(ii) Helitrons are transposons which donot have TIR but show small motifs at the ends. Autonomous elements code for helicase and use rolling circle replication (iii) Mavericks are transposons with TIR, and they are capable of coding for a number of proteins. It has been suggested that they are related to double stranded DNA viruses. Mechanism of their transposition has not been clarified.

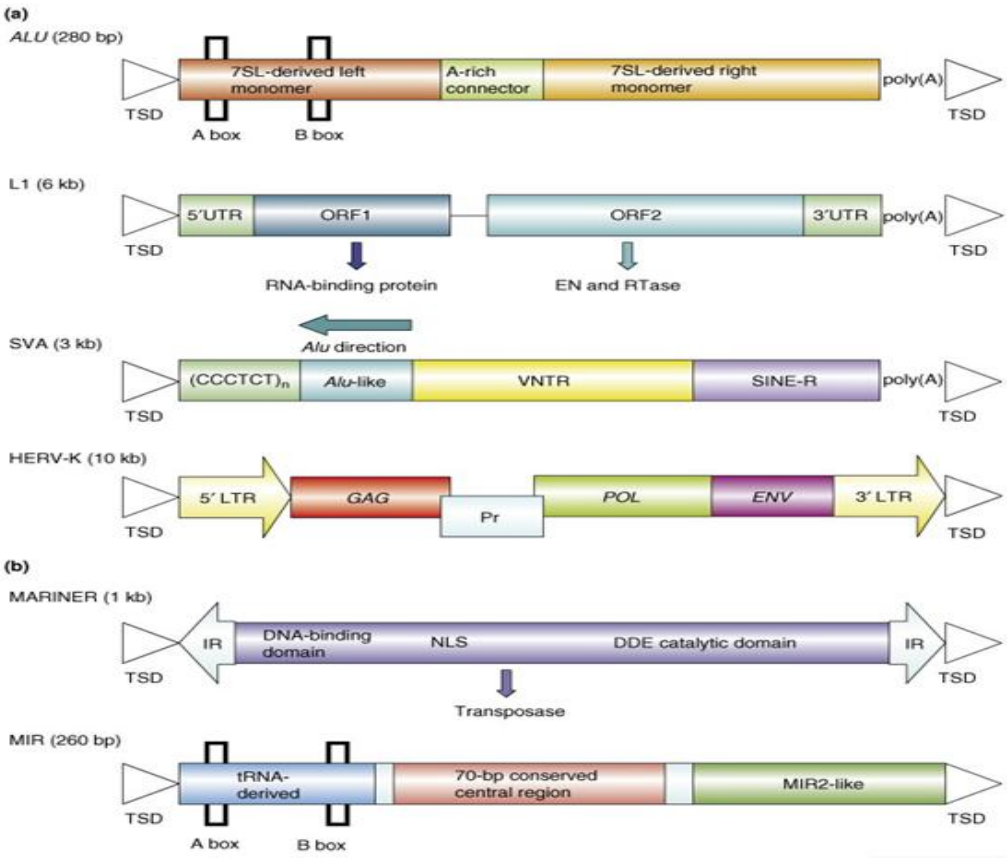

Fig.8.4 Types of Transposable Elements in the Human Genome

Major types of DNA transposons have a very ancient origin and have persisted over a long evolutionary time. This is suggested by the studies that most of the super families of DNA transposons can be superimposed on two or more eukaryotic supergroups and that they were already differentiated in the eukaryotic ancestor.A lot of variation is seen among different species regarding relative abundance of DNA transposons and retrotransposons. Retrotransposons, (LINES, SINES) predominate in humans accounting for 44% of the genome and are currently active, while DNA transposons contribute only about 3% to the genome, with no evidence of activity during the past 40-50 million years. However it
has been suggested that DNA transposons were active during early primate evolution ~ 37 My ago.

For DNA transposons, using cut and paste mechanism, there are two ways by which their copy numbers can increase. In the first mechanism, during DNA replication, as the element moves from a newly replicated chromatid to an unreplicated site, the transposon gets replicated twice, increasing one copy as shown for maize Ac elements. In the second mechanism, as the excision of the element leaves a double strand break, homologous recombination tries to repair the gap by introducing the element at the donor site. Sister chromatids may serve as the template for gap repair if transposition occurs during the S phase of the cell cycle. This way copy numbers may increase as seen for P elements of Drosophila.

8.6 GENOME ANALYSIS

After an organism has been selected, genome projects involve three components: the sequencing of DNA, the assembly of that sequence to create a representation of the original chromosome, and the annotation and analysis of that representation.

Historically, sequencing was done in *sequencing centers*, centralized facilities (ranging from large independent institutions such as Joint Genome Institute which sequence dozens of terabases a year, to local molecular biology core facilities) which contain research laboratories with the costly instrumentation and technical support necessary. As sequencing technology continues to improve, however, a new generation of effective fast turnaround benchtop sequencers has come within reach

of the average academic laboratory. On the whole, genome sequencing approaches fall into two broad categories, *shotgun* and *high-throughput* (or *next-generation*) sequencing

Shotgun sequencing

Shotgun sequencing is a sequencing method designed for analysis of DNA sequences longer than 1000 base pairs, up to and including entire chromosomes. It is named by analogy with the rapidly expanding, quasi-random firing pattern of a shotgun. Since gel electrophoresis sequencing can only be used for fairly short sequences (100 to 1000 base pairs), longer DNA sequences must be broken into random small segments which are then sequenced to obtain *reads*. Multiple overlapping reads for the target DNA are obtained by performing several rounds of this fragmentation and sequencing. Computer programs then use the overlapping ends of different reads to assemble them into a continuous sequence.

For much of its history, the technology underlying shotgun sequencing was the classical chain termination method or 'Sanger method', which is based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication. Recently, shotgun sequencing has been supplanted by high-throughput sequencing methods, especially for large-scale, automated genome analyses. However, the Sanger method remains in wide use, primarily for smaller-scale projects and for obtaining especially long contiguous DNA sequence reads (>500 nucleotides). Chain-termination methods require a single-stranded DNA template, a DNA primer, a DNA polymerase, normal deoxynucleoside triphosphates (dNTPs), and modified nucleotides (dideoxyNTPs) that terminate DNA strand elongation. These chain-terminating nucleotides lack a 3'-OH group required for the formation of a phosphodiester bond between two nucleotides, causing DNA polymerase to cease extension of DNA when a ddNTP is incorporated. The ddNTPs may be radioactively or fluorescently labeled for detection in DNA sequencers. Typically, these machines can sequence up to 96 DNA samples in a single batch (run) in up to 48 runs a day.

Assembly:

Sequence assembly refers to aligning and merging fragments of a much longer DNA sequence in order to reconstruct the original sequence. This is needed as current DNA sequencing technology

cannot read whole genomes as a continuous sequence, but rather reads small pieces of between 20 and 1000 bases, depending on the technology used. Third generation sequencing technologies such as PacBio or Oxford Nanopore routinely generate sequencing reads >10 kb in length; however, they have a high error rate at approximately 15 percent. Typically the short fragments, called reads, result from shotgun sequencing genomic DNA, or gene transcripts (ESTs).

Finishing:

Finished genomes are defined as having a single contiguous sequence with no ambiguities representing each replicon.

Annotation:

The DNA sequence assembly alone is of little value without additional analysis. Genome annotation is the process of attaching biological information to sequences, and consists of three main steps:

1. Identifying portions of the genome that do not code for proteins
2. Identifying elements on the genome, a process called gene prediction, and
3. Attaching biological information to these elements.

Automatic annotation tools try to perform these steps *in silico*, as opposed to manual annotation (a.k.a. curation) which involves human expertise and potential experimental verification. Ideally, these approaches co-exist and complement each other in the same annotation pipeline (also see below).

Traditionally, the basic level of annotation is using BLAST for finding similarities, and then annotating genomes based on homologues. More recently, additional information is added to the annotation platform. The additional information allows manual annotators to deconvolute discrepancies between genes that are given the same annotation. Some databases use genome context information, similarity scores, experimental data, and integrations of other resources to provide genome annotations through their Subsystems approach. Other databases (e.g. Ensembl) rely on both curated data sources as well as a range of software tools in their automated genome annotation pipeline. *Structural annotation* consists of the identification of genomic elements,

primarily ORFs and their localisation, or gene structure. *Functional annotation* consists of attaching biological information to genomic elements.

8.7 GENETIC REGULATION OF CELL DIVISION IN YEAST AND EUKARYOTES

Genetic regulation in eukaryotic:

Although prokaryotes (i.e., non-nucleated unicellular organisms) divide through binary fission, eukaryotes undergo a more complex process of cell division because DNA is packed in several chromosomes located inside a cell nucleus. In eukaryotes, cell division may take two different paths, in accordance with the cell type involved. Mitosis is a cellular division resulting in two identical nuclei is performed by somatic cells. The process of meiosis results in four nuclei, each containing half of the original number of chromosomes. Sex cells or gametes (ovum and spermatozoids) divide by meiosis. Both prokaryotes and eukaryotes undergo a final process, known as cytoplasmatic division, which divides the parental cell into new daughter cells. The series of stages that a cell undergoes while progressing to division is known as cell cycle. Cells undergoing division are also termed competent cells. When a cell is not progressing to mitosis, it remains in phase G0 ("G" zero). Therefore, the cell cycle is divided into two major phases: interphase and mitosis. Interphase includes the phases (or stages) G1, S and G2 whereas mitosis is subdivided into prophase, metaphase, anaphase and telophase.

The cell cycle starts in G1, with the active synthesis of **RNA** and proteins, which are necessary for young cells to grow and mature. The time G1 lasts varies greatly among eukaryotic cells of different species and from one tissue to another in the same organism. Tissues that require fast cellular renovation, such as mucosa and endometrial epithelia, have shorter G1 periods than those tissues that do not require frequent renovation or repair, such as muscles or connective tissues.

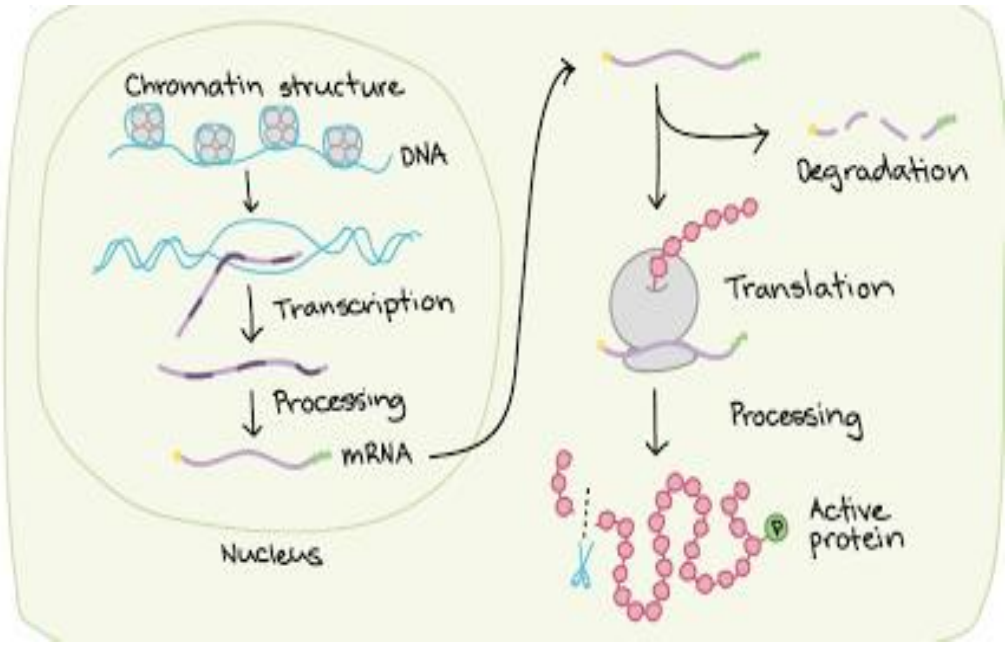

Fig 8.5 Eukaryotic Gene regulation

The cell cycle is highly regulated by several **enzymes**, proteins, and **cytokines** in each of its phases, in order to ensure that the resulting daughter cells receive the appropriate amount of genetic information originally present in the parental cell. In the case of somatic cells, each of the two daughter cells must contain an exact copy of the original genome present in the parental cell. Cell cycle controls also regulate when and to what extent the cells of a given tissue must proliferate, in order to avoid abnormal cell proliferation that could lead to dysplasia or tumor development. Therefore, when one or more of such controls are lost or inhibited, abnormal overgrowth will occur and may lead to impairment of function and disease.

Cells are mainly induced into proliferation by growth factors or hormones that occupy specific receptors on the surface of the cell membrane, and are also known as extra-cellular ligands. Examples of growth factors are as such: epidermal growth factor (EGF), fibroblastic growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), or by hormones. PDGF and FGF act by regulating the phase G2 of the cell cycle and during mitosis. After mitosis, they act again stimulating the daughter cells to grow, thus leading them from G0 to G1. Therefore, FGF and PDGF are also termed competence factors, whereas EGF and IGF are termed progression

factors, because they keep the process of cellular progression to mitosis going on. Hormones are also pro-mitotic signals. For example, thyrotrophic hormone, one of the hormones produced by the pituitary gland, induces the proliferation of glands cells. Another pituitary hormone, known as growth hormone or somatotrophic hormone (STH), is responsible by body growth during childhood and early adolescence, inducing the lengthening of the long bones and protein synthesis. Estrogens are hormones that do not occupy a membrane receptor, but instead, penetrate the cell and the nucleus, binding directly to specific sites in the DNA, thus inducing the cell cycle.

Anti-mitotic signals may have several different origins, such as cell-to-cell adhesion, factors of adhesion to the extracellular matrix, or soluble factor such as TGF beta (tumor growth factor beta), which inhibits abnormal cell proliferation, proteins p53, p16, p21, APC, pRb, etc. These molecules are the products of a class of genes called tumor suppressor genes. Oncogenes, until recently also known as proto-oncogenes, synthesize proteins that enhance the stimuli started by growth factors, amplifying the mitotic signal to the nucleus, and/or promoting the accomplishment of a necessary step of the cell cycle. When each phase of the cell cycle is completed, the proteins involved in that phase are degraded, so that once the next phase starts, the cell is unable to go back to the previous one. Next to the end of phase G1, the cycle is paused by tumor suppressor **gene** products, to allow verification and repair of DNA damage. When DNA damage is not repairable, these genes stimulate other intra-cellular pathways that induce the cell into suicide or apoptosis (also known as programmed cell death). To the end of phase G2, before the transition to mitosis, the cycle is paused again for a new verification and "decision": either mitosis or apoptosis.

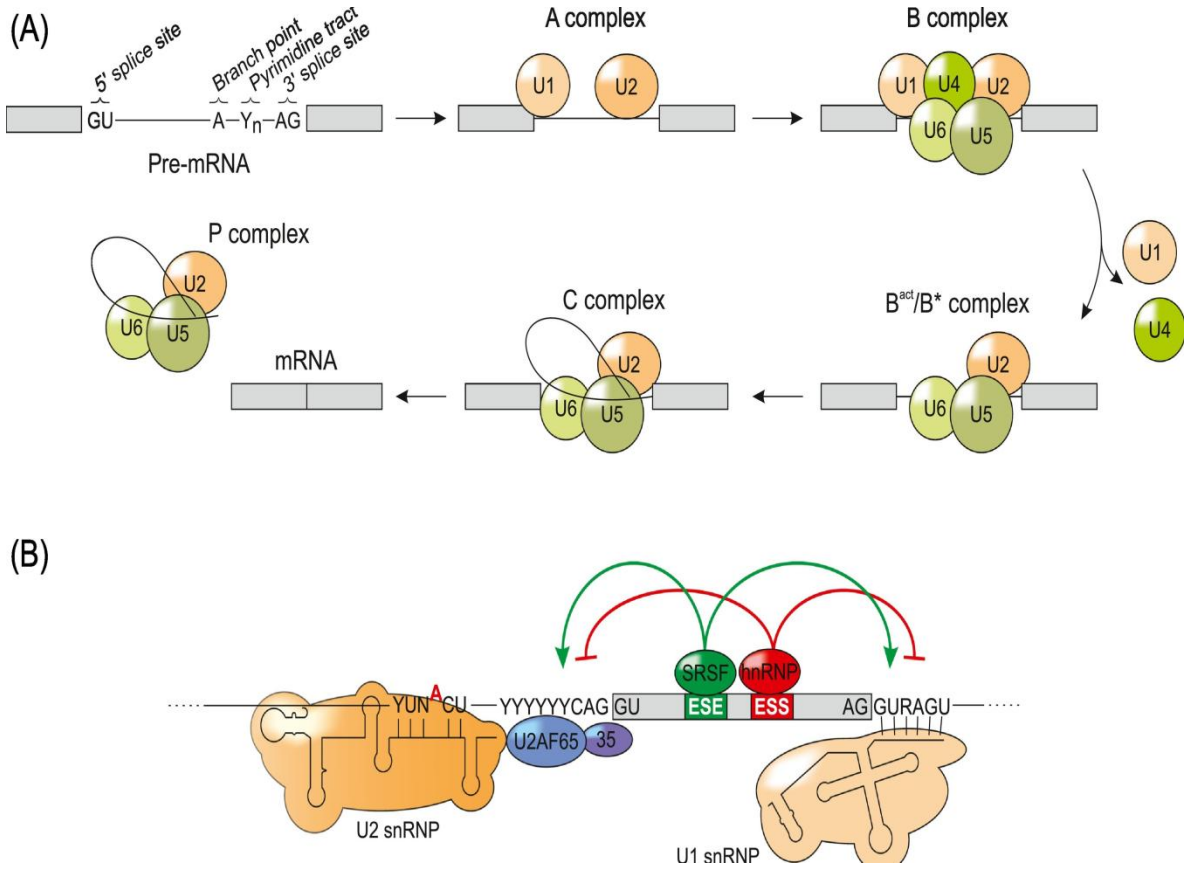

Fig 8.7 Eukaryotic Gene regulation

Along each pro-mitotic and anti-mitotic intra-cellular signaling pathway, as well as along the apoptotic pathways, several gene products (**proteins and enzymes**) are involved in an orderly sequence of activation and inactivation, forming complex webs of signal transmission and signal amplification to the nucleus. The general goal of such cascades of signals is to achieve the orderly progression of each phase of the cell cycle.

Interphase is a phase of cell growth and metabolic activity, without cell nuclear division, comprised of several stages or phases. During Gap 1 or G1 the cell resumes protein and RNA synthesis, which was interrupted during mitosis, thus allowing the growth and maturation of young cells to accomplish their physiologic function. Immediately following is a variable length pause for DNA checking and repairs before cell cycle transition to phase S during which there is synthesis or semi conservative replication or synthesis of DNA. During Gap 2 or G2, there is increased RNA

and protein synthesis, followed by a second pause for proofreading and eventual repairs in the newly synthesized DNA sequences before transition to Mitosis.

At the start of mitosis the chromosomes are already duplicated, with the sister-chromatids (identical chromosomes) clearly visible under a light **microscope**. Mitosis is subdivided into prophase, metaphase, anaphase and telophase.

The identification and detailed understanding of the many molecules involved in the cell cycle controls and intracellular signal **transduction** is presently under investigation by several research groups around the world. This knowledge is crucial to the development of new anti-cancer drugs as well as to new treatments for other genetic diseases, in which a gene over expression or deregulation may be causing either a chronic or an acute disease, or the impairment of a vital organ function. Scientists predict that the next two decades will be dedicated to the identification of gene products and their respective function in the cellular microenvironment. This new field of research is termed **proteomics**.

Genetic regulation in prokaryotic:

Although prokaryotes do not have an organized **nucleus** and other complex organelles found in eukaryotic cells, prokaryotic organisms share some common features with **eukaryotes** as far as cell division is concerned. For example, they both replicate **DNA** in a semi conservative manner, and the segregation of the newly formed DNA molecules occurs before the cell division takes place through cytokinesis. Despite such similarities, the prokaryotic genome is stored in a single DNA molecule, whereas eukaryotes may contain a varied number of DNA molecules, specific to each species, seen in the interphasic nucleus as **chromosomes**. Prokaryotic cells also differ in other ways from eukaryotic cells. Prokaryotes do not have cytoskeleton and the DNA is not condensed during mitosis. The prokaryote chromosomes do not present histones, the complexes of histonic proteins that help to pack eukaryotic DNA into a condensate state. Prokaryotic DNA has one single promoter site that initiates replication, whereas eukaryotic DNA has multiple promoter sites. Prokaryotes have a lack of spindle apparatus (or microtubules), which are essential structures for chromosome segregation in eukaryotic cells. In prokaryotes, there are no membranes and organelles dividing the cytosol in different compartments. Although two or more DNA molecules

may be present in a given prokaryotic cell, they are genetically identical. They may contain one extra circular strand of genes known as plasmid, much smaller than the genomic DNA, and **plasmids** may be transferred to another prokaryote through bacterial **conjugation**, a process known as horizontal **gene** transfer.

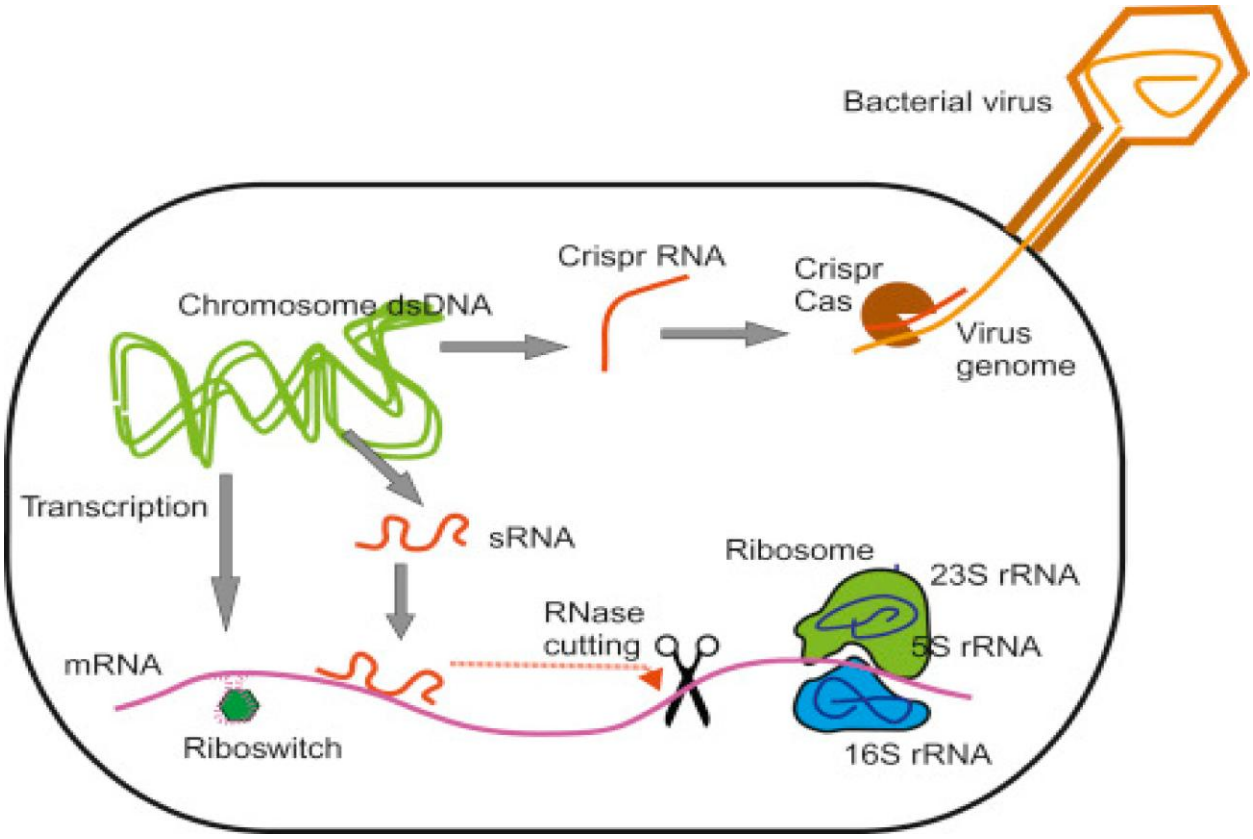

Fig.8.8 Gene regulation in Prokaryotes

The prokaryotic method of reproduction is asexual and is termed binary fission because one cell is divided in two new identical cells. Some prokaryotes also have a plasmid. Genes in **plasmids** are extra-chromosomal genes and can either be separately duplicated by a class of gene known as **transposons** Type II, or simply passed on to another individual. Transposons Type I may transfer and insert one or more genes from the plasmid to the cell DNA or vice-versa causing mutation through genetic **recombination**. The chromosome is attached to a region of the internal side of the membrane, forming a nucleoide. Some bacterial cells do present two or more nucleoides, but the genes they contain are identical.

The prokaryotic **cell cycle** is usually a fast process and may occur every 20 minutes in favorable conditions. However, some **bacteria**, such as *Mycobacterium leprae* (the cause of **leprosy**), take 12 days to accomplish replication in the host's leprous lesion. Replication of prokaryotic DNA, as well as of eukaryotic DNA, is a semi-conservative process, which means that each newly synthesized strand is paired with its complementary parental strand. Each daughter cell, therefore, receives a double-stranded circular DNA molecule that is formed by a new strand is paired with an old strand.

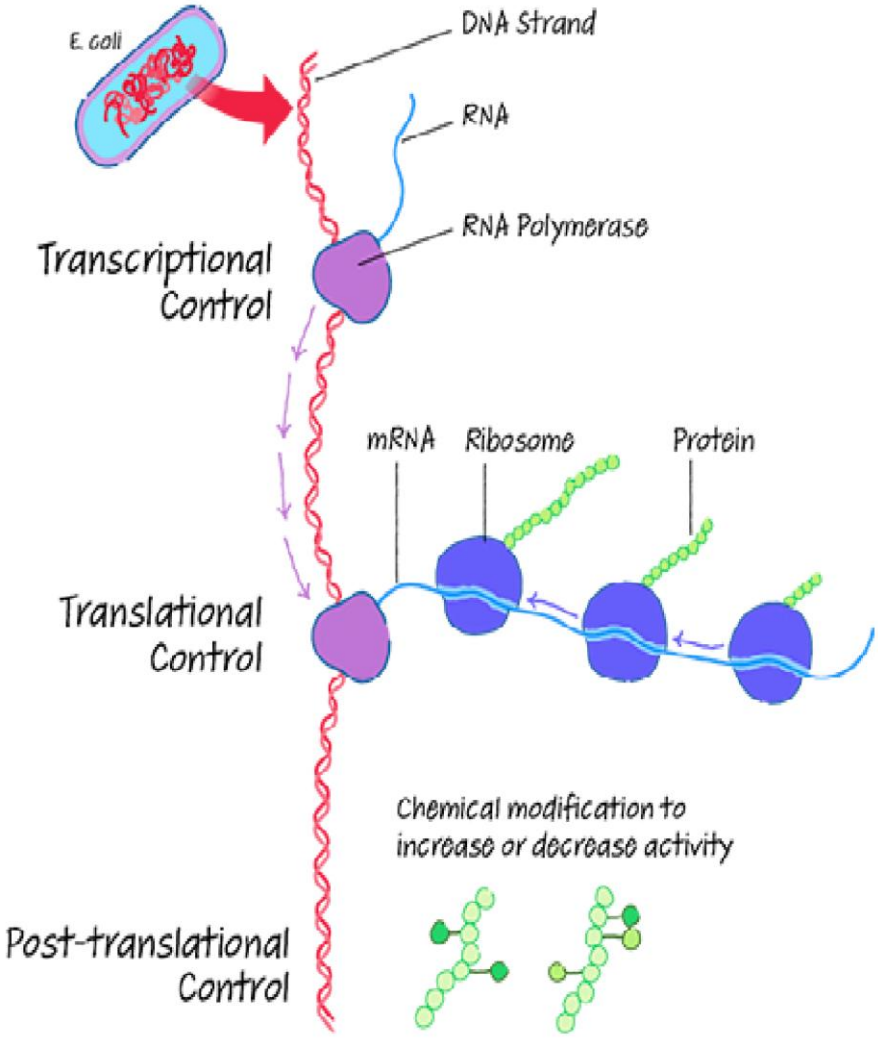

Fig.8.9 Gene expression in Prokaryotes

The cell cycle is regulated by genes encoding products (i.e., **enzymes** and proteins) that play crucial roles in the maintenance of an orderly sequence of events that ensures that each resultant daughter cell will inherit the same amount of genetic information. Cell induction into proliferation and DNA replication are controlled by specific gene products, such as enzyme DNA polymerase III that binds to a promoter region in the circular DNA, initiating its replication. However, DNA polymerase requires the presence of a pre-existing strand of DNA, which serves as a template, as well as **RNA** primers, to initiate the polymerization of a new strand. Before replication starts, timidine-H^3, (a DNA precursor) is added to a Y-shaped site where the double helices were separated, known as the replicating fork. The DNA strands are separated by enzyme helicases and kept apart during replication by single strand proteins (or ss DNA-binding proteins) that binds to DNA, while the enzyme topoisomerase further unwinds and elongates the two strands to undo the circular ring.

DNA polymerase always makes the new strand by starting from the extremity 5' and terminating at the extremity 3'. Moreover, the two DNA strands have opposite directions (i.e., they keep an anti-parallel arrangement to each other). Therefore, the new strand 5' to 3' that is complementary to the old strand 3' to 5' is synthesized in a continuous process (leading strand synthesis), whereas the other new strand (3' to 5') is synthesized in several isolated fragments (lagging strand synthesis) that will be later bound together to form the whole strand. The new 3' to 5' strand is complementary to the old 5' to 3'. However, the lagging fragments, known as Okazaki's fragments, are individually synthesized in the direction 5'to 3' by DNA polymerase III. RNA polymerases produce the RNA primers that help DNA polymerases to synthesize the leading strand. Nevertheless, the small fragments of the lagging strand have as primers a special RNA that is synthesized by another enzyme, the primase. Enzyme topoisomerase III does the proofreading of the newly transcribed sequences and eliminates those wrongly transcribed, before DNA synthesis may continue. RNA primers are removed from the newly synthesized sequences by ribonuclease H. Polymerase I fills the gaps and DNA ligase joins the lagging strands.

After DNA replication, each DNA molecule is segregated, i.e., separated from the other, and attached to a different region of the internal face of the membrane. The formation of a septum, or

dividing internal wall, separates the cell into halves, each containing a nucleotide. The process of splitting the cell in two identical daughter cells is known as cytokinesis.

8.8 MOLECULAR BASIS OF CELLULAR CHECK UP

As we just learned, the cell cycle is a fairly complicated process. In order to make sure everything goes right, there are checkpoints in the cycle. Let's learn about these and how they help control the cell cycle.

The length of the cell cycle is highly variable, even within the cells of a single organism. In humans, the frequency of cell turnover ranges from a few hours in early embryonic development, to an average of two to five days for epithelial cells, and to an entire human lifetime spent in G_0 by specialized cells, such as cortical neurons or cardiac muscle cells. There is also variation in the time that a cell spends in each phase of the cell cycle. When fast-dividing mammalian cells are grown in culture (outside the body under optimal growing conditions), the length of the cycle is about 24 hours. In rapidly dividing human cells with a 24-hour cell cycle, the G_1 phase lasts approximately nine hours, the S phase lasts 10 hours, the G_2 phase lasts about four and one-half hours, and the M phase lasts approximately one-half hour. In early embryos of fruit flies, the cell cycle is completed in about eight minutes. The timing of events in the cell cycle is controlled by mechanisms that are both internal and external to the cell.

Regulation of the Cell Cycle by External Events:

Both the initiation and inhibition of cell division are triggered by events external to the cell when it is about to begin the replication process. An event may be as simple as the death of a nearby cell or as sweeping as the release of growth-promoting hormones, such as human growth hormone (HGH). A lack of HGH can inhibit cell division, resulting in dwarfism, whereas too much HGH can result in gigantism. Crowding of cells can also inhibit cell division. Another factor that can initiate cell division is the size of the cell; as a cell grows, it becomes inefficient due to its decreasing surface-to-volume ratio. The solution to this problem is to divide.

Whatever the source of the message, the cell receives the signal, and a series of events within the cell allows it to proceed into interphase. Moving forward from this initiation point, every parameter required during each cell cycle phase must be met or the cycle cannot progress.

Regulation at Internal Checkpoints:

It is essential that the daughter cells produced be exact duplicates of the parent cell. Mistakes in the duplication or distribution of the chromosomes lead to mutations that may be passed forward to every new cell produced from an abnormal cell. To prevent a compromised cell from continuing to divide, there are internal control mechanisms that operate at three main cell cycle checkpoints. A checkpoint is one of several points in the eukaryotic cell cycle at which the progression of a cell to the next stage in the cycle can be halted until conditions are favorable. These checkpoints occur near the end of G_1, at the G_2/M transition, and during metaphase fig.8.10.

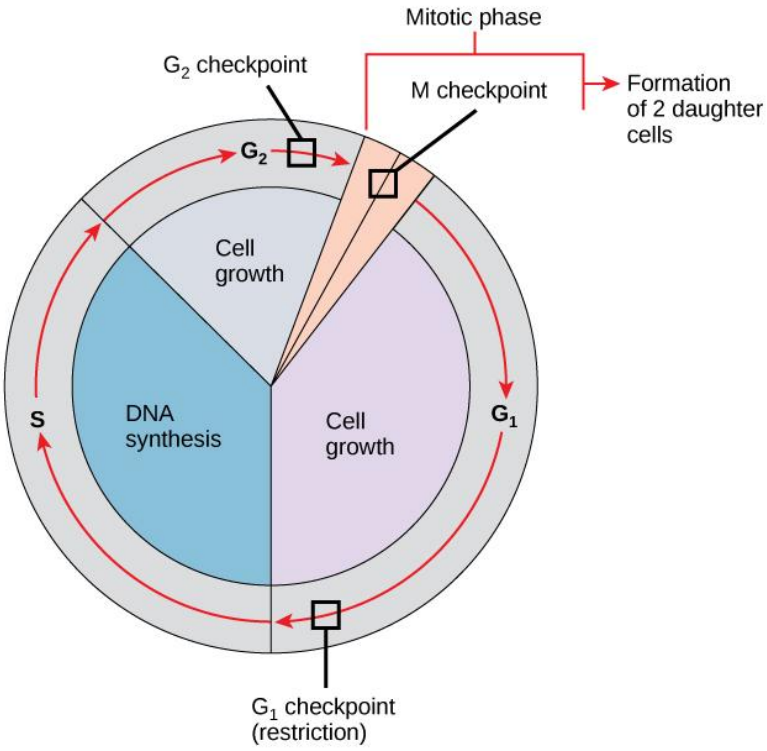

Fig.8.10 Cell Cycle Check Points

The G_1 Checkpoint

The G_1 checkpoint determines whether all conditions are favorable for cell division to proceed. The G_1 checkpoint, also called the restriction point (in yeast), is a point at which the cell irreversibly commits to the cell division process. External influences, such as growth factors, play a large role in carrying the cell past the G_1 checkpoint. In addition to adequate reserves and cell size, there is a check for genomic DNA damage at the G_1 checkpoint. A cell that does not meet all the requirements will not be allowed to progress into the S phase. The cell can halt the cycle and attempt to remedy the problematic condition, or the cell can advance into G_0 and await further signals when conditions improve.

The G_2 Checkpoint

The G_2 checkpoint bars entry into the mitotic phase if certain conditions are not met. As at the G_1 checkpoint, cell size and protein reserves are assessed. However, the most important role of the G_2 checkpoint is to ensure that all of the chromosomes have been replicated and that the replicated DNA is not damaged. If the checkpoint mechanisms detect problems with the DNA, the cell cycle is halted, and the cell attempts to either complete DNA replication or repair the damaged DNA.

The M Checkpoint

The M checkpoint occurs near the end of the metaphase stage of karyokinesis. The M checkpoint is also known as the spindle checkpoint, because it determines whether the entire sister chromatids are correctly attached to the spindle microtubules. Because the separation of the sister chromatids during anaphase is an irreversible step, the cycle will not proceed until the kinetochores of each pair of sister chromatids are firmly anchored to at least two spindle fibers arising from opposite poles of the cell.

8.9 MOLECULAR BASIS OF NEOPLASIA

Neoplasia (nee-oh-PLAY-zhuh) is the **uncontrolled, abnormal growth of cells or tissues in the body**, and the abnormal growth itself is called a neoplasm (nee-oh-PLAZ-m) or tumor. It can be benign (bee-NINE) or malignant.

The Molecular Basis of Cancer-Cell Behavior

Oncogenes and tumor suppressors—and the mutations that affect them—are different beasts from the point of view of the cancer gene hunter. But from a cancer cell's point of view they are two sides of the same target. The same kinds of effects on cell behavior can result from mutations in either class of genes, because most of the control mechanisms in the cell involve both inhibitory (tumor suppressor) and stimulatory (proto-oncogene) components. In terms of function, the important distinction is not the distinction between a tumor suppressor and a proto-oncogene, but between genes lying in different biochemical and regulatory pathways.

Some of the pathways important in cancer carry signals from a cell's environment (as discussed in Chapter 15); others are responsible for the cell's internal programs, such as those that control the cell cycle or cell death still others govern the cell's movements and mechanical interactions with its neighbors . The various pathways are linked and interdependent in complex ways. Much of what we know about them has been learned as a byproduct of cancer research; conversely, study of these basic aspects of cell biology has transformed our understanding of cancer.

In the first section of this chapter, we summarized in general terms the properties that make a cancer cell a cancer cell and listed the kinds of misbehavior a cancer cell displays. In this section, we consider how each of the characteristic properties arises from mutations that have been identified in cancer-critical genes affecting specific regulatory pathways. For some parts of this problem the answers are straightforward. For other parts, large mysteries remain.

We begin with a brief general discussion of how we determine the cellular function of cancer-critical genes. We then review what is known about how these cancer-critical genes control the relevant cell behaviors. Finally, we turn to the development of colon cancer as an example of how tumors evolve through the accumulation of mutations that lead from one pattern of bad behavior to another that is worse.

Most cancer-critical genes code for components of the pathways that regulate the social behavior of cells in the body—in particular, the mechanisms by which signals from a cell's neighbors can impel it to divide, differentiate, or die. In fact, many of the components of cell-signaling pathways were first identified through searches for cancer-causing genes, and a full list of proto-oncogene

products and tumor suppressors includes examples of practically every type of molecule involved in cell signaling—secreted proteins, transmembrane receptors, GTP-binding proteins, protein kinases, gene regulatory proteins, and so on Many cancer mutations alter signal pathway components in a way that causes them to deliver proliferative signals even when more cells are not needed, switching on cell growth, DNA replication, and cell division inappropriately. Mutations that inappropriately activate a receptor tyrosine kinase, such as the EGF receptor, or proteins in the Ras family, which lie downstream from such growth factor receptors, act in this way.

Mutations in Genes That Regulate Apoptosis Allow Cancer Cells to Escape Suicide:

To achieve net cell proliferation, it is necessary not only to drive cells into division, but also to keep cells from committing suicide by apoptosis. There are many normal situations in which cells proliferate continuously, but the cell division is exactly balanced by cell loss. In the germinal centers of lymph nodes, for example, B cells proliferate rapidly but most of their progeny are eliminated by apoptosis. Apoptosis is thus essential in maintaining the normal balance of cell births and deaths in tissues that undergo cell turnover. It also has a vital role in the cellular reaction to damage and disorder. Cells in a multicellular organism commit suicide when they sense that something has gone wrong—when their DNA is severely damaged or when they are deprived of survival signals that tell them they are in their proper place. Resistance to apoptosis is thus a key characteristic of malignant cells, essential for enabling them to increase in number and survive where they should not.

A number of mutations that inhibit apoptosis have been found in tumors. One protein that blocks apoptosis, called Bcl-2, was discovered because it is the target of a chromosome translocation in a B-cell lymphoma. The effect of the translocation is to place the Bcl-2 gene under the control of a regulatory element that drives over expression, which allows survival of B lymphocytes that would normally have died.

Of all the cancer-critical genes involved in control of apoptosis, however, there is one that is implicated in cancers in an exceptionally wide range of tissues. This gene, called p53, stands at a crucial intersection in the network of pathways governing a cell's responses to DNA damage and other stressful mishaps. Control of apoptosis is only part of the gene's function—though a very

important part. As we shall now explain, when p53 is defective, genetically damaged cells do not merely fail to die; worse still, they wantonly continue to proliferate, accumulating yet more genetic damage that can lead toward cancer.

Mutations in the *p53* Gene Allow Cancer Cells to Survive and Proliferate despite DNA Damage

The *p53* gene—named for the molecular mass of its protein product—may be the most important gene in human cancer. This tumor suppressor gene is mutated in about half of all human cancers. What makes *p53* so critical? The answer lies in its triple involvement in cell-cycle control, in apoptosis, and in maintenance of genetic stability—all aspects of the fundamental role of the *p53* protein in protecting the organism against cellular damage and disorder.

In contrast with Rb, very little *p53* protein is found in most of the cells of the body under normal conditions. In fact, *p53* is not required for normal development: transgenic mice in which both copies of the gene have been knocked out appear normal in all respects except one—they usually develop cancer by the age of 3 months. These observations suggest that *p53* may have a function that is required only occasionally or in special circumstances. Indeed, when normal cells are deprived of oxygen or exposed to treatments that damage DNA, such as ultraviolet light or gamma rays, they raise their concentration of *p53* protein by reducing the normally rapid rate of degradation of the molecule. The *p53* response is seen also in cells where oncogenes such as *Ras* and *Myc* are active, generating an abnormal stimulus for cell division.

In all these cases, the high level of *p53* protein acts to limit the harm done. Depending on circumstances and the severity of the damage, the *p53* may either drive the damaged or mutant cell to commit suicide by apoptosis a relatively harmless event for the multicellular organism—or it may trigger a mechanism that bars the cell from dividing so long as the damage remains unrepaired. The protection provided by *p53* is an important part of the reason why mutations that activate oncogenes such as *Ras* and *Myc* are not enough by themselves to create a tumor.

The p53 protein exerts its cell-cycle effects, in part at least, by binding to DNA and inducing the transcription of *p21*—a regulatory gene whose protein product binds to Cdk complexes required

for entry into and progress through S-phase. By blocking the kinase activity of these Cdk complexes, the *p21* protein prevents the cell from entering S phase and replicating its DNA.

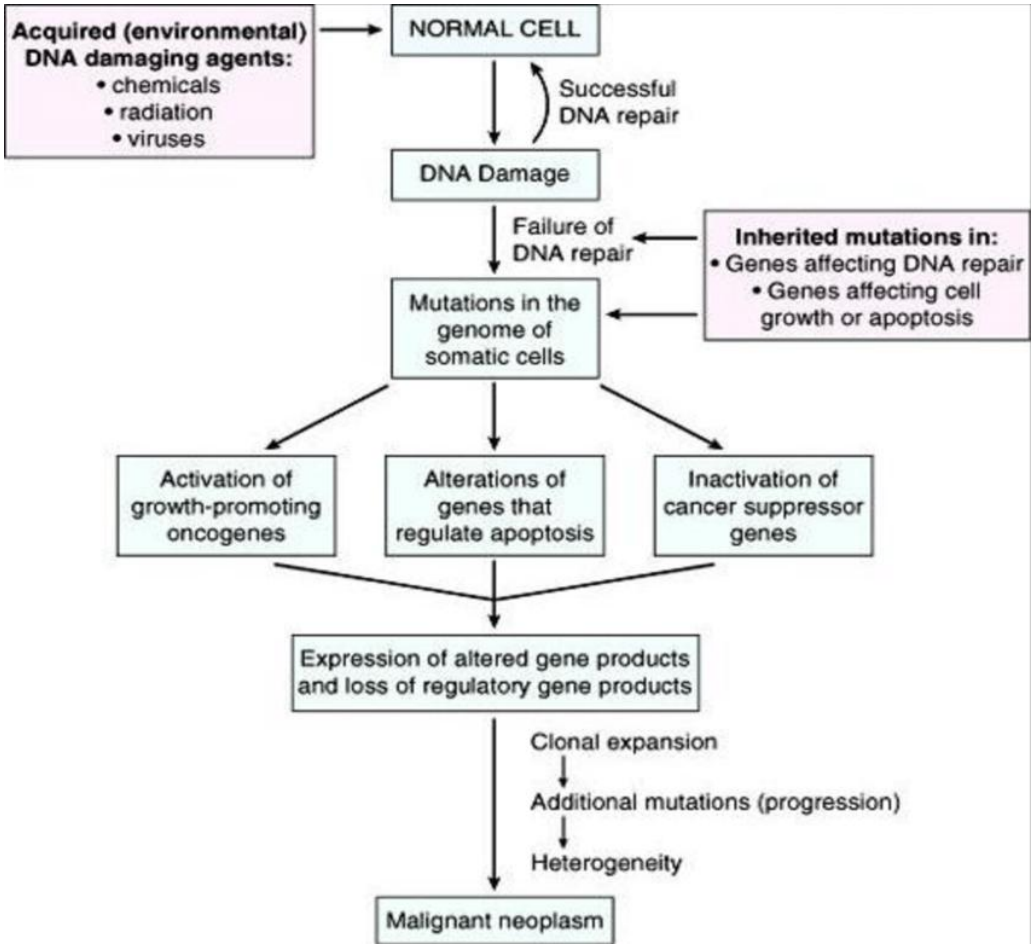

Fig.8.11Molecular basis of Neoplasia

Cells defective in *p53* fail to show these responses. They tend to escape apoptosis, and if their DNA is damaged—by radiation or by some other mishap—they carry on dividing, plunging into DNA replication without pausing to repair the breaks and other DNA lesions that the damage has caused. As a result, they may either die or, far worse, survive and proliferate with a corrupted genome. A common consequence is that chromosomes become fragmented and incorrectly rejoined, creating, through further rounds of cell division, an increasingly disrupted genome as explained in Such chromosomal mayhem can lead to both loss of tumor suppressor genes and activation of oncogenes, for example by gene amplification. In addition to being an important

mechanism for activating oncogenes, gene amplification can also enable cells to develop resistance to therapeutic drugs.

Telomere Shortening May Pave the Way to Cancer in Humans

The mouse is the most widely used model organism for the study of cancer, yet the spectrum of cancers seen in mice differs dramatically from that seen in humans. The great majority of mouse cancers are sarcomas and leukemias, whereas more than 80 percent of human cancers are carcinomas—cancers of epithelia where rapid cell turnover occurs. Many therapies have been found to cure cancers in mice; but when the same treatments are tried in humans, they usually fail. What could be the reason for the difference between mouse and human cancer, an important part of the answer may lie in the behavior of telomeres and the relationship between telomere shortening, replicative cell senescence, and genetic instability.

As we saw earlier, most human cells seem to have a built-in limit to their proliferation: they show replicative senescence, at least when grown in culture. Replicative cell senescence in humans is thought to be caused by changes in the structure of telomeres—the repetitive DNA sequences and associated proteins that cap the ends of each chromosome. These telomeric DNA sequences are synthesized and maintained by a special mechanism that requires the enzyme telomerase In most human cells, other than those of the germ line and some stem cells, expression of the gene coding for the catalytic subunit of telomerase is switched off, or at least not fully activated. As a result, the telomeres in these cells tend to become a little shorter with each round of cell division. Eventually, the telomeric cap on the chromosome end can become shortened to the point where a danger signal is generated, arresting the cell cycle. The signal is similar, in function at least, to the one that arrests the cycle when an uncapped DNA end is created by an accidental double-strand chromosome break. The effect in both cases is to prevent cell division so long as the cell contains broken or inadequately capped DNA. In the cell with the chromosome break, this allows time for DNA repair; in the normal senescent cell, it seems that it simply puts a stop to cell proliferation. As we discussed at the beginning of the chapter, it is not clear how often cells in normal human tissues run up against this limit; but if a self-renewing cell population does undergo replicative senescence, any rogue cell that undergoes a mutation that lets it carry on dividing will enjoy a huge competitive advantage much more than if the same mutation had occurred in a cell in a non-

senescent population. Viewed in this light, replicative senescence might be expected to favor the development of cancer.

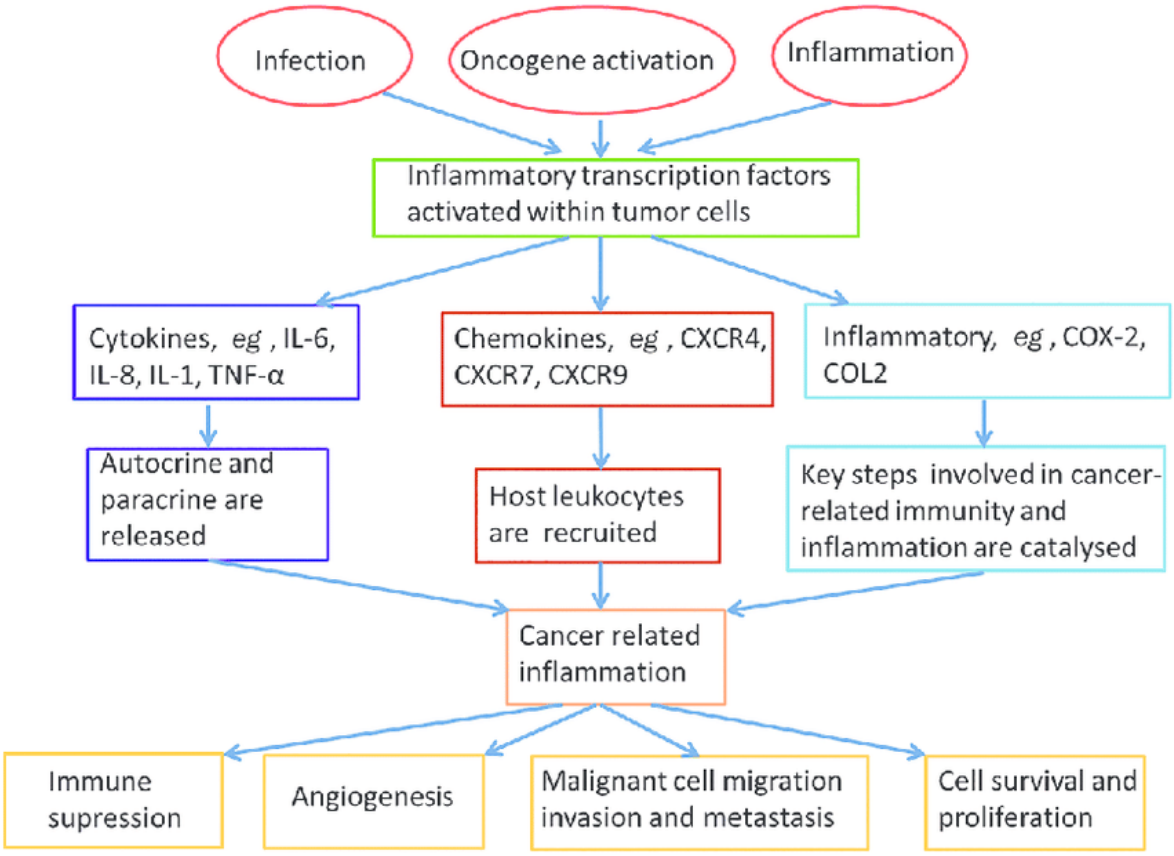

Fig.8.12 Molecular basis of Cancer related Inflamation

Mice have telomeres much longer than those of humans. Moreover, unlike humans, they keep telomerase active in their somatic cells, and mouse telomeres therefore do not tend to shorten with increasing age of the organism. It is possible, however, to use gene knockout technology to make mice that lack functional telomerase. In these mice, the telomeres become shorter with every generation, but no untoward consequences are seen until, in the great-great-grandchildren of the initial mutants, the telomeres become so short that they disappear or cease to function. Beyond this point, the mice begin to show various abnormalities, including an increased incidence of cancer. This raises the possibility that natural telomere shortening helps to engender many human tumors.

8.10 SUMMARY

Genomes of eukaryotes are not fixed but continue to change due to horizontal transfer of genes by bacteria and viruses. This may allow genes to be transferred across different species, genera or even across different domains, which is not possible for vertical gene transfer.

Comparative genomic studies suggest that HGT has played an important role in the evolution of genome of a number of eukaryotic groups. HGT on the grandest scale has been responsible for the evolution in eukaryotes of mitochondria and plastids, by endosymbiosis of a proteobacterium and a cyanobacterium respectively.Transposons, in eukaryotes, have played important role in genome dynamics, by various mechanisms; either producing insertional mutagenesis or leading to enhanced expression of some genes , or even leading to creation of novel genes and new regulatory networks There are many examples to suggest that several host genes have been derived from transposases such as Rag1/2 complex (needed for immune response), Tram (Zbed) located in the pseudoautosomal region of X and Y chromosomes in human, Cenp-b (DNA binding protein centromere function),SETMAR (for radiation resistance and DNA repair).

Each step of the cell cycle is monitored by internal controls called checkpoints. There are three major checkpoints in the cell cycle: one near the end of G_1, a second at the G_2/M transition, and the third during metaphase. Positive regulator molecules allow the cell cycle to advance to the next stage. Negative regulator molecules monitor cellular conditions and can halt the cycle until specific requirements are met.

Cancer is the result of unchecked cell division caused by a breakdown of the mechanisms that regulate the cell cycle. The loss of control begins with a change in the DNA sequence of a gene those codes for one of the regulatory molecules. Faulty instructions lead to a protein that does not function as it should. Any disruption of the monitoring system can allow other mistakes to be passed on to the daughter cells. Each successive cell division will give rise to daughter cells with even more accumulated damage. Eventually, all checkpoints become nonfunctional, and rapidly reproducing cells crowd out normal cells, resulting in a tumor or leukemia (blood cancer).

8.11 TERMINAL QUESTIONS AND ANSWERS

Question No.1 Explain the Gene regulation in Prokaryotic & Eukaryotic cell.

Question No.2.Write a short note on Genome analysis?

Question No.3.Explain the various model of Prokaryotic Genome?

Question No.4. Explain the Molecular basis of Neoplasia?

Question No.5. What do you understand by the Molecular basis of cellular check-up?

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