IMB 102

Handout of Molecular Biology

Prof. Dr. Mohamed AbdelAziz
Prof.Dr. Hanaa Abdel Kader
&
Prof.Dr. Ahmed El Demery

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Introduction

The human body is an extraordinary organization of cells and tissues that function as independent yet coordinated systems in conditions of health and in illness and disease. The purpose of Molecular Biology module is to provide an overview of important processes at the cellular level. In this regard, the cell has maximized the ability to produce many more times the number of proteins than the number of genes through multiple modification processes. However, all of this requires precision in multiple steps. Many errors, if not detected or repaired with the cell survival, can lead to uncontrolled growth and cancer. Cancer and cancer dormant genes are crucial to be understood.

First of all the study starts with the building unit of the nucleus which is DNA and it is formed of two chromosomes with millions of nucleotides.

The Authors

Unit 1

CYTOGENETICS

Nucleus

All cells in the human body have a nucleus, **except mature red blood cells**

Size: Approximately 7–8μm in diameter

Shape: Rounded, oval, flat, horse-shoe, kidney shaped, segmented or lobulated.

**Site:** Central, eccentric, peripheral &may be basal in position.

Number: Single (mononucleated), two nuclei (binucleated), or many nuclei (multinucleated).

LM: The nucleus appears as a prominent basophilic structure within the cell as it contains nucleic acids (DNA & RNA), it may be:

1- **Vesicular nucleus:** Pale stained as it has extended chromatin.

2- **Condensed nucleus:-**Darkly stained due to its condensed chromatin.

EM: The nucleus is composed of:

1- Nuclear membrane.
2- Nuclear chromatin.
3- Nuclear sap.

4- Nucleolus.

3- Nucleolus

4- Nuclear sap

2-Nuclear membrane
I-Peripheral chromatin
II-Chromatin Island
III-nucleolus associated chromatin

1- Nucleur membrane:
I-outer membrane

II-inner membrane
III-nuclear pore

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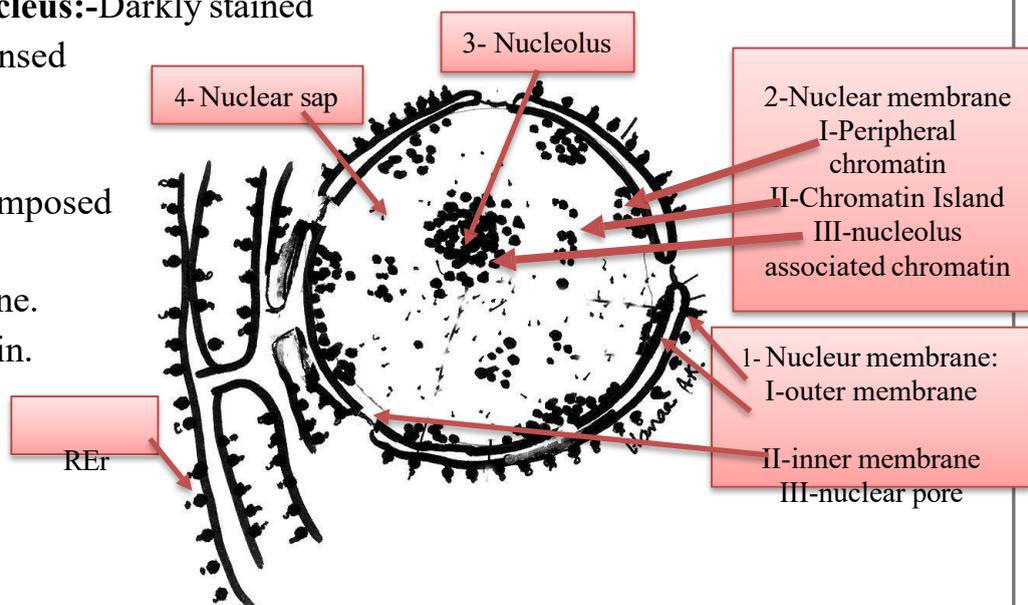
Nuclear Membrane: **LM:** It appears as a blue line because of the presence of chromatin on its inner surface& ribosomes on its outer surface.

> **EM:** It is a **double walled** membrane. Its two layers are separated by a **perinuclear space**. Nuclear **pores** are present at intervals along the nuclear membrane &they are covered by diaphragms. They allow macromolecules to pass in between the nucleus & cytoplasm in both directions.

1- Nuclear Chromatin:

It consists of nucleoprotein (DNA+ protein) that forms the chromosomes of the cell which carry the genetic materials. Chromosomes are only visible during cell division.

LM: Chromatin appears as a **basophilic mass** having two types according to cell activity

1- Euchromatin:
* It is the extended type of chromatin (fine granules).
* It is invisible & pale in staining.
* It carries the active genes.
* Active cell has a pale nucleus with extended chromatin.

2- Heterochromatin:
* It is present in a condensed form (coarse granules of chromatin)
* It is darkly stained.
* It carries the inactive genes.
* Inactive cell has a dark nucleus with condensed chromatin

Classification of chromatin according to site:
1- **Peripheral chromatin** adherent to nuclear membrane.
2- **Nucleolus associated** present around the nucleolus.
3- **Chromatin island** scattered between the nucleolus & nuclear membrane.

3- Nucleolus:
- It is a dense region of RNA &protein & appears as dark basophilic structure within the nucleus.
- The nucleus may contain one, two or no nucleoli according to its activity.
- **Functions:**
  - **Formation of** ribosomes which leave the nucleus to cytoplasm through nuclear pores

- They are responsible for protein synthesis.
- **Protein forming cells** have well defined **one or two nucleoli.**

4- Nuclear Sap:

- It is the colloidal fluid filling areas between nucleolus & chromatin islands, it acts as a transport medium to carry ribosomes from the nucleus to cytoplasm.

- **Functions of nucleus:**

1- It contains chromosomes which carry the genetic information.

2- It controls all the vital processes within the cell, as protein formation.

3- It forms the different types of RNA (m RNA, t RNA, r RNA).

Human chromosome

- **Biochemical structure of chromosome**

Each chromosome is formed of 2 identical chromatids.

- **Each chromatid consists of:**

1- A single DNA molecule.

2- Histone proteins: are basic proteins rich in Histidine and Arginine amino acids.

Histones are in contact with the microgrooves of DNA.

Five types of histones are present H1, (H2A, H2B), H3 & H4.
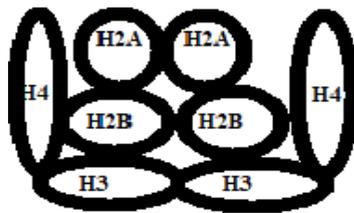
Each chromatid is formed of many nucleosomes.

Nucleosome structure:

Histone octamers (H2A,H2B, H3 &H4)2 around which DNA is wrapped
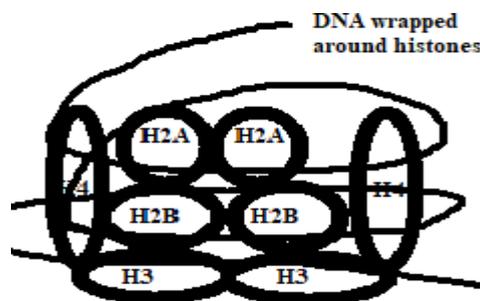
Multiple nucleosomes are connected through linker DNA (H1).
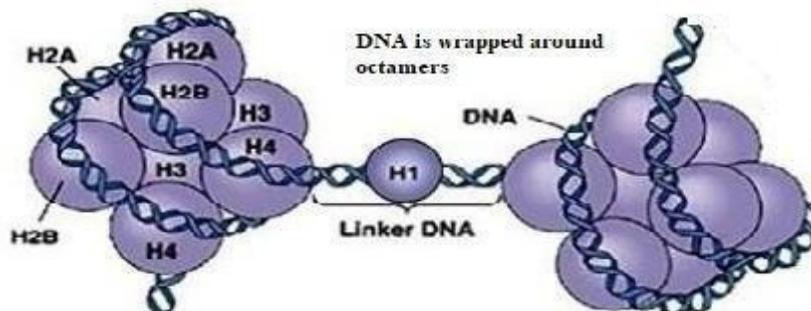
3- Non histone proteins:

Connected to major groove of DNA.

They are needed for replication, transcription and gene expression.

A solenoid is formed of:

- *Six nucleosomes* associated with the *H1 histone*.
- This solenoids form scaffold (framework) that coil further to form the chromosome.
- The histone tails have many modifications that play important regulatory roles in transcription activation or repression, chromosome condensation, DNA repair, and gene silencing.
- Nucleotide arrangement of chromosomes: Nucleotide arrangement can be categorized in several ways based upon the repetition of a particular sequence with chromosomes.

A model of chromatin structure 30 nm fibers
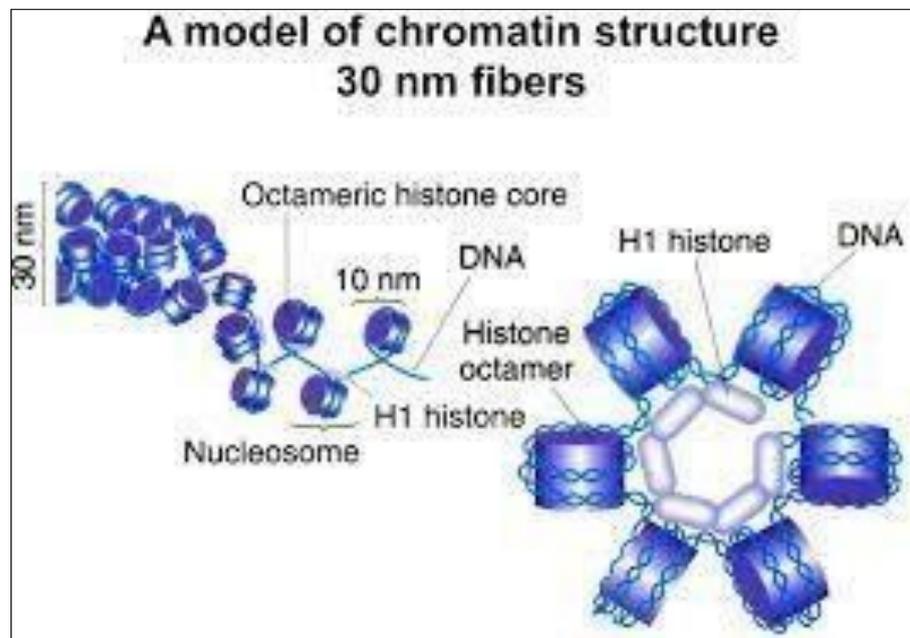
Human chromosome
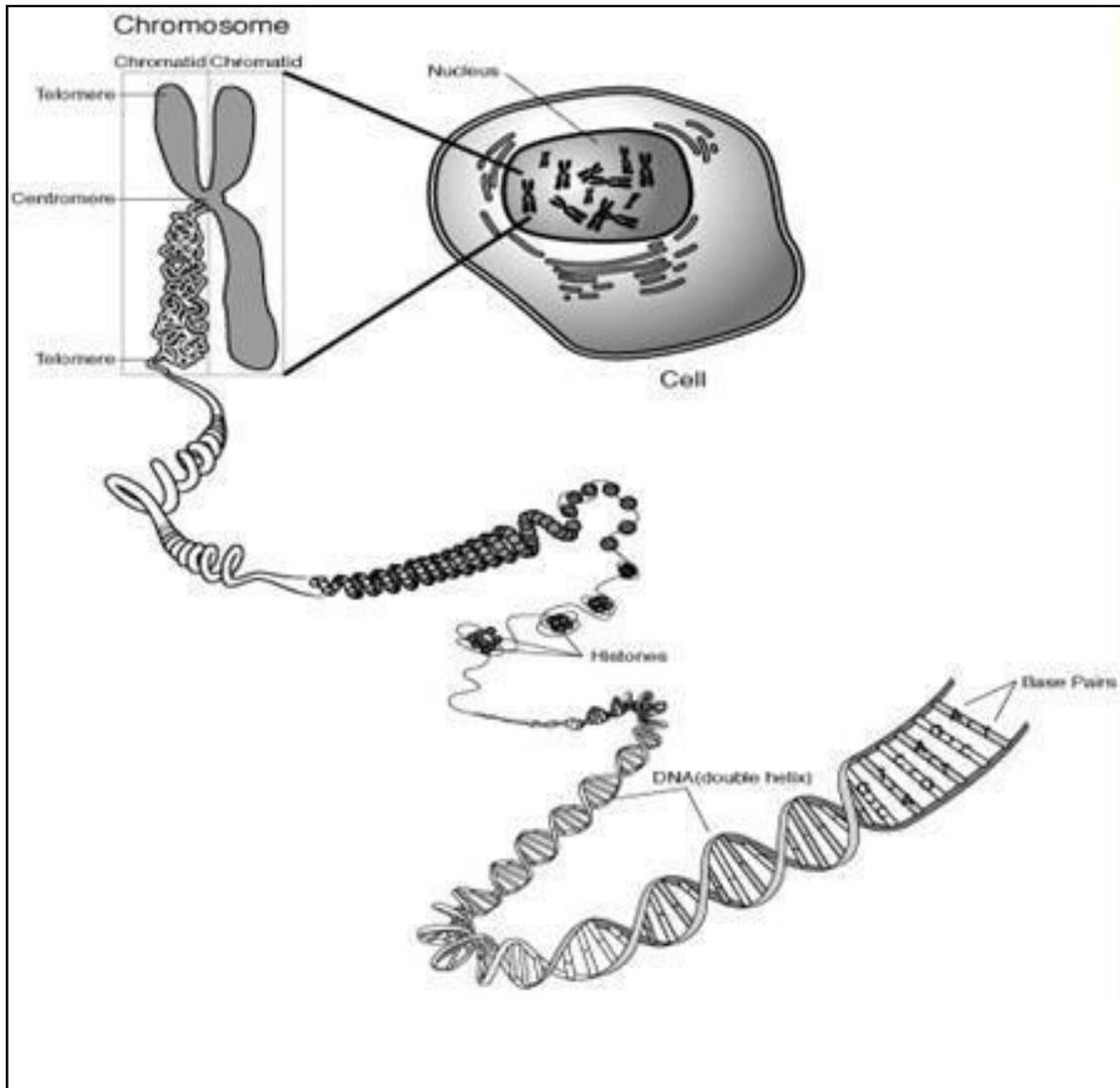
Chromosomes are **chromatin fibers** that become so **condensed** and tightly coiled during **mitosis and meiosis**   so they are **visible** with the light microscope.

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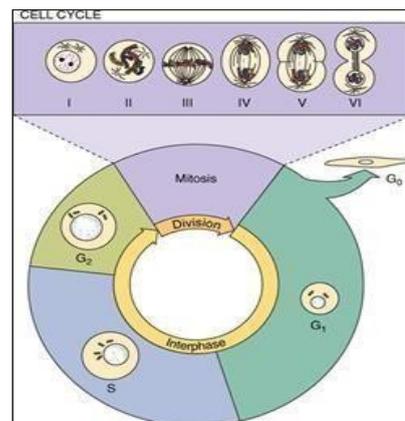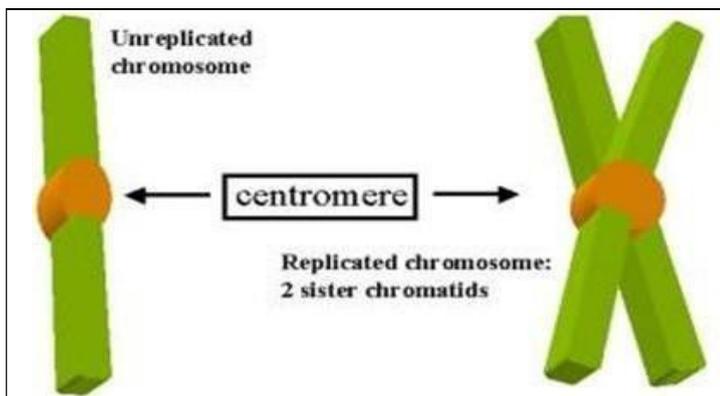
- **Each chromosome** is formed of **2 chromatids** connected at **centromere**.

- **Each chromatid** is formed of **DNA molecule** coiled around **histone** and **non-histone proteins.**

- **Kinetochores** are 2 **disc of protein** located at **centromere** to which the **spindle fibers** are attached during cell division.

- **Genes** are segments of DNA molecules responsible for the formation of specific proteins.

- **Telomeres** regions of repeated sequence at the ends of chromosomes which protects the end of the chromosome from destruction.

Cell Cycle

Cell cycle is a **group of events** that **prepares** the cell for **dividing into two daughter cells**.

- It is formed of **interphase** and **mitosis.**

Interphase:
- Period between **2 successive divisions**.
- **Very long**.
- Divided into **3 phases**:

G1 (gap 1) phase:
- Varies in duration depending on the **rate** of cell division &**specialization**.
- The **more** specialized the cell the **longer** the **G1-phase** &the less the rate of division. In **bone** tissue, G_1 lasts 25 hours.
- The most important phase for cell growth
- At this stage the cell takes a decision   either to progress to **S- phase** &division or to enter resting stage G0

It is characterized by:
1. Growth of cell.
2. RNA and protein synthesis.
3. Chromosome is formed of **one thread** of DNA **(s-chhromosome Or chromatid)**

S (synthesis) phase: **8 hours**, characterized by:
1. Duplication of centrioles.
2. Duplication of DNA.
3. Chromosome is formed of **double thread**s of DNA **(d-chromosome)**
4. Each cell contains **46 d- chromosomes**

G2 (gap 2) phase: About **4 hours**, It is the phase of final preparation of the cell to enter mitosis. It is characterized by:

1. **Synthesis of RNA and proteins** essential to cell division.
2. **Energy** for mitosis is stored.
3. **Tubulin** is synthesized for building microtubules required for mitosis,
4. Check point to make sure of **DNA replication &correction** of any DNA errors.

Highly specialized cells leave the cycle at G1 &go to **G0** stop mitosis either permanent or temporary

Permanently (e.g., neurons, muscle cells) or **temporarily** (e.g., peripheral lymphocytes)

- Cells that have left the cell cycle are said to be in a **resting stage OR stable phase**, the **G$_0$ (outside) phase.**

- Cells which leave the cell **cycle temporarily** can **return** to the cell cycle later time.

- Some specialized cells **cannot divide** (**end cells**) e.g. blood cells &sperm, they are replaced from stem cells.

- **Stem cells** are **undifferentiated cells.**

- They can give either **one** type [**unipotential**] of cells as male germ cells.

- OR more than one type as in blood cells [**multipotential**]

- **Cell death:** This occurs either by **necrosis** or **apoptosis.**

Mitosis

It is the division of the nucleus to produce **2 daughter cells genetically identical** to mother cell. It is formed of **4 stages:**

Prophase:

1. Chromosomes become shorter and thicker each one is formed of **2 chromatids held together at the centromere**.
2. The **nuclei disappear** as **nuclear envelope disappears**

3. The **microtubule**s are arranged to form a **mitotic spindle.**

4. **Centrioles move to opposite poles** of the cells due to growth of microtubules

   Metaphase:

   1. **Chromosomes**, line up **in the equator** of the spindle.

   2. **kinetochore attaches the centromere of each chromosome** to the mitotic spindle

Anaphase:

1. The **centromeres divide** and the **spindle fibers shorten** pulling chromosomes to opposite poles.

2. The **chromatids separate** and are now called **daughter chromosomes**.

Telophase:

1. A **cleft develops** at the equatorial plane of the mother cell dividing the **cytoplasm and its organelles into two**.

2. The chromosomes become **extended, uncoiled so invisible**.

3. **Nuclear envelope &nucleoli reappear.**

NB: Each cell has **46 chromosome** formed of **one thread** of DNA OR **one chromatid (s-chromosome)**
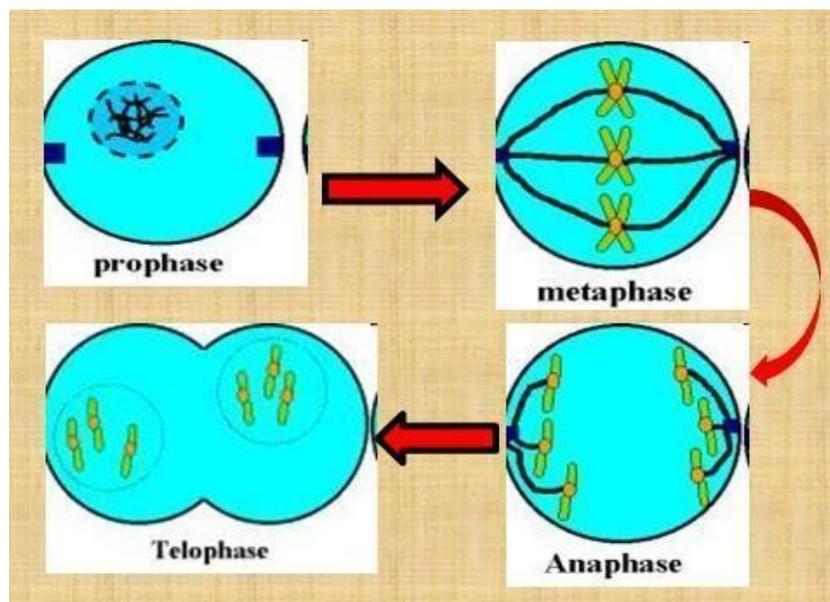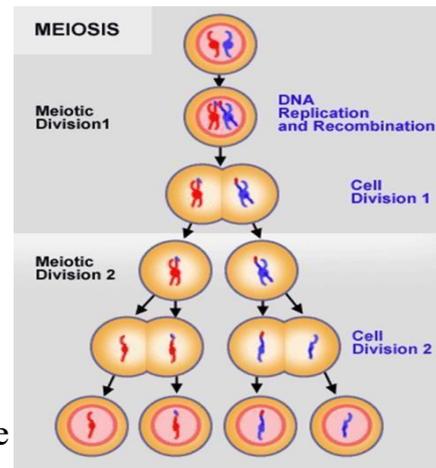
Meiosis

- **A special type** of cell division ends by formation of gametes (**sperm or ova**).

- **Each gamete has haploid number of chromosomes [23 chromosomes]**.

- It consists of **two successive cell divisions called Meiosis I & Meiosis II.**

- It ends by formation of 4 cells each cell has **23 s-chromosomes**

Meiosis I

Prophase I: Lasts a long time and is subdivided into **five stages:**

1- **Leptotene.** The chromosomes appear as thin, unassociated threads.

2- **Zygotene**. Chromosomes are arranged in 23 pairs. Each pair contains 1 maternal and 1 paternal chromosome.

3- **Pachytene.** Chromosomes are thicker and shorter; crossing over appear[chiasmata] resulting in exchange of genetic material between homologous chromosomes.

4- **Diplotene**. Chromosomes separate

5- **Diakinesis.** Chromosomes are condensed the nucleolus & nuclear envelope disappear, so the chromosomes are free into the cytoplasm.
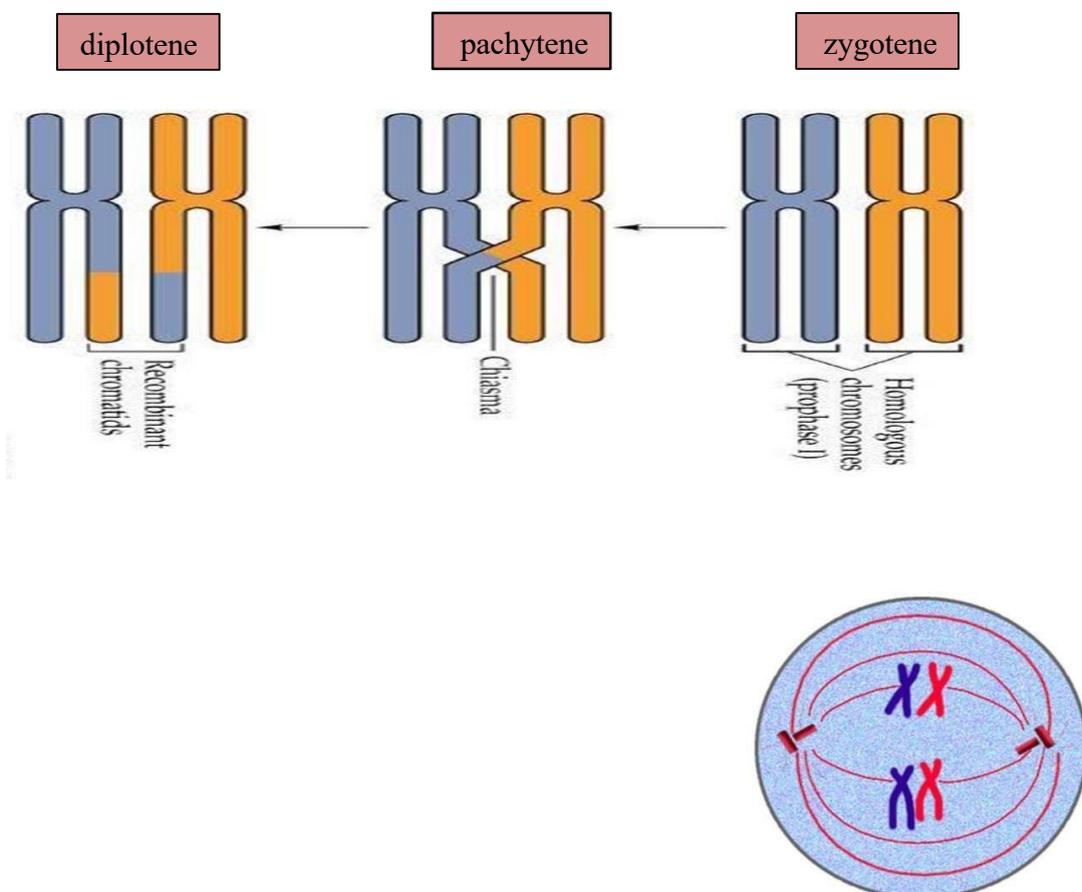
Metaphase I: The 23 pairs arrange themselves in the equatorial plane

Anaphase I: Each chromosome of the bivalent moves towards one pole of the cell.**Telophase I:** two daughter cells are formed. Each cell possesses **23 chromosomes**,

Chromosome 1   Chromosome 4   Chromosome 14
Metacentric   Submetacentric   Acrocentric

the **haploid (1n)** number, but because each chromosome is composed of **two chromatids**, the DNA content is still diploid. Each of the two newly formed daughter cells enters meiosis II
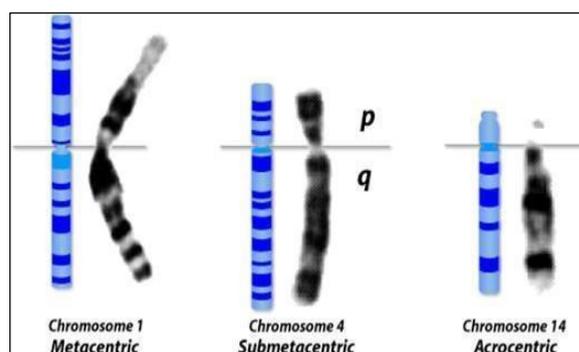
Meiosis II : It is very similar to mitosis and is subdivided into:**Prophase II:** the mitotic spindle starts to form.**Metaphase II:** the 23 d- chromosomes are arranged at the equatorial plane.**Anaphase II:** Each chromatid moves towards one pole of the cell.**Telophase II:** Four daughter cells from the original diploid cell.

Each of the four cells contains 23 s chromosome

	Mitosis	**Meiosis**
Site	Occurs in **somatic cells**	Occurs in **germ cells**
Number of divisions	**One** division.	**Two** divisions.
Separation of chromosomes	Each chromosome splits into **2 chromatids**.	In 1st division each **chromosome** of bivalent move towards one pole.
Crossing over	**No** crossing over	Crossing over (**genes exchange**
Daughter cells	**2diploid** cells those are **genetically identical**.	**4 haploid** cells showing **Genetic variation**.

Classification of chromosomes:
1. According to genes:
   A. **Autosomes:** 22 pairs, carry genes that control somatic characters.
   B. **Sex chromosomes**: 1 pair determines the sex.

2. According to position of centromere:

Centromere

p arm

q arm

Telocentric   Acrocentric   Submetacentric   Metacentric

   A. **Metacentric:** centromere is at the center.
   B. **Submetacentric:** centromere is midway between center and end of chromosome.

   C. **Acrocentric:** centromere is near to one end. Chromosome has one very long arm &short one.
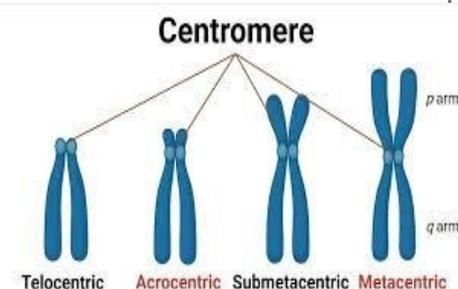
D. **Telocentric:** centromere is at the very end of chromosome, not found in human present in mice.

3. **According to length:** 7 groups in descending order of length (A,B,C,D,E,F and G).

Karyotype
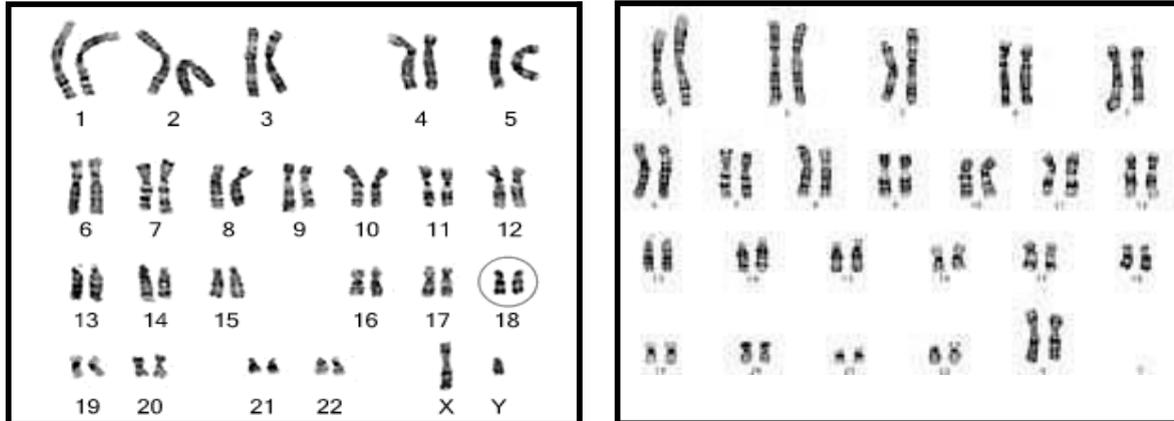
- **Definition:** Study of the **number** and **type** of **chromosomes** according to **position of centromere and length.**

- **Steps:**

    1. Add heparin to a blood sample.

    2. **Separate WBCs** by centrifugation.

    3. **Phytohaemagglutinin** is added to stimulate division of WBCs.

    4. Stop division by adding **colchicine** .

    5. A **hypotonic solution** is added to rupture cells so chromosomes disperse.

    6. Samples are spread on glass slides, fixed and stained with **Giemsa** stain.

    7. Chromosomes are **photographed**.

    8. Individual **chromosomal** photographs **are matched in pairs**.

Banding of chromosomes:

- **Staining of chromosomes with certain stains to show characteristic patterns of horizontal bands.**

- Each chromosome has its **own banding pattern** so can be easily identified in Karyotyping.

The Banding also permits the   **recognition of structural abnormalities in chromosomes**

Sex Chromatin(Barr Body)

- **Darkly stained body** present in nuclei of female cells
- **Inactive X chromosome** which is **normally** present **in female cells**
- It appears in **epithelial cells** of buccal cavity (60% of cells) and **b. Neutrophils** (6 % of cells) in females.
- Barr body is normally **absent** in **male** &in **female** with only **one X** chromosome **(XO)**
- Barr body is normally **present** in **female** & in **male** with **extra X** chromosome**(XXY)**
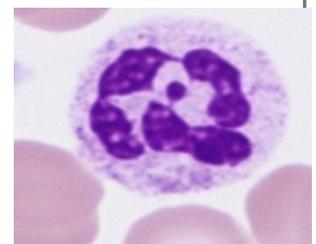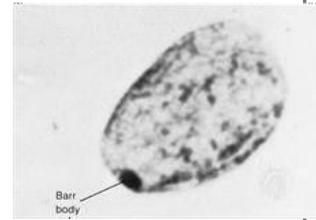
  Two Barr bodies are only present in **female with multiple X syndrome**

Significance of Chromosomal Examination:

1. **Diagnosis of genetic sex** in doubtful cases of **hermaphrodism.**
2. **Identification of foetal sex** by staining cells obtained from amniotic fluid.
3. **Diagnosis of sex chromosome abnormalities** as in Turner's syndrome (XO) and Klinefilter syndrome (XXY).
4. **Diagnose causes** of primary amenorrhea, repeated abortion and infertility.
5. **Diagnosis of structural abnormalities** in chromosomes as deletion in mental retardation and translocation in chronic myeloid leukaemia.
6. **Diagnosis of numerical abnormalities** as in mongolism.

Chromosomal Aberrations (Abnormalities)

Definition: It means any deviation from normal number or structure.

This may occur in autosomes or in sex chromosome pair

Causes of chromosomal aberrations:

1- **Radiation**: causes chromosomal damage &non-disjunction

2- **Viral infection**: e.g. germinal measles that cause fragmentation of chromosomes.

3- **Pregnancy in old age**: increase the risk of non-disjunction

4- **Drugs**: as colchicine (cytotoxic drugs) prevent formation of mitotic spindle.

5- **Autoimmune diseases** that causes non-disjunction

2 types;

1. **Numerical:**
   a. Euploidy.
   b. Aneuploidy.
2. **Structural:**
   a. Deletion.
   b. Duplication.
   c. Ring Chromosome.
   d. Inversion.
   e. Translocation.
   f. Isochromosome.

Numerical Aberrations in Chromosomes

a. **Euploidy**: Presence of **exact multiple of haploid (n)** number of chromosomes.

Origin of triploidy

- **Triploidy:3n**

- **Tetraploidy: 4n.**

- **Polyploidy: 5n or more.**

   Causes: one or both gametes are not haploid.

b. **Aneuploidy**: presence **of an extra or missing** chromosome.

- **Trisomy:** presence of **3 copies** of one chromosome. (Down syndrome).

- **Monosomy: presence of 1 copy** of one chromosome. (Turner's syndrome).
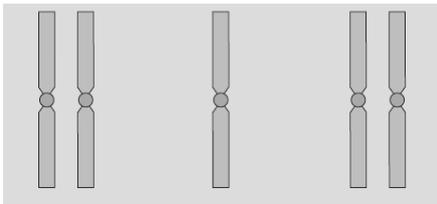
Causes of aneuploidy:

1. **Non disjunction**:

a. **Primary: Failure of separation** of homologous chromosomes during **first meiotic division**.

b. **Secondary: Failure of separation** of sister **chromatids** during **second** meiotic division.
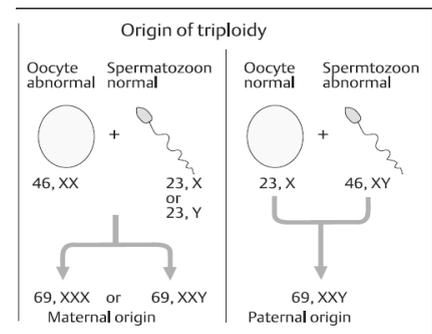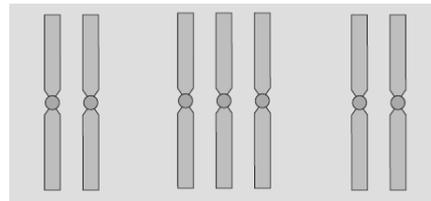
Mosaic: non disjunction occurs in **mitosis** after many normal divisions so the **cells of the body** have **more than one karyotype** (46, **47,45).**
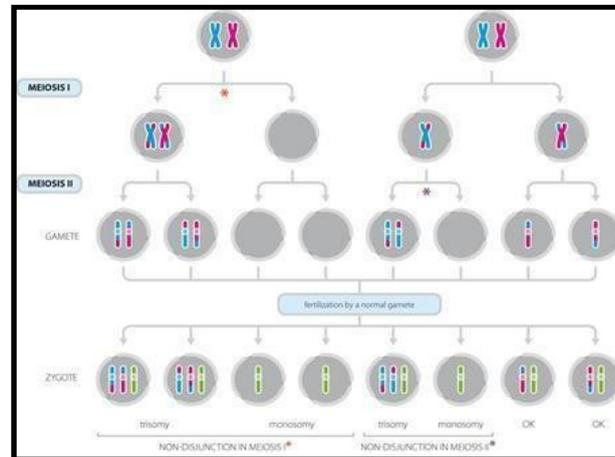
2. **Anaphase delay:**

> describes the **delayed movement** of one chromosome during <u>anaphase</u>. The lagging chromosome will be <u>lost</u> and there will be **one normal** cell and one cell with <u>monosomy</u>

3. **Failure of duplication** of chromatid during **S stage**.

Numerical aberrations in Autosomes

Down Syndrome (Trisomy 21) (Mongolism):

- Cells have **47 chromosomes**.

- There are **3 copies** of chromosome **21 (Trisomy 21).**

- The child has **mongol features** (slanting eyes, thick protruded tongue, mental retardation, small genital organs).

- **Causes:**

    1. Non disjunction.

    2. Translocation (21 & 14).

Numerical Aberrations in Sex chromosomes

1. Klinefelter's syndrome (47,xxy):

a) **Male** having **additional X** chromosome.
b) **Positive sex chromatin** due to **extra X chromosome**.

c) **Cause**: **non disjunction** of **X** chromosomes during **1ˢᵗ meiotic division** of oocyte. The **ovum** with **2 X** chromosomes is fertilized with **sperm** containing **y chromosome**.

4. Multiple X syndrome (47, XXX):

a) **Female** having **additional X** chromosome.

b) **2 sex chromatins** due to **extra X** chromosome.

c) **Same cause** as in Klinefilter syndrome but the ovum is fertilized by **sperm having X chromosome.**

5. Turner's syndrome (45, XO):

a) **Female** with only **one X chromosome**.

b) **No sex chromatin** due to **missing** of one of X chromosomes.

c) Due to **non-disjunction of X** chromosomes during **first meiotic** division in oocyte.The ovum with **no X is** fertilized with a **sperm having X** chromosome.

Structural Aberrations of chromosomes

a. **Deletion:** Loss of a part of chromosome either at its end [**terminal**] or between two breaks in one arm [**interstitial**].

Wolf-syndrome: partial deletion of the short arm of **chromosome 4**

Cri du chat syndrome : partial deletion of short arm of **chromosome 5**.
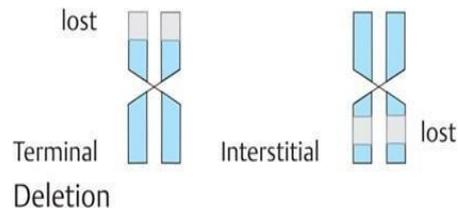
b. **Duplication: addition of an extra piece to a chromosome as a result of unequal crossing over.**

c. **Ring Chromosome: 2 breaks**, **loss of broken** part then **reunion in a ring form**.
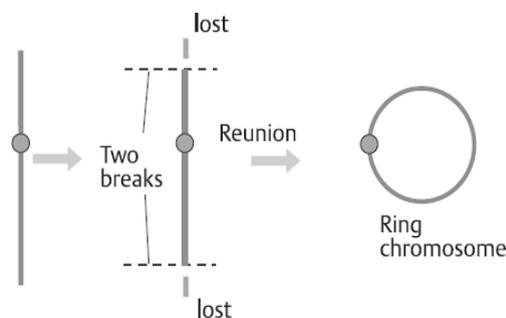
d. **Inversion**: 2 breaks in the chromosome followed by rejoining in an inverted form. It is of 2 types:

   a- **Pericentric:** breaks occur on **either side of the centromere**.

   b- **Para centric:** the breaks occur on **one side of the centromere.**

e. **Translocation**: 2 types:

a) **Centric fusion**: occur in **acrocentric chromosomes** as **chromosome21 & 14**. By **fusion of the long arm** of chromosome **21** and the **long arm** of chromosome**14**. The **short arms** of both chromosomes are l**ost** but this is insignificant.

b) **Reciprocal translocation:**

   An **exchange of chromosomal material** between **two chromosomes**. Since usually **no** chromosomal material is lost or added, it is **insignificant**.

   The Philadelphia chromosome due to **reciprocal translocation** between a chromosome **22** and a chromosome **9**. It is used to diagnose **chronic myeloid leukemia.**
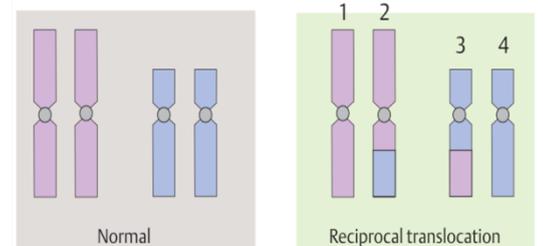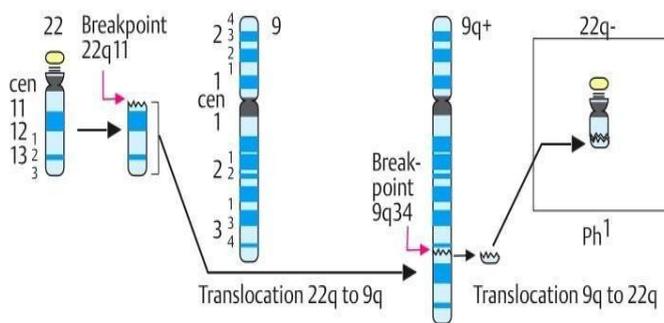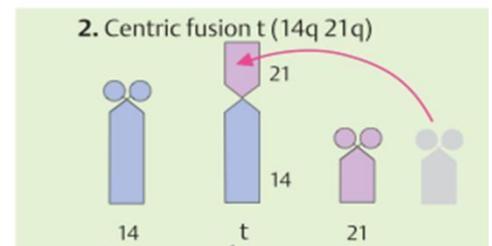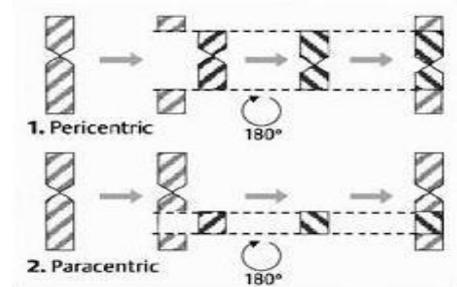
Isochromosomes:

- **Due to** abnormal division of the **centromere**
- **Transverse** instead of longitudinal
- Resulting in **unequal chromosomes**

One with **2 long arms** and the other with **2 short arms**
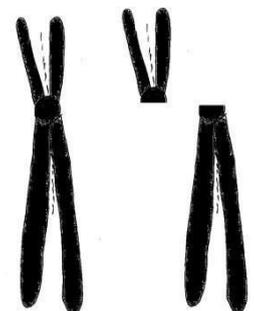
Unit (2)
Chemistry of Nucleotides

ILOs:
- By the end of this unit the student will be able to:
- Enumerate different types of purines and pyrimidines and mention their structure.
- Discuss in details the chemical structure of chromosomeand its components, DNA and RNA .
- Mention different free nucleotides and enumerate their biomedical importance.
- Mention different types of RNA.
- Discuss in detailed mechanisms involving apoptosis.

Nucleotides are the building units of nucleic acids.

Nucleic Acid
Nuclease

Nucleotides
Nucleotidase

Nucleosides   **Phosphates**

Nucleosidase

Nitrogenous bases   **Pentose Sugar**

Purines   **Pyrimidines**   **Ribose**   **Deoxyribose**
                                                    in RNA   in DNA
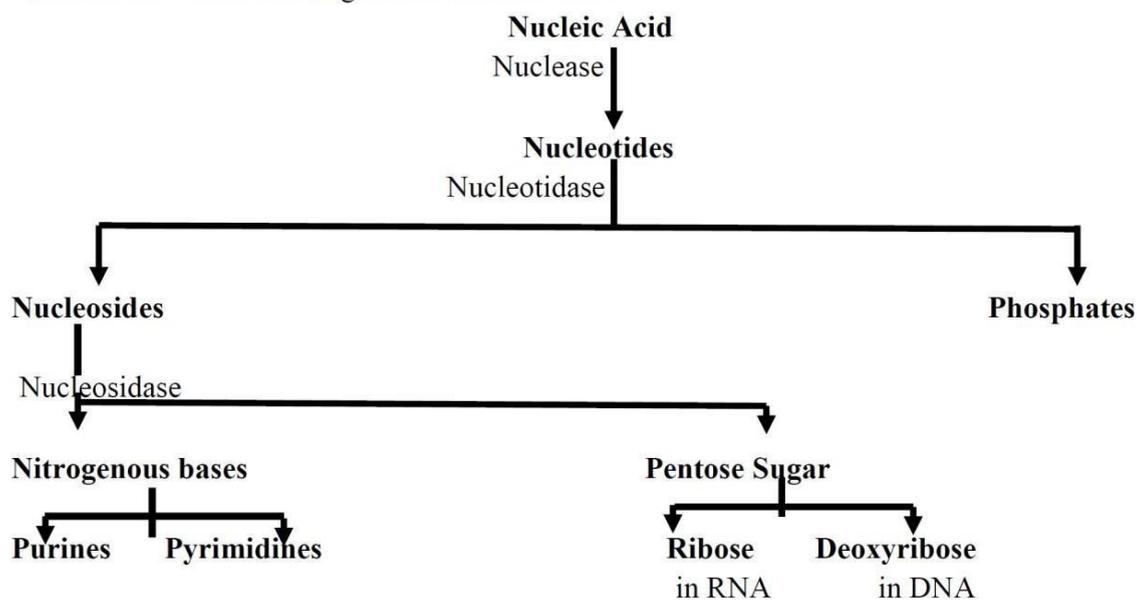
Nitrogenous Bases

A) Purines:
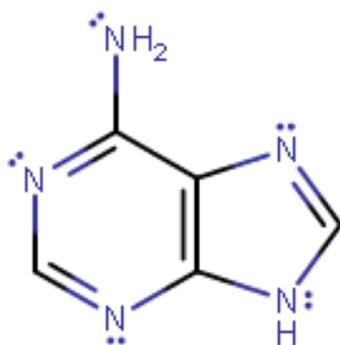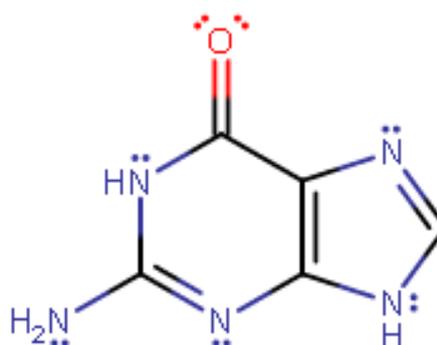
Adenine (A)   Guanine (G)

B) Pyrimidines:
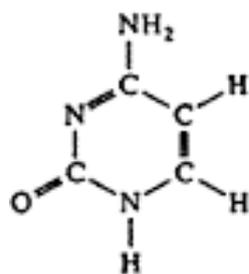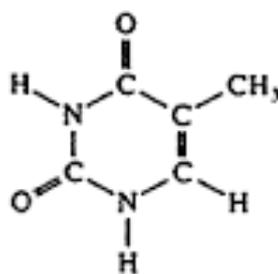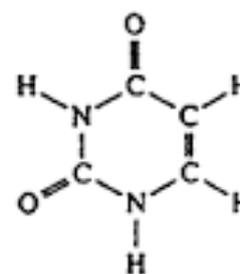
Cytosine   Thymine   Uracil

DNA contains: Adenine, Guanine, Cytosine & Thymine.

RNA contains: Adenine, Guanine, Cytosine & Uracil.

Nucleotides & Nucleosides

A nucleotide is formed of nucleoside + phosphate.

A nucleoside is formed of nitrogenous base + Pentose

Nucleic Acids

Deoxy-riboNucleic Acid (DNA)

Site: Nucleus and small part is present in the mitochondria.

Function:

1. It carries the genetic information.
2. Synthesis of RNA.

Structure:

Deoxyribonucleic acid (DNA) is composed of :

1- Four nucleotides formed from one of the four nitrogenous bases (adenine [A], thymine [T], cytosine [C], and guanine [G]).

2- Deoxy ribose **sugar** attached to carbon number 5.

3- One or more phosphate *groups*.

Nucleotides are joined by phosphodiester bonds to form polynucleotide strand.

The backbone of the structure is an alternating structure of phosphate and sugar residues. DNA has *two antiparallel strands* that provide a *Right-handed helical conformation, or B form.*

The helical turns create major and minor grooves that are sites of protein binding.
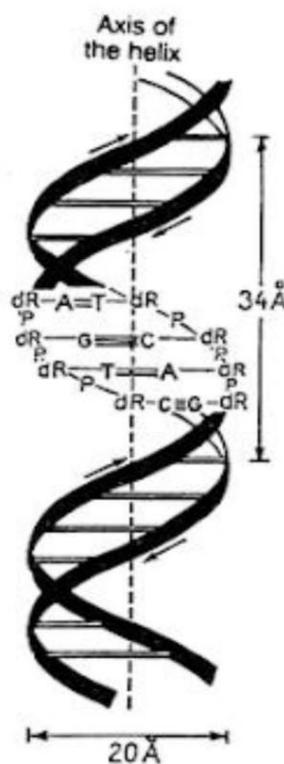
In order to accommodate DNA within the small space in the nucleus, helical DNA is coiled. Coiling is facilitated by the formation of the Nucleosome structure which is composed of eight histone proteins and the DNA encircling the histones.

Denaturation of DNA:

It is a process in which nucleic acids lose all structures and keep its primary structure. This occurs by application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent (e.g., alcohol or chloroform), radiation or heat. The increase of the number of hydrogen bonds increases the melting point.

Melting point:

It is the temperature for breaking of half hydrogen bonds between 2 strands.

Adenine (A) — Thymine (T)

Guanine (G) — Cytosine (C)
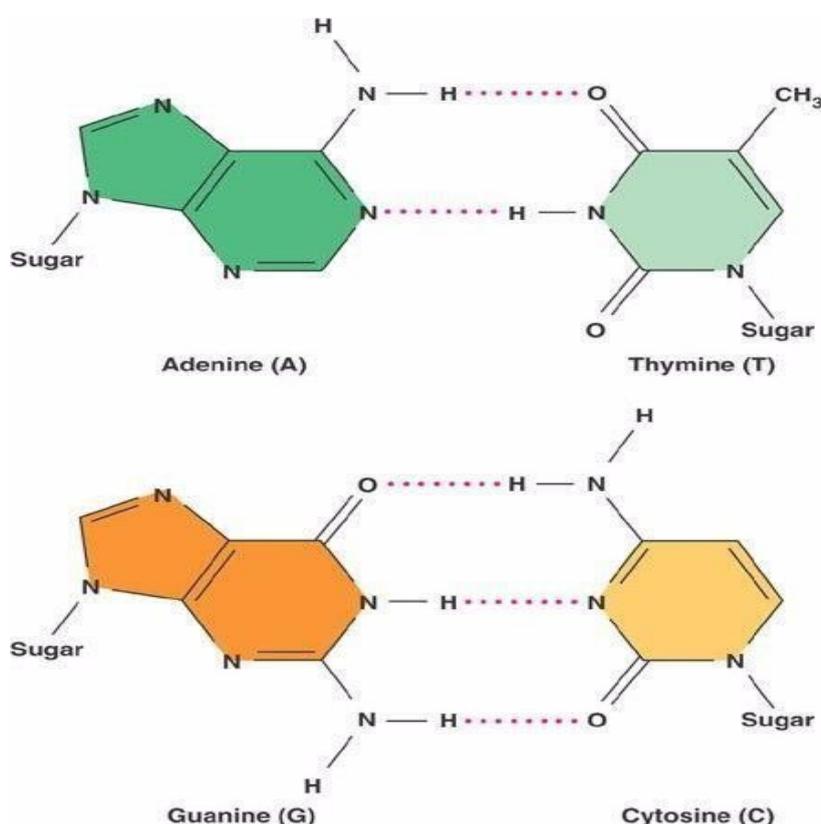
N.B.: As adenine binds with thymine by 2 hydrogen bonds and cytosine binds guanine by three hydrogen bonds.

So; CG rich areas in DNA have higher melting point than AT areas.

Free Nucleotides of Medical Importance

- **Free Adenosine nucleotides**

  1- Adenosine triphosphate(ATP), it is consumed in energy reactions.

  2- Cyclic adenosine Monophosphate(cGMP),it is a second messenger for hormones

  3- S-adenosyl methionine (SAM),it is active methyl donor

  4- Phosphoadenosine phosphosulfate(PAPS),it is used for synthesis of sulfur containing compounds.

  5- Nicotinamide containing nucleotides(NAD and NADP)and Flavin containing nucleotides(FAD and FMN), acting as hydrogen carriers.

  6- CoA-SH it is a carrier of fatty acids

- **Free Guanosine nucleotides**

  1- Guanosine triphosphate(GTP)

  2- Cyclic guanosine Monophosphate (cGMP).it is a second messenger for hormones.

- **Free Cytidine nucleotides**

  Cytidine diphosphate (CDP) for phospholipids synthesis.

- **Free Uridine   nucleotides**

  Uridine diphosphate (UDP) for synthesis of glycogen and glucuronic acid.

- **Mitochondrial DNA**

  - It forms about 1% of total DNA in the cell.

  - Mitochondrial inheritance involves genes found within mitochondria of the cell. Mitochondria has circular DNA that is not one of the 46 chromosomes found in the nucleus.

  - Characters of mitochondrial DNA :
    - Mitochondrial inheritance contains 37 genes and is inherited *only from the mother* and represent. Males do not share mitochondria with offspring.
    - Unlike nuclear chromosome DNA, mitochondrial *DNA has poor mechanisms to repair damage* and relies primarily on the
      proofreading function of DNA polymerase gamma (POLG) to repair errors in replication.
      - POLG is very sensitive to mutations, and thus, mutations develop and are sustained from generation to generation of cell divisions.
      - Mitochondria also do not have a coordinated mechanism of replication a condition known as (replicative segregation). In

which, some daughter cells may receive a greater percentage of mutated mitochondria than do others.

- *Mutations of Mit. DNA leads to Myopathies and Neurological diseases..*

N.B. Some other mitochondrial disease, may origin from both mitochondrial and nuclear mutations. As ATP generation requires two major processes:
1- The citric acid cycle (its enzymes are encoded in the nucleus.
2- Oxidative phosphorylation. Its enzymes are encoded in the mitochondria

- **Prokaryote DNA**

It is present in bacteria and all areas are coding with no nuclear membrane small nuclear circular DNA is named as plasmid

Ribonucleic Acid (RNA)

- There are 3 types of RNA:
- Messenger RNA (mRNA) .
- Transfer RNA (tRNA)
- Ribosomal RNA (rRNA)

- **Messenger RNA (mRNA):**

  - It forms **5% of** total RNA.It is transcribed from a DNA template, as a single strand and carries coding information **(exons)** interrupted by non coding **(interons)**.

  - The sequence of nucleotides arranged into ***codons*** *(consisting of three bases)*.

  - Each codon stands for a specific amino acid. Number of codons are 64 for 20 a.a. The **1ˢᵗ codon is AUG** and it encodes for methionine amino acid. (AUG) is always the first amino acid in eukaryotic proteins. As eukaryotic mRNA are *monocistronic* (i.e. only code for one protein).Stop codons are UAA, UGA and UAG.

  - There is no tRNA with anticodons complementary to the codons that codes for the stop signal.

- **Functions:** It carries genetic information from DNA to direct protein synthesis.

- **Transfer RNA (tRNA):**
  - It is RNA that transfers a specific amino acid to polypeptide chains.
  - tRNA has:

    1) **The *acceptor arm*** at 3' terminal site for amino acid attachment at sequence (CCA). Each type of tRNA molecule can be attached to only

one type of amino acid.

2) **Anticodon loop** formed of three bases that can base pair to the corresponding three base codon region on mRNA. It has hypoxanthine base that can pair with cytosine, adenine or uracil in mRNA.

3) **Loop I (loop D)** contains dihydrouracil.

4) **Loop IV** It has *variable region* and abnormal base (pseudouridine) thymine loop.

Ribosomal RNA (rRNA):

- It is formed of 2 subunits; 40S (formed of 35 polypeptides) and 60S subunits (formed of 50 polypeptides) they fuse together to form 80S. S is the Svedberg unit of sedimentation.

- **Functions:**

It is forms the predominant part of complete ribosome (site of peptide bond formation and protein synthesis).
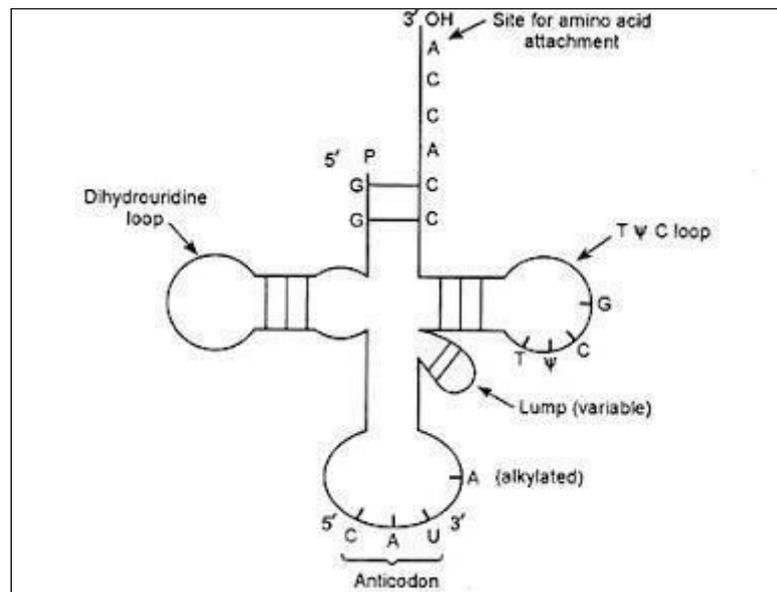
Small nuclear RNA:

- They have high content of uridine .They are responsible of processing of mRNA and excising interons .They are 2 major groups

- Group I: act as splicesomes and remove introns from a transcribed pre-mRNA a type of primary transcript.

- Group II: named as U7 small nuclear RNA responsible for synthesis of histones needed for mRNA processing.

Splicesomes added to mRNA

removal of interon
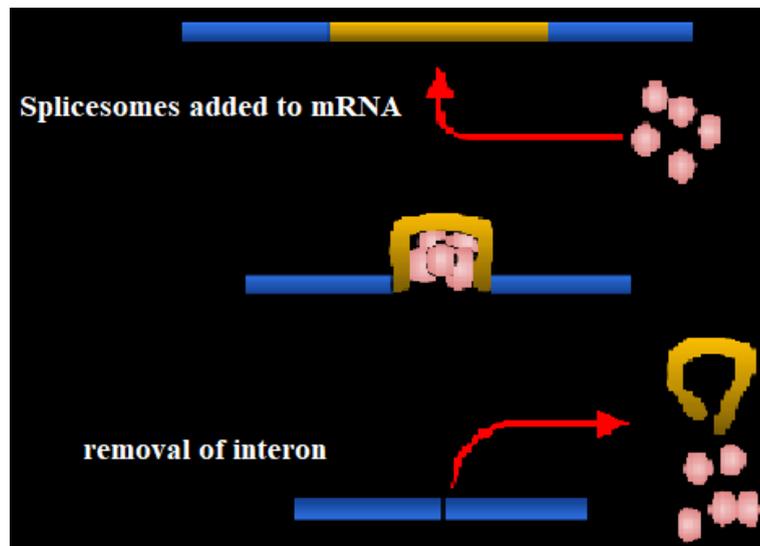
- **Nucleic acids of Viruses**
  - *DNA virus*es like Hepatitis B are less liable to mutations because they **have Proofreading** of DNA polymerase and correct any wrong base, so it is easy to form an effective vaccine against any one of them.
  - *RNA viruses* as HIV ,Hepatitis C and influenza viruses are liable to mutations and not corrected so there is **no Proofreading**. So it is difficult to form a vaccine.

Comparing DNA and RNA	
• DNA	• RNA
• **Double** stranded	• **Single** stranded
• Sugar = **deoxyribose**	• Sugar = **ribose**
• Bases: A-T, C-G	• Bases: A-U, C-G
• Found only **in nucleus**	• Found **in and out** of the nucleus
• Can repair itself	• Cannot repair itself

Apoptosis

- Apoptosis is **programmed cell death or Cell** Suicide.
- It is due to:

      1) Receptor mediated like FAS and tumor necrosis factor

      2) Chemical or physical factors exposure to factors as chemotherapy
                     or irradiation,hypoxia and change of hormones.

- **Role of apoptosis in human life:**
    - Menstrual cycle. hormonal changes lead to apoptosis of uterine cells.
    - Embryonic development and morphogenesis.
    - Aging is enhanced with increase in apoptosis.
    - Inhibiton of apoptosis leads to cancer.

- **Mechanism of apoptosis**

  1) **Damage of DNA** leads to **activation of P53**,

  2) P53 inhibits cell cycle and allow repair.

  3) If DNA changes <u>cannot be repaired</u>. P53 causes **Activation of Bax gene to release Bax Proteins.**

  4) Bax **Proteins release cyotchrome C from mitochondria.**

  5) **C**ytochrome C produces activation of **Caspases**.*Caspases cut proteins of cells between cystein and aspartic acid.*

  7) **Caspases activate DNA endonucleases to split DNA and formation of characterstic ladder appearance .**
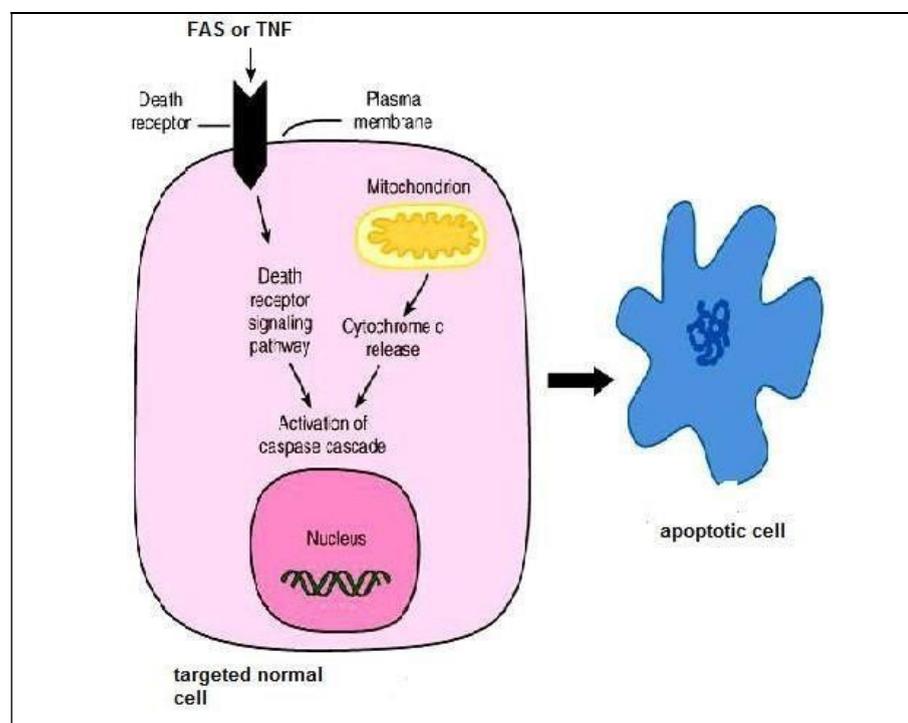
Unit (3)
Replication &Transcription and Translation

ILOs:
- By the end of this unit the student will be able to :
- Define replication and mention its different enzymes and steps in both prokaryote and eukaryote.
- Define DNA repair and its steps and sequences of defects in DNA repair.
- Define transcription and mention its enzymes ,steps and processing of each type of RNA in both prokaryote and eukaryote.
- Mention definition of translation (protein synthesis) steps and processing of protein.

Replication

- It is the process of DNA synthesis it occurs in S phase .old strands act as a template to the new ones.2 complementary strands are formed according to the base pairing rule. The process is *(semiconservative)* : two daughter cells are formed with one original and one newly formed. This is important to transfer genetic information in correct way.

- **Structural maintenance of chromosome (SMC)**
Proteins, binds to chromosomes during G1. They are critical to replication and hold the replicated chromosomes, chromatids, together.

- **Cohesion:**
The process of holding the identical sister chromatids and homologous chromosomes together. Cohesion molecules attached along the chromosome and the centromere become the association between the chromatids and are maintained through protein modification. They are important for:
    1- Metaphase alignment
    2- Mitotic spindles attach to cohesion molecules of the kinetochore
    3- Remain associated near the centromere until metaphase.

Cohesion disappears during chromatids separation

- **Replication in Prokaryote**
Prokaryote DNA is a double circular one. Replication occurs in 2 steps.

1) **Separation of two strands:**
    - **Ori site** it is the site of origin of replication. they contain A-T bases sequence.
    - **DNA A proteins** bind DNA at the ori site and produce unwinding of DNA. Forming V shaped region called replication fork.
        - **Prokaryote enzymes of replication**
        - **Helicase:** it interacts with dna-A protein (unwinds) and separates the 2

strands by breaking hydrogen bonds, this process requires energy(ATP).
- **Single stranded binding proteins (SSBP);** They bind each strand to prevent base pairing and reformation.
- **Topoisomerase I** break phosphodiester bonds between phosphate and deoxyribose back bone in ***one strand*** releases supercoiling ahead of replication and allows DNA to rotate then phosphodiester is reformed.

2) Synthesis of two new strands
- **Primase** it is responsible for formation of RNA primers. It is 5 nucleotides in length. It is formed in 5'-3' direction. it needs ribonucleotides (ATP,CTP,GTP,UTP).
- **DNA polymerase III:**
  - It reads the original nucleotide sequence from 3'to 5' direction. It forms the new strands *(leading and lagging strands)* in 5'-3' direction by using deoxy-ribonucleotides (d ATP,d CTP,d GTP,d TTP). The new strands are complementary and it has a ***proofreading power* (the ability to correct wrong bases)**. It needs the RNA primer.
  - **Leading strand** is formed in the same direction of helicase enzyme formed in 1 piece. it is formed from 5-3 direction.
  - **Lagging strand** is formed in pieces (okazaki fragments). It is formed from 5'-3'direction but in the opposite direction of helicase. Many RNA primers are needed for lagging.
  - **Okazaki fragments** small fragments formed of RNA primer and small piece of NEW DNA in lagging strand.
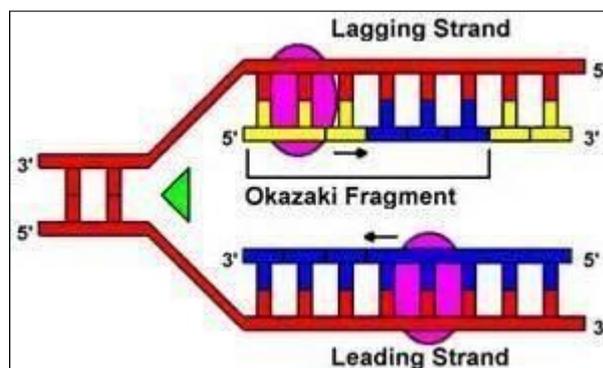
- **DNA polymerase II** it is responsible for repair in prokaryotes

3) Excision of RNA primer and replacement by DNA by DNA polymerase I:
- *DNA ligase* connects DNA fragments

- **Topoisomerase II** separates ***two interlocked strand*s** release circular
  bacterial DNA at end of replication
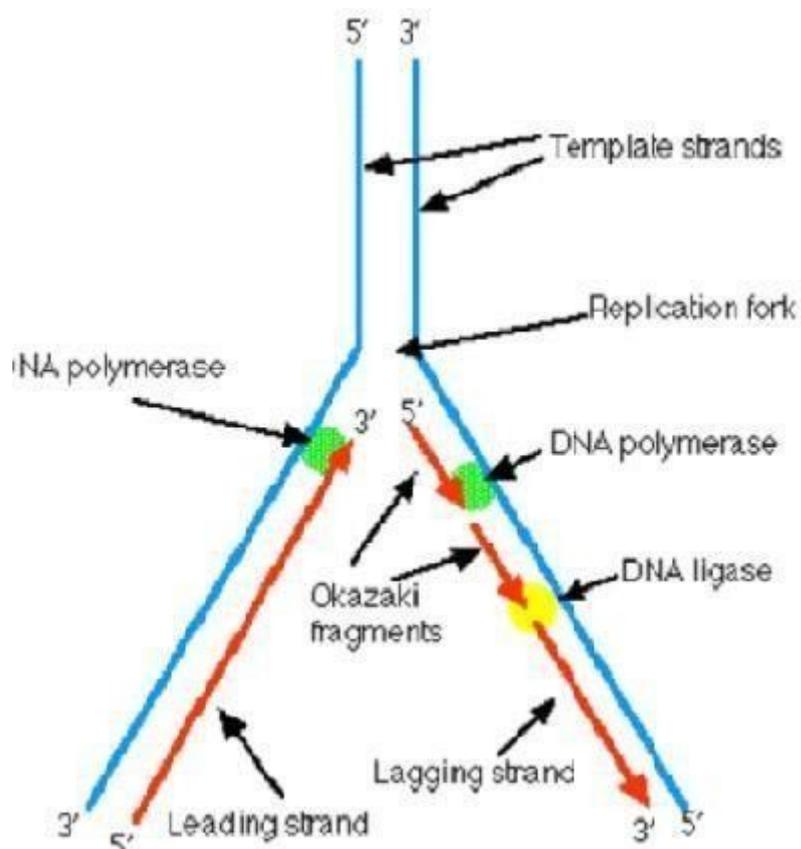
Eukaryote Enzymes of Replication

- In eukaryotic cells, DNA (deoxyribonucleic acid) synthesis occurs at specific sites that move through the genome called replication forks. Multiprotein complexes at these forks catalyse the synthesis of two new strands of DNA using parental strands as templates to produce two complete copies of the parental DNA .
- The eukaryotic replication fork machinery must deal with the chromatin and chromosome structure of eukaryotic genomes .

- **The process must be able :**

    1- To replicate DNA in the context of a complex cell cycle.

    2- It also must be able to deal with the constant threat of mutations that could arise due to replication of damaged DNA.

- **Steps of Eukaryote Replication**
    - There are different steps and enzymes needed for replication in Eukaryotes

    - **First step:**
        - **Separation of DNA** occurs at **multiple origins of replication** and **multiple replication forks.**

        - **DNA polymerase α-primase complex:** it forms RNA primers and short DNA stretch connected to this strand.

        - **DNA polymerase β** it is for repair of DNA.

        - **DNA polymerase δ :** it is for synthesis of lagging strand and the Okazaki fragments.

        - **DNA polymerase ε :** it is used for synthesis of leading strand.

        - **DNA polymerase γ:** for mitochondrial DNA.

        - **RnaseH :** it is used for removal of RNA primer.

- **DNA ligase :** it joins ends of adjacent DNA segments.

- **Topoisomerases:** they are used for removal of positive supercoil in front of replication fork.

- **Telomers & Telomerase:**

- Telomeres are repetitive sequences present in the ends of chromosomes.
- Their replication in the terminus is very difficult. This results in telomeres shortening with repetitive mitosis.

- Function: They are associated with binding proteins that protect the cell against apoptotic actions like (fusion of chromosomes, inappropriate recombination, and degradation).

- *Originally,* telomerase is a reverse transcription enzyme. They are responsible for maintenance of the length of telomeres by addition of guanine-rich repetitive sequences.

A. Telomere shortening

- The process of shortening telomeres is associated with down- regulation of telomerase in somatic cells. In normal cells, telomere shortening leads to senescence (process of deterioration with age.).
- Tumor cells, however, bypass the senescence phase, and, even though telomeres may shorten, telomerase activity increases to stabilize the length and confers immortality to cancer cell progression. These cells can proliferate indefinitely.

B. Telomerase up-regulation

- Changes occur in DNA and chromosome structure that lead to instability of gene function and chromosome integrity as well as multiple cellular alterations. *Telomerase up-regulation is the final step in establishing and maintaining a cancer phenotype.* Therapies are under development that target telomerase activity in cancer cells.
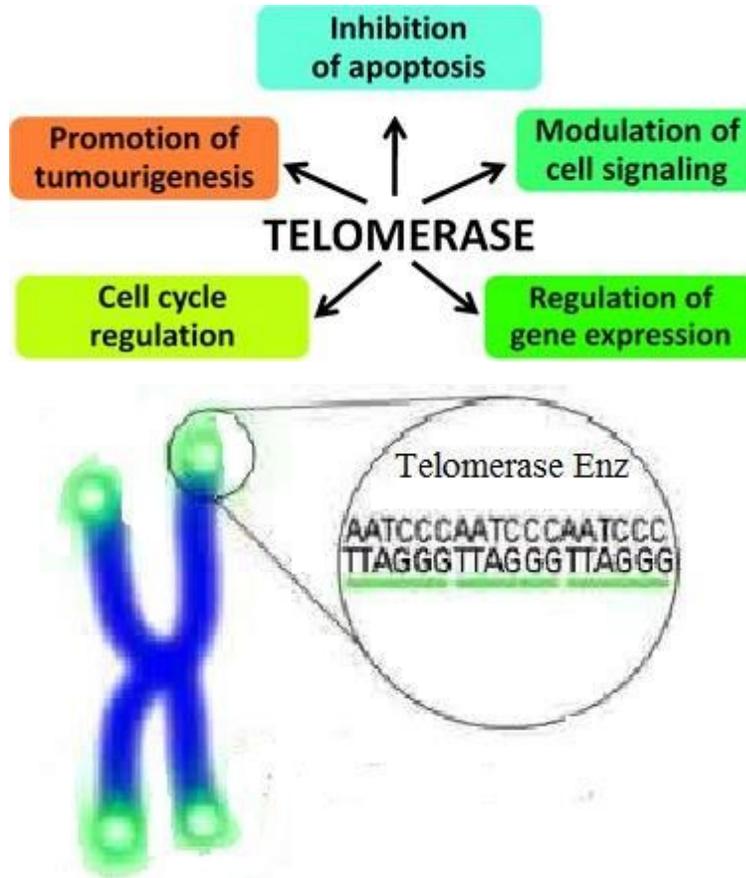
- **Factors reduce time of replication:**
   1) The presence of multiple replication origins and forks.
   2) The presence of great number of polymerases.

Comparison between Enzymes of Replication in Prokaryote and Eukaryote

	Prokaryote	Eukaryote
Primer	Primase	DNA polymerase α
Leading strand	DNA polymerase III	DNA polymerase ε
Lagging strand	DNA polymerase III	DNA polymerase δ
Mitochondrial DNA	Not present	DNA polymerase γ
DNA Repair	DNA polymerase II	DNA polymerase β
Telomerase	Not present	Present
Excision of primer and filling gaps	DNA polymerase I	Rnase H

DNA Repair

- Damage of DNA may result from physical, chemical and environmental factors.
- The process of DNA repair is a continuous process that needs:
- DNA Repair is an active, alert system for detection of wrong bases and its correction.

- **Steps of DNA repair**
  - Process of repair starts with scanning of newly formed strands by specific proteins.
  - The template strand has methylated adenine (as a reference)to differentiate it from the newly formed strand .
  - Detection of wrong base by endonuclease in the non methylated strand.
  - Wrong base is cut by exonuclease with removal of faulty DNA.
  - The defect is filled by DNA polymerase and ligase.

- **Hereditary DNA repair diseases:**
  1. Xeroderma pigmentosa : it results in skin cancer and premature aging.
  2. Ataxia telangiectasia:
     - In this condition the inactivate CDC25 (cell division cycle 25 protein prevents activation of CDC2, thereby preventing transition from G2 to M phase.
     - Double-stranded DNA breaks are recognized by the ATM (ataxia– telangiectasia mutated) gene protein and becomes activated.
     - This leads to activation and phosphorylation of p53.
     - Once activated, p53 regulates other genes that affect repair, arrest growth, or induce apoptosis.
  3. Familial Adenomatous Polyposis (FAP), which caused an increased risk for colon cancer in which hundreds to thousands of precancerous polyps preceded the development of colon cancer. Mutations and alteration of transcriptional cofactors for genes including cyclin D1, cyclins , and CDK complex, drive many of the early

events of cell division.

Gene Expression (Transcription)

An mRNA copy of the gene is made in the nucleus.

Amino acids are linked to one another at the ribosome to form the protein encoded by the mRNA.
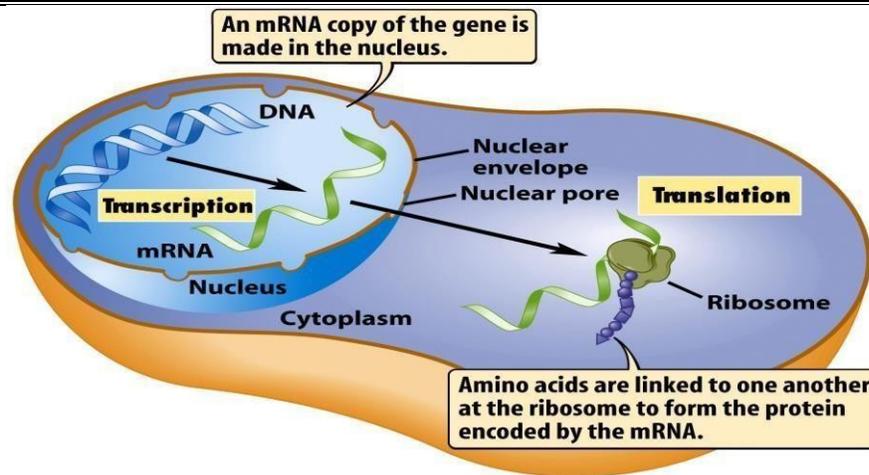
Figure 13-2 Discover Biology 3/e
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1) Transcription in Prokaryotes

- **Step 1: Initiation**
Initiation in prokaryotes:
  - There is only (1) RNA polymerase for synthesis of 3 types RNA. Prokaryote RNA polymerase is formed of sigma factor and 2α and 2β subunits _ and Sigma factor(σ) it recognizes and binds the promoter at TATA box then RNA polymerase binds the start point.
  - Antibiotic (Rifampicin) binds β subunitand inhibits RNA synthesis.
  - There are two boxes for identification of initiation of transcription in prokaryotes:

    1. TATA Box: it is a specific sequence of nucleotides located 10 bases upstream of start

    2. TTGACA BOX : it is a specific sequence of nucleotides located 35 bases upstream of start point. it determines frequency of transcription.
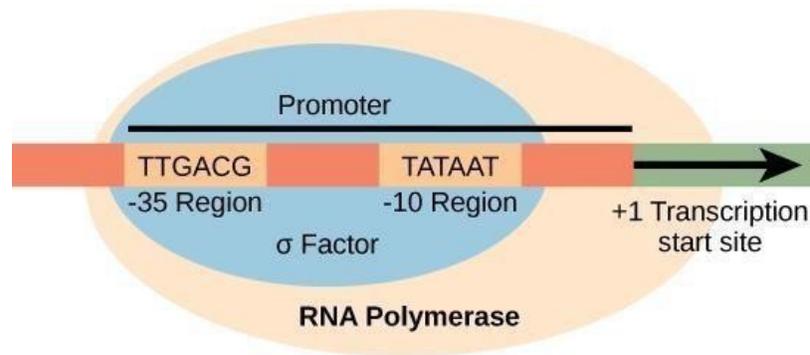
- **Step 2: Elongation**
    - RNA polymerase creates +ve supercoil in front of its movement and –ve supercoil behind it.
    - **Gyrase enzyme** release +ve supercoil in front of RNA polymerase.
    - **Topoisomerase** enzyme release – ve supercoil behind RNA polymerase.

- **Step 3: Termination**
    - **Rho factor dependant:** a specific termination protein.
        - The simplest stop signal is a palindromic GC region immediately followed by a T-rich region. The palindromic GC-rich region forms a hairpin structure in the RNA due to the base pairing.
        - There are also proteins that assist in terminating RNA transcription
        - One such protein discovered in prokaryotes is the rho (ρ) protein This binds to the newly made RNA at C-rich G-poor regions and scans along the RNA towards the RNA polymerase in an ATP- dependent manner When it catches up with the RNA polymerase, it breaks the DNA/ RNA association and thus terminates transcription.
    - **Rho independent** RNA forms hairpin preventing attachment of RNA polymerase. In absence of hair pin loop another mechanism involves the presence of (Histone mRNAs) that are processed by a histone-specific mechanism to end after a highly conserved

RNA hairpin element.

- **Transcription bubble is formed of** template DNA ,RNA polymerase, newly formed RNA

2) Eukaryote Transcription

- There are 3 polymerases in eukaryotes
- **RNA polymerase I** for synthesis of **r RNA**
- **RNA polymerase II** for synthesis of **m RNA**
- **RNA polymerase III** for synthesis of **t RNA**

- **Step 1:**

- **Preinitiation complex**: RNA polymerase II is composed of 12 subunits and is the target of regulation by multiple transcription factors that specify which genes are transcribed.
- The promoter is the binding site for transcription factors that form a preinitiation complex (PIC).
- RNA polymerase II does not bind directly to the promoter sequence but to the PIC.

https://www.slideshare.net/adurganaveen/transcription-57863890
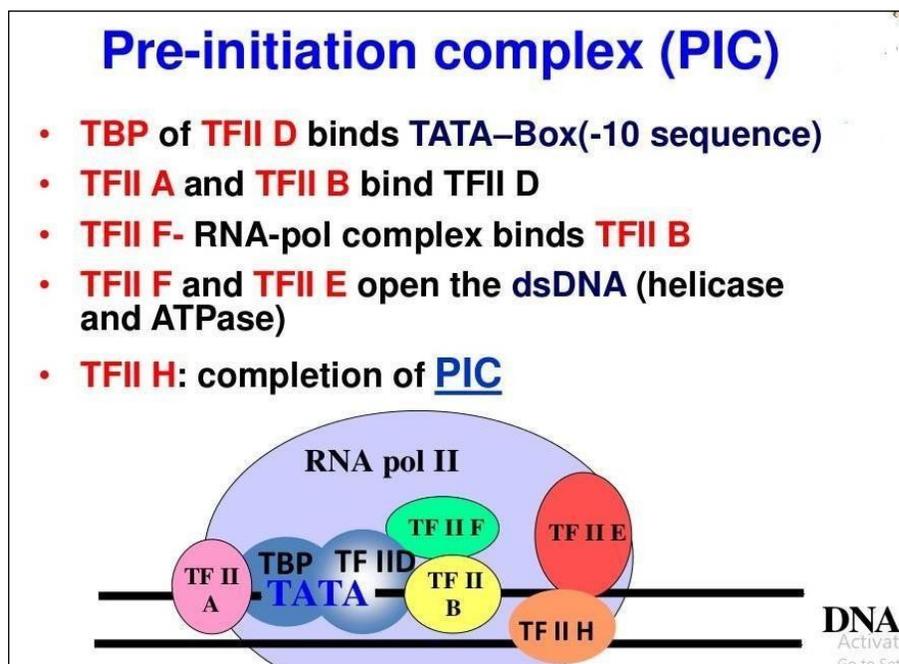
- **Step 2: Initiation**
  - **TATA box** is present-25 bases upstream from initiation of transcription it determine site of initiation of transcription. The first step is the binding of the TATA box-binding protein (TBP) and TBP-associated factors (TAFs, collectively known as TFIID) to the TATA box.
  - **CAAT box** (CCAATC) is present 40 bases from initiation of transcription.
  - **GC box** (GGCGGG) is present 200 bases from initiation of transcription (determine frequency of transcription.

- **Transcription factor IID (TFIID)**
  - The first step is the binding of the TATA box-binding protein (TBP) and TBP-associated factors (*TAFs, collectively known as TFIID*) to the TATA box.
  - Then recruitment of other transcription subunits namely TFIIA, TFIIB, TFIIF This is followed by RNA polymerase II itself and, finally, TFIIE and TFIIH
  - Unwinding of a short stretch of the DNA double helix to reveal the single-stranded that it will be transcribed .The rate of transcription initiation by the the end of this process is named as : ***Basal Transcription Apparatus***
  - ***Enhancer sequences*** can, when bound, also modify the rate of initiation complex formation. The rate of transcription is controlled by the stability
  of the com plex, which can dissociate easily from the promoter
  - Transcription starts by phosphorylation of the enzyme by kinase.

3) Elongation

RNA synthesis requires unwinding of DNA then the enzyme
RNA polymerase forms new RNA strand.

4) Termination

- The "end" of the newly formed RNA occurs between AAUAAA sequence followed by GU-rich sequence separated by about 40-60 nucleotides in the emerging RNA. Once both of these sequences have been transcribed, a protein called Polyadenylation Specificity Factor (CPSF) in humans binds the AAUAAA sequence and a protein called Cleavage Stimulation Factor (CstF) in humans binds the GU-rich sequence.
- The Poly(A) Polymerase enzyme which catalyzes the addition of a 3′ poly-A tail on the pre-mRNA is part of the complex that forms with CPSF and CstF.

Post transcriptional Modification of mRNA

Processing of mRNA

- **Capping**
  - *A 7-methylguanosine* is linked to the first transcribed nucleotide through a *5′-5′* triphosphate bridge to form a 5' methyl cap. After this occurs by a capping enzyme and an RNA (guanine-7-) methyltransferase.
  - Capping is **important for**
    1) Protection of 5 end from exonuclease.
    2) 5' methyl cap binds to a translation factor (eIF4E), which is the first step of mRNA recruitment to the 40S ribosome subunit.

- **Polyadenylation**
  - It is the process of addition of poly adenine nucleotides to the end of mRNA(tail) at its 3 end. Polyadenylation occurs during and/or immediately after transcription of DNA into RNA. It is about 100-200 bases of Adenine bases. (This reaction is catalyzed by polyadenylate polymerase)
  - Polyadenylation is **important for**:
    1) Protection of 3 end from exonuclease.

2) It is important for transcription termination, export of the mRNA from the nucleus and translation.

- ## RNA Splicing

  - It means excision of interons and joining of exon ends. It requires small nuclear RNA (splicesomes) this process is important for gene rearrangement coding for different proteins.
  - Five specific snRNAs are essential for binding to more than 100 non-small nuclear ribo- nuclear protein-splicing factors to form small nuclear ribonucleo- proteins (snRNPs).
  - Assembling and capping of snRNAs with trimethylguanosine to be different from other mRNAs.
  - Adding of several uridine-rich proteins (U1, U2, U4, U5, and U6), to the cap to reenter the nucleus.
  - *Alternative splicing*: An extra level of complexity is added to splicing by alternative splicing in which an mRNA can be spliced differently, thereby leading to a different assortment

Processing of t RNA

- It has a clover leaf appearance and it is synthesized by RNA polymerase III it processed by :
  1) Removal of extension at 5
  2) Adding of CCA at 3`
  3) Excision of interons at anticodon
  4) Modification of some bases by methylation of Uracil

Processing of r RNA

- It is formed by RNA Polymerase I and 5s by RNA polymerase III.
- It undergoes processing and cleavage in the nucleus into 28S,5.8S and 18S.

- **Comparison between Transcription in Prokaryote and Eukaryote**

		Prokaryote	eukaryote
enzyme		One enzyme formed of 2α & 2β and sigma factor	3 enzymes RNA polymerases I &II &III
	Initiation	TATA Box and TTGACA box located upstream of start	TATA &CAAT & GC Boxes located upstream of initiation
		Sigma factor recognize promoter at TATA and binds it	TFII D binds the TATA box at site of initiation
	Elongation	<u>Gyrase enzyme</u> release +ve supercoil in front of RNA polymerase <u>Topoisomerase</u> enzyme release – ve supercoil behind RNA polymerase	RNA polymerase binds with TFIID forming (pre-initiation complex). <u>RNA</u> synthesis; unwinding of DNA then RNA polymerase forms new RNA strand
	Termination	<u>Rho factor dependant</u> specific termination protein <u>Rho independent</u> RNA forms hairpin preventing attachment of RNA polymerase.	Specific sequence (AAUAAA)appears in RNA then RNA polymerase is dissociated and dephosphorylated

Protein synthesis (Translation)

- **Genetic Code:**
    - It is the nucleotide sequence of mRNA representing the code of amino acids.
    - Sequence of bases in codons of mRNA is similar to that of the coding strand of DNA except for T in DNA is replaced by U in RNA.
    - Each codon is formed of 3 successive N_2 bases (U, C ,A or G).
    - There are $4^3 = 64$ Possible Codons.

- **Characteristics of Genetic Codes:**

    1- Specificity:
    - Each amino acid is coded by a specific codon.
    - Initiation codon, (AUG) codes for one amino acid : Methionine.
    - Termination operates by the non sense codons (UAA, UAG or UGA). They don't code for amino acids and they cause termination for synthesis of polypeptide chain.

    2- Degenercy:
    It is the presence of more than one codon for single amino acid (synonym codons) for example: Arginine is coded by 6 codons.

    3- Universality:
    Genetic codes are usually universal with few exceptions e.g. mitochondrial RNA reads 4 codons being different from cytoplasmic RNA.

    4- Reading frame:
    One reading frame will produce a functional protein (it is determined by initiation codon AUG).

- **Wobble Hypothesis:**
    - This is the mechanism by which tRNA can

recognize amino acid. The first 2 nitrogenous bases
are essential, but the third base is flexible.
- Explanation of Wobble Theory:
    - Unusual base pairs can form because of flexibility
      of the bases in the anticodon of t RNA.
    - The pairing of the first base of anticodon with
      the third base of the codon follows less rigid
      requirement than the first 2 bases.

- Steps of Protein Synthesis:

1- Synthesis of Aminoacyl - tRNA:
This takes place in 2 steps:
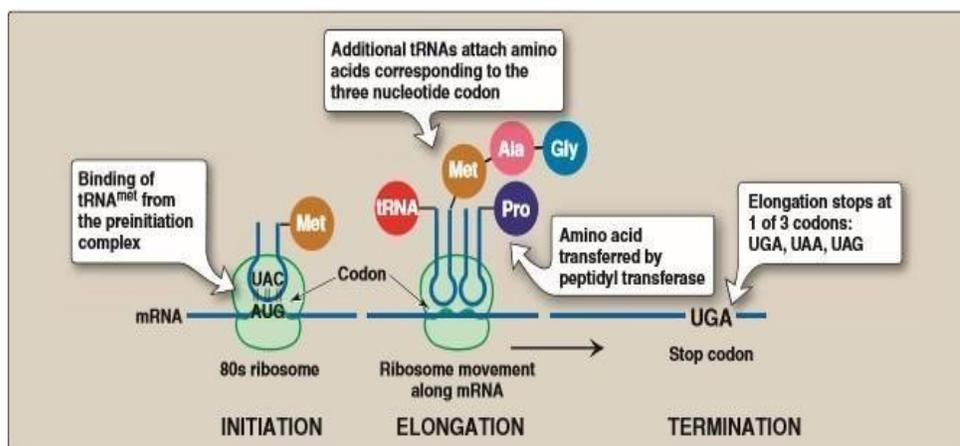
- Initiation:

Steps:
- **Dissociation Of Ribosomal Subunits:** this needs IF-1
  &IF-3 bind to 40S.
- **Formation Of 43 Preinitiation complex**: IF-2 and
  GTP and methionyl tRNA*(ternary complex)*
- **Formation of 48S subunits** IF-4 bind 43S and methyl
  GTP of mRNA and move on m RNA to find AUG OF
  initiation codon.
- **Formation of 80S fubunit.** Formed of 60S and 48 S
  requires hydrolysis of GTP and release of IF1,2,3
- **60S is formed of P site occupied by tRNA carrying**

Methionine and <u>A site</u> for the second codon ready to accept the next aminoacyl- tRNA.

- <u>Elongation</u>

1- Elongation factor 1 (EF1)binds aminoacyl- tRNA to A site and hydrolysis of GTP.

2- Elongation factor 2 translocates the peptidyl t RNA from A site to P site and accept a new aminoacyl t RNA energy reqired to elongation is 4 ATPs.

During elongation, the peptidyl–tRNA is protected from hydrolysis in the peptidyl transferase center.

- <u>Termination</u>

- It needs releasing factor (RF) it can recognize termination codons. the stop codons which are :(UAA,UGA,UAG) can't protect the peptidyl transferase enzyme.
- When release factors recognize a stop codon, the ester bond bridging the polypeptide with the terminal nucleotide of peptidyl–tRNA is hydrolyzed, and the polypeptide chain is released from the tRNA.
- This factor needs GTP for its hydrolysis.

Posttranslational Processing of Proteins

- **Trimming** digestive enzymes as pepsin and trypsin are secreted as inactive form and are activated on need. Also,insulin hormone is secreted as inactive form with an extension peptide. the extension is removed to be active.

Adding of chemical groups (covalent modification)

1- **Hydroxylation** in collagen fibres proline and lysine are hydroxylated

2- **Glycosylation** occurs for proteoglycans and glycoproteins, carbohydrates are added to OH group of serine or

Threonine. This modification serves several functions including protein folding, conformation, distribution, stability, and activity. It is active in tissues forming mucin.

3- **Phosphorylation** Protein phosphorylation is used for signal transduction and affects all basic cellular processes. The phosphate group is added to OH group of serine or Threonine, in some cases it is added to lysine amino acid.

- The unmodified form of the proteins can be regenerated by protein phosphatases. *There are two reasons phosphorylation in cells:*

- *The first* is that it is easy to convert from the phospho to dephospho forms of the protein to affect regulatory control.

- *The second* is that phosphorylation is added to serine or threonine and used to change functional properties, which changes the three-dimensional structure of a protein (enzymes) and affects its activity.

- *An example* is : the p53 tumor suppressor protein. This protein has a lysine- rich region that can be modified by phosphorylation.

4- **Carboxylation**: It is the activation for blood clotting factors and carboxylation of osteocalcin of bone synthesis.

5- **Methylation**: *S-adenosylmethionine*, SAM (methyl donor) can regulate transcription, protein modifications can activate or repress the function of the

protein. For example, in histones H3 and H4, arginines and lysines when methylated can either repress or activate transcription;RNA stability through bound proteins, cell signaling, or enzymatic activity.

6- **Ubiquitination:** It is the formation of a covalent bond between ubiquitin and the highly reactive amino group of a

lysine.

- Proteins with *many ubiquitins* are selectively targeted to an ATP-dependent protease enzyme.

- Proteins with few ubiquitins are targeted for endocytosis and proteolysis in a lysosome.

7- Acetylation: It is the transfer of an acetyl group to nitrogen through reversible and irreversible mechanisms through the addition of an acetyl group by N-acetyltransferase enzymes.

- *The purpose of this modifications* is to alter protein–protein interactions.

- As histones are acetylated at the N-terminal lysine there is facilitation of transcription while deacetylation of chromatin decreased transcription.

- These histone acetyltransferases (HATs) is an important mechanism for con-densed chromatin structure to unwind for transcription of down-stream genes.

8- Farnesylation: Some proteins can be attached to an unsaturated carbon of fatty acid (known as a farnesyl anchor) which inserts the protein on either side of membrane bilayer associated with lipids into the plasma membrane.

- **Regulation of gene expression**

Regulation of gene expression occurs in both prokaryote and eukaryotes

- **Regulation of gene expression in prokaryotes**

There are 2 types of genes

- **Constitutive genes**

They are genes that act in continuous manner they act in a slow rate.

- **Inducible genes**

They are the genes that act only when stimulated and

normally they are repressed.

- **Lac operon**

  In absence of inducer

| p | i | p | | z | y | a | Absence of inducer |

repressor binds to the operator region and prevents RNA polymerase from transcribing the operon

repressor mRNA

repressor

  In presence of inducer

| p | i | p | o | z | y | a | Presence of inducer |

repressor mRNA   *lac* mRNA

β-galactosidase   permease   transacetylase
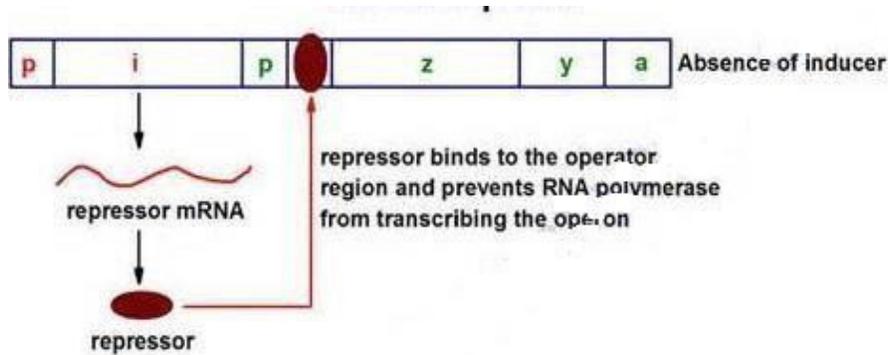
+ inducer   (inactive repressor)
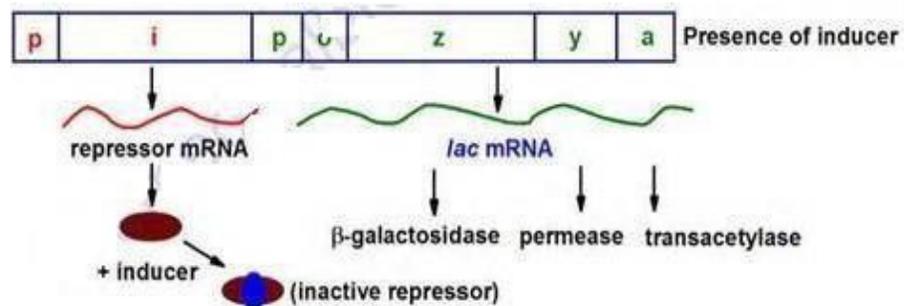
- **Regulation of Gene Expression in Eukaryotes**
    - There are many levels to control genes in eukaryotes:
    - First level at chromosomes
    - Second level: at the transcription rates.
    - Third level at RNA processing.
    - Forth level at the translation of proteins.

First level: Chromosomal regulation of Gene Expression:

There are many mechanisms of regulation at the chromosomes:

1- Gene Amplification:
    - It involves the increase in number of genes for certain function.
    - Gene amplification process occurs at the level of increased rRNA expression.
    - Some drugs may cause gene amplification as methotrexate. It is used to treat cancer.

- Mechanism of action of methotrexate is inhibition of dihydrofolate reductase gene. Through this action tetrahydrofolate is not formed and DNA synthesis is inhibited with inhibition of cell growth and multiplication. Cancer cells respond by increasing the number of its dihydrofolate reductase gene.

- The patient can't benefit from methotrexate drug and this is one of drug resistance forms.

2- Gene Diminution:

- It is the reduction in gene size or number during maturation of cells as red blood cell development.

3- Gene Rearrangement:

- It is the recombination of different units of certain gene to react as if it is a new gene.
- This occurs in genes of antibodies.
- There are two chains of immunoglobulin (light and heavy) each one has its constant, variable and joining regions.
- Each region is formed of many subunits the recombination of these subunits leads to formation of thousand types of immunoglobulins.

4- Epigenetic Regulation :

- It is a regulatory mechanism of gene expression by reducing the DNA packing around the positively charged histones.
- There are different mechanisms to activate or inhibit transcription:

    - It is the binding of acetylated compounds to histones and this will reduce its positive charges resulting in decrease of DNA wrapping.
    - This will allow the transcription factors to bind DNA and increase in the gene expression.

I Activating transcription:

Histone Methylation;

- It is the binding of acetylated compounds to histones and this will reduce its positive charges resulting in decrease of DNA wrapping.
- This will allow the transcription factors to bind DNA and increase in the gene expression.

II Inhibiting transcription

Cytosine Methylation;

- It is a biochemical process where a methyl group is added to the cytosine in DNA nucleotides. DNA methylation typically occurs in a CpG dinucleotide, (*it is a nucleotide where cytosine and guanine are separated by only one phosphate*).
- DNA methylation is essential for normal development and it is impaired in carcinogenesis.

Second level: Regulation affecting the rate of transcription

- **1- DNA regulatory regions:**
  The regulatory regions for transcription include:
  A- basal regulatory element (they are present in any gene):
    - They include TATA box which direct the RNA polymerase enzyme to initiate transcription.
    - Also, CAAT box and GCGC boxes direct the frequency of transcription.

  B-Specific regulatory element:
    - They are specific sequences responsible for amplification of certain genes.They are present either before the target gene (upstream) or after

the target gene (downstream).

- Best example of specific regulation occurs for immunoglobulins.
- **Enhancers** increase The rate of immunoglobulin transcription .
- **Silencers** decrease The rate of immunoglobulin transcription**.**
- **Hormone responsive element:** Certain tissues regulation occurs in response to hormones.

Protein factors initiate transcription

- They are domains (*specific proteins with specific structure act as regulatory factors in transcription)*.
- They include:

1- Receptors of hormones (steroids and thyroids).

2- Ligand binding domain: they transfer hormones to the nucleus.

3- DNA binding domain: they bind DNA to hormone response element.

4- Transcription activation domain: they enhance transcription.

Third Level: Regulation of Post Transcription

It is the regulation of RNA processing:

1- Alternative splicing:

It results in formation of different types of proteins.

Example: There are 7 types of α-tropomyosin produced by this mechanism.

2- Regulation of DNA stability:

By capping and polyadenine tail of mRNA.the half life of RNA depend on the length of ply A tail.

Forth Level: Regulation Of Translation

Regulation of initiation of protein synthesis by phosphorylation :

1- Phosphorylation of initiation factor 2 (IF2):

prevents formation of 43 Spreinitiation complex and inhibits protein synthesis.

2- **Phosphorylation of IF4:** enhances the rate of initiation of protein synthesis.

Cancer genes

Unit (4)

Proto-oncogenes and Oncogenes

ILOs:
- By the end of this unit, the student will be able to :
- Define, enumerate and mention actions of oncogens and protoncogenes and tumor suppressor genes.
- Define and enumerate mutations with its different causes

Proto-oncogenes and Oncogenes

- **Oncogenes** are cancer producing genes.
- **Proto-oncogenes** are genes present as dormant genes and may be changed to oncogenes under certain conditions.
- **Mechanisms of conversion of protooncogenes to oncogenes**
  There are many mechanisms for proto-oncogene transformation.

1- Promoter insertion:
   Some viruses invade cells and integrated in the genome.

2- Enhancer insertion:
   Some viruses invade cells and inserted in the enhancer of the genome leading to its conversion to oncogene.

3- Mutations :
   It can affect the product of certain gene expression.

4- Chromosomal translocation :
   A piece of a chromosome may be separated and translocated to another gene. If this piece is under control of another regulatory element the result is huge units of the gene products and may end with cancer.
   Example: *In Chronic myeloid leukemia; part of chromosome 9 is translocated to chromosome number 22.there will be increased activity of tyrosine kinase enzyme and WBCs become*

cancerous (Philadephia Chromosome)

5- Gene Amplification:

The amount of proto-oncogene play role in cell transformation to oncogene.

- **Mechanism of action of oncogene:**
  - For the individual, but this is not the case with translocations.
  - A well-known example of a translocation is t(8;14)(q24;q32) found in Burkitt lymphoma.
- The MYC gene, which is an important and normally expressed transcription factor, is translocated to the region of the immunoglobulin heavy chain gene. Normally, MCY is activated by mitogenic signals that are part of the MAPK/extracellular signal–regulated kinase (ERK) pathway and has many functions that include cell proliferation, cell growth, and apoptosis.
- For most cells, MCY is expressed at low levels.
- In the new position, MYC comes under the control of a promoter for a highly expressed gene and is like- wise highly expressed as a result. Increasing MYC increases cell proliferation.

- **The first example of this was the discovery of the Philadelphia chromosome** (named for the city it was discovered).
  - This rearrangement is created by a translocation,. The ABL1 gene, which encodes a tyrosine kinase, is translocated into the BCR gene, thereby creating a gene, ABL1-BCR. Mutations in ABL1 are associated with chronic myelogenous leukemia. The two genes translocated together form a new chimeric protein that typically produces a 210-kDa protein and demonstrates an increased tyrosine kinase activity. Kinases transfer phosphates from ATP to proteins and activate the proteins.

- Amplification: Genes, as well as other sequences, can be amplified by redundant replication of DNA.
- The amplifications are often observed karyotypic abnormalities called double minute (DM) chromosomes.

- **Double minute chromosomes** are minichromosomes without centromeres and homogeneous staining regions (HSRs), which lack normally observed bands when stained.

- **Examples** are members of the ATP-binding cassette (ABC) gene family. These membrane proteins transport a wide variety of molecules from the cell including drugs. Overexpression of causes resistance to certain drugs used in therapies.

   1- They *may act as growth factor.*

   2- They *may act as DNA binding factors*

   3- They *may act as protein kinase* and activate cell proteins.

   4- They *may act as adenyl cyclase* and activate the cell.

   5- Alteration of telomerase activity

- **Tumor suppressor Genes**
  - They are genes produce proteins prevent development of cancer.
  - Any mutation of these genes (inactivation) result in carcinogenesis.
  - Tumor suppressor genes facilitate normal cell function in a non-mutated state. Their function is to suppress the formation of tumors.
  - Once the gene is mutated, this function is altered, allowing a tumor to develop.

  - **Counter to oncogene function, tumor suppressor genes demonstrate loss- of-function mutations that occur via impairment of both gene alleles.**
    - Four groups of genes are included among tumor suppressor genes: those that control the cell cycle, growth promotion,

DNA repair, and apoptosis.
- Mutations in any of these genes can lead to an abnormal cell cycle with unrepaired mutations and an inability of cell mechanisms to eliminate such cells.
- Mutations in these genes increase an individual's risk for cancer rather than directly cause the cancer progression.

- **Two-hit hypothesis:** The loss-of-function property of tumor suppressor genes is sometimes confusing because it requires a loss of function of both alleles of the gene, also termed the two-hit hypothesis. In this respect, it demonstrates recessive mendelian inheritance in which both alleles are mutated for the expression of an abnormal phenotype. The cell is able to function within normal limits with one functioning allele.

- Thus, a tumor is more likely to be expressed earlier when a mutated tumor suppressor allele is inherited than when it occurs spontaneously. An individual with an inherited tumor suppressor mutation may demonstrate what appears to be dominant mendelian inheritance even though both alleles are affected for expression to occur.

1- Apoptosis Gene:
- The gene mutations most frequently observed in cancer cells are those in the TP53 gene that alter the function of p53 in apoptosis.
- Genomic damage activates p53 in the cell cycle, and it is p53 that is
   responsible for initiating apoptosis. Two things can occur: p21 arrests the cell cycle so repair can occur, and GADD45 is expressed and promotes apoptosis if repair fails.
- If p53 is mutated, cells with altered DNA proliferate and cancers progress.
- In Li-Fraumeni syndrome, a p53 mutation is inherited, increasing the

individual's risk for different cancers within the same family.
- Mutation of p53 results in increased possibility of cancer.

2- Retinoblastoma:

- It is active when dephosphorylated.
- Phosphorylation of retinoblastoma gene leads to its inactivation and release of transcriptional factors will lead to RNA and protein synthesis.
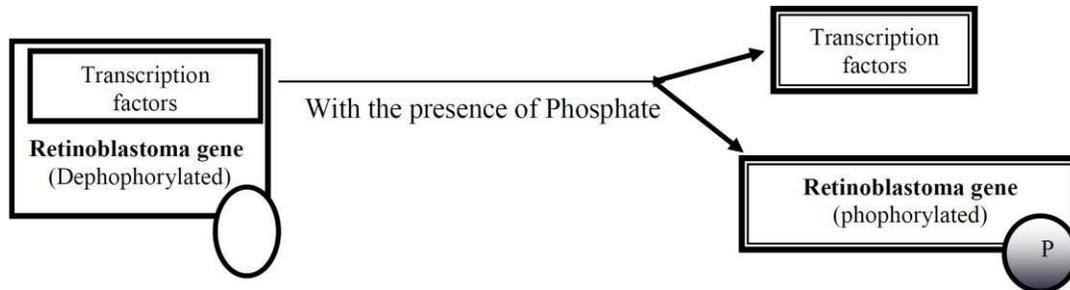
- Several types of mutations are frequently reported with retinoblastoma: sequence mutations, hyper-methylation of the promoter, and chromosome 13 deletions. The latter situation is associated with the term loss-of-heterozygosity, which is common with other tumor suppressor mutations.
- Two mutated alleles are required for the expression of retinoblastoma. If one allele is mutated and the other allele is deleted, electrophoretic analysis appears to demonstrate two mutated alleles with the same mutation.

3- Growth promotion genes:

- One of the earliest cancers investigated was familial adenomatous polyposis (FAP), which caused an increased risk for colon cancer in which hundreds to thousands of precancerous polyps preceded the development of colon cancer.
- Mutations were identified in the adenomatous polyposis coli (APC) gene and are now associated with other conditions that also manifest colonic polyposis including Gardner syndrome.

4-DNA repair genes:

- Cells have several mechanisms to repair DNA that is damaged during growth and proliferation. These are important because DNA is prone to errors during replication. When an error is not corrected, such as when an appropriate **glycosylase** does not replace an incorrect or damaged base in base excision repair, the corresponding protein may have a modified structure and function.
- Daughter cells will have the same error, and the accumulation of cells

  with errors can lead to apoptosis or to an aberrant cell line such as a tumor.

- ## Tumor Markers:

  - Some cancers produce specific proteins.
  - Elevation of these proteins in blood indicates the presence of cancer.
  - **Examples:**
    1. **Alpha feto protein:** Increased in cancer of liver.
    2. **Carcinoembryonic antigen:** It is high in cancer of colon, lungs and

                                    breast.
    3. **CA 15.3:** Breast cancer.
    4. **Prostate specific antigen:** Cancer prostate.

  - **Uses of tumor markers:**

    A- Screening for common cancers on a population basis. Example: elevated prostate specific antigen suggests prostate cancer.

    B- Monitoring of cancer survivors after treatment.

    C- Diagnosis of specific tumor types, particularly in certain brain tumors and other instances where biopsy is not feasible.

  - **Limitation of uses of tumor markers**

- Some people have a small amount of these markers in their blood.
- The levels of these markers tend to get higher than normal only when there's a large amount of cancer present.
- Some people with cancer never have high tumor marker levels.
- Because cancer is many different diseases, no single tumor marker can be used to look for all types of cancer.

Gene mutations

- **Definition:** Mutation is a permanent damage of DNA sequence or structure

- **Causes:**

1. **Error of DNA polymerase and replication** (a wrong base is added and not deleted or repaired by proofreading)

2. **Error in DNA recombination** (DNA is physologicaly rearranged as gene of immunoglobulin if not corrected this leads to mutation)

3. **Modification of bases:** It may be **due to:**

   A) **Chemical modification** the effect of different chemicals may be hazardous as:

      1) Nitrous acid produces oxidative deamination of bases converting adenine to hypoxanthine.

      2) Dimethyl sulfate.

   B) **Spontaneous modification** of bases (spontaneous deamination of cytosine will convert it to uracil) & (spontaneous depurination)

4. **Irradiation**: ultraviolet rays and ionization radiation as xrays lead to mutation and abnormal base pairing

- **Types of mutations**

   1) Point mutation: It is the replacement of a base by another. it is one of 2 forms

      1- Transitions: Replacement of a purine base with another purine or replacement of a pyrimidine with another pyrimidine

2- Transversions: Replacement of a purine with a pyrimidine or vice versa.

This leads to:

1- **Nonsense Mutations:** a codon is changed to stop cofon leads to premature stop of protein synthesis.

Example is thalasemia, where the formed Hb is shorter than normal heamoglobin or hemoglobin may be absent and leads to intrauterine fetal death.

Missense Mutations: codon a of certain amino acid is changed and another amino acid is added in the protein for a stop, **example**, **sickle-cell anemia** is caused by a (single) point mutation of amino acid,where valine replaces glutamic acid in 6th amino acid in β chain.

2- **Silent Mutations:** the changed code is fortunately a code for the same amino acid.

2) Insertions or deletions of bases:

This type of mutation produces one of the following

a- **Frame shift mutation**

There is a deletion or insertion of one or two bases which will alter the reading frame of the codon ending with abnormal protein.

b- **Deletion or insertion of three or multiple of three**

This leads to deletion or insertion of an amino acid to the protein formed.

Unit (5)
Recombinant DNA Technology

ILOs:
- By the end of this unit the student will be able to :
- Define recombinant DNA, importance and different techniques.
- Mention different vectors.
- Mention in details different molecular screening test with their importances.

Biomedical importance of recombinant DNA:

1. Study of different mutations

2. Diagnosis of genetic diseases

3. Help in preparation of proteins for treatment of
   diseases as interferon and insulin

4. Help in preparation of vaccines as Hepatitis B

5. Help in preparation of certain genes as Adenosine deaminase gene.

DNA Cloning

- It is a method for in vivo amplification of certain genes it is
  one of recombinant DNA molecule.
- A clone is identical host cells that carry identical recombinant DNA .

- **Steps of cloning:**

   1. Separation and preparation of certain gene (the target gene).

   2. Insertion of the gene into the carrier (the vector).

   3. In vivo multiplication of the vector in a host cell.

   4. Separation of the vector from the host cell.

   5. Separation of the gene from the vector .

   6. Separation and Preparation of a gene by one of the following:

   7. By using of the restriction endonuclease enzyme :it is

an enzyme that cuts DNA at specific sequence with its regulatory element.DNA fragment of the whole genome is named as genetic library. it is present in bacteria and protect it from viral infection: restrict virus. The bacteria are protected from the enzyme by methylation of its DNA. Restriction endonuclease cleaves both strands of DNA at specific sequence: palindrome (symmetrical inverted repeat). EcoRI was the first enzyme

isolated from bacteria Escherichia coli. The enzyme cut DNA in the form of either staggered cut with overlapping ends or blunt cuts with straight ends cut.

8. In case of staggered cut was cut by the same enzyme can form complementary base pairs and ligase enzyme join the cut ends to form a chimeric DNA (recombinant DNA).

9. Blunt DNA can be joined by ligase enzyme but with adding Poly A at one strand and Poly T at the other strand.

10. By using m RNA with reverse transcriptase enzyme: that converts RNA into DNA.it is complementary to RNA so it is (cDNA).it doesn't contain regulatory elements nor interons (m RNA reverse transcriptase cDNA)

11. by using DNA synthesizer with DNA fragment less than 200 bases. 12.Insertion of the gene in the vector

- Vectors

A vector is a DNA molecule to which target DNA is recombined and cloned.it has a specific nucleotide sequence that can be recognized by the restriction

endonuclease and capable to replicate in host cells.

- <mark>**Commonly used vectors:**</mark>

1. **Plasmids** small circular double stranded DNA molecule it carries genetics of antibiotic resistance.plasmids replicate independent of chromosomal DNA used for DNA segment less than 10KB in size.

2. **Viruses**: Retrovirus or adenovirus are commonly used after removing their pathogen{ (may be single stranded or double stranded) with a protein coat}.1-transfer the virus to target DNA,2- allow the target replication, 3-target cell lysis 4-Recombined DNA is released.

3. **Bacteriophage**: It is a virus that can infect the bacteria and cone DNA up to 20KB.

4. **Cosmids**: They are plasmids containing part of a phage,used for clone of large DNA up to 50KB .

5. In vivo multiplication of the vector in a host cell The plasmid is inserted in the host cell. Select the plasmid that carry on or more antibiotic resistance.and allow culture in the presence of antibiotic.

6. Separation of the vector from the host cell Separation occurs by lysis of the host cell.

7. Separation of the gene from the vector. The same restriction endonuclease is used to cut the amplified target gene DNA purification occurs by electrophoresis.

Molecular Screening and Testing

- Diagnosis in medicine has evolved from a science of observation and association to one of certainty with the advent of important technologies in the late 20th and early 21st centuries.

- The use of different molecular testing have improved diagnostics for physicians and patients.

A. Chromosome identification:

- To visualize variations in chromosome structure and number, chromosomes are condensed to the metaphase or pro-metaphase mitotic stage during the M phase of the cell cycle and then suspended in this condition by disruption of the microtubules necessary to segregate the chromatids. Techniques of chromosomal identification include:

- Simple staining to fluorescent labeling and whole chromosome painting.

- The choice of technique used for chromosome study is dependent on the information needed: chromosome number, banding anomalies, sex, and rearrangements. The needed outcomes of a study dictate the need for low- or high-resolution techniques.

B. Polymerase chain reaction:

- The most important technologic advances for the study of human disease and the molecular interpretations of diagnoses are those that facilitated rapid evaluation of nucleic acids.

- **Polymerase chain reaction** (PCR), **uses the concept of DNA replication**, which requires:

1- **Denaturation** through use of high temperatures).
   The denaturing of a double-stranded, antiparallel

DNA held together by hydrogen bonds. The bonds are broken by **heat.**

2- **Taq polymerase** is used for **Elongation**, adding the nucleotides for strand elongation. The process of denaturing, binding of primers, and extension with Taq polymerase can be done repetitively in a very short time.

With the completion of the genome sequencing project, primers can be synthesized for any region of interest in the genome. Sequences of DNA

can be amplified by PCR and used with a number of other procedures or viewed directly after electrophoretic separation.

- **Applications of PCR:**

  - PCR is a fabulous diagnostic tool. It is already widely used in **the detection of genetic diseases**. The amplification of all or part of a gene responsible for a genetic disease makes it possible to reveal the deleterious mutations (s), their positions, their sizes, and their natures.

  - **PCR can still be used to detect infectious diseases (viral, bacterial, parasitic, etc.**), as is already the case for AIDS, hepatitis C, or chlamydia infections. Although other diagnostic tools are effective at detecting these diseases, PCR has the enormous advantage of producing very reliable and rapid results from minute biological samples in which the presence of the pathogen is not always detectable with other techniques.

  - **PCR** has forensic applications as the coding sequences correspond to the genes and are therefore translated into proteins. The noncoding sequences are not translated, represent a large proportion of eukaryotic genomic DNA (up to 98%). The coding sequences are highly

homologous in individuals of the same species. Indeed, the species is characterized by characters and common traits that are guaranteed by its genes. This technique is important for **solving of disputed paternity.**

C. Sequencing:

- Nucleic acid sequencing provides the order of nucleotides in a region of interest for diagnostics. Automation of sequencing with the Sanger chain termination method decreased the time for sequencing and improved output.

- A combination of PCR to amplify DNA of a gene suspected in a patient presentation and sequencing can provide a definitive diagnosis of a specific disease and become informative for screening other family members **for presymptomatic or early disease detection.**

D. Single nucleotide polymorphisms:

- Analysis of the enormous amount of data resulting from sequencing the human genome brought the realization that single nucleotides might vary in a population with or, perhaps, without adverse consequence to the individual. Prior to this realization, it was known that single changes, or

6. point mutations, could cause disease.

7. - Different alleles were associated with disease and severity of disease. In the case of SNPs, defined as any single nucleotide change occurring in 1% or more of the population, it was recognized that some disease was not associated with genes. Studies revealed SNPs could occur within introns, exons, or outside of a gene regularly in a population. Additional studies demonstrated associations existed between particular SNPs and disease. Information about SNPs has become important in understanding processes such as response to particular drugs.

8. - **For example**, SNPs can identify high versus low responders to

a treatment or rapid versus slow metabolizers of a drug. SNPs are also being associatedwith some complex diseases with a multifactorial etiology such as diabetes mellitus and gestational diabetes. The future of SNPs is becoming the beginning for molecular personalized medicine. Commercial laboratories are performing DNA analyses for individuals to better understand their own DNA profile.