NUCLEUS

- ✓ Nu*cleus is a membrane bound organelle containing chromosomes and nucleolus.* It controls all the cellular activities. So it is referred to as the *controlling centre o*f the cell. It functions as the *heart* of a cell. The nucleus acts as the *brain o*f a cell. It is the *administrative office* of the cell.
- ✓ It was first discovered b*y Robert Brown* (1831) in piant cells. The study of nucleus is term*ed karyology*. Nucleus is the *largest organelle o*f the cell.
- ✓ The nucleus is present in all *eukaryotic cells*. However, it is absent from *RBC* of man and some *lens cells* of eye. The *sieve tubes* of plants also lack nucleus.
- ✓ In eukaryotes, the nucleus is surrounded by a *nuclear membrane*. But in prokaryotes. the nucleus is not surrounded by a nuclear membrane. Such a nucleus without a nuclear membrane is called a *nucleoid or incipient nucleus.*
- ✓ The nucleus occurs in two phases. They are *mitotic phas*e nucleus and in wu*cleus. The nucleus which is involved in cell division* is called *mitotic pha*se During interphase, the nucleus is involved in metabolic activities. This phase is also call *phase.*
- ✓ Generally, a cell contains only one nucleus. But sometimes two or more nucic.. Rased on the number of nucleus, the cells are classified into 4 types. They are.
  1. *Anucleate cell*
  2. *Mononucleate cell*
  3. *Binucleute cell*
  4. *Multinucleate cell*

❖ Anucleate cell: In *anucleare cells*, the nucleus is absent. E*g. RBC of ma.*

❖ Mononucleate cell: In *mononucleate cells,* a single nucleus is present. Eg I*mobu, alpical cell, e*tc.

❖ Binucleate cell: *In bimucleate cell, t*wo nuclei are present. Ofthese, one nucleus is small and called m*icronucleus* and the other nucleus is large and called *macronucleus*. Eg. *Paramecium.*

❖ Multinucleate cell: *Multinucleate cell c*ontains many nuclei. Eg. *Opalina.* The multinucleate animal cells are called sy*ncytial cells. (*Eg. *Epidermal cells of 1scaris*) and the multi-nucleate plant cells are called *coenocyte*s.

✓ The position of the nucleus in a cell is variable. Usually it is situated in the centre of the cell But in adipose cells and eggs rich in yolk, the nucleus is forced to lie on the periphery. In glandular cells and in *Icetabuluria,* it lies in the *basal region*. In some plant cells and epithelial cells, it is *peripheral.*

✓ The shape of the nucleus varies considerably. In most of the cells, it is *spherical i*n shape

✓ In cylindrical cells, it *is elliptical.* In human neutrophils, it *is trilobed. In* macronucleus is kidney shaped. The nucleus of spinning gland cells of inse*c*ts is In V*orticella, it is horse-shoe* shaped. In *Stentor*, it *is beaded.*

✓ The size ofthe nucleus is not constant. It varies from 11 to 25 um. The size of the nucleus is directly proportional to the volume of cytoplasm. The more the volume of the cytoplasm, the larger is the size of the nucleus.

✓ *R. Hertwig* has formulated a relationship between the nuclear volume and the cytoplasmic volume which is called the *nucleocytoplasmic index* (NP).

Structure of the Interphase Nucleus

Interphase is the period of the cell between two divisions. It is the longest phase. typical human cell, the interphase lasts for 89 hrs and the period of division lasts for 1 interphase cell is metabolically active. But it is the *resting phase* as it is not dividing. *The nucleus of the interphase cell* is called *interphase nucleus.*

The interphase nucleus is surrounded by an envelope called *nuclear membr* nuclear membrane encloses a granular semi solid substance called *nucleoplasm. It i*s *nuclear sap, nuclear matrix* or *karyolymph*. The nucleoplasm contains c*hromatin reticulum* and *nucleolus.*

❖ Nuclear Membrane

✓ The nucleus is separated from the cytoplasm by a semi permeable membranes *membrane*. It is also called *nuclear envelope o*r *karyotheca.*

✓ It is *double layered* and *lipoprotein* in nature as the plasma membrane.The outer layer is called *ecto karyotheca* and the inner layer is called *endokaryothica*. They are separated by *a perinuclear space* which is about 150 to300 A. Each layer is about *70 to 80*A thick.

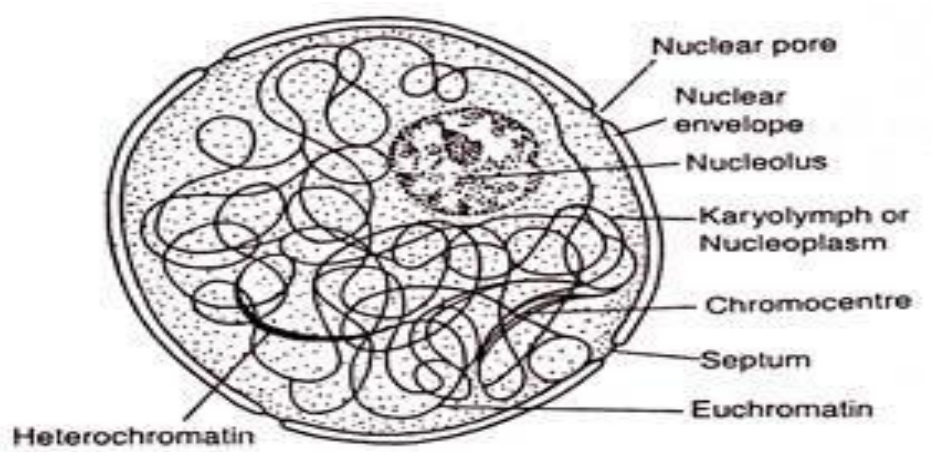


Fig. 8.1 Structure of a nucleus.

- ✓ The nuclear membrane has a *fluid mosaic s*tructure similar to plasina membrane
- ✓ The inner nuclear membrane is lined by a librous inalcrial called *oncleur lamina*. Ili composed of a filament protein calle *lamin,*
- ✓ The outer membrane is also surrounded by lamin, but they are not wellorganised like the nuclear lamina.
- ✓ The outer nuclear inembrane is beset with *ribosomes.* The outer membrane *c*ommunicate'. with endoplasmic reticulum at several points.
- ✓ The outer inembrane is often continuous with incombranes oftheriolyi, endoplasmic reticulum, mitochondrion and plasma membrane. The outer membrane is *roughow*ing to the presence of ribosomes, while the inner membrane is smooth.
- ✓ *The nuclear membrune contuins muny pore*s called *nuclear pores*.

❖ **Nucleoplasm**

The nucleus is filled with a homogenous, transparent acidophilic substance known as th*e nucleoplasm or nuclear sap* or *karyolymph.* There are one or more definite structures called *nucleoli. The chromatin threads* remain suspended in the nucleoplasm. In addition, there may be larger bodies which stain like chromatin threads and hence they are known as *chromatin nucleoli or false nucleoli.* The nuclear sap contains organic and inorganic substances like *nucleic acids, proteins, enzyme*s and *minerals.*

❖ **Chromatin Reticulum**

There are lightly stained thread-like bodies embedded in the nucleoplasm called the *chromonemata,* which form a network called the *chromatin reticulum.* They represent ***chromosomes***. ***Chro*me** in Greek means *colour* because they are coloured during staining.

The chromatin network readily stains with basic dyes. The chromatin net work is condensed to form thick ribbon-like bodies call*ed chromosome*s during cell division. At certain stages of cell division, the chromatin reticulum may show bead-like structures call*ed chromomeres*.

❖ **Nucleolus**

✓ *Fontona (*1874) discovered the presence of round oval bodies call*ed nucleoli* embedded in the nucleoplasm. Nucleoli are distinct in the interphase nucleus. They disappear at prophase, reinain indistinct during metaphase and anaphase and reappear only during telophase.

✓ Nucleoli occupy a fixed position. They are often associated with the nucleolar organizing portion of the chromosomes. The number of nucleoli varies from species to species. It depends on the number of chromosome sets. The size of the nucleoli is related to the synthetic activities of the cell. Under the light microscope, the nucleolus appears as a fluid or semi solid body of omogenous consistency. Under the electron microscope, it shows the following parts:

1. Granular Portion: It occurs at the periphery of the nucleolus. It consis granules of 150 to 200 Å diameter. It is composed of RNA and proteins.

2. Fibrillar Portion: It consists of many fibrils of 50 to 80 A long. These fibrila *nucleolonema,* formed ofribonucleo proteins.

3. Amorphous Portion: This portion has low electron density and it is found only nucleoli.

4. Nucleo**lus Asso**ciated Chromatin: It consists of fibrils of 100 Å thickne around the nucleolus extending into it. It contains DNA.

The important function of the nucleolus is the synthesis of ribosomal RNA and protein RNA produced inside the nucleolus passes first into nucleoplasm and from there it is passe the cytoplasm.

❖ **Chromocentres**

In certain cells, such as salivary gland cells o*f Drosophila* and S*ciara* one or more areas of nucleus stain very dark with basic dyes. Such areas are called *chromocentre*s. The chromocentres differ from the heterochromatin by their large size.

Chemistry of the Nucleus

Nucleus mainly consists of *nucleoproteins*. Besides, enzymes, inorganic salts and lipids occur in smaller amounts. The nucleoproteins are resolved into three groups.

1. *Basic protein*s

2. *Acidic proteins*
3. *Nucleic acids*

Nucleic acids are the most important constituents of the nucleus. They are of two types, namely *deoxyribo nucleic acid* and *ribonucleic acid.* DNA is present in chromatin net and RNA is present in the nucleolus and in small quantity in chromosomes. Lipids occur in the formof lipoprotein and phospholipids. It comprises about 3.1% of the total weight of the nucleus. Numerous enzymes have been observed. A few important enzymes present in the nucleus are nucleoside phosphorylase, ribonuclease, etc. The inorganic compounds usually found in the nucleus are salts of *calcium, iron* and z*in*c.

Functions of Nucleus

- ✓ **Metabolism**: Nucleus controls majority of the activities of cells. It is a regulatory organelle in cell metabolism.
- ✓ **Heredity:** Since the nucleus contains DNA molecules in its chromosomes, it plays a significant role in heredity.
- ✓ **Differentiation:** It controls cell differentiation during the embryonic development. The presence of nuclear enzymes such as DNA polymerase, DPN synthetase, etc. points to the fact that DNA replication and transcription (synthesis of RNA) occur mainly in the nucleus.
- ✓ **RNA Synthesis**: The synthesis of ribosomal RNA takes place in the nucleolus.
- ✓ **Exchange of Materials**: Nuclear membrane is concerned with the exchange of materials between the cytoplasm and nucleoplasm.
- ✓ **Support:** Nuclear membrane provides a surface for the attachment of structural elements of the cytoplasm such as microtubules and microfilaments.
- ✓ **Genetic code**: Nucleus contains the master plan for protein synthesis.

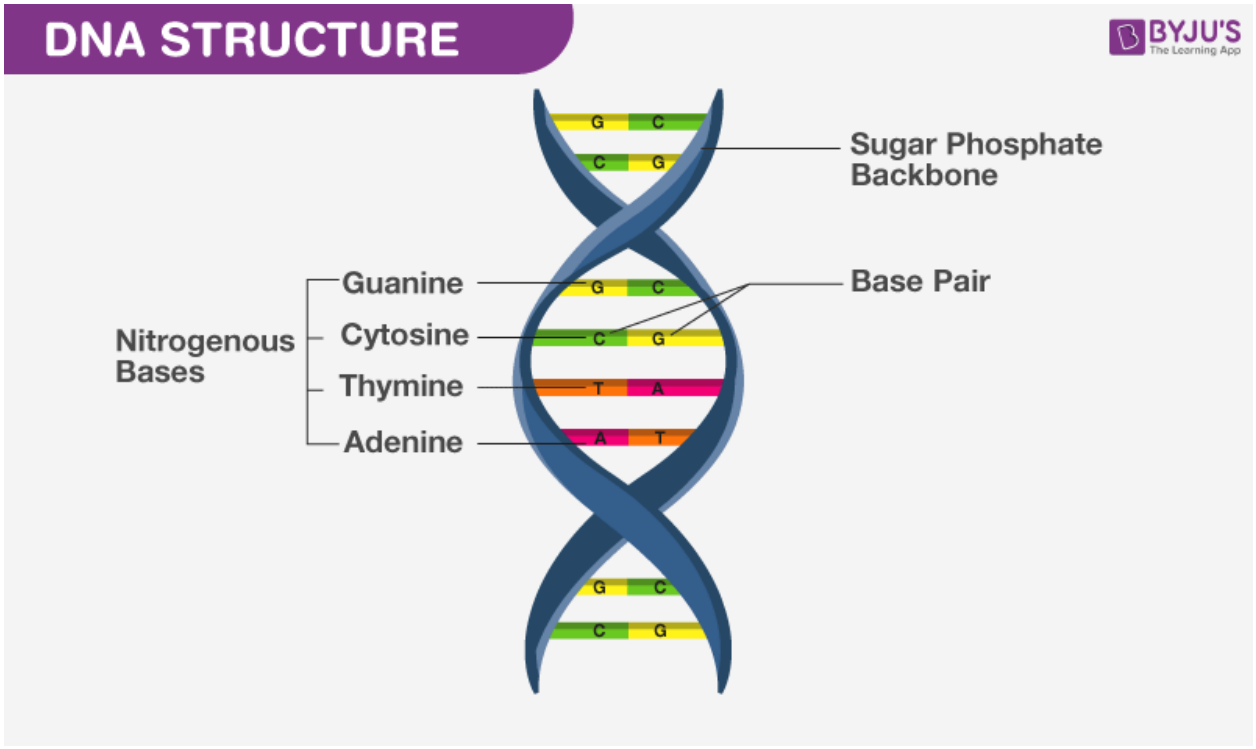
Deoxyribonucleic Acid (DNA)

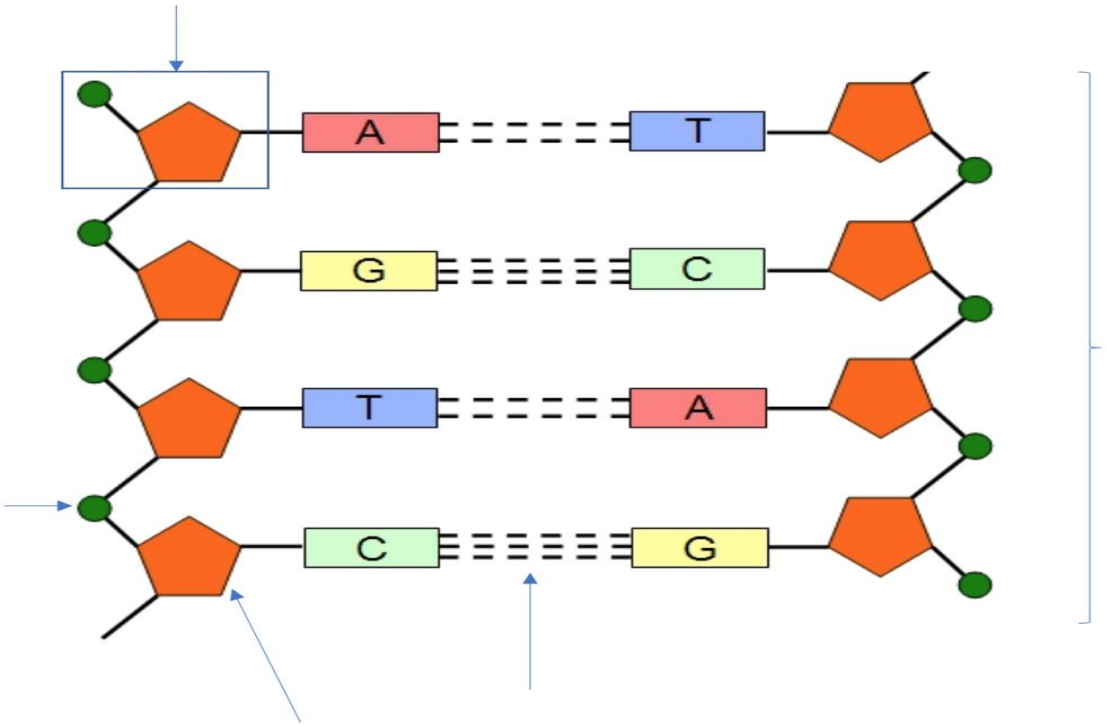
- ✓ *Deoxyribonucleic acid (DNA) is the molecule of heredity. It functions as the genes.*
- ✓ DNA is present in all cells except plant virus. In eukaryotic cells, DNA is present in the chromosomes of nucleus. In addition, the mitochondria and plastids also contain DNA.
- ✓ In *eukaryotic nucleu*s, the DNA is in the form of *a double helix.* In bacteria, mitochondria and plastids the DNA molecules are *circular.* In viruses and bacteriophages, they ar*e coiled.*
- ✓ The number of DNA molecules in eukaryotic cells corresponds to the number of chromosomes per cell.

Watson and Crick Model of DNA

(Structure of DNA)

- ✓ Watson and Crick in 1953 designed the structure of *D*NA. It is call**ed theWatson and** *Crick model of D*NA. They were awarded with *Nobel Prize* in 1962 for this work.
- ✓ According to Wats*on* and *Crick*, DNA is in the form of a *double helix.*
- ✓ ***D*NA is a *nucleic acid*. It is a *macromolecule*. It is made up of two chains. Each chain is a *polynucleotide chain*. Each po**lynucleotide **is made up of many small units called *nucleotid*es. Each nucleotide is made up of three chemical components, namely a phosphorie** *acid, a deoxyribose suga*r and a *nitrogen bas*e.
- ✓ The nitrogen bases ar*e adenine, guanine, thymine* and cy*tosine*.


DNA STRUCTURE
BYJU'S
The Learning App
Sugar Phosphate Backbone
Base Pair
Nitrogenous Bases
Guanine
Cytosine
Thymine
Adenine
G
C
C
G
T
A
A
T


A
T
G
C
T
A
C
G

The nucleotides of DNA are named according to the type of nitrogen bases present. As there are four types of nitrogen bases, DNA contains four types of nucleotides, namely

- ❖ AMP - Adenosine monophosphate (Adenylic acid)
- ❖ GMP - Guanosine monophosphate (Guanylic acid)
- ❖ TMP - Thymidine monophosphate (Thymidylicacid)
- ❖ CMP - Cytidine monophosphate (Cytidylic acid)
- ✓ In each nucleoside, C- I of pentose sugar is attached with nitrogen atom of the nitrogen base by a glycosidic bond. A pho acid molecule is linked with the sugar of nucleoside to form a nucleotide.
- ✓ Many nucleotides are linked together to form a *polynucleotide chain*. Two nucleotides are joined by *a phosphodiester bond.* It is formed between the sugar of one nucleotide and til phosphate component of another nucleotide.
- ✓ Each DNA molecule has two polynucleotide chains. The nucleotides of adjacent ch linked. Adenine is always linked with thymine (A-T). Similarly guanine of one chain is with cytosine *(G-C).*
- ✓ The linking between purines and pyrimidines is brought about by *hydrogen bond*s.cd are two hydrogen bonds between A and T(A=T) and 3 hydrogen bonds between a °C).
- ✓ The amount of adenine is equivalent to the amount of thymine and the amount of equivalent to the amount of cytosine.
- ✓ The two chains of *a D*NA are *complementar*y to each other. If the sequence of bas *chain* i*s A, G, A, T,* G, C, then the sequence of base in the sec*ond chain* is *T, C, T,* A, C,
- ✓ At one end of the polynucleotide chain, the 3 rd carbon of the sugar is free and it is not linked to any nucleotide. This end is called 3 *prime (*3) *end.* At

the other end, the 5 th carbon of the sugar is free and this end is called 5 *prime (5) end.*

- ✓ The 3'end of one chain lies close to the 5'end of the other chain and never in the reverse condition. Hence the two strands of a DNA are called *antiparallel.* One chain is upside down to the other.
- ✓ The DNA molecule is in the form of *a spiral stair case* (ladder).
- ✓ The DNA molecule is in the form of *a double helix.* The two polynucleotide chains are coiled around each other to form *a double helix.*
- ✓ The width (diameter) of the DNA helix is 208.
- ✓ The DNA helix has two external grooves, namely *major groove and minor groove.* The major groove is wide and deep.
- ✓ The minor groove is shallow and narrow. The distance between two nucleotides is 3.4 Å.
- ✓ *Watson* and *Crick m*odel explains the structure of B-DNA (right handed helix. But under physiological conditions, the DNA does not always exist in the B-form. Some regions are in the form of right handed helix and some other regions are in the form of left handed helix (A-DNA). This is the latest invention about the structure of DNA.
- ✓ The distance between two nucleotides is 3.4 Å.
- ✓ The DNA with right handed coiling is called *B DNA.*

Chemical Composition of DNA

DNA is made up of three chemical components, namely,

1. Sugar 2. Phosphoric acid and 3. Nitrogenous bases.

Sugar

- ✓ The sugar present in the DNA is called *deoxyribose.* It is a pentose sugar which five carbon atoms (CH, O.). It contains one O atom less than the ribose sugar Tu carbon of deoxyribose

bonds with *two hydrogen* atoms; but in ribose sugar, the seco atom bonds with one hydrogen atom and one hydroxyl group (OH).

Phosphoric Acid (H,PO.)

- ✓ Phosphoric acid links consecutive nucleotides by joining their pentose sugars with a phos*phate diest*er bond. This bond links carbon 5 in one nucleoside with carbon 3' in the next nucleoside.

Nitrogenous Bases

- ✓ These are N. containing organic compounds. They are of two types, name *pyrimidines*
- ✓ Purines: Purines are two-ringed N. compounds. They include *adenin*e an Pyrimidines: These are single ringed N. compound**s.** They include thymine and cytosine

Properties of DNA

- ✓ The Size of DNA molecule: The size of DNA molecule varies from organism to organism. It depends upon the size of the chromosome found in each living cell. The size basically depends upon the number of nucleotides present in each DNA molecule. The size of DNA molecule ranges from 0.7 mm to 40mm (4 cms).
- ✓ Fragility of DNA molecule: The DNA molecule is highly fragile. The fragility of DNA molecule is determined by its length. Larger molecules break easily. But smaller molecules are not susceptible to breakage.
- ✓ The DNA molecule is highly susceptible to breakage during handling operations, such as mixing, pipetting, pouring, etc. So the smaller molecules with less than 2 x 10 daltons molecular weight are isolated without damage. The large-sized DNAs (above 2 x 10 daltons) undergo breakage during their extraction.

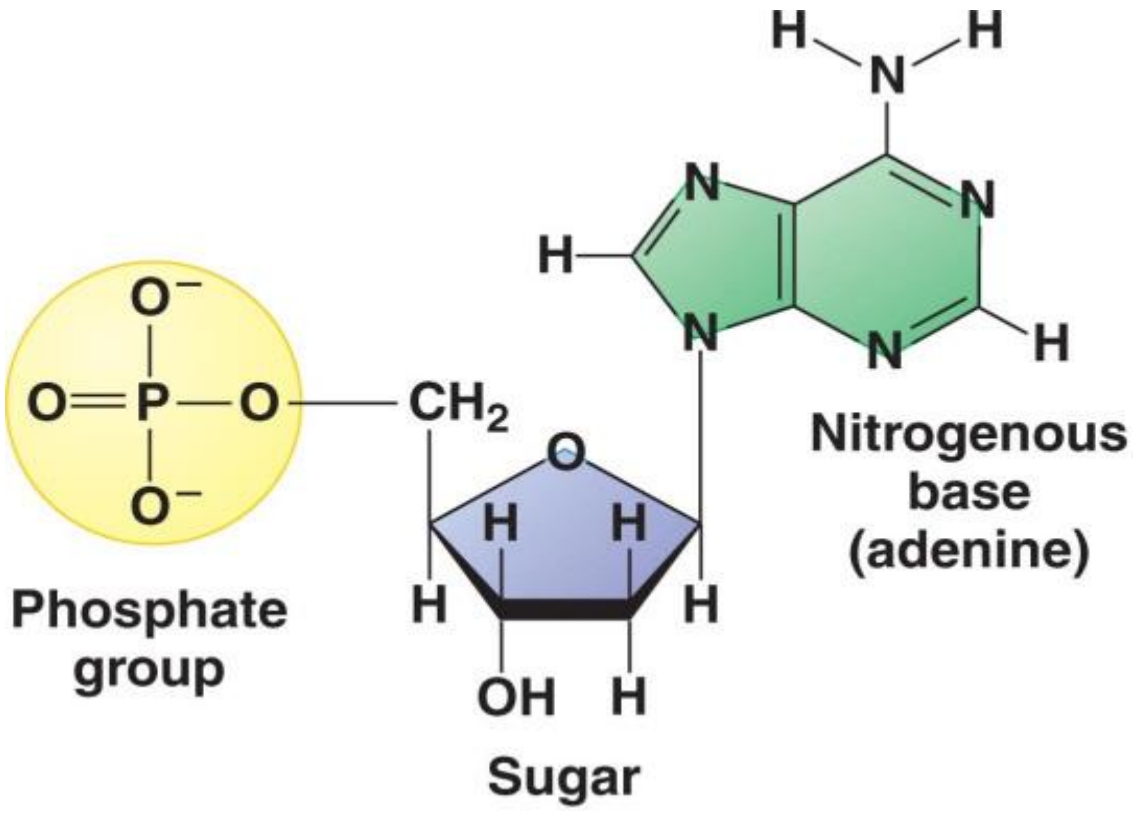
- ✓ Denaturation: Denaturation refers to the separation of the two strands of a DNA. Denaturation is brought about by high temperature, acid or alkali.
- ✓ Denaturation is brought about by the breakdown of hydrogen bonds between base pairs. Since G-C base pairs have 3 hydrogen bonds and A-T pairs have only 2 hydrogen bonds, the region of DNA containing G-C base pairs is more stable and it requires high temperature or pH for denaturation
- ✓ Denaturation begins in the regions rich in A-T base pairs and progressively extends to the regions of increasing G-C content.
- ✓ **Renaturat**ion: The denatured single stranded DNA can **be made into double stranded** UNA by cooling or by neutralizing the medium. This process is called *renaturation.*
- ✓ Effect of pH on DNA: The DNA is stable around the neutral pH in the solution. Further incred ease in PH (alkali treatment) causes strand separation and finally denaturation occurs above pH 11.3.
- ✓ Stability: The DNA is a highly stable molecule. The stability is due to two forces: a. Hydrogen bonding between the bases. b. Hydrophobic interactions between bases.
- ✓ Hyperchromic Effect: DNA molecule absorbs light energy. This is a property of individual bases The intact DNA absorbs les ct DNA absorbs less light energy as its bases are packed into a double helix.
- ✓ A denatured DNA molecule absorbs more light as its bases in single strands are The increase in the absorption of light occurs eventhough the amount of DNA remains This phenomenon of increased light absorption is calle*d hyperchromic effect.*
- ✓ A single stranded DNA does not show the hyperchromic effect. This phenom nyperchromic effect can be used to distinguish single or double stranded DNAs in the sample.

Functions of DNA

- ✓ DNA plays an important role in all biosynthetic and hereditary functions of all living
- ✓ DNA acts as the carrier of genetic information from generation to generation is very stable macromolecule in almost all living organisms and it is immortal.
- ✓ DNA controls all developmental processes of an organism and all life activities
- ✓ DNA synthesizes RNAs.
- ✓ DNA has the genetic information for protein synthesis.

Nucleotides

- ✓ Nucleotides are defined as phosphoric acid esters of nucleosides.
- ✓ A nucleotide is made up of three components, namely *a nitrogen base, a pento*se su and a *phosphoric acid.*
- ✓ The pentose sugar may be a *ribose sugar or a deoxyribose sugar.* Accordingly, the nucleotides are grouped into two types, namely *ribonucleotide and deoxyribonucleotide*.
- ✓ The nitrogen base may be *a purine or pyrimidine.* The purines are two types, namely *adenine* and *guanine.* The pyrimidines are of three types, namely *thymine*, *cytosine* and *uracil.*
- ✓ The nucleotides are named according to the purines and pyrimidines. They are the following
- ✓ Adenylic acid or Adenosine monophosphate (AMP)
- ✓ Guanylic acid or Guanosine monophosphate (GMP)
- ✓ Thymidylic acid or Thymidine monophosphate (TMP)

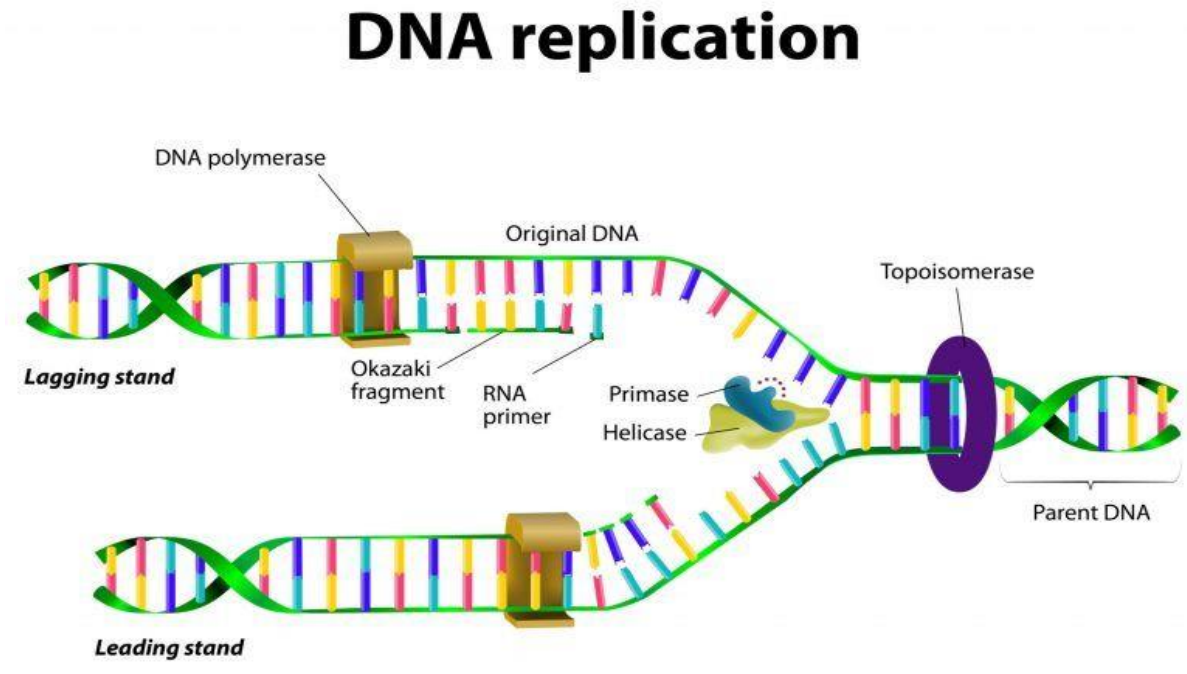


Biological Significance of Nucleotides

- ✓ Components of Nucleic acid: Nucleotides form the main component of nucleic acids.
- ✓ Genetic material: Deoxyribonucleotides of DNA function as the genetic material. They transmit hereditary characters from parents to offspring.
- ✓ Source of High energy: Nucleotides functions as the source of high energy. Eg. *ATP*, UTP, *CTP*, etc.
- ✓ **Oxidative phosphorylatio**n: ATP is involved in oxi**dative phosphorylation.** 5. Coenzymes: Certain nucleotides function as coenzymes. Eg. *UDP*G, **CoA, FMN, FAD,**
- ✓ 6Vitamins: Certain nucleotides function as vitamin-B. Eg. *FMN*, *FAD, NAD,* etc.

Replication of DNA

- *Replication is the duplication process by which a DNA molecule produces exact copies of its own structure.*
- Replication is the *copying of* DNA. DNA has the ability for *self duplication.*
- DNA replication occurs i*n all living organisms. Eg. Prokaryotes, Eukaryotes, Viruses,* Pla*nts, Animals, Bacteria, Mitochondrial DNA, Chloroplast DNA, Plasmids, etc.*
- The mechanism of replication is same from bacteria to man.
- The machinery of replication is working millions of years without much change during Evolution.
- Replication occurs both in *single stranded DNA* and *double stranded DNA* In replication, the parent DNA strands function as *templates.* The newly synthesized strand is *complementary* to the parental strand.
- The DNA replication is a *semi conservative proces*s because, ofthe two strands pro one strand is the parental strand and the second strand is newly synthesized.



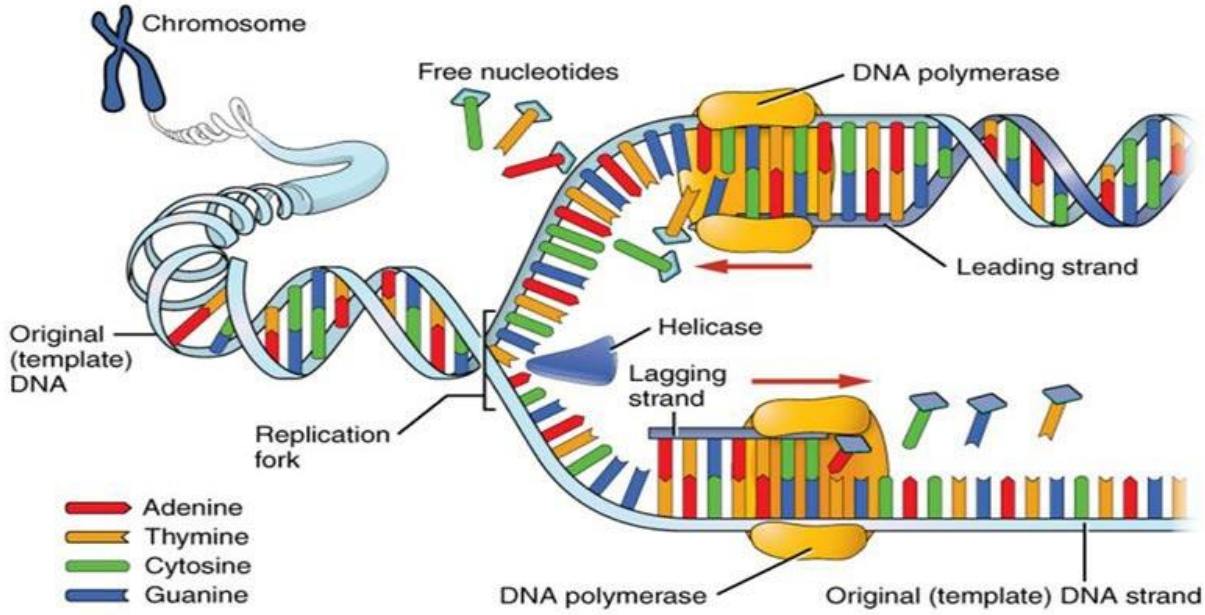
- The replication is *semi discontinuou*s because in one strand the DNA is synthesi short fragments and in the other strand it is synthesized continuously.
- Replication i*s bidirectional* because it occurs in opposite directions in the two stran Replication involves three steps, namely.
- *Initiation*
- *Elongation*
- *Termination*
- During replication *deoxyribonucleotides* are added one by one resulting in the formation of a *polynucleotide chain.*
- The formation of polynucleotide chain by the addition of nucleotides is called *polymeriz*ation The addition of nucleotides is catalyzed by an enzyme *DNA polymerase*. The synthesis occurs always from the 5' to the *3'direction.*

DNA Replication in Prokaryotes

- *Replication is the duplication of DNA.*
- Among Prokaryotes, DNA replication is clearly studied in *E. coli*.
- DNA replication occurs in the bacteria*l chromosomes.*
- DNA has the ability for *self duplication.*
- The DNA replication is a *semi conservative* process.
- The replication i*s bidirectional*.
- It is *semi discontinuous.*
- The synthesis occurs always from the 5to the *3 direction.*

- In replication, the parent strands function as templates. The newly synthesized strand is complementary to the parental strand.
- During replication, deoxyribonucleotides are added one by one resulting in the formation leotide chain. This process is called polymerisation.
- The addition of nucleotides is catalysed by an enzyme called DNA polymerase III.
- In E. coli , 1500 nucleotides are added per second. This speed can be compared to many times, the rotation of an electric fan.
- E.coli takes 40 minutes to complete DNA replication. Replication involves 3 steps namely.
- Initiation - Separation and unwinding of DNA strands
- Elongation – Linking of nucleotides
- Termination – Stopping of replication.
- E.coli has a circular DNA hence it exhibits circular model of DNA replication.
- In E.coli, replication starts from a single point called origin (Ori)
- The enzyme helicase binds to the site of origin. It breaks the hydrogen bonds between the two DNA strands.
- As a result the two DNA strands open and separate at the origin.
- The enzyme helicase unwinds the DNA strands.
- The separation of DNA strands produces an "eye' like structure on the **origin called replication fork.**The replication fork *is (theta)*shaped.
- The *replication fork m*oves on both directions from the origin.

- *Single stranded binding proteins* (SSBP) bind to the separated DNA strands. They hold the DNA strands and prevent them from folding.



- The unwinding creates twisting on the double helix.
- The enzyme *DNA gyras*e binds to the double helix near the replication fork. It removes the *twist*s caused by the unwinding action of helicase in the following ways.
- Creating twists called *super coiling* in the DNA much like that in a rubber band.
- *Catenation*- make knots.
- *Decatenation*-*r*emove knots
- *Cutting o*f DNA strands
- *Rejoining* of DNA strands.
- *Single stranded binding proteins (*SS*BP*) bind to the separated DNA strands. They prevent folding
- At the point of separation, a *replicating fork* is formed. The for**k appears in the form** *of a 0 (theta).*

- The separated DNA strands act as *templates.*
- DNA synthesis requires a *RNA primer.* The primer is a short RNA *polynucleo chain.*
- **The primer RNA is sy**nthesized by the DNA template close to the origin of replicati is synthesized b*y RNA polymerase.*
- **Synthesis of the new DN**A strand takes place by the addition of DNA nucleotides to ribonucleotide of the RNA primer. This is catalyzed by the enzyme *DNA polymerase*
- **Addition of nucleotides le**ads to the elongation of the primer nucleotide in the direction.
- **On one parent DNA stra**nd, the daughter strand is synthesized as a continuous su This strand is call*ed leading strand* because it is synthesized first.
- Each Okazaki fragment starts with an RN*A primer.*
- ter the RNA primers are removed by the enzym**e *polymera*s*el*.**
- The enzyme DN*A liga*se joins the Okazaki fragments into a continuous polynucleotide
- Replication occurs in both directions from the point of origin.
- The newly formed chain is exactly *complementar*y to the template chain.
- The two separated DNA strands function as *templates.* One strand is 5' → 3' and the other strand is 3'+5'.
- The DNA synthesis always occurs in the 5' → *3' direction.*
- So the synthesis is in the *opposite directions* in the two strands.
- The replication stops when the replication fork of the two sides meet at a site called *terminu*s (*ter).* Terminus is situated exactly opposite to origin.

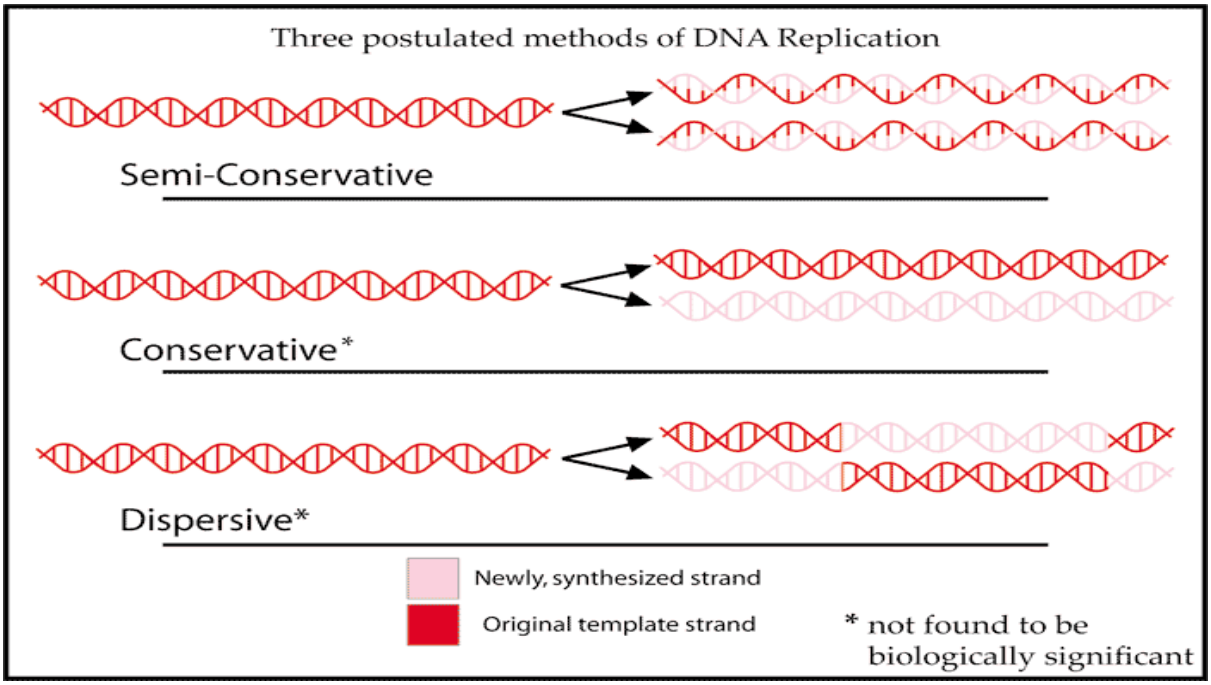
Types of DNA Replication

There are three types of DNA replication. They are following:

- ❖ Semi conservative method
- ❖ Conservative method
- ❖ Dispersive method

Semi ConservativeMethod

- ➢ **This method of DNA replication was pr**oposed b*y Watson and Crick*. In this method, one d of the DNA molecule serves as a template for the synthesis of a new complementary **chain.**
- ➢ **So** during replication one strand ofthe parent molecule is incorporated in the **molecule and anot**her strand is assembled a new. As one strand ofthe parent molecule: in the daughter molecule, this method is called s*emi conservative method*. This universally accepted and is experimentally supported by Meselson and Stahl.



Conservative Method

- This method was proposed by *Cavalieri an*d *Rosenberg.* According to this method, the DNA strands do not separate. The two strands act as a template and produce a new daughter double strand. Thus of the two double helixes formed, one would be entirely ofold material and the other double helix would be entirely of new material.

Dispersive Method

- According to this method, the parent DNA molecule breaks down into small p Each piece synthesizes a small DNA molecule. Then the daughter DNA molecule is asso by the linking of the old and new pieces at random. As the pieces of parent DNA molecule remain scattered in the daughter DNA molecule, this method is called dispersive method of replication.

PROTEIN SYNTHESIS

- The construction of protein molecules in the cell by sequentially arranging amino acids as per the genetic code sequence of mRNA is called protein synthesis. The amino acids are linked together to produce a linear polypeptide chain. The polypeptide chain is a unit of a protein molecule. Protein synthesis occurs inside the cytoplasm. Protein synthesis occurs inside the cytoplasm.
- The following cellular materials are needed to build a protein molecule:

- DNA (gene)
- RNA polymerase
- mRNA
- Aminoacyl synthetase

- tRNA
- Methionyl transferase
- rRNA
- Translocase
- Ribosomes
- Amino peptides
- Mg 2+ or Mn2+
- Protein factors
- Amino acids
- ATP GTP, UTP, CTP

MECHANISM OF PROTEIN SYNTHESIS

- The DNA is transcribed into an mRNA and the mRNA inturn is translated into a protein, This is the central dogma of protein synthesis (Crick, 1958). Most of the living beings except somne viruses follow this central dogma during protein synthesis.

  - **Transcription**
  - **Translation**
  - **Post-translational processing**

TRANSCRIPTION

- The formation of RNA complementary to a DNA strand is called transcription. In this process, the RNAs required for protein synthesis are synthesized on DNA strands. The three kinds of RNAs, namely mRNA, RNA and rRNA are synthesized on different regions of DNA.

- The enzyme, RNA Polymerase 1, II and III are involved in the synthesis of rRNA. mRNA and tRNA respectively in the cukaryotes. In prokaryotes, only one type of RNA polymerase is present to synthesize all the three classes of RNA.

- In the DNA double helix, one of the strands serves as a template to produce RNAs. On the template strand, a special region called promoter region is present. This region contains a set of nucleotide sequences initiating transcription. This region where the initiator sequences are present is called initiation site. In this region, the transcription is started by the RNA polymerase.

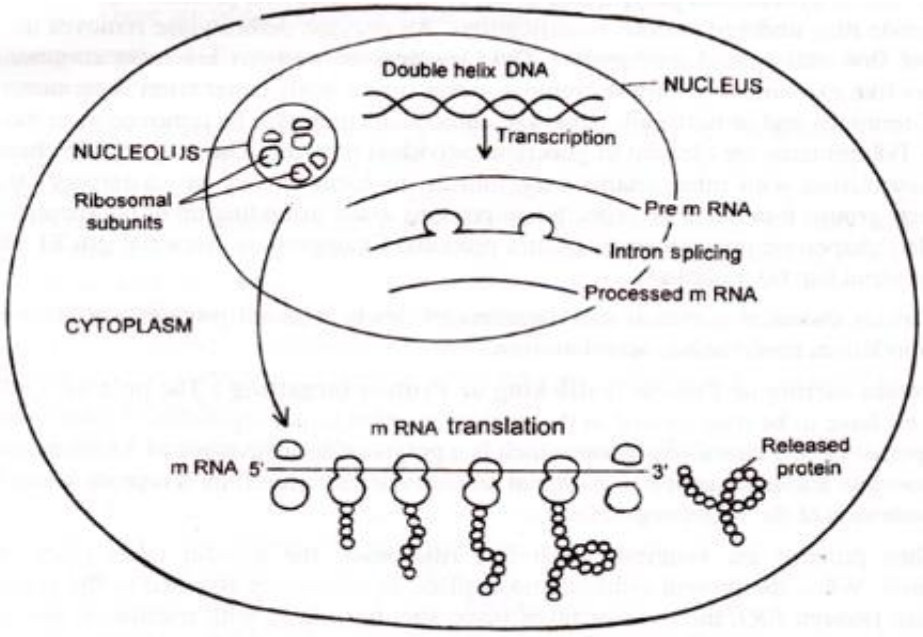


Fig. 12.11. Transcription, mRNA processing and mRNA translation.

- RNA polymerase attaches to the initiator site with the help of Mg2+.The RNA polymerase attachment makes a local opening in the double helix. From one of the unwound DNA strands, the polymerization of new RNA occurs. GTP or ATP forms the first

synthesized base. To this base ATP, CTP, GTP and UTP are added and the RNA elongates.

- The new RNA strand grows in the 5' to 3 ' direction as the enzyme moves along the DNA. The movement of the enzyme along the DNA is halted when an extended sequence of Poly A3' nucleotide (AAAAAAA) is encountered. This site of halt of the RNA elongation is called pause site. Protein factors like rho and SF help to terminate transcription.

- The RNA produced by transcription is inactive and is called pre-RNA or primary transcript or nascent RNA. It is active only after processing.

TRANSLATION

- Translation is a process by which the base sequence of mRNA is translated into amino acid sequence of a polypeptide chain. In short, translation is the synthesis of polypeptide chain.

- Translation involves the following steps:

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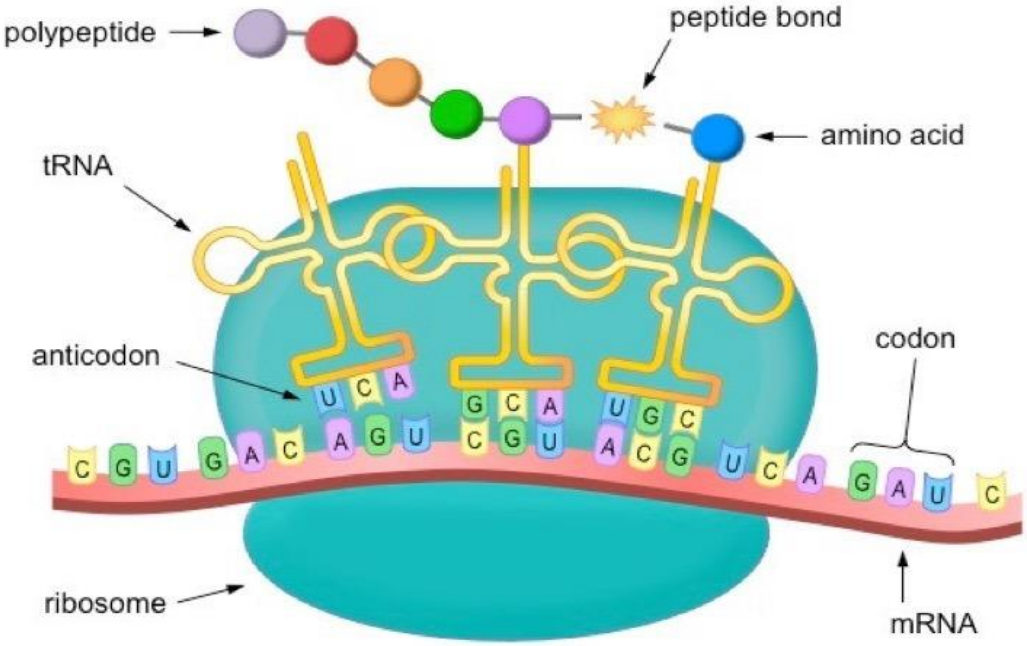
  - Activation of amino acid.
  - Attachment of activated amino acid with tRNA.
  - Initiation of polypeptide chain.
  - Elongation of polypeptide chain.
  - Termination of polypeptide chain.



Activation of Amino Acid :

- ❖ Amino acids, the building blocks of proteins, are present in the cytoplasm. The amino wids are activated by ATP with the help of the enzyme aminoacyl synthetase. Aminoacyl is specific in activating each amino acid.
- ❖ The activated amino acid is called aminoacyl adenylate or aminoacyl AMP. Attachme**nt of Activated Amino Acid with tRNA**

Attachment of activated amino acid with tRNA:

- ❖ The activated amino acid is attached to the acceptor arm oftRNA. The loading of an activated amino acid to the tRNA is called aminoacylation of tRNA. This reaction is catalyzed by an

enzyme called aminoacyl tRNA synthetase. The product formed is called aminoacyl RNA complex.

Initiation of Polypeptide Chain:

- Protein synthesis is initiated by the selection and transfer of the first amino acid to ribosome. This process requires ribosome sub units, amino acyl-tRNA complex, mRNA and initiation factors (IF). Initiation of polypeptide chain involves the following steps:

- The 30 S ribosome binds with the initiation factor-3 (IF-3) forming 30S-1F3 compler. The 30S - IF3 complex binds to mRNA forming 30S - IF3 - mRNA complex. The 1F3 helps the 30S to bind with mRNA. In the 30S - IF3 - mRNA complex, the mRNA is attached to the 30S ribosome in such a way that the first codon AUG ofmRNA is inserted in the Psite ofribosome. In prokaryotes, the first codon of mRNA will be AUG and it codes for the amino acid methionine.

Elongation of Polypeptide Chain :

- Elongation refers to addition of amino acids one by one to the first amino acid methionine, as per the sequence of codons in the mRNA. It involves the following steps:

- The second codon in the mRNA is recognized and the aminoacyl-tRNA containing the corresponding anticodon is activated by an elongation factor, EF Tu, It moves to the 705 ribosome and fits into the A-site. Here the anticodon oftRNA base pairs with the second codon of Mrna

- A peptide bond is formed between the first amino acid (methionine) of P-site and the second amino acid of A-site. The peptide bond links the two amino acids to form a dipeptide. The peptide bond synthesis is catalysed by the enzyme peptidyl transferase.

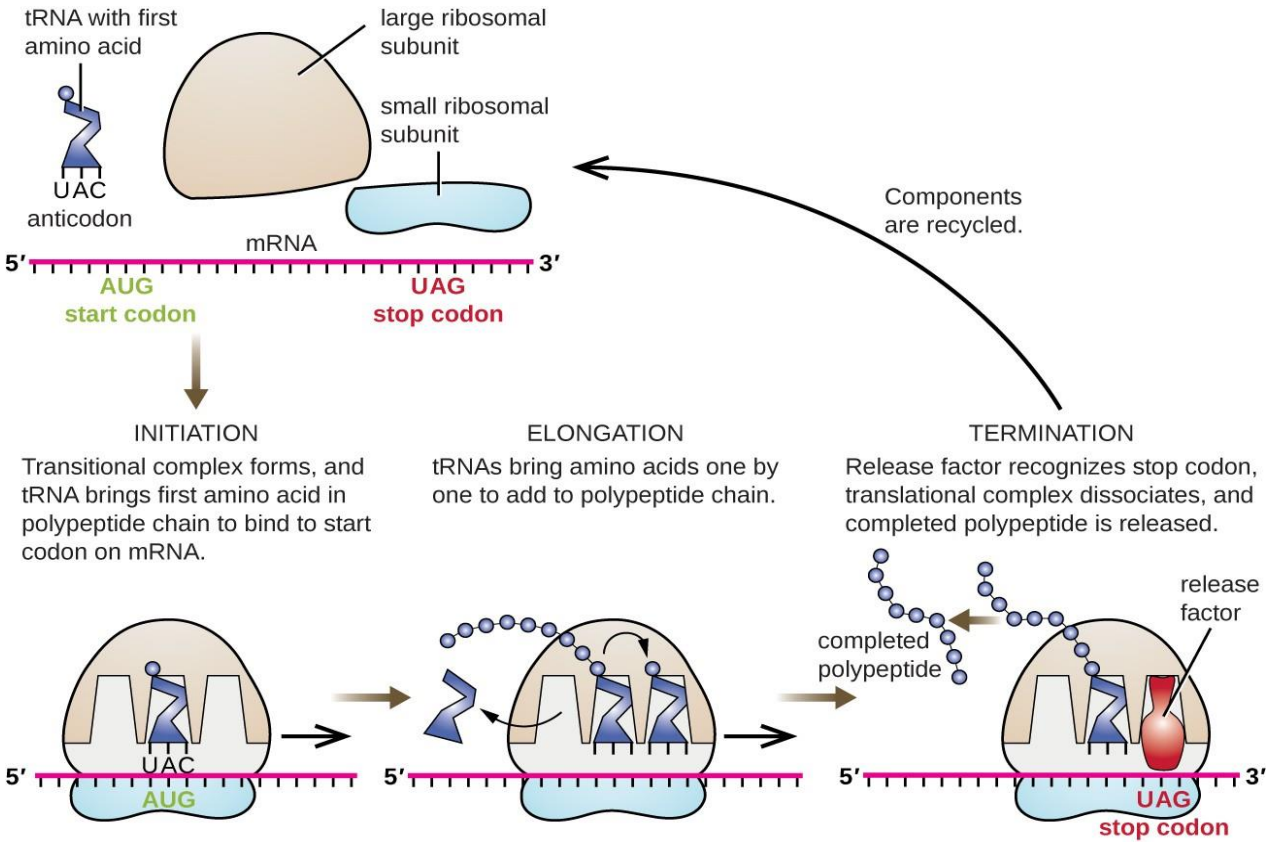
- After the formation of peptide bond, the methionine and tRNA are separated hun enzyme called tRNA deacylase. The process of separation of an amino acid from tRNA called deacylation.

- Now the tRNA at the A site has a dipeptide (two aminoacids with a peptide bond) Hence it is called a dipeptidyl tRNA.

- Now the ribosome moves on the mRNA in the 5'-3' direction for a distance ofone codon. This process is called translocation and it is catalysed by an enzyme called translocase.

- When the ribosome moves one codon away, the dipeptidyl tRNA is still attached to the second codon of mRNA. Hence the movement of ribosome shifts the dipeptidyl tRNA from the Asite to the P site.

- The deacylated tRNA (first tRNA) is shifted to the next site Exit site (E site). In arvotes, the *E-site is absent* and hencethe deacylated tRNA leaves the ribosome and enters



- The third codon is recognized and the aminoacyl tRNA containing the corresponding anticodon moves to the 70S ribosome and fits into the A site. The anticodon base pairs with the codon.
- A peptide bond is formed between the third amino acid of site A and the second amino acid of dipeptide present in the P site. Thus a tripeptide is formed.
- The second tRNA and the second amino acid are dissociated. The secondo shifted from P site into the E-site.

- The third aminoacyltRNA is shifted from A site to P site. The fourth codon occupies the A site and in this way the process is repeated.
- The amino acids are added one by one as per the codon in the mRNA and hence the tripeptide is converted into a *polypeptide chain.* The polypeptide chain elongates by the addition of more and more amino acids.
- As each ribosome synthesizes a polypeptide chain, each mRNA is used to synthesize many copies of polypeptide chains.

Termination of Polypeptide Chain:

- Termination is the completion of polypeptide chain. By termination, a polypeptide chain is finished and released. The polypeptide chain is completed when a stop codon comes to occupy the A-site.
- 2. The stop codon or termination codon may be UAA (amber) or UAG (ochre) or UGA (opal) codons.
- Termination is helped by releasing factors RF-1, RF-2 and RF-3. The exact mechanism in termination is still unknown. The terminated polypeptide chain is released from the ribosome.
- The polypeptide chain is released. The 70S ribosome dissociates into 30S and 50S. 5. After the release of polypeptide chain, the 70S unit dissociates into 50S and 30S subunits. These subunits are again used in the formation ofanother initiation complex.

POST TRANSLATION PROCESSING

- The polypeptide chain released after translation is inactive. It is processed to make it active. In the processing, the initiating amino acid methionine is removed along with methionine.

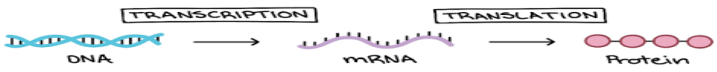
❖ In prokaryotes, v2 amino acids are added a few more amino acids are removed from N-terminal of the polypeptide chain. Then is carried out by deformylase and amino peptidase. **Rate of Protein Synthesis**

❖ Antibiotic drugs, other chemicals and toxins affect one or more steps involved in protein synthesis. Using the inhibitors, the details of DNA and RNA synthesis can be studiat The inhibitory impact of antibiotic drugs helps to kill the disease causing bacteria in manor in other animals.

❖ The important inhibitors of protein synthesis are streptomycin, neomycin, tetracycline erythromycin, chloramphenicol, puromycin, fusidic acid, abrin, ricin cycloheximide, etc.

CENTRAL DOGMA OF PROTEIN SYNTHESIS

❖ The general belief about protein synthesis that is considered to be the truth, is called central dogma of protein synthesis**.** The DNA contains the information for protein in the form of nucleotide sequences. This information is copied by mRNA. The mRNA synthesis from DNA is called transcription. The nucleotide sequence of mRNA determines the sequence of amino acids.



❖ Aminoacids are linked as per the sequence of nucleotides of mRNA is called translation. This is the most accepted fact and hence the central dogma of protein synthesis.