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CONCEPT TUTORIALS



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Ch-6

Genetic Material - The substance which stores biological information in a coded form, transfers it to the next gen. and causes its expression in the offspring is called genetic material.

Requirements of Genetic Material:-

1. It should be present in every cell
2. Its amount should be the same in all somatic cell
3. It should be able to duplicate itself, forming its carbon copies.
4. It should be able to pass its copies into the progeny.
5. It should be able to store information in the coded form.
6. It should be able to develop inheritable changes to allow adaptation and evolution.

Experimental Evidence for DNA's Role as Genetic Material:-

(1) Griffith's and Avery's Transformation Experiment

Griffith's Experiment:- He experimented with Streptococcus Pneumoniae. There are two different strains of this bacterium (1) a smooth, virulent strain (S-strain) (2) a rough, harmless strain (R-strain)

He injected live, rough bacteria (R-strain) into mice, the mice did not die.

He injected S-strain with heat killed, the mice did not die.

He injected S-strain live, the mice die.

Now, he injected live rough bacteria (R-strain)

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and heat killed smooth strain (S-strain) to mice. Now the mice die. In dead mice, he found strain of live smooth bacteria.

He concluded that there is some transforming agent, which transfers the killed smooth strain into live smooth strain.

Avery's Experiment :- This scientist extract separate the extract of smooth, virulent bacteria into protein, DNA and Carbohydrates fractions. Each fraction was separately added to a culture medium containing live rough bacteria. Only the culture that received the DNA fraction of the extract from virulent bacteria produced smooth bacteria.

This proved that DNA was the transforming agent.

Hershey and Chase Experiment

These scientists experimented on viruses that infect bacteria (bacteriophage). They performed their experiments with the bacterium E. coli and bacteriophage T₂.

First they grow T₂ with E. coli in the presence of radioactive sulphur ³⁵S. Protein contains T₂ bacteriophage has two things i.e. DNA and protein. Protein contains sulphur, and incorporated incorporated with radioactive sulphur.

Secondly they grow T₂ with E. coli in the presence of radioactive phosphorus ³²P. Now DNA contains phosphorus. DNA incorporated with radioactive phosphorus ³²P.

When T₂ attaches or infects bacteria E. coli, the radioactive phosphorus ³²P DNA enters into

E. coli, the radioactive protein remains outside. This shows that bacteriophage DNA enters the bacterial cell, not proteins.

Structure of DNA → In prokaryotic cells, the DNA is circular and occurs in cytoplasm whereas in eukaryotic cells, the DNA is linear and occurs in nucleus. It is also called nuclear DNA.

A small amount of DNA is present in mitochondria and plastids. This is called extra-nuclear or organelle DNA.

Quantity:- The content of DNA is constant in all the cells of a given species. Just before cell division, the amount of DNA is doubled, the daughter cells have half the amount of DNA, as they contain half the number of chromosomes.

Chemical Composition:-

DNA is the largest biomolecule and largest macromolecule. DNA is a long double chain of deoxyribonucleotide. The two deoxyribonucleotide chains are twisted around a common axis to form a right handed double helix.

The deoxyribonucleotide unit consists of three different molecules:-

1. Phosphate (PO_4)
2. 5-Carbon deoxyribose sugar ($\text{C}_5\text{H}_{10}\text{O}_5$)
3. Nitrogenous base.

Purines are Adenine (A) and Guanine (G)

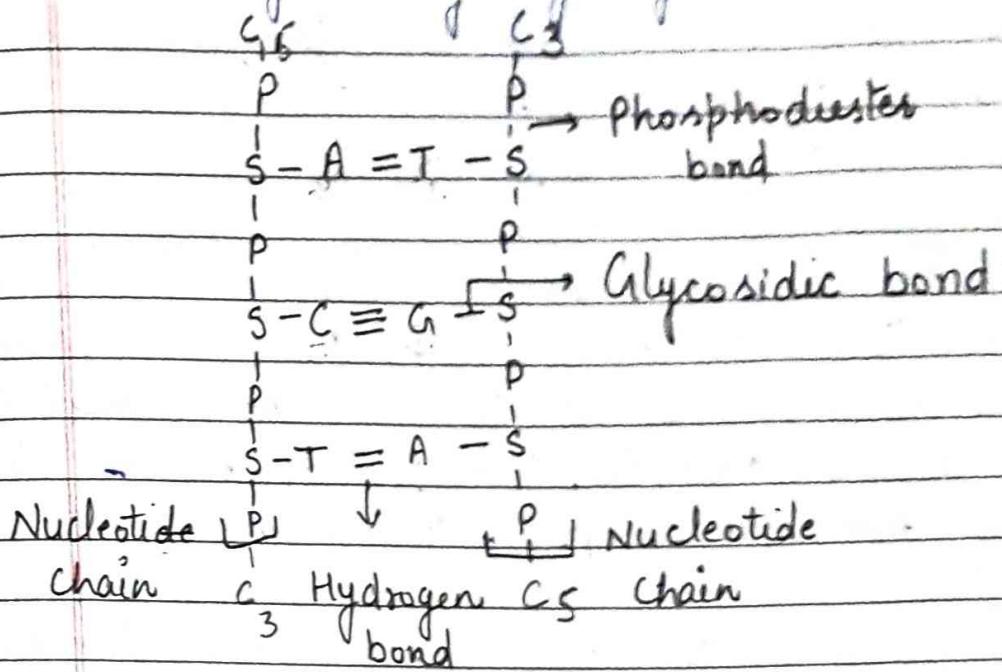
Pyrimidines are Thymine (T) and Cytosine (C)

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In each chain, the phosphate component of one nucleotide unit is joined to the sugar component of other nucleotide unit by a bond called phosphodiester bond.

These bonds form the backbone of the DNA double helix. The nitrogenous base molecules are joined to sugar molecules by glycosidic bonds.

The two deoxyribonucleotide chains are held together by hydrogen bonds.



Adenine of one chain is always joined to Thymine of other chain by 2 hydrogen bonds ($\text{A} = \text{T}$)

Cytosine of one chain is always joined to Guanine by 3 hydrogen bonds ($\text{C} \equiv \text{G}$)

Thus, there are only four possible base pairs

$$\text{A} = \text{T}, \text{T} = \text{A}, \text{C} = \text{G}, \text{G} \equiv \text{C}$$

Antiparallel Direction of DNA :- The two chains of DNA molecule run in opposite or antiparallel directions. Thus, two chains are parallel but their $5' \rightarrow 3'$ directions are opposite.

Chargaff's Rule for DNA :-

1. The purines and pyrimidines are always in equal amounts i.e. $A + G = T + C$
2. The amount of adenine is always equal to that of thymine and the amount of guanine is always equal to that of cytosine i.e. $A = T$ and $C = G$
3. The base ratio $A + T / G + C$ may vary from one species to another.
4. The deoxyribose sugar and phosphate component occurs in equal proportions.

Denaturation and Renaturation of DNA :-

If DNA molecule is exposed to a high temp. or treated with an acid or an alkali, the weak hydrogen bonds between $A = T$ and $C \equiv G$ will break and the two strands of DNA will break, and this process is known as Denaturation or melting.

When the denatured DNA is incubated at a low temp., the two separated strands reassociate to form a DNA duplex. This process is known as renaturation.

Absorbance :- An intact DNA molecule absorbs less light energy, as it is double helix and a denatured DNA or unwinding DNA absorbs more light as its base in a single strand are exposed.

Optical Rotation :- DNA is dextrorotatory i.e. it rotates the polarised light to the right.

Watson and Crick Model of DNA:-

According to Watson and Crick Model, the DNA molecule consists of two long, parallel chains, which are joined together by short crossbars at regular intervals.

The two chains are spirally coiled around a common axis and form a double helix. A double helix is a diameter 2nm (nanometer). Major groove is about 22 Å (Angstrom) and a minor groove is about 12 Å.

One complete spiral of helix is 3-4 nm long and has 10 base pairs. The gap between two base pairs is 3.4 Å . The sugar and phosphate components form backbone of DNA. The helix is right handed.

Watson and Crick also shows that DNA molecule can replicate itself and remains stable from generation to generation.

Sense and antisense strands :- The strand which exists or contains genetic information is called sense strand. The other complementary strand is called antisense or missense chain.

The antisense or missense chain plays important role in the replication of DNA.

Cistron :- A part of sense strand that codes for a polypeptide chain is called a cistron. Each cistron consists of many codons.

Histones :- In eukaryotes, DNA is associated with a set of positively charged basic proteins called histones. Histones are low molecular weight proteins rich in basic amino acids lysine and arginine.

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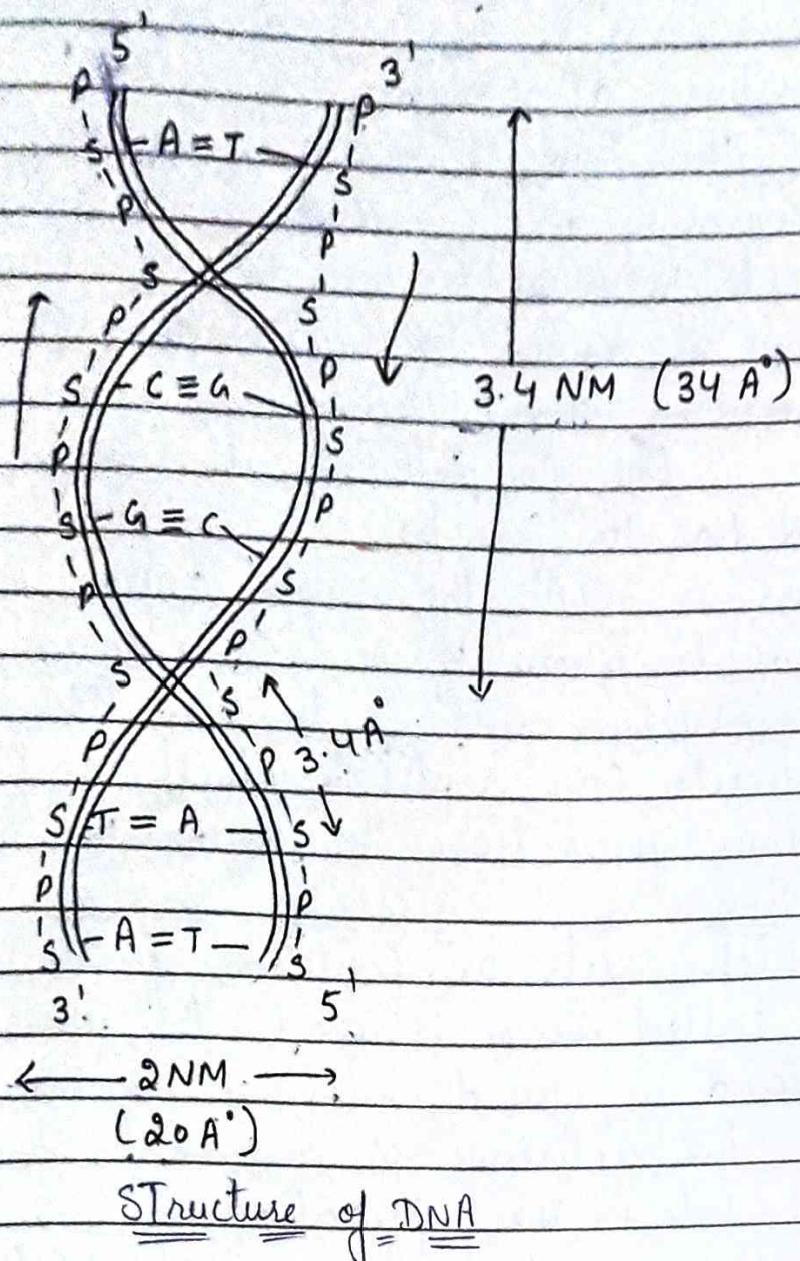
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The DNA and histones are loosely bound together in about 1:1 ratio to form deoxyribo nucleoprotein also called chromatin.



A positively charged R groups of these amino acids bind strongly to the negatively charged phosphate group of DNA.

The histones are organised to form a unit of eight molecules called histone octamer. Histone octamer consists of two molecules each of four different histones - H₂A, H₂B, H₃ and H₄.

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A molecule of fifth histone called H₁ attaches to the DNA near the bead. This complex structure consisting of negatively charged DNA wrapped around the positively charged histone octamer is called nucleosome.

Each nucleosome is connected to the next by a short DNA linker. A nucleosome and a linker are together referred to as a chromatosome.

Types of DNA :- (1) Linear and circular DNA :-

DNA molecules are of 2 types linear and circular. Linear DNA is found in eukaryotic cells. It is associated with proteins.

Circular DNA is found in prokaryotic cells. It is not associated with protein.

(2) Trophic and Genetic DNA :- The ciliates have separate trophic (macronucleus) and genetic (micronucleus). The macronucleus controls the vegetative functions and micronucleus controls the sexual reproduction.

(3) A, B, C, D and Z DNA :-

A contains 11 base pairs per turn of the helix.

B-DNA occurs under the physiological conditions in the living cells. It has the specifications given in the foregoing description of DNA.

C-DNA has 9 base pairs per turn of the helix.

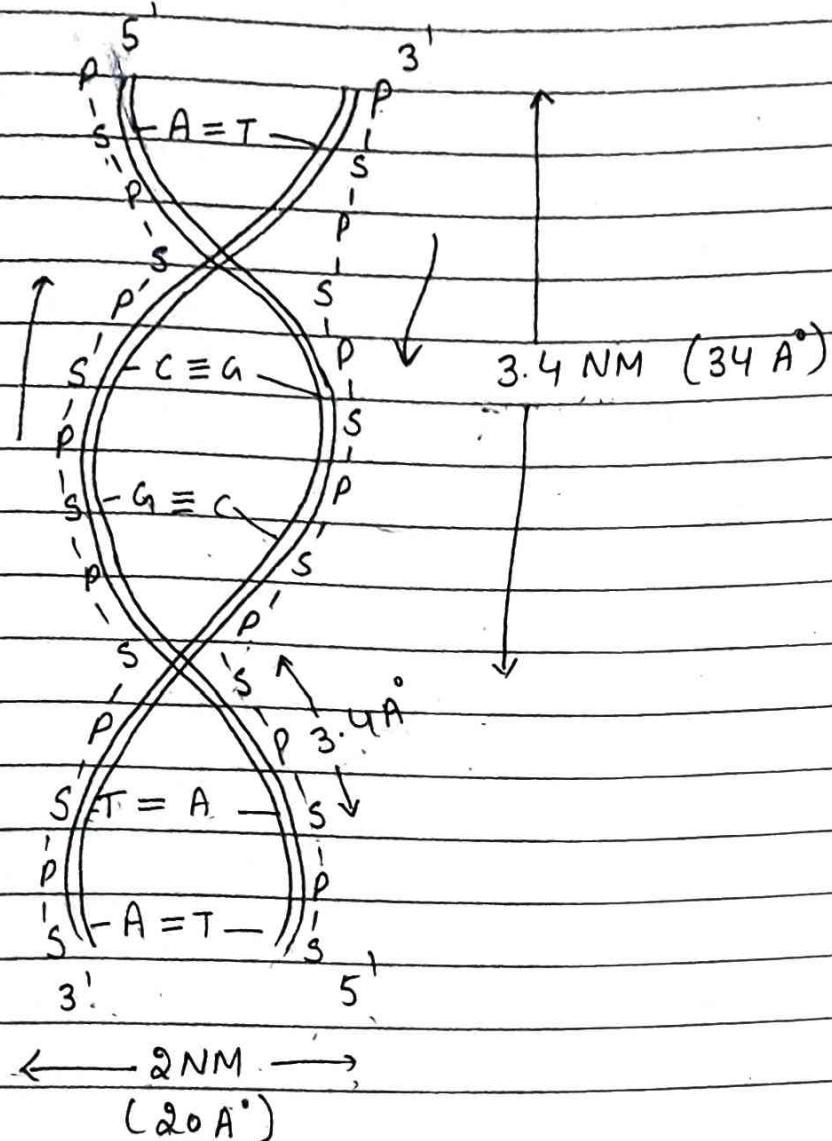
D-DNA has only 8 base pairs per turn of helix.

Z-DNA is left handed, 12 base pairs per turn of helix.

(4) Coding and non-Coding DNA :- (a) Non-Coding DNA :-

Greater part of DNA in eukaryotic cells does not code for RNAs. This is known as non-coding DNA. In non-coding DNA, many have

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Structure of DNA

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Sequences repeated several times. These repeated sequences collectively known as Repetitive DNA. Some repetitive DNA are not at fixed sites, these are known as jumping genes.

- (2) Coding DNA - The coding genes lie as islands in the sea of non functional DNA sequences. These are of two types -
 - (a) Protein Coding DNA
 - (b) DNA Coding for rRNA, t-RNA, and Histones.

Functions of DNA :-

1. It is the genetic material and carries hereditary characters from parents to young ones.
2. It enables the cell to maintain, grow and divide.
3. It produces RNAs by transcription for use in protein synthesis.
4. It contributes to the evolution of the organism by undergoing gene mutations.
5. It brings about differentiation of cells during development.

Replication and OR Synthesis of DNA

OR

DNA ACTS AS SEMI CONSERVATION MODE OF
REPLICATION :-

Meselson and Stahl Experiment : They grew E. coli bacteria in a medium containing the heavy nitrogen isotope N^{15} for many generations. New generation DNA was heavier than normal bacteria. Now, this new generation with heavy DNA grown in isotope

N. The first generation extracted and centrifuged. They were hybrid N^1-N^2 , i.e. all were half heavy. This experiment shows that DNA shows semiconservative mode of DNA replication.

Synthesis of DNA :- The unique process of making an identical copy of a double stranded DNA, using existing DNA as a template for the synthesis of new DNA strands is called DNA replication.

Mode of Replication :- DNA shows semiconservative mode of replication.

Procedure :-

(1) Activation of Deoxyribonucleotides :-

Four types of deoxyribonucleotides monophosphates i.e. AMP, GMP, CMP, TMP found floating free in the nuclear sap. They serve as raw material for DNA synthesis.

These deoxyribonucleotides remain inactive. Firstly they union with ATP and becomes active. Now they are ATP, GTP, CTP, TTP. The process of union of deoxyribonucleotides with ATP is called phosphorylation and the enzyme which helps is called phosphotransferase.

(2) Exposure of Parent DNA :- The parent DNA double helix uncoils and splits into single DNA strands by breakdown of hydrogen bonds.

Enzymes called helicases help in unwinding the helix. Other enzymes named SSB (Single stranded DNA binding protein) also called helix stabilizing protein and other enzymes named topoisomerases helps in uncoiling. The entire DNA chain does not split in

Q. The DNA strands starts separating at a specific point called origin of replication. Unzipping of the double stranded DNA forms a Y shaped structure called replication fork

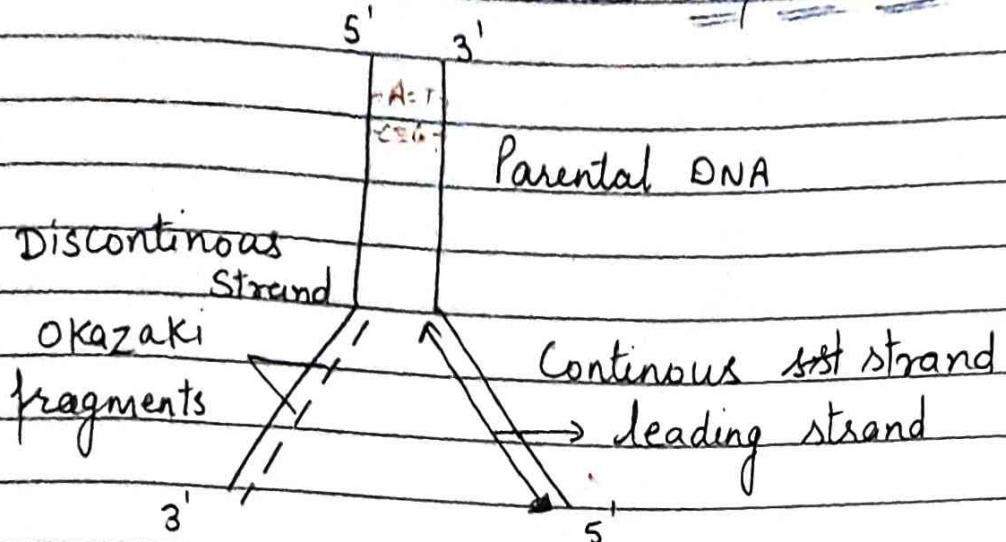


Diagram of replication fork

(3) Formation of RNA Primers :- A short chain of RNA is formed on the DNA template at the 5' end. This is called RNA Primers. The enzyme Primase catalyzes the polymerization of RNA building blocks (A, U, G, C) into the primers.

The RNA primer is formed because the enzyme DNA polymerase cannot initiate the synthesis of a new DNA strand.

The primers are later removed and the gaps, so left are filled with deoxyribonucleotides to make the DNA strand continuous.

(4) Base Pairing :- The deoxyribonucleoside triphosphate get joined by hydrogen bonds, according to Watson and Crick rule i.e A-T, T-A, C-G, G-C

(5) Conversion to deoxyribonucleoside Monophosphate

The deoxyribonucleotide triphosphates joined to each single DNA chain break off their inner high energy bonds and set free with the help of an enzyme pyrophosphatase.

(6) Formation of new DNA chains :- Now, there is formation of new DNA chains with the help of enzyme DNA polymerase and by metal ion Mn^{++} or Mg^{++} .

This produces two double chains, which are identical to each other as well as to the original mother chain.

Leading and Lagging strands :-

This new strand of DNA, that is formed continuous stretch in the 5'-3' direction is called leading strand.

On the other hand, other parent strand, short DNA segments are formed. These segments are called Okazaki segments. This discontinuous strand is called lagging strand. The short DNA segments are joined with the help of an enzyme called DNA ligase.

(7) Editing and DNA Repairing :- Sometimes, wrong bases do get in. These are removed by DNA polymerase.

The abnormal regions of DNA are cleaved by enzymes nucleases.

The DNA ligase joined the new and old

segments of the strand under repair. This makes DNA strand normal.

- (8) Helix formation:- Each daughter double DNA molecule becomes spirally coiled to form a double helix.

Structure and Role of RNA :-

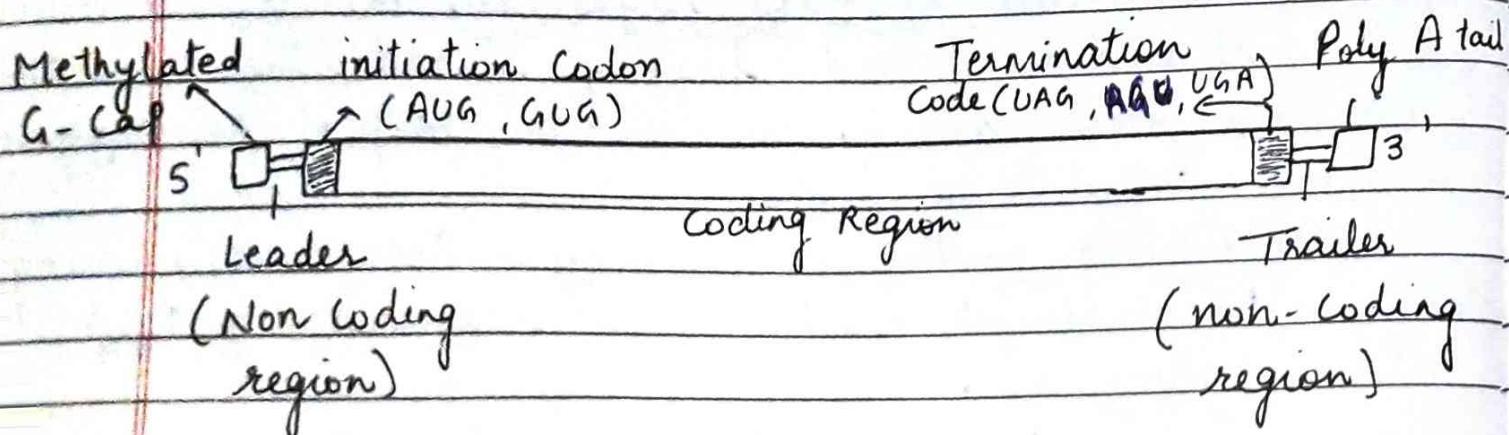
RNA molecule is a long, single stranded polymer of ribonucleotides. Each nucleotide unit is composed of three smaller molecules :-

- (1) a phosphate group
- (2) a 5-carbon ribose sugar
- (3) a nitrogen containing base.

Type: RNA is of three types

- (1) m-RNA:- The m-RNA carries the message (information) from DNA about the sequence of particular amino acids to be joined to form a polypeptide. It is also called informational RNA or template RNA.

Because of the variation in size in mRNA population in a cell, the m-RNA is also called heterogeneous nuclear RNA or hn RNA.



Structure of m-RNA Chain

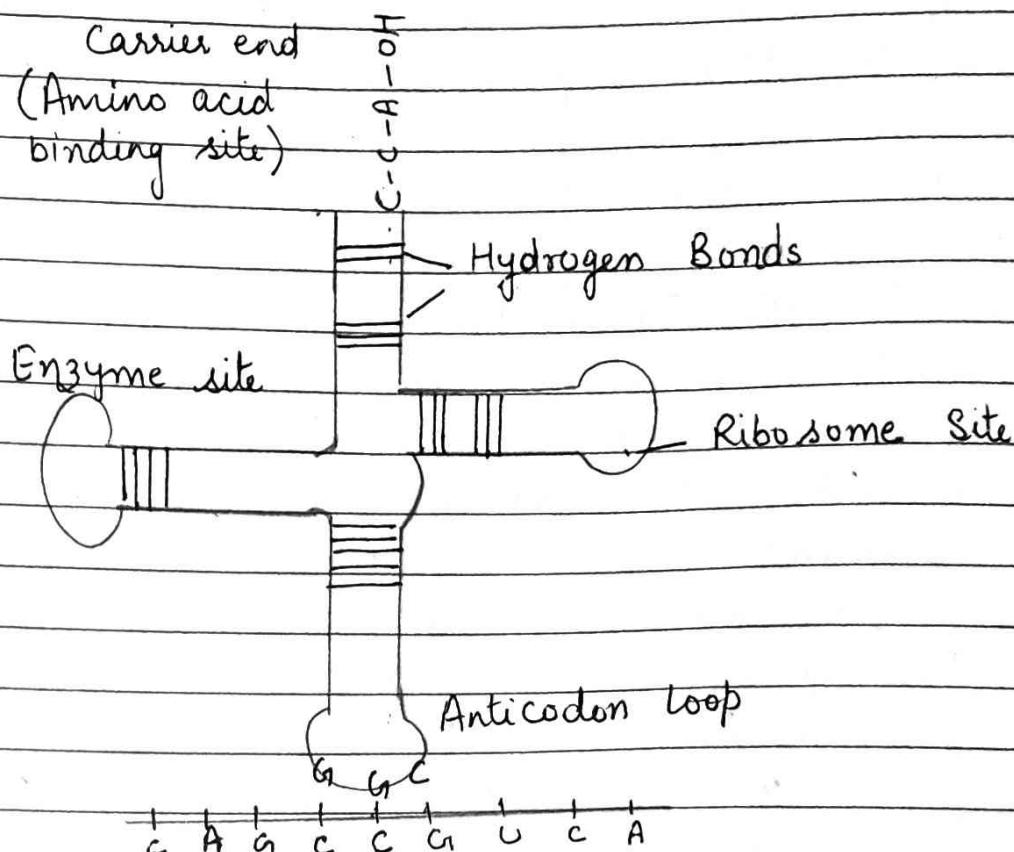
(2) t-RNA:- The tRNAs form about 15% of the total RNA of a cell. Its molecule is the smallest of all the RNA types. It has four regions:-

(a) Carries end → This is the 3' end of the molecule. It has a base triplet CCA with -OH at the tip. The -COOH of amino acid joins the -OH.

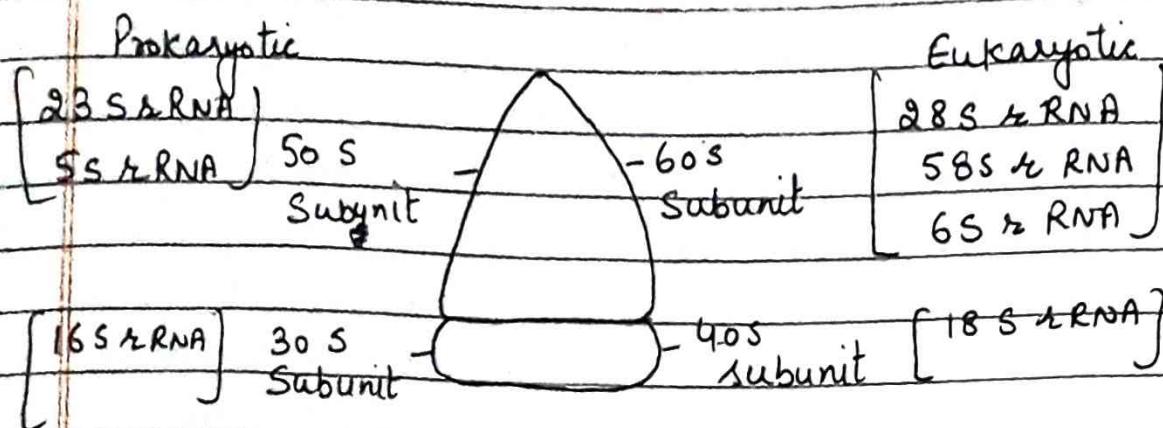
(b) Recognition End:- It has 3 unpaired ribonucleotides. A base triplet on m-RNA chain is called a codon, and its complementary bases on the triplet on t-RNA molecule is termed an anticodon.

(c) Enzyme site:- It is on one side of the molecule. It is meant for a specific charging enzyme which catalyses the union of a specific amino acid to t-RNA molecule.

(d) Ribosome site:- It is on the other side of the molecule. It is meant for attachment to a ribosome.



(3) t-RNA - The t-RNA molecule is greatly coiled. In combination with proteins, it forms the small and large subunits of ribosome.



Structure of α -RNA

TRANSCRIPTION :-

Transcription is a process of making a copy of genetic information stored in a DNA strand into a complementary strand of RNA with the aid of RNA polymerase.

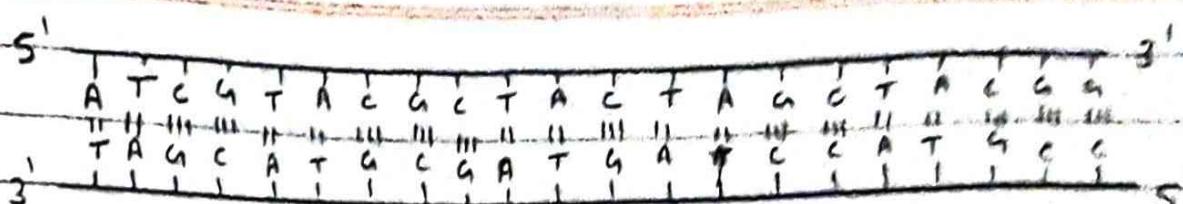
Transcription unit :- It comprises of three regions:-

- Promoter
- The structural gene
- A Terminator

The promoter situated on the starting point of DNA, structural gene situated at centre and terminator is situated at the end.

The two strands of the DNA have opposite polarity i.e. one strand has 5'-3' and other strand has 3'-5' strand.

3'-5' strand is also called Complementary strand and 5'-3' is called Coding strand.



A DNA Structure

Material Required:- The process of transcription requires :-

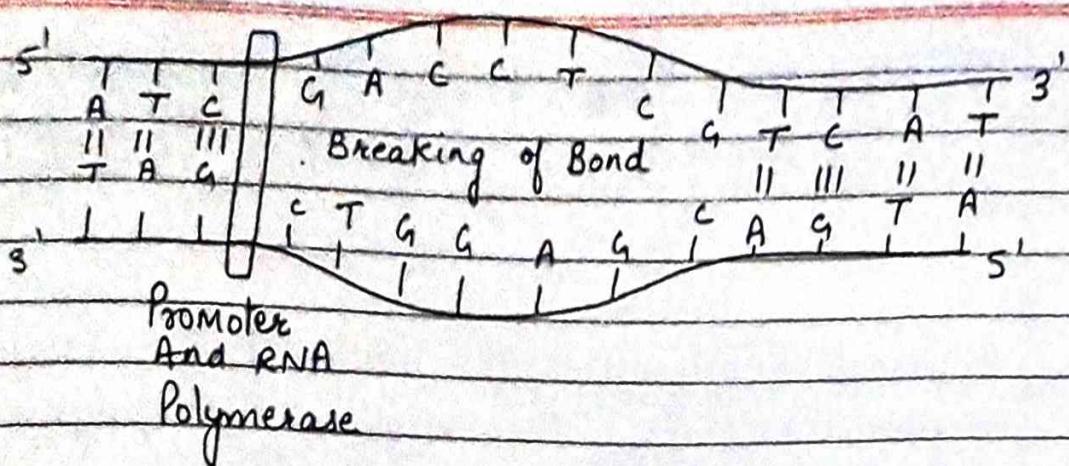
- (1) The enzyme RNA polymerase
- (2) A DNA Template
- (3) All four types of ribonucleoside triphosphate (ATP, CTP, GTP, UTP)
- (4) Mg^{2+} or Mn^{2+} as cofactors

Procedure :- (1) Binding of RNA polymerase to DNA Duplex :-

Histone coat that is the protective covering of DNA removes and the polynucleotide sequences exposed. The RNA polymerase enzyme binds to a specific site called promoter. The enzyme recognize the promoter by its sigma (σ) subunit.

Transcription factors and RNA polymerase bond to the promoter together, forming a transcription initiation complex.

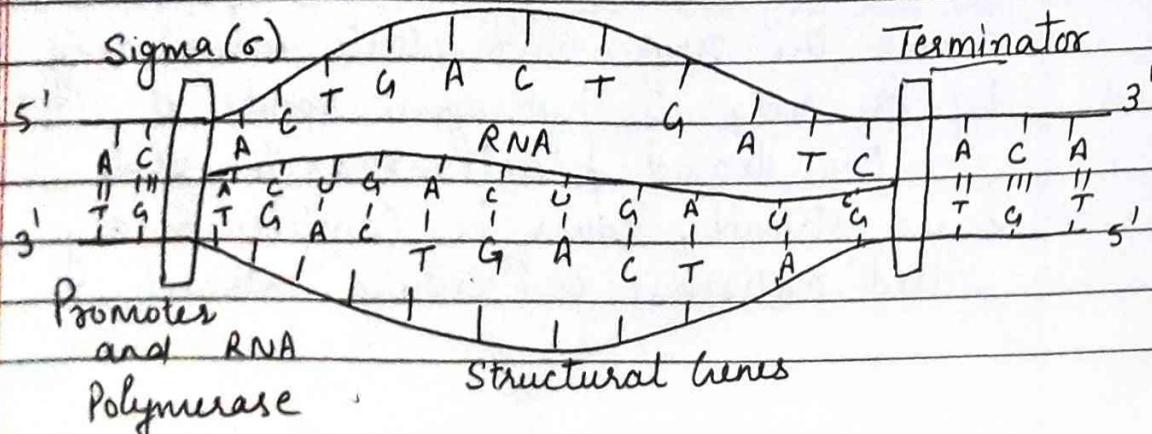
- (2) Exposure of RNA Bases:- The RNA polymerase moves along the DNA and starts unwinding of DNA by breaking its hydrogen bonds of A-T, C-G and G-C. One strand functions as template called sense strand, other is complementary, so called antisense or ^{non}Coding strand.



(3) Base Pairing :- For the synthesis of RNA, some raw material are needed. These are adenosine triphosphate (ATP), guanosine Triphosphate (GTP), Cytidine Triphosphate (CTP) and Uridine triphosphate (UTP). These enzymes are activated by the enzyme phosphatase and they changed into adenosine monophosphate (AMP), guanosine monophosphate (GMP), Cytidine monophosphate (CMP) and Uridine monophosphate (UMP).

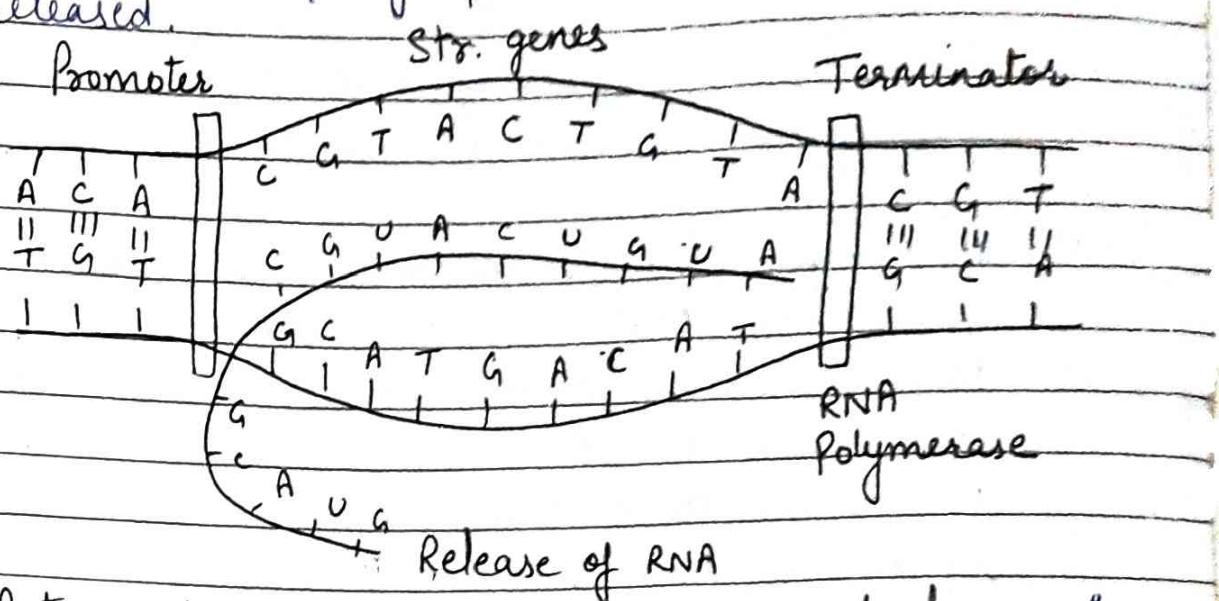
(4) Conversion to Ribonucleoside Monophosphate :-

The various ribonucleoside Triphosphate on the linking to DNA template chain break off their high energy bonds. This triphosphate RNA converted into RNA monophosphate. It undergoes hydrolysis by the enzyme phosphatase.



(5) Formation of RNA chain :- Each ribonucleoside monophosphate joined to DNA template chain. This process requires RNA Polymerase that is already present along with Mg^{++} or Mn^{++} .

(6) Separation of RNA chain :- There is formation of hybrid DNA RNA Complex. When the RNA polymerase reaches at terminator signal, it leaves the DNA. The fully formed RNA chain is now released.



(7) Return of DNA Segment to original form :- As the RNA chain grows, the transcribed region of the DNA molecule gets hydrogen bonds and assume the original helical form.

(8) Processing of RNA's :- The forms of RNAs originally transcribed from DNA are called Primary transcript. They undergo extensive changes, termed processing or maturation or post transcriptional modifications of RNAs.

In RNA Processing (1) Larger RNA's are cut

- into smaller RNA's by ribonuclease P enzyme.
- (2) unwanted nucleotides are removed by enzymes named nucleases.
 - (3) useful regions are rejoined by ligase enzyme.
 - (4) certain nucleotides are added at the ends called terminal addition.
 - (5) Molecule may fold on itself to assume proper shape.
 - (6) Some nucleotide may be modified.

Three different types of RNA polymerases are used in the process of transcription.

- (1) Polymerase I → It is present in nucleolus and synthesize 18 S and 28 S rRNA's.
- (2) Polymerase II → It is found in nucleoplasm and synthesize mRNA.
- (3) Polymerase III → It is present in nucleoplasm and synthesize t-RNA.

Translation

Translation :- It is the mechanism by which the triplet base sequence of an m-RNA guides the sequence of amino acids to form a polypeptide on ribosomes.

Machinery for protein synthesis :-

Protein synthesis requires amino acids, DNA, RNAs, ribosomes and enzymes.

- i) Amino acids : amino acid acts as raw material for protein synthesis. The proteins of living organisms

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need about 20 amino acids are located in cytoplasmic matrix or amino acid pool.

(2) DNA :- DNA act as specificity control. This specificity control is exercised by DNA through which sequence of 3 nitrogenous bases in DNA double helix form the biochemical or genetic code.

(3) m-RNA :- m-RNA serves as the intermediate between DNA and proteins in a cell.

(4) Ribosomes as protein factories :- ribosomes are called protein factories. Each ribosome consist of large and small subunits. Mostly the subunits of ribosomes remains separately, but during the formation of protein, these subunits joined. Many ribosomes line up on the m-RNA chain during protein synthesis. This row of active ribosomes is called a polyribosomes.

A ribosome has one binding site for m-RNA and three binding sites for tRNA.

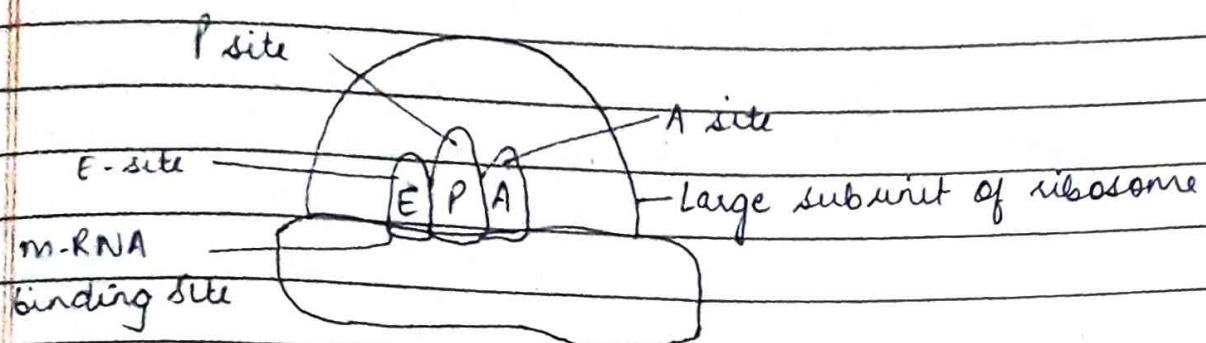
- (1) P-site (Peptidyl-t-RNA site)
- (2) A-site (Amino acyl-t-RNA site)
- (3) E-site (Exit site)

Mechanism of Protein Synthesis :-

- (1) Activation of amino acids :- all the amino acids remains inactive in amino acid pool. For the activation of amino acids, they react with ATP and form amino-acid-AMP complex and pyrophosphate.

in the presence of Mg^{2+} An enzyme aminoacyl-tRNA synthetase helps to form this complex.

The amino acid-AMP enzyme complex is also called activated amino acid.



Binding sites of a ribosome

(2) Charging of t-RNA:- The amino acid AMP complex joins to the amino acid binding site of its specific t-RNA, where its -COOH group bonds to -OH group of the terminal base triplet CCA. This reaction is catalysed by the same aminoacyl tRNA synthetase enzyme.

The resulting t-RNA aminoacid complex is called a charged tRNA.

Activation of ribosomes:- The mRNA chain joins the small ribosomal subunit by first codon through base pairing. This combination of two is called Initiation Complex. It's combination also requires proper conc. of Mg^{2+} .

Assembly of Amino acids (Polypeptide formation) :-

It involves 3 stages:-

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IMPORTANT NOTES