VII B

TRANSCRIPTION (Protein synthesis)

IN PROKARYOTES

Transcription is the process by which non-genetic RNA molecules i.e. except RNA viruses are synth synthesized by using the base sequences of one strand of DNA as a template. It is a polymerization reaction that is catalyzed by enzymes called DNA dependent RNA polymerases or simply RNA-polymerases. The mechanism of RNA synthesis differ in prokaryotic and eukaryotic organisms.

Transcription in Prokaryotic Organism:

The essential chemicals and apparatuses of

1. RNA Polymerase enzyme -

(a) In E. Coli and other prokaryotes, a simple type of RNA polymerase is respect responsible for the synthesis of all kinds of RNA. It consist consists of six polypeptide chains i.e. six subunits.

(i) 2 identical alpha subunits (α)

(ii) One chain of beta (β)

(iii) Beta dash (β')

(iv) Omega (ω) and

(v) Sigma (σ) subunit.

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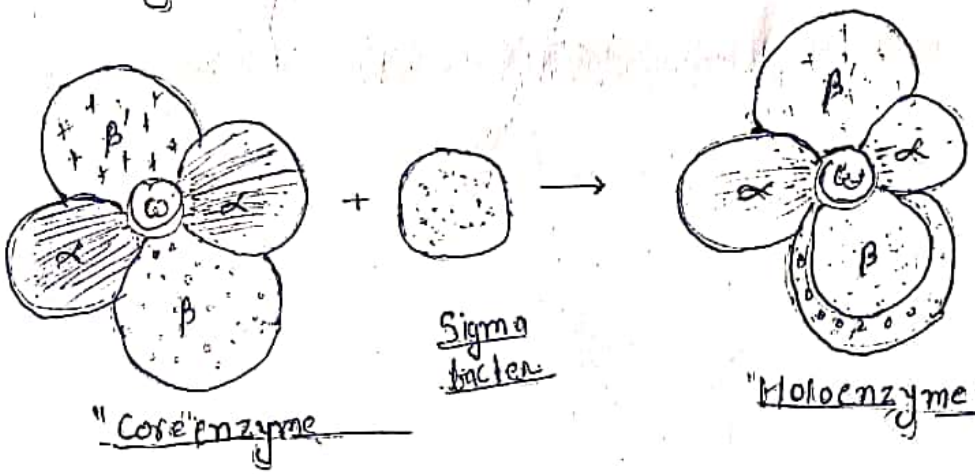
(b) The first 5 subunits are known as core enzyme ($2\alpha, \beta, \beta', \omega, \phi$) with the attachment of σ-subunit. the complete RNA polymerase is formed. holo-enzyme.



(c) Some basic characteristics of the subunits are as follows:—

(i) α-subunit is a promoter binding.

(ii) β is nucleotide binding.

(iii) β' is DNA template binding.

(iv) The function of ω is still not clear.

(v) σ-factor starts initiation.

2. The precursor in the synthesis of RNA are the four ~~four ribonucleotide~~ 5'-triphosphates i.e. ATP, GTP, CTP and UTP.

3. Only one of the two strands of DNA helix is transcribed. In other words, it serves as template.

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i) This template may be in region or any strand of DNA. This strand is called the sense strand.

ii) The other strand of DNA which is not transcribed is called antisense strand.

iii) The region of the sense strand of DNA which is actually transcribed into RNA is called the coding region.

4. Mg^{++} is required for all nucleic acid polymerisation.

Transcription of RNA includes —

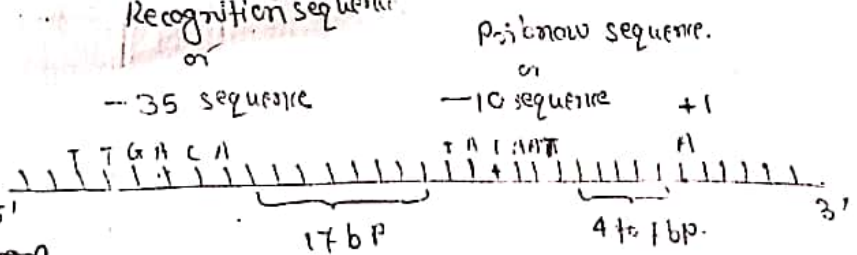
a. Initiation
b. Elongation
c. Termination.

a. Initiation:

1. The first step is binding of RNA polymerase to the sense strand of DNA molecule.

2. Binding occurs at particular sites called promoters. Promoters have specific sequence of 20 to 200 bases at which interactions occur.

The special promoter regions have been identified that appear in all organisms.



Mid points of -10 or -35 sequence occur at about 10 to 35 nucleotides before transcription - initiation complex.

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(a) In a region of 5-10 base pairs before the coding region is a sequence of seven bases that read TATAATG with minor variation.

In E. Coli i.e. bacteria, this region is called "Pribnow box."

(i). The centre of Pribnow box lies usually 10-base pairs upstream of the coding region.
It contains T and is called -10 sequence which recognised by RNA polymerase during the binding reaction.

(ii) It is belived that, this box orient the RNA polymerase as such that synthesis proceeds from 5'-3' direction.

(iii) It is also the region at which double helix opens to form the open promoter complex.

(b) Another important promoter region is located approximately 35 bases upstream from the coding region.

(i) This is called -35 sequence and consists of a base consensus sequence.

(ii) It is considered to be the actual site of the binding of the RNA polymerase.

3(a). The σ subunit of RNA polymerase first binds to the -35 sequence in a highly specific interaction. This huge enzyme can come in contact

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with pribnow box. It is the region where the double helix opens to form the open promoter complex.

b) RNA polymerase induces the conformational change in DNA ~~helices~~ helix and DNA strand open to denaturation. The open ~~pre~~ promoter complex is highly stable and is the active intermediate in chain initiation.

Thus the binding site of RNA polymerase include promoter region, initiation as well as elongation sites.

c) At the initiation site only purine type phosphates namely ATP and GTP bind but it is usually ATP. In other words, ATP is the first nucleotide in the growing RNA chain and thymine is the first DNA base that is transcribed.

d) i- The initiation nucleoside triphosphate binds to the enzyme in the open promoter complex and

ii- also forms a hydrogen bond with the complementary DNA base (T).

+a. In polymerisation reaction, a 3' OH group of one nucleotide reacts with the 5' triphosphate of a second nucleotide.

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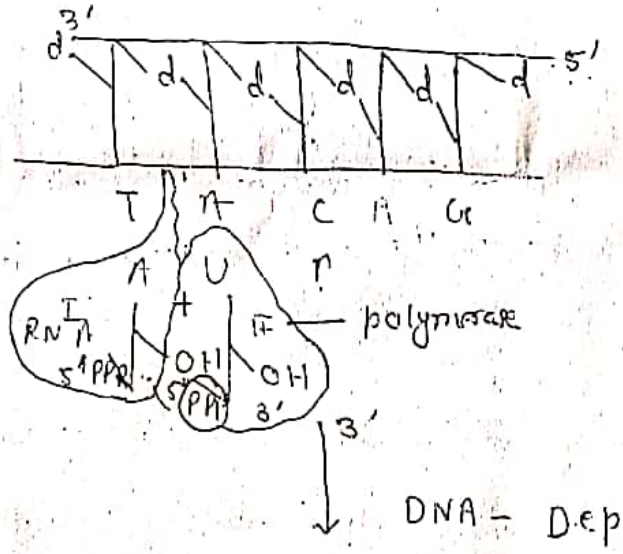
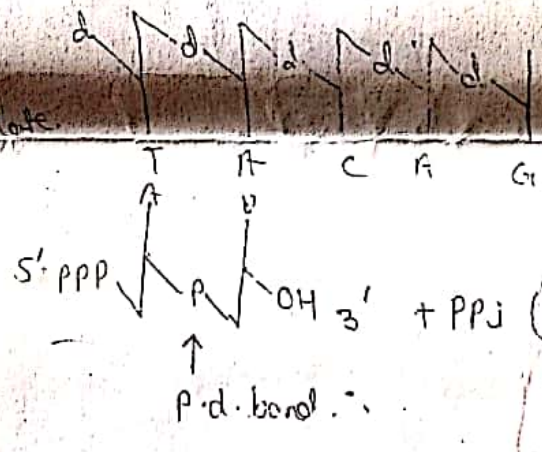
b. A pyrophosphate (PPi) is removed and a phosphodiester bond is formed between two nucleotides.



d = Sugar deoxyribose.

DNA - Dependent RNA polymerase.



5a) Next the ribonucleotide triphosphate form a hydrogen bond with the next base in the DNA strand

b) The two nucleotides are then joined together.

c) The first base of RNA nucleotide is released from the initiation site of RNA polymerase and the initiation is completed. The dinucleotide remains H-bonded to the DNA.

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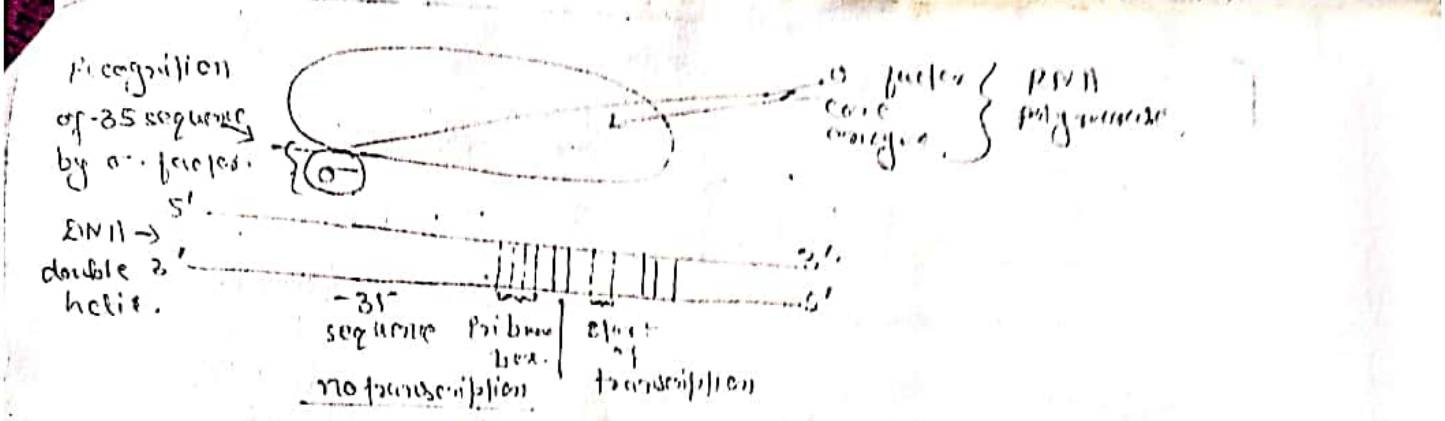
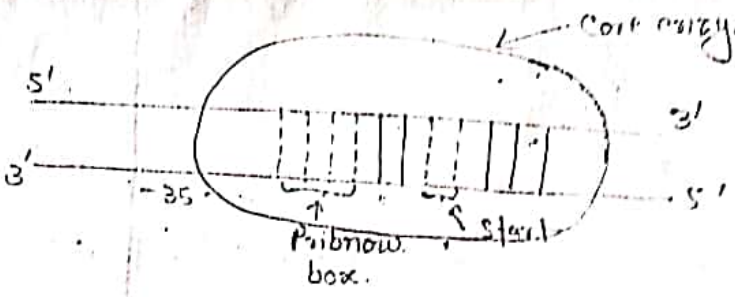
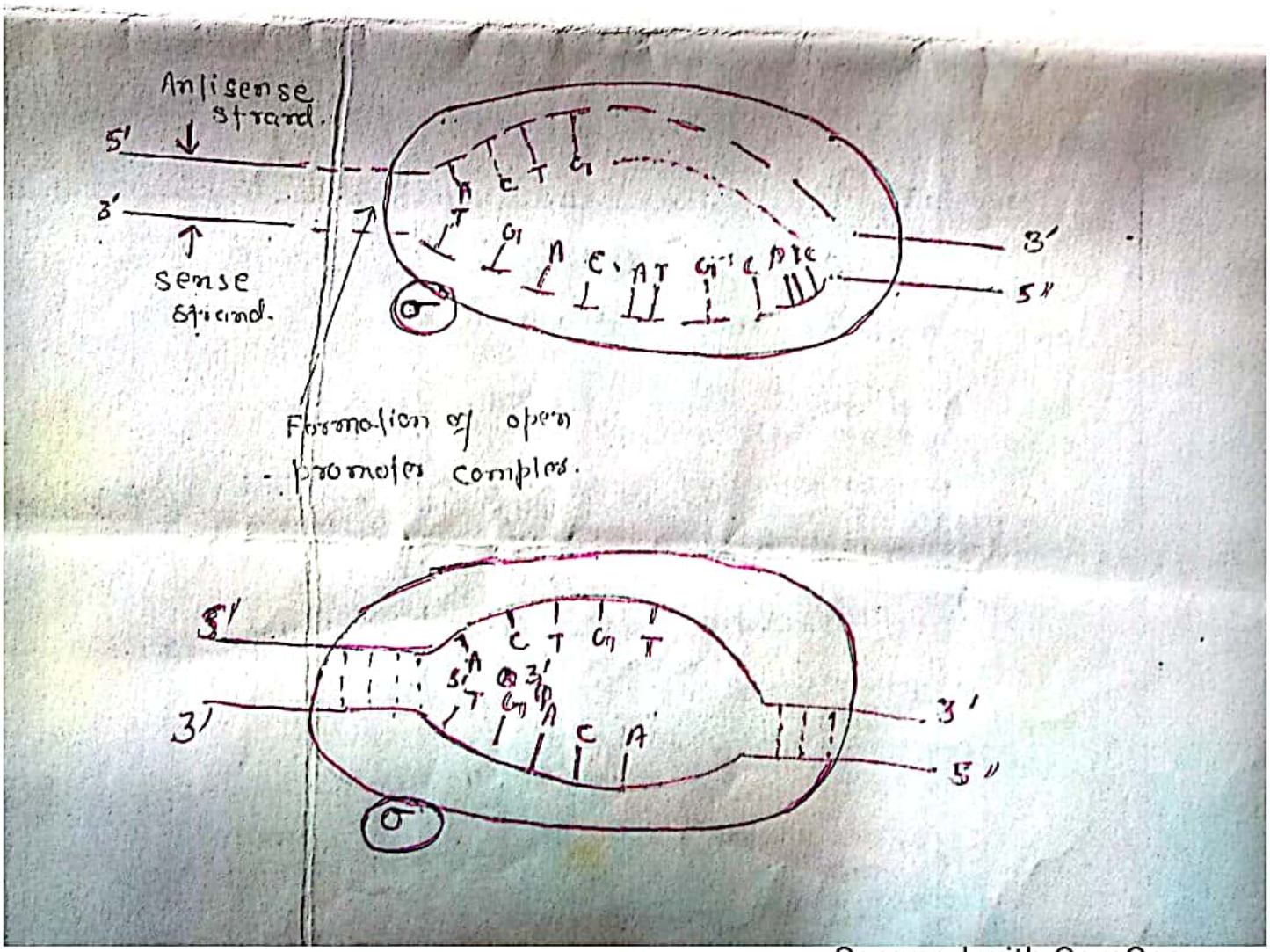
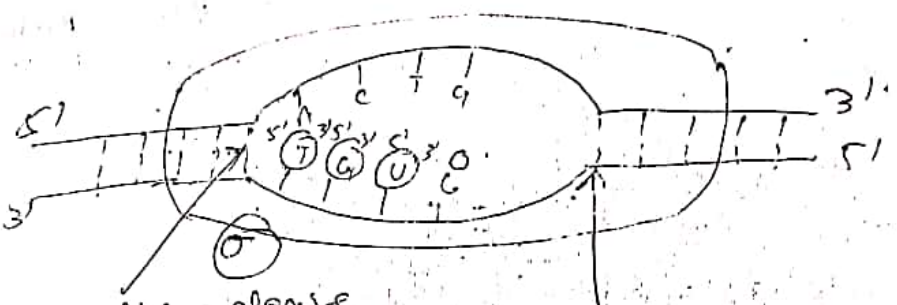
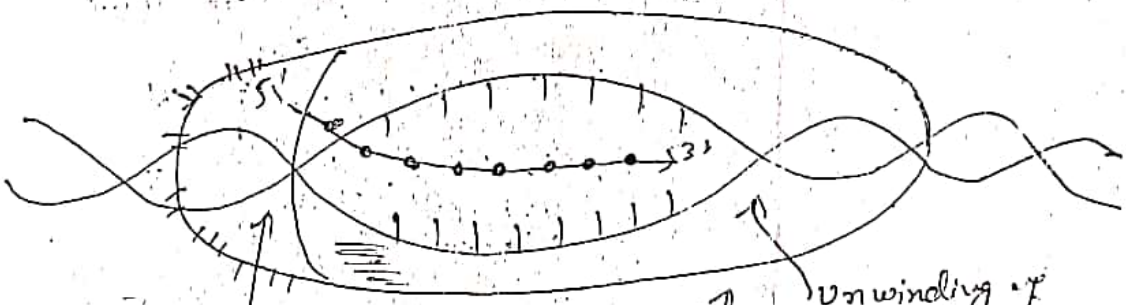




Movement of RNA polymerase to pribnow box.







Elongation :—

The elongation phase begins when the RNA polymerases release the DNA attached RNA PST base and moves along the DNA chain.

a) The sequence of base in RNA molecules is determined by the base sequence of the DNA.

b) The RNA chain grows in 5' → 3' direction. In other words, nucleotides are added only to the 3'OH end of the growing chain.

c) Several nucleotides are added in the growing chain and when approximately 8

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nucleotides are added. σ factor dissociates from the RNA polymerase.

d) Elongation is carried out by the core enzyme which move along the DNA template and binds ribonucleotide triphosphate.

e) The open region of the DNA extends only over a few base pairs of DNA. As newly synthesized RNA is released from its H. bonds with the DNA, DNA helix is reformed as helix recloses just behind the enzyme.

f) The promoter itself is not transcribed

Termination:

Twenty termination sequences have so far been determined and each has the characteristics. Termination region consists of the following three important regions :—

1. First, there is an inverted repeat base sequence containing a central non-repeating segment.
2. The second region is a sequence having a high G+C content.
3. A third region, sometimes absent, is a sequence of A-T Pairs in the DNA helix that yield in the RNA a sequence of six to eight uri-

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often, followed by adenine.

But according to De-Roberts, there are only two types of termination events —

(a) those that depend only on the DNA base sequence and

(b) those that require the presence of termination protein called rho (ρ) factor with ATPase activity. It causes the release of completed RNA mol. The final step in the termination process is the dissociation of the core enzyme from the DNA.

The dissociated core enzyme again interacts with a free sigma factor to reform the holoenzyme and is ready to initiate from transcription again

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Difference between prokaryotic and eukaryotic Transcription :—

I. RNA polymerase :—

In eukaryotes there are three major classes of RNA polymerase which are designated as I, II and III and are found

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the nucleus.

The three polymerases have different proportion:

Enzyme	Location	Product of abundance.
1) RNA Polymerase I	Nucleolus	r-RNA (50-70%) (except 5S r-RNA)
2) RNA polymerase II	Nucleoplasm	hn-RNA (m-RNA) (20-40%)
3) RNA Polymerase III	Nucleoplasm	t-RNA (~10%) (and 5S rRA)

II

(i) Promoter :- The promoters of genes of RNA polymerase II contain three distinct regions which are centered at sites lying between -25 bp and -100 bp.

(a) One of these three regions is the TATA or Goldberg Hogness box which is 7 bp long. It is located 20 bp upstream to the starting point.

(b) CAAT box sequence lies between -70 and -80 base pairs and induced GGT/AC AATCT base composition.

(c) Another sequence called GC box (GGGC-CCGG) is found in one or more copies of -60 or -100 bp upstream in many mutation.

(ii) Apart from what it contain regulators called enhancers or silencers.

Enhancer :-

Eukaryotic promoters also consists of

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sites located 100 to 200 base pairs upstream which interact with proteins other than RNA polymerase. They regulate the activity of promoter. These sites are called enhancers, since they lead upto 200 fold increase in the rate of transcription of an affected gene.

© Silencers:— There are other regulatory sites known as silencers which repress gene expression

III. Transcription factors:—

Various proteinous transcription factors (TFS) are also involved in the formation of a pre initiation complex or transcription, complex which are needed for initiation of transcription. ② Generally each of RNA polymerase is bound to its own set of transcription factor (TFS) are not part of the RNA polymerase. After the formation of this complex distribution of transcription occurs. ③ The following are the six main transcription factors in initial in order:— (i) TFIID (ii) TFHA and TFIIB (iii) TFAH F (iv) TFII E TFII H and TFII J.

Elongation Factor:—

There are certain accessory factors in eukaryotic transcription called elongation factors which enhance the overall activity of RNA polymerase II and lead to increase in the elongation rate. At least two such proteins are known —

i) The TF I F ii) The TF II S

In eukaryotes, the actual termination of RNA polymerase —

The synthesized RNA are further processed to form r-RNA, m-RNA & t-RNA.

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