For P.G. -2nd semester Zoology

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Q: => Give an account of gene expression at molecular level.

Ans: => Gene expression is accomplished by a series of events. The information present in DNA is converted into molecules that determine the metabolism of the cell. During the process of gene expression DNA is first copied into an RNA molecules which determines the amino acid sequence of molecule of protein. The RNA molecules are synthesized by using a portion of base sequences of single strand of double stranded DNA. This single strand is called template. Hence, formation of an RNA transcript is facilitated by an enzyme, RNA polymerase & process of synthesis of an RNA molecule corresponding to a gene is called transcription. Production of an amino acid sequence from an RNA base sequence is called translation. After completion of translation protein are synthesized. Therefore, gene expression refers to protein synthesis through two major events, transcription & translation.

Central Dogma

Dogma can't directly order for the synthesis of amino acid

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but it forms its transcripted which is then translate into protein. F Crick (1958). suggested that there is unidirectional flow of information's from DNA to RNA to protein as shown below.

DNA Transcription → RNA Translation → Protein.

This sequential transfer of information from DNA to protein via RNA is known as central dogma.

Transcription (RNA Synthesis)

The tRNA, mRNA & rRNA are involved in the process of transcription. However an enzyme transcriptase i.e. DNA dependent RNA polymerase is required for the synthesis of RNA by using ribonucleotide diphosphates i.e ATP, GTP, CTP & UTP.

RNA polymerase

It is found in both prokaryotic & eukaryotic. In prokaryotes only a single enzyme, RNA polymerase governs the synthesis of the all cellular RNAs, whereas in eukaryotes for the synthesis of a cellular type is

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RNA polymerase involved.

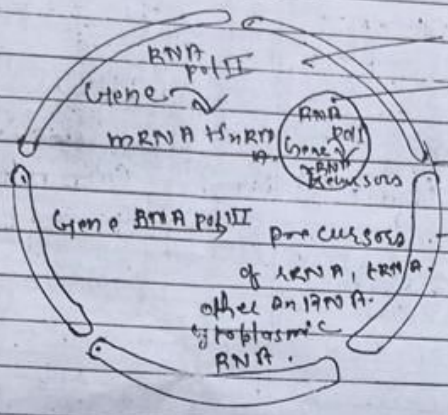

fig: fun' of RNA polymerase I, II & III in eukaryotes.

1. Types of RNA polymerase.

There are different eukaryotic RNA polymerases that are transcribed by three different sets of genes.

a. RNA polymerase I (RNA pol I)

It is located in the nucleolus & synthesizes precursors of most r-RNAs. It is sensitive to α-amanitin.

b. RNA pol II

It is located in the nucleoplasm & synthesizes mRNA precursors & some small nuclear RNAs. It is very sensitive to α-amanitin.

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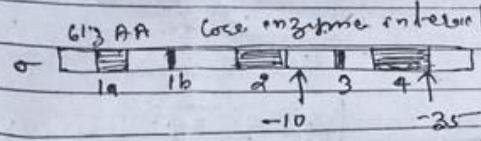
c. RNA Pol III.

It is located in the nucleoplasm. It synthesises the precursors of tRNA, 5S rRNA & other small nuclear & cytoplasmic RNAs. It is moderately sensitive to α-amanitin.

In addition to antibiotic Rifampicin commonly inhibits β-Sub-unit of prokaryotic RNA polymerase.



Conserved region. promoter Recognition.

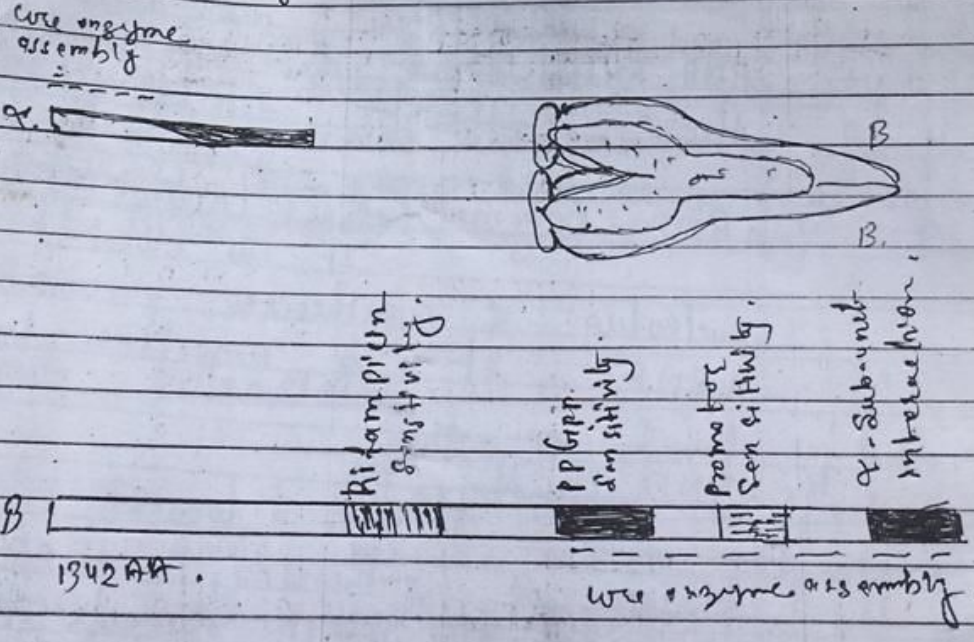


Fig: Functional map of RNA polymerase Sub-unit (Ishihama 1992)

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II The site of transcription.

Transcription begins at the promoter site of only one strand of ds DNA is transcribed.

III Process of transcription.

The process of transcription is accomplished in the following three main steps. chain initiation, chain elongation & chain termination.

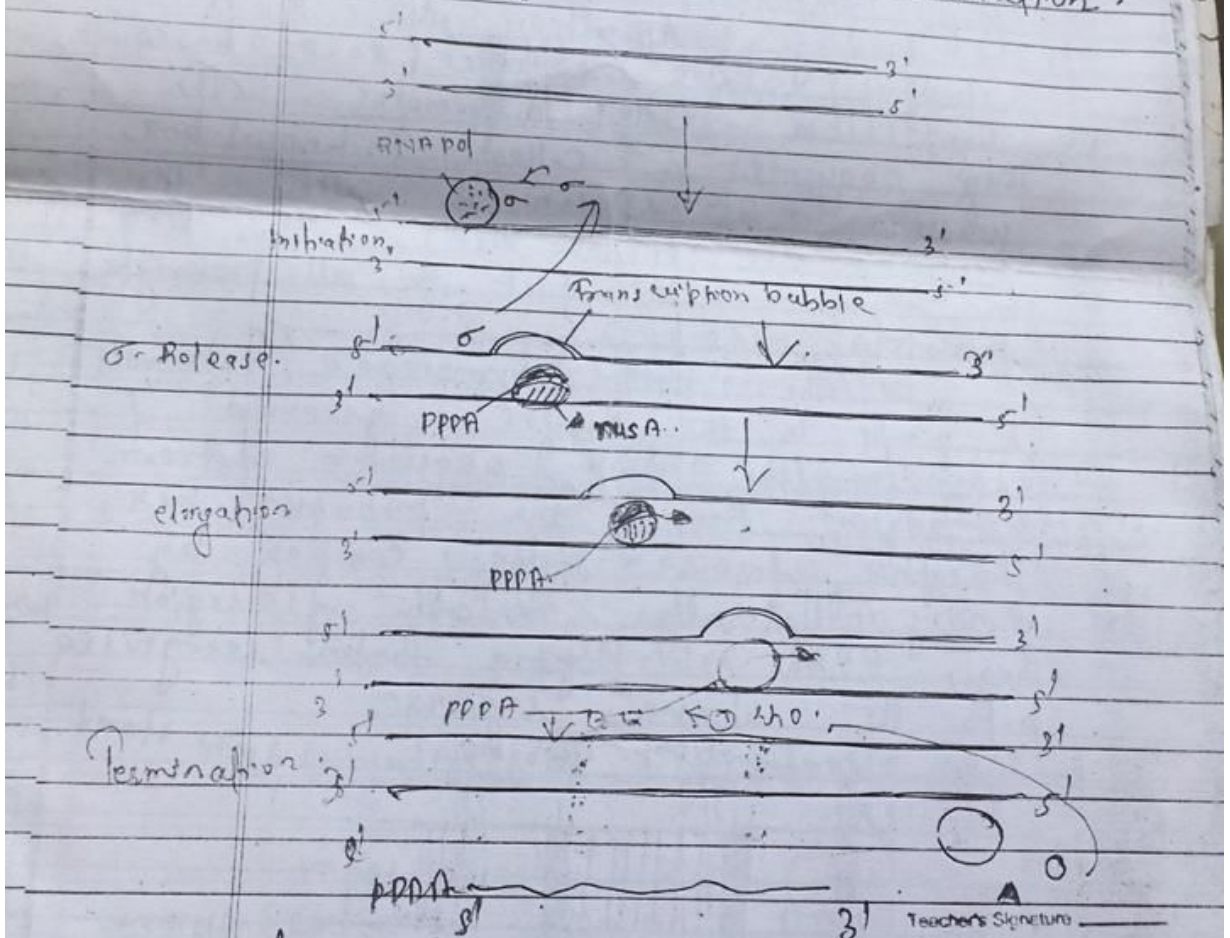


Fig :- The process of transcription.

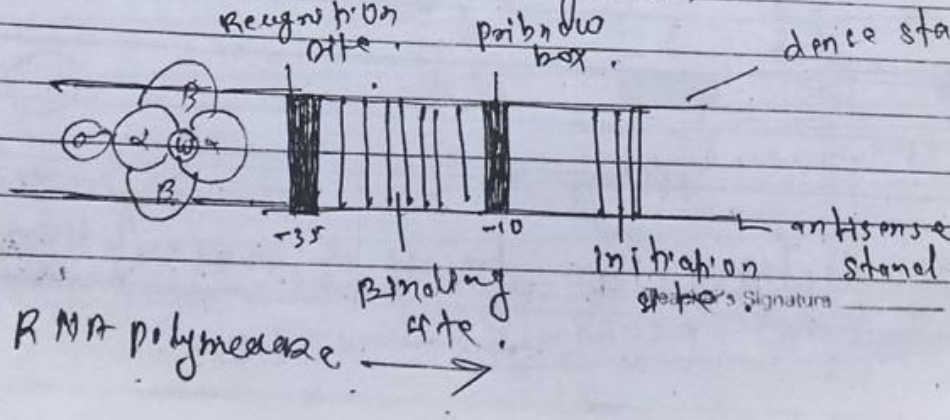
2. Chain Initiation.

During the process of transcription, divalent metal ions (Mg^{++} or Mn^{++}) RNA polymerase & template required the initiation the RNA chain.

1. Promoter Recognition.

RNA polymerase play important role in recognition & binding of initiation site. A specific base sequence (20-200 bases long) is called a promotor. The -10 sequence is called Pribnow's box, whereas -35 region is called the recognition site (Pribnow 1975). This box is found as a part of all prokaryte promotors.

The RNA polymerase interacts 5 groups in the major grooved & recognises the proper sequence upstream (-35 region) from the pribnow's box, thereafter forms stable complex by moving laterally to the -10 region. Thus, the sigma factor recognised both the above regions.



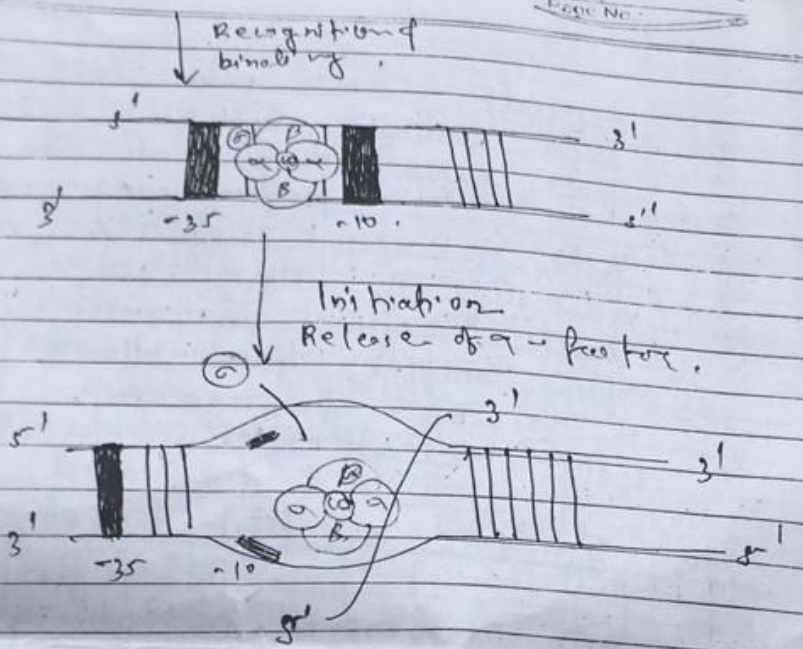


fig :- DNA template showing recognition binding & initiation sites.

ii. Binding of RNA polymerase to promoter

Cyclic AMP receptor protein (CRP) is the binding site for protein rather than RNA polymerase (Freifelder 1987.

iii Unbinding of DNA double helix.

The torsional stress imposed by super coiling makes the certain area of DNA easier to

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separate RNA polymerase. Hence super coiling affects the expression of some genes more than the others.

Binding of omega (ω) factor of RNA polymerase results in unwinding of DNA helix. Thus a short segment of DNA opens. The open complex then allows tight binding of the RNA polymerase & subsequent initiation of RNA synthesis.

iv. Synthesis of first base of RNA chain.

The first base of RNA synthesized is always in a form of purin. either triphosphate guanine (pppG) or adenine (pppA). Initiation ends after the formation of first-inter nucleotide bond (5'pppGpN)

3 chain elongation.

chain elongation occurs by the core enzyme that moves along the DNA template. After beginning the elongation transcription goes on at a rate between 30-60 nucleotides per second at 37°C. In general the elongation step include the step.

i. Nucleotide triphosphate binding
ii Bond formation between the nucleotide.

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& the 3'-OH of nascent RNA chain.

iii- iv. Pyrophosphate release
Translocation of polymerase along the DNA template.

The activated ribonucleoside triphosphates i.e ATP, UTP, GTP & CTP are added according to nucleotide of one strand of DNA template.

The incoming nucleotide forms hydrogen bond to a DNA base. Reaction occurs bet' 3' end of RNA molecule and P in the triphosphate group. (Hanna & Means 1983) leading to removal of phosphate (PPi). The PPi is then hydrolysed to inorganic phosphate (Pi).

2. Release of σ factor.

The release of σ (Hgms) can combined to any of the enzymes & thus reused for the initiation of new chain (Travis & Burgess 1969)

Direction of transcription.

The ribonucleoside triphosphate attach to the free nucleotide of the template & chain growth takes place in 5'-3' direction.

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Evidences

In newly formed RNA triphosphate group at 5' - free. oH group on 3" ends.

iii. Proof Reading of mRNA.

The RNA - polymerases releases & moves at the 3' end of the gene. (Most acid factor 1995)

iv. Protein - protein interaction - RNA Synthesis

There are several regulatory factor that directly contact the RNA polymerases at the promoter region of template.

(i) Chain termination.

The process of termination of RNA chain ends & the events.

- Cessation of elongation.
- Release of transcript from the tertiary Complex.
- dissociation of polymerase from the DNA template.

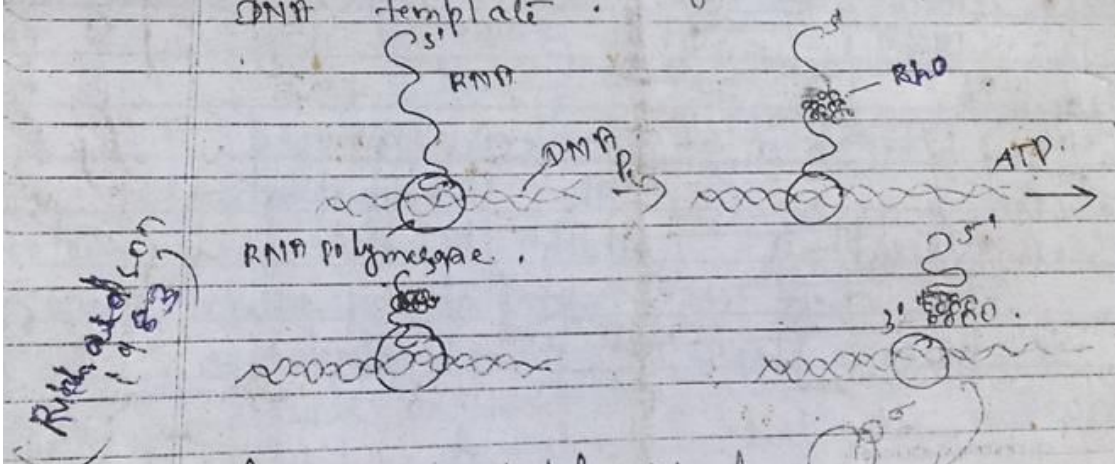

fig :- A model for Rho factor mediated release of RNA chain