COURSE: B.Sc (Semester: II), Department of Botany
PAPER: BOT CC 408; Molecular Biology
TOPIC: TRANSCRIPTION IN PROKARYOTES

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TRANSCRIPTION

INTRODUCTION:

- The first stage of gene expression in which there is formation of RNA on DNA template is known as TRANSCRIPTION.
- RNA synthesis take place by an enzyme known as **RNA PYMERASE**.
- RNA synthesis take place by using template strand 3'-5'direction.
- RNA synthesis is a copy of non-template strand in 5'-3'direction.
- Other name uses to describe non-template strand are sense strand or coding strand.
- RNA molecule synthesized is called TRANSCRIPT.
- During transcription, RNA is synthesized by polymerization of ribonucleotide tri-phosphate sub unit (ATP, UTP, GTP, CTP).
- The 3'OH of one ribonucleotide react with 3' phosphate of another to form phospho-di-ester bond.

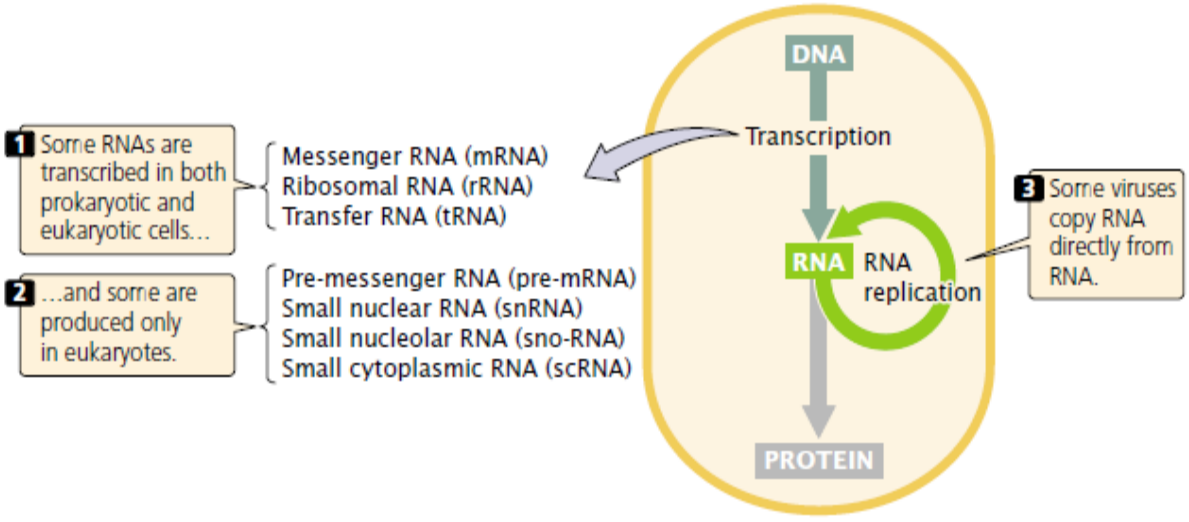

Fig. 1: All cellular types of RNA are transcribed from DNA.

- RNA differs from DNA in that it possesses a hydroxyl group on the 2_-carbon atoms of its sugar, contains uracil instead of thymine, and is normally single stranded.

Required component for Transcription:

- Like replication, transcription requires three major components:

1. a DNA template;
2. the raw materials (substrates) needed to build a new RNA molecule; and
3. the transcription apparatus, consisting of the proteins necessary to catalyze the synthesis of RNA.

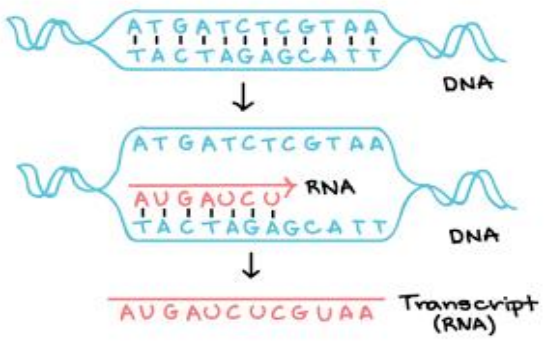

Fig.2: RNA synthesis is a copy of non-template strand.

The transcription unit:

- A transcription unit is a stretch of DNA that codes for an RNA molecule and the sequences necessary for its transcription.
- A transcription unit is a piece of DNA that encodes an RNA molecule and the sequences necessary for its proper transcription. Each transcription unit includes a promoter, an RNA-coding region, and a terminator.
- The **promoter** is a DNA sequence that the transcription apparatus recognizes and binds. It indicates which of the two DNA strands is to be read as the template and the direction of transcription.
- The promoter also determines the transcription start site, the first nucleotide that will be transcribed into RNA. In most transcription units, the promoter is located next to the transcription start site but is not, itself, transcribed.
- The second critical region of the transcription unit is the **RNA-coding region**, a sequence of DNA nucleotides that is copied into an RNA molecule.
- A third component of the transcription unit is the **terminator**, a sequence of nucleotides that signals where transcription is to end. Terminators are usually part of the coding sequence; that is, transcription stops only after the terminator has been copied into RNA.

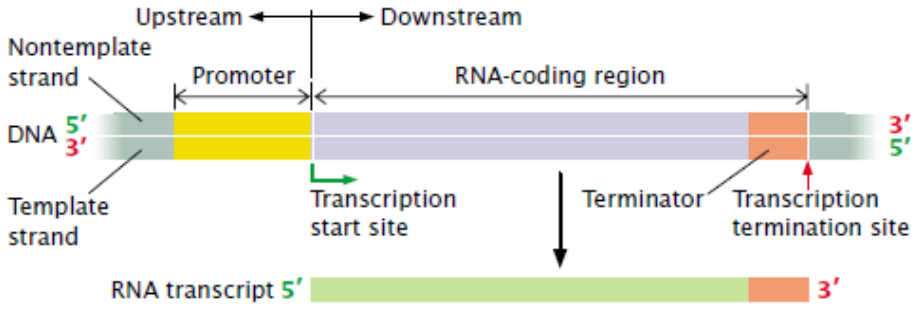

Fig. 3: A transcription unit includes a promoter, an RNA-coding region, and a terminator.

The Substrate for Transcription

- RNA is synthesized from **ribonucleoside triphosphosphates** (rNTPs). In synthesis, nucleotides are added one at a time to the 3_-OH group of the growing RNA molecule.
- Two phosphates are cleaved from the incoming ribonucleoside triphosphate; the remaining phosphate participates in a phosphodiester bond that connects the nucleotide to the growing RNA molecule.

$$RNA_n + rNTP \longrightarrow RNA_{n+1} + PP_i$$

- The overall chemical reaction for the addition of each nucleotide is: where PPi represents two atoms of inorganic phosphorus. Nucleotides are always added to the 3' end of the RNA molecule, and the direction of transcription is therefore 5':3'.

The Transcription Apparatus

RNA polymerase carries out all the required steps of transcription.

- The action of RNA polymerase is enhanced by a number of accessory proteins that join and leave the polymerase at different stages of the process.
- Each accessory protein is responsible for providing or regulating a special function. Thus, transcription, like replication, requires an array of proteins.

Bacterial RNA polymerase: Bacterial cells typically possess only one type of RNA polymerase, which catalyzes the synthesis of all classes of bacterial RNA: mRNA, tRNA, and rRNA. Bacterial RNA polymerase is a large, multimeric enzyme (meaning that it consists of several polypeptide chains).

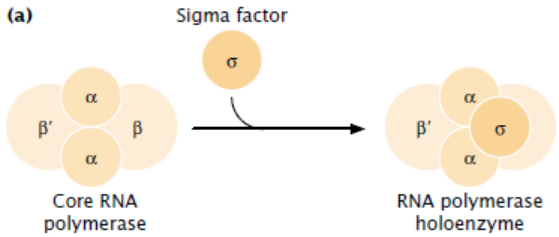

Fig. 4: In bacterial RNA polymerase, the core enzyme consists of four subunits: two copies of alpha (α), a single copy of beta (β), and single copy of beta prime (β).

- The core enzyme catalyzes the elongation of the RNA molecule by the addition of RNA nucleotides. (α) The sigma factor (σ) joins the core to form the holoenzyme, which is capable of binding to a promoter and initiating transcription.
- After sigma has associated with the core enzyme (forming a **holoenzyme**), RNA polymerase binds stably only to the promoter region and initiates transcription at the proper start site. Sigma is required only for promoter binding and initiation; when a few RNA nucleotides have been joined together, sigma detaches from the core enzyme.

TRANSCRIPTION IN PROKARYOTES

Bacterial cells possess a single type of RNA polymerase, consisting of a core enzyme and other subunits that participate in various stages of transcription.

Transcription can be conveniently divided into three stages:

1. **initiation,** in which the transcription apparatus assembles on the promoter and begins the synthesis of RNA;
2. **elongation**, in which RNA polymerase moves along the DNA, unwinding it and adding new nucleotides, one at a time, to the 3_ end of the growing RNA strand; and
3. **termination,** the recognition of the end of the transcription unit and the separation of the RNA molecule from the DNA template.

INITIATION: Initiation includes all the steps necessary to begin RNA synthesis, including
(1) promoter recognition,
(2) formation of the transcription bubble,
(3) creation of the first bonds between rNTPs, and
(4) escape of the transcription apparatus
from the promoter.

- A promoter is a DNA sequence that is adjacent to a gene and required for transcription. Promoters contain short consensus sequences that are important in the initiation of transcription.
- The spacing and location of these nucleotides relative to the transcription start site are similar in most promoters. These short stretches of common nucleotides are called **consensus sequences.**

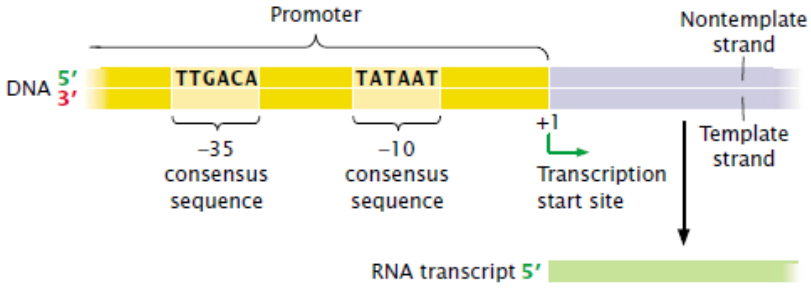

Fig. 5: In bacterial promoters, consensus sequences are found upstream of the start site, approximately at positions _10 and _35.

- After the holoenzyme has attached to the promoter, RNA polymerase is positioned over the start site for transcription (at position +1) and has unwound the DNA to produce a single-stranded template.
- The orientation and spacing of consensus sequences on a DNA strand determine which strand will be the template for transcription, and thereby determine the direction of transcription.
- To begin the synthesis of an RNA molecule, RNA polymerase pairs the base on a ribonucleoside triphosphate with its complementary base at the start site on the DNA template strand.
- No primer is required to initiate the synthesis of the 5_ end of the RNA molecule. Two of the three phosphates are cleaved from the ribonucleoside triphosphate as the nucleotide is added to the 3_ end of the growing RNA molecule.

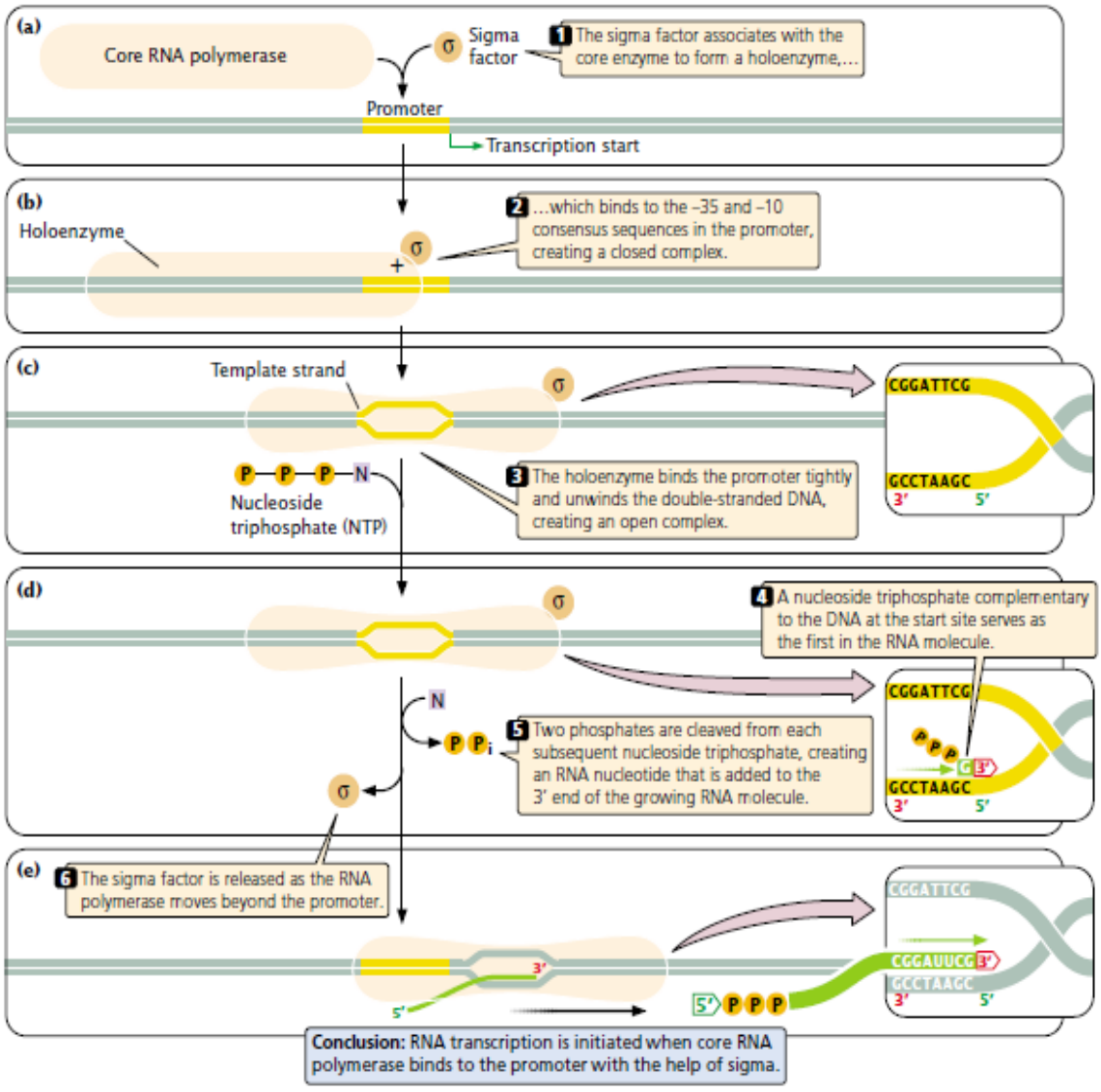

Fig. 6: Transcription in bacteria is carried out by RNA polymerase, which must bind to the sigma factor to initiate transcription.

ELONGATION:

After initiation, RNA polymerase moves downstream along the template, progressively unwinding the DNA at the leading (downstream) edge of the transcription bubble, joining nucleotides to the RNA molecule according to the sequence on the template, and rewinding the DNA at the trailing (upstream) edge of the bubble.

Transcription takes place within a short stretch of about 18 nucleotides of unwound DNA—the transcription bubble.

TERMINATION:

- RNA polymerase moves along the template, adding nucleotides to the 3_ end of the growing RNA molecule until it transcribes a terminator. Most terminators are found upstream of the point of termination. Transcription therefore does not suddenly end when polymerase reaches a terminator.
- At the terminator, several overlapping events are needed to bring an end to transcription: RNA polymerase must stop synthesizing RNA, the RNA molecule must be released from RNA polymerase, the newly made RNA molecule must dissociate fully from the DNA, and RNA polymerase must detach from the DNA template.

Bacterial cells possess two major types of terminators:

1. **Rho-dependent terminators** are able to cause the termination of transcription only in the presence of an ancillary protein called the **rho factor.**
2. **Rho-independent terminators** are able to cause the end of transcription in the absence of **rho**.

Rho-independent terminators have two common features.

- First, they contain inverted repeats (sequences of nucleotides on one strand that are inverted and complementary). When inverted repeats have been transcribed into RNA, a hairpin secondary structure form.
- Second, in rho-independent terminators, a string of approximately six adenine nucleotides follows the second inverted repeat in the template DNA. Their transcription produces a string of uracil nucleotides after the hairpin in the transcribed RNA.

The presence of a hairpin in an RNA transcript causes RNA polymerase to slow down or pause, which creates an opportunity for termination. The adenine–uracil base pairings downstream of the hairpin are relatively unstable compared with other base pairings, and the formation of the hairpin may itself destablize the DNA–RNA pairing, causing the RNA molecule to separate from its DNA template. When the RNA transcript has separated from the template,RNA synthesis can no longer continue.

Rho-dependent terminators have two features:

- DNA sequences that produce a pause in transcription; and
- a DNA sequence that encodes a stretch of RNA upstream of the terminator that is devoid of any secondary structures.

This unstructured RNA serves as binding site for the rho protein, which binds the RNA and moves toward its 3_ end, following the RNA polymerase.

When RNA polymerase encounters the terminator, it pauses, allowing rho to catch up.

The rho protein has helicase activity, which it uses to unwind the RNA–DNA hybrid in the transcription bubble, bringing an end to transcription.

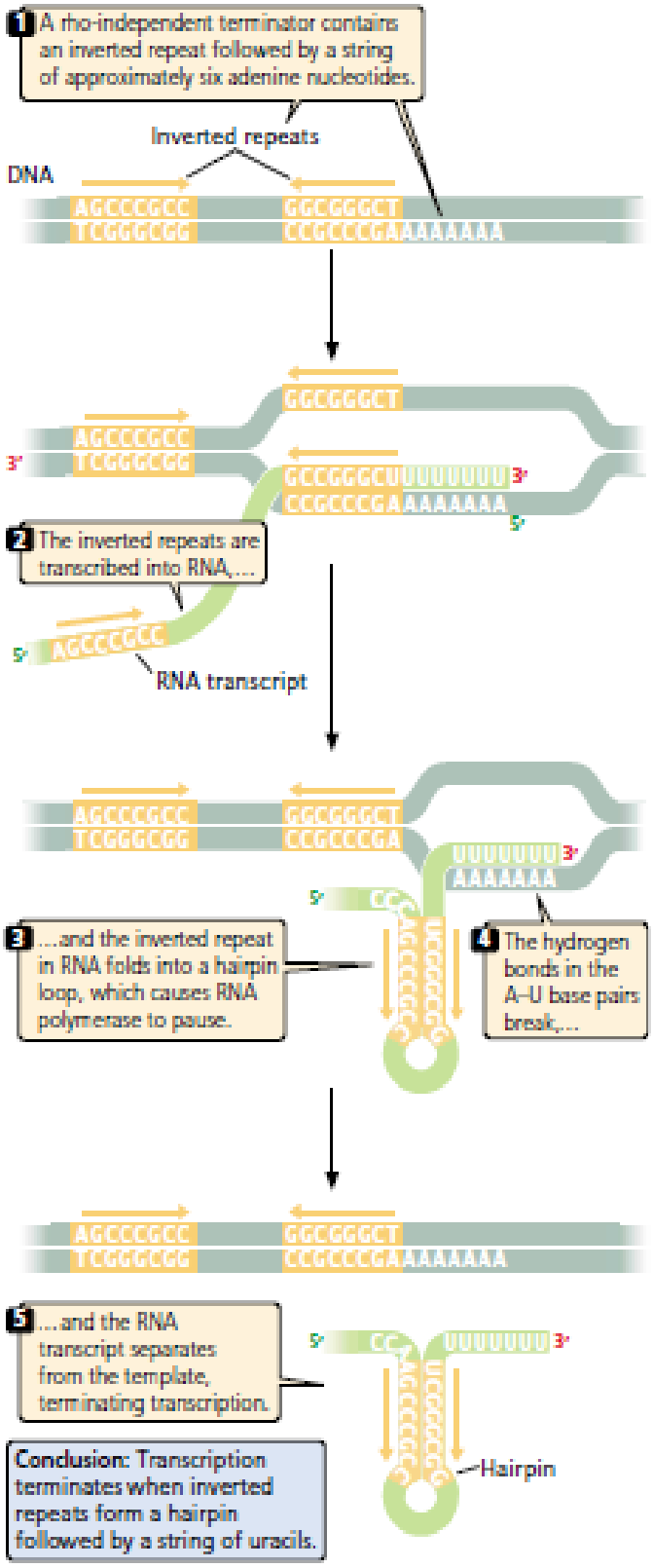

Fig. 7: Termination by bacterial rho-independent terminators is a multistep process.

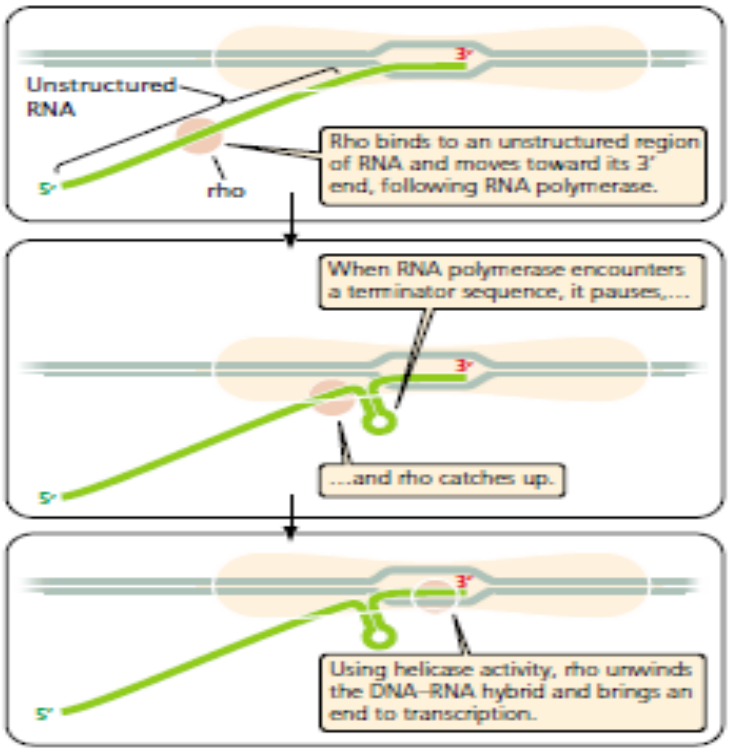

Fig. 8: The termination of transcription in some bacterial genes requires the presence of the rho protein.