

Transcription in Prokaryotes and Eukaryotes

4.1 INTRODUCTION

The prokaryotes include bacteria and archaea. They lack membrane bound nuclei and other organelles. A chromosome of DNA of bacteria is covalently closed circular DNA which is without histone proteins and is called nucleoid. Transcription in prokaryotes (and in eukaryotes) requires DNA double helix to form mRNA. It is the first stage of gene expression involving the synthesis of RNA from a DNA template with the help of enzyme called RNA polymerase. The difference between the process of transcription and replication is that Transcription occurs only at the **selected position of genome** while in replication **entire genome is copied**.

4.2 TRANSCRIPTION IN PROKARYOTES

Ribonucleic acid (RNA) is of three types i.e., mRNA, rRNA and tRNA. m-RNA transfer genetic information, so it works on genetic level whereas rRNA and tRNA works on functional level, rRNA has a role in ribosome formation while tRNA helps in transfer of amino acid to the m-RNA.

Let's discuss some terminology which one should know before studying the process of transcription.

- **Template strand:** Template strand of the DNA is the strand that is used for the synthesis of mRNA. It has polarity in 5' to 3' direction.
- **Coding strand:** DNA is a double-stranded molecule. Each strand carries all the genetic information needed for future generations and is complementary to the other strand i.e., template strand. The product of transcription process is almost identical to this **coding strand** which is also called is **non-template strand**.

The only difference is that m-RNA contains Uracil (U) in place of Thymine (T) because in RNA Adenine (A) binds with Uracil (U) with two hydrogen bonds.

- **Transcription bubble:** The double-helix of DNA must unwind at the region where the gene has to be transcribed. The region where DNA is opened up is called 'transcription bubble'.

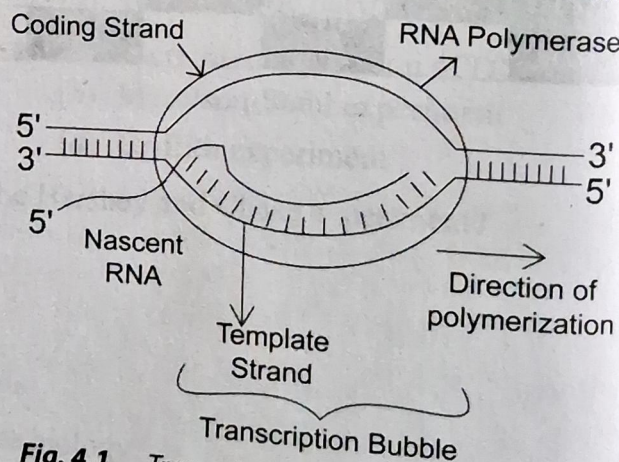


Fig. 4.1. Transcription bubble of prokaryotes.

- **Initiation site:** The site present on the DNA from where the first RNA nucleotide is transcribed. It is denoted as +1 site or called as initiation site.
- **Upstream and downstream site:** The nucleotides that come before the initiation site are given **negative numbers** and are called as **upstream site** whereas the nucleotides present after the initiation site are given positive numbers and are called as **downstream site**.

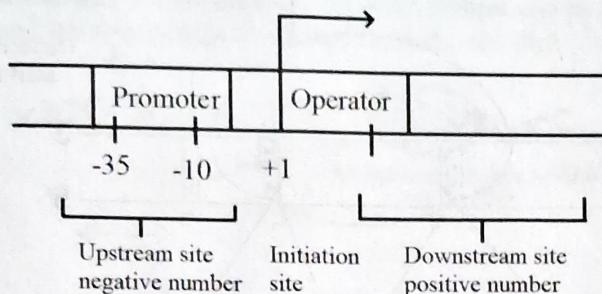


Fig. 4.2. Location of upstream & downstream sites in prokaryotes.

Prokaryotes do not have membrane enclosed nuclei. Therefore, the process of transcription and translation occurs simultaneously in the cytosol. The process of transcription is an **enzymatic process** and produces **polycistronic mRNAs** that covers more than one gene which codes for more than one protein. The enzyme involved in transcription is **RNA polymerase**. The beauty of RNA polymerase is that it can initiate the process of transcription by itself unlike DNA polymerase which required a primer to initiate replication.

The mechanism of transcription completes in three major steps

- Initiation
- Elongation
- Termination

(i) Initiation: The transcription is initiated by RNA polymerase holoenzyme from a specific point called **promoter** sequence.

RNA polymerase is composed of five subunits i.e., two copies of small subunit which is of 37 KDa in size, two large subunits of β and β' . This forms the core polymerase. The core polymerase along with σ -factor is called holo-enzyme i.e., RNA polymerase holoenzyme. This σ -factor binds with promoter and signals the polymerase to initiate transcription.

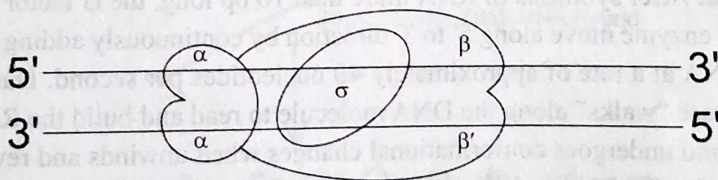


Fig. 4.3. Structure of RNA-polymerase holoenzyme.

A promoter region in prokaryotes consists of two conserved sequences 5' TTGACA 3' at -35 element and 5' TATAAT 3' at -10 element. Both these sequences are upstream to the site from where transcription begins. The binding of holoenzyme to two conserve sequence of promoter form closed complex, since the DNA is base paired. A promoter is a DNA sequence onto which the transcription machinery binds and initiates transcription. After formation of

closed complex, the RNA polymerase separates the hydrogen bonded base pairs in the region start site, forming an "open complex". The separated region extends for about 10-12 bp. After the RNA polymerase transcribes approx 10-12 base pairs the σ -factor is released. Thus, σ -factor acts as an "initiation factor".

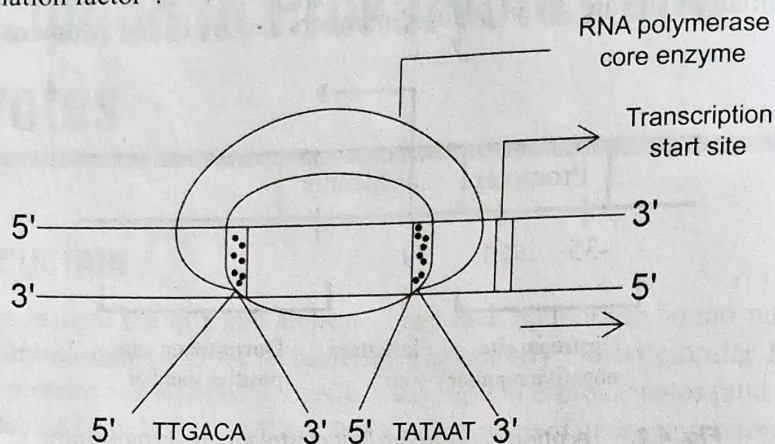


Fig. 4.4. Two-conserved regions of prokaryotes.

RNA polymerase can initiate a new chain synthesis using a DNA template and the first base which is added is usually Adenine (A) or Guanine (G) i.e., a purine. The RNA polymerase always binds a new RNA strand in 5' to 3' direction and catalyzes the formation of phosphodiester bonds between ribonucleotides 'precursors' i.e., ATP, GTP, UTP and CTP i.e., it can only add RNA nucleotides to the 3'OH end of the strand.

TABLE 4.1 Subunits of RNA polymerase of prokaryotes.

Prokaryotic RNA polymerase holoenzymes				
S. No.	Subunit	Size in KDa	No of molecule	Function
1.	2α	37	Two	Chain initiation and interacts with regulatory protein
2.	β	151	1	Chain initiation & elongation
3.	β'	156	1	DNA binding
4.	σ	70	1	Promoter recognition

(ii) **Elongation:** After synthesis of RNA more than 10 bp long, the σ factor is released. The RNA polymerase enzyme move along 5' to 3' direction by continuously adding nucleotides and synthesizing mRNA at a rate of approximately 40 nucleotides per second. During elongation, the RNA polymerase "walks" along the DNA molecule to read and build the RNA molecule in 5' to 3' direction and undergoes conformational changes when unwinds and rewinds the DNA. Elongation proceeds until RNA polymerase triggers to a special sequence called a **termination signal**.

(iii) **Termination:** Once a gene is transcribed and newly made mRNA is released a series of overlapping events are needed to bring to an end the transcription. The RNA polymerase is detached from the DNA template, releasing newly synthesized mRNA.

The bacterial cell possesses two major types of termination signals. One is **protein-based** and the other is **RNA - based**. Rho-dependent termination is controlled by rho-protein.

(i) Rho dependent termination

(ii) Rho-independent termination

(i) Rho-dependent termination: It contains rho-helicase protein that binds at RuT sites (Rho-utilization site) on RNA, which is an ATP-dependent protein and moves towards where the RNA polymerase binds using energy from ATP hydrolysis. Once rho-protein reaches the RNA polymerase site then it encounters DNA-RNA hybrid and at that time rho-helicase unwinds the duplex and RNA transcript is released from the complex.

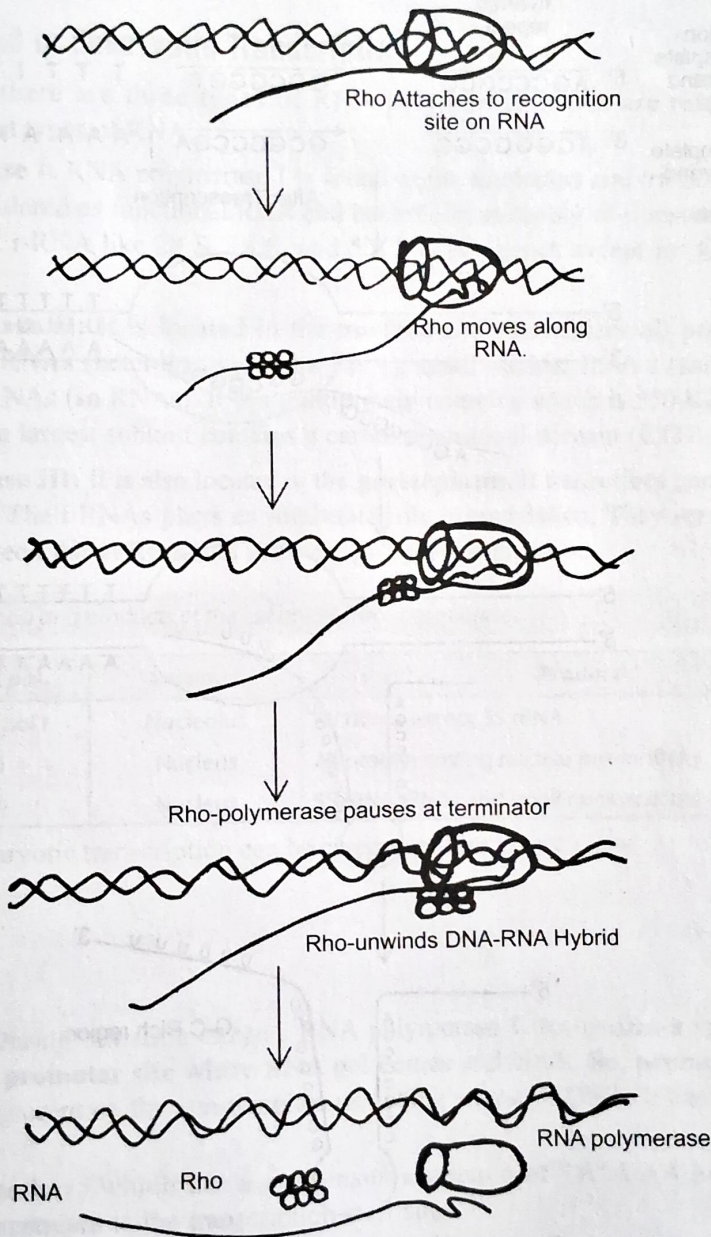


Fig. 4.5. Rho-dependent termination in prokaryotes.

(ii) Rho-independent termination: Rho independent termination is controlled by specific sequences in the DNA template strand. The terminator DNA contains inverted repeats. As the

RNA polymerase reaches the end of transcription, it encounters a G-C rich nucleotide region and this region folds back itself, as C and G are complementary nucleotides resulting in a stable **hairpin** formation which causes the polymerase to stall. This inverted repeat is followed by a string of approx. 6 Adenine nucleotides on template DNA and their transcription produces a string of **uracil nucleotides** on RNA forms a hairpin structure. The presence of hairpin in RNA transcript slows-down the RNA polymerase and RNA gets separated from RNA-DNA heteroduplex.

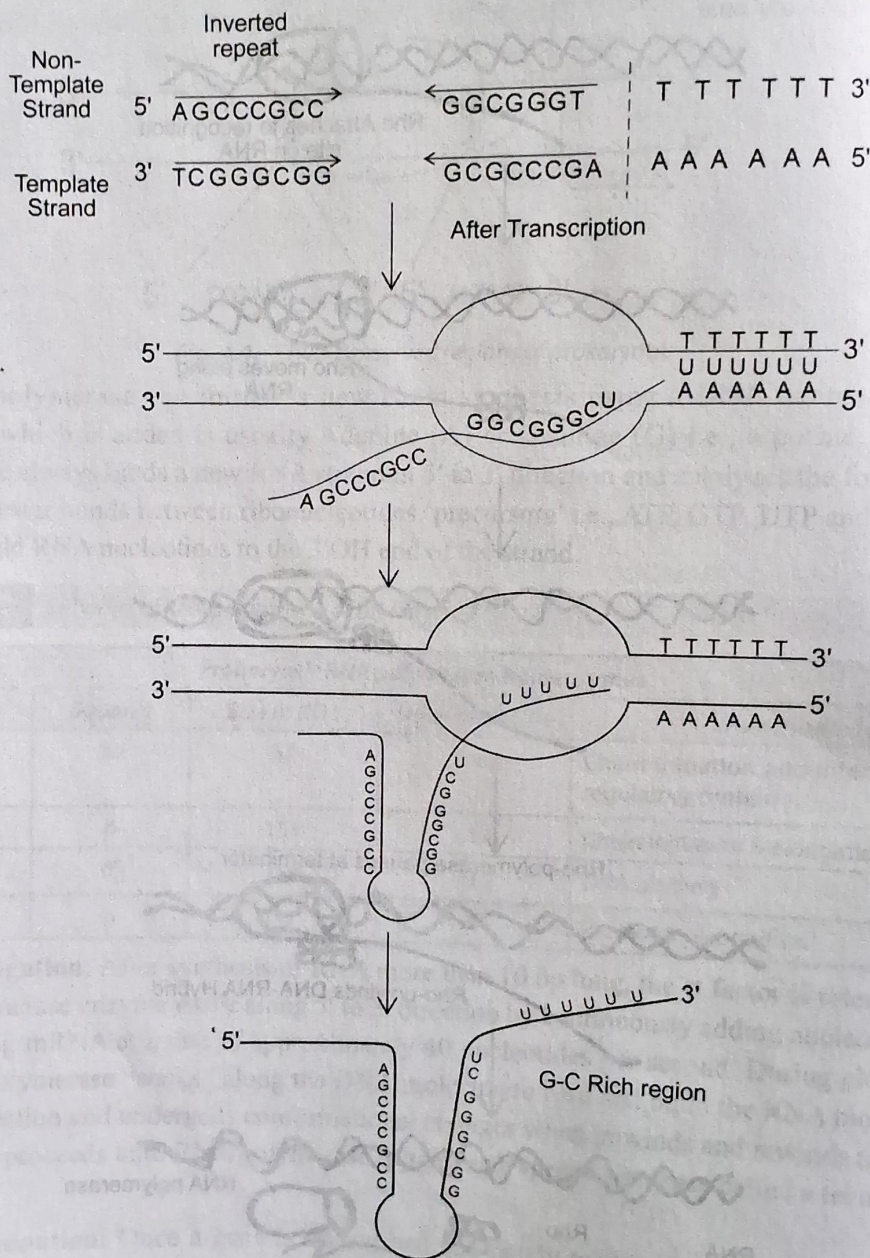


Fig. 4.6. Rho-Independent termination.

Upon termination, the process of transcription is complete. As we earlier discussed that in prokaryotes transcription and translation occurs simultaneously. As soon as the termination occurs the prokaryotic transcript would already have been used to synthesize protein.