TRANSCRIPTION AND RNA PROCESSING

Transcription And Post-transcriptional Processing In Prokaryotes

Transcription is defined as the synthesis of RNA molecule using DNA as a template that results in the transfer of the information stored in double-stranded DNA into a single-stranded RNA; which is used by the cell to direct the synthesis of proteins.

Cellular RNAs include:

- Messenger RNA (mRNA)
- Ribosomal RNA (rRNA)
- Transfer RNA (tRNA)
- Several small nuclear RNAs (snRNAs).

All are transcribed from DNA. The first three RNAs are involved in protein synthesis and snRNA is involved in mRNA splicing.

Basic Requirements for Replication

- Template
- Substrates
- Enzymes

Template

- A single-strand of DNA acts as a
- The DNA strand that is transcribed to RNA molecule is referred to as **template strand.**
- The other DNA strand is referred to as the **coding strand** of the gene.

Substrate

1. ATP
2. GTP
3. CTP
4. UTP

Enzyme

- DNA dependent RNA polymerase, called RNA polymerase (RNAP).
- Prokaryotes have single RNA polymerase (RNAP) that
- transcribes all three RNAs, i.e. mRNA, rRNA, and tRNA
- Eukaryotes have three RNA polymerases, designated I, II and III.

1. **RNA Polymerase I**: Synthesis of rRNA.

2. **RNA Polymerase II**: Synthesis of m-RNA and sn RNA.

3. **RNA Polymerase III**: Synthesis of tRNA.

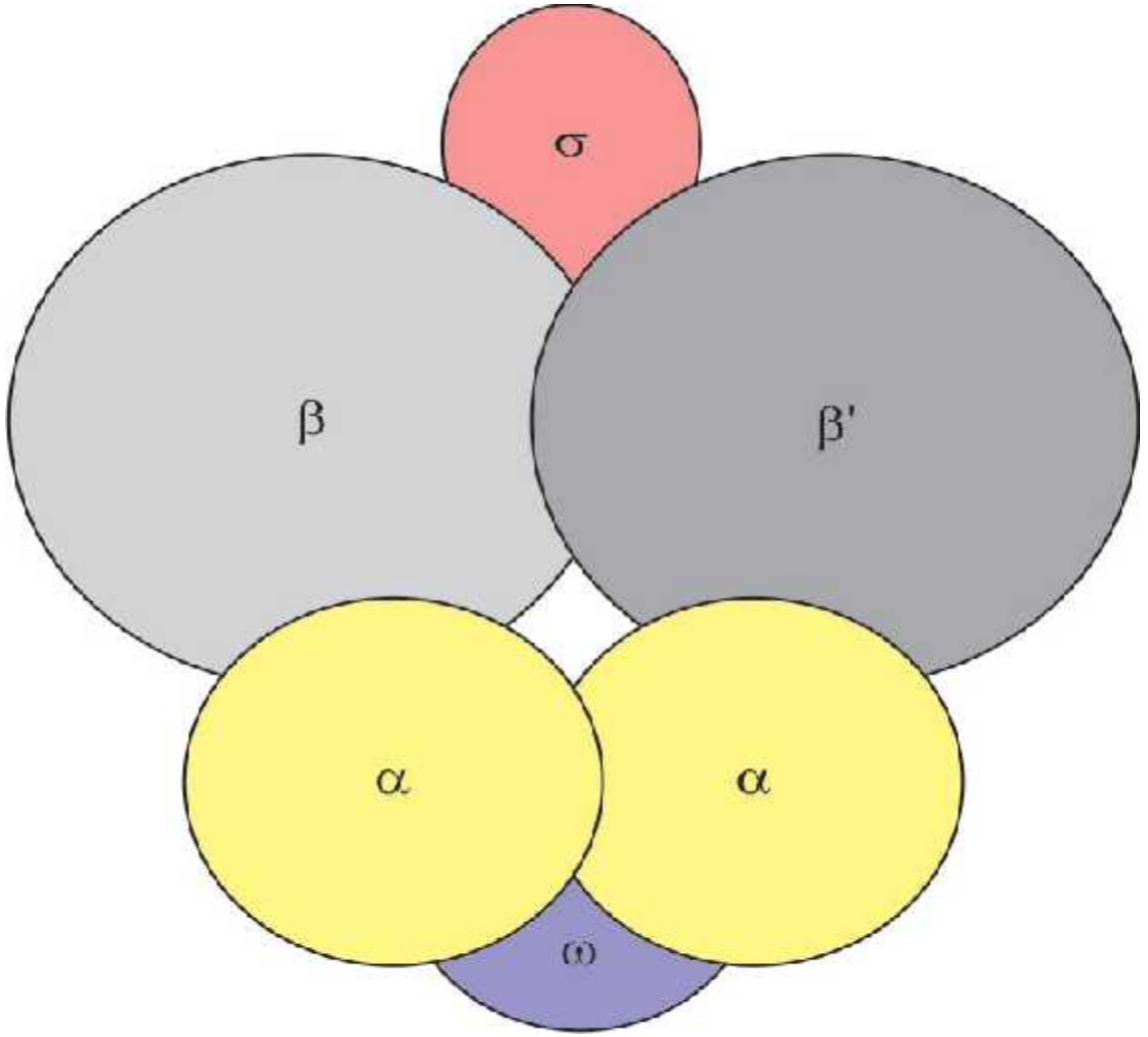
4. **Mitochondrial RNA polymerase (mtRNAP):** Synthesis of all the three types of RNA in mitochondria.

RNA polymerase (RNAP)

- RNAP contains five subunits (2α, β′, β, ω) which form the core enzyme.
- The active enzyme, the holoenzyme contains core enzyme and a sixth subunit called **sigma (σ) factor.**
- σ subunit binds to core enzyme and directs enzyme to specific binding sites on the DNA.

- RNA polymerases lack proofreading 3′ to 5′ exonuclease activity as that of DNA polymerases.
- RNA polymerase requires Mg^{2+} as well as Zn^{2+} for its activity.

Components of prokaryotic holoenzyme RNA polymerase.



Stages of Transcription

1. Initiation
2. Elongation
3. Termination

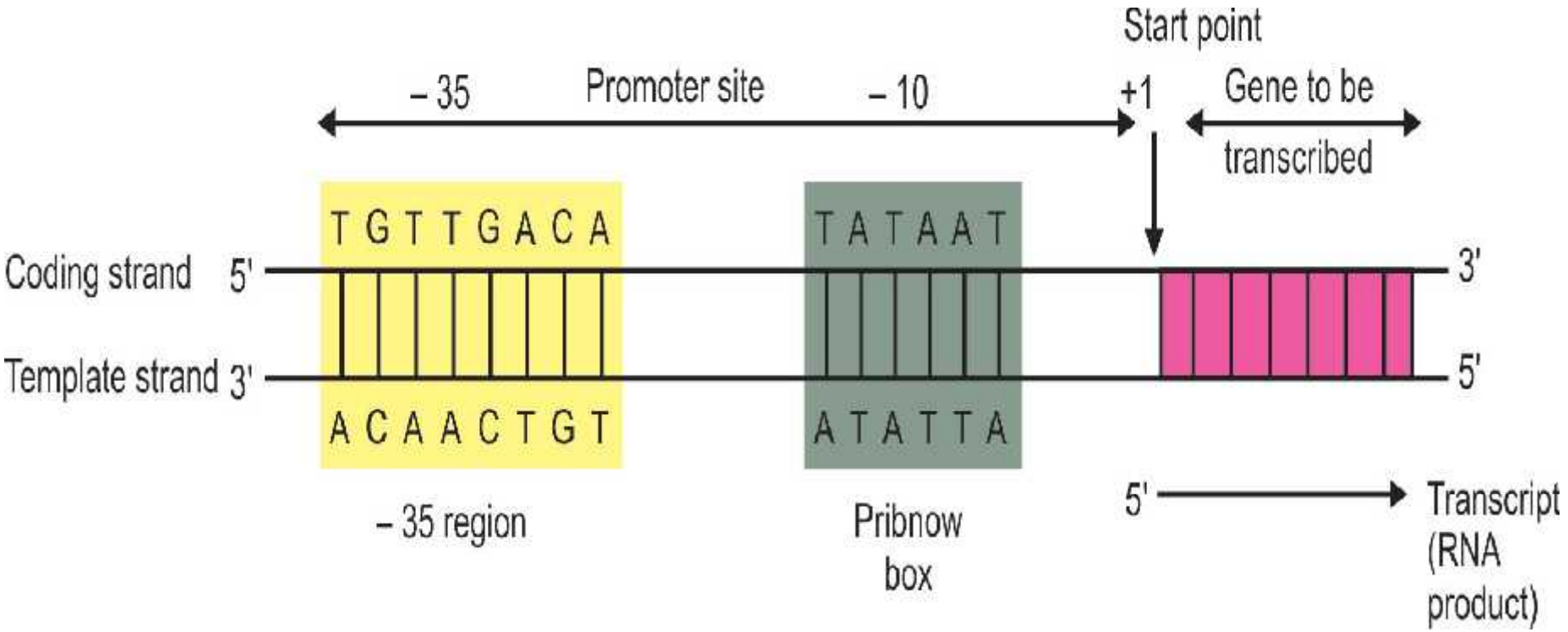
Initiation

- Initiation of transcription involves binding of RNA polymerase (core enzyme + σ factor) to DNA template at *promoter site.*
- The sigma factor facilitates the RNA polymerase to recognize and bind to promoter sequences.
- Promoters are characteristic sequences of DNA which are different in prokaryotes and eukaryotes

Prokaryotic genes have two promoter sequences.

1. Pribnow box (–10 region) has the nucleotide sequence TATAAT, found 10 base pairs away from (upstream) the start point.

2. –35 region, has nucleotide sequence TTGACA, found 35 base pairs away from (upstream) the start point.



Prokaryotic promoter sites for transcription.

- Promoter sequences directs RNA polymerase to initiate transcription at a particular point known as start point or initiation site.
- The binding of the RNA polymerase to the DNA template results in unwinding of DNA double helix.

- RNA polymerase then begins to synthesize a transcript of DNA sequence and short piece of RNA is made.
- As with replication, transcription is always in the 5′ to 3′ direction.
- The first base is usually a purine nucleotide.
- By the time 10 nucleotides have been added, the σ factor dissociates

Elongation

- Sigma is released and the RNAP move along the template stand and continues the elongation of the transcript.
- The process of elongation of the RNA chain continues until a termination signal is reached

Figures A to E: Process of transcription in prokaryotes

A: Recognition of promoter by sigma factor

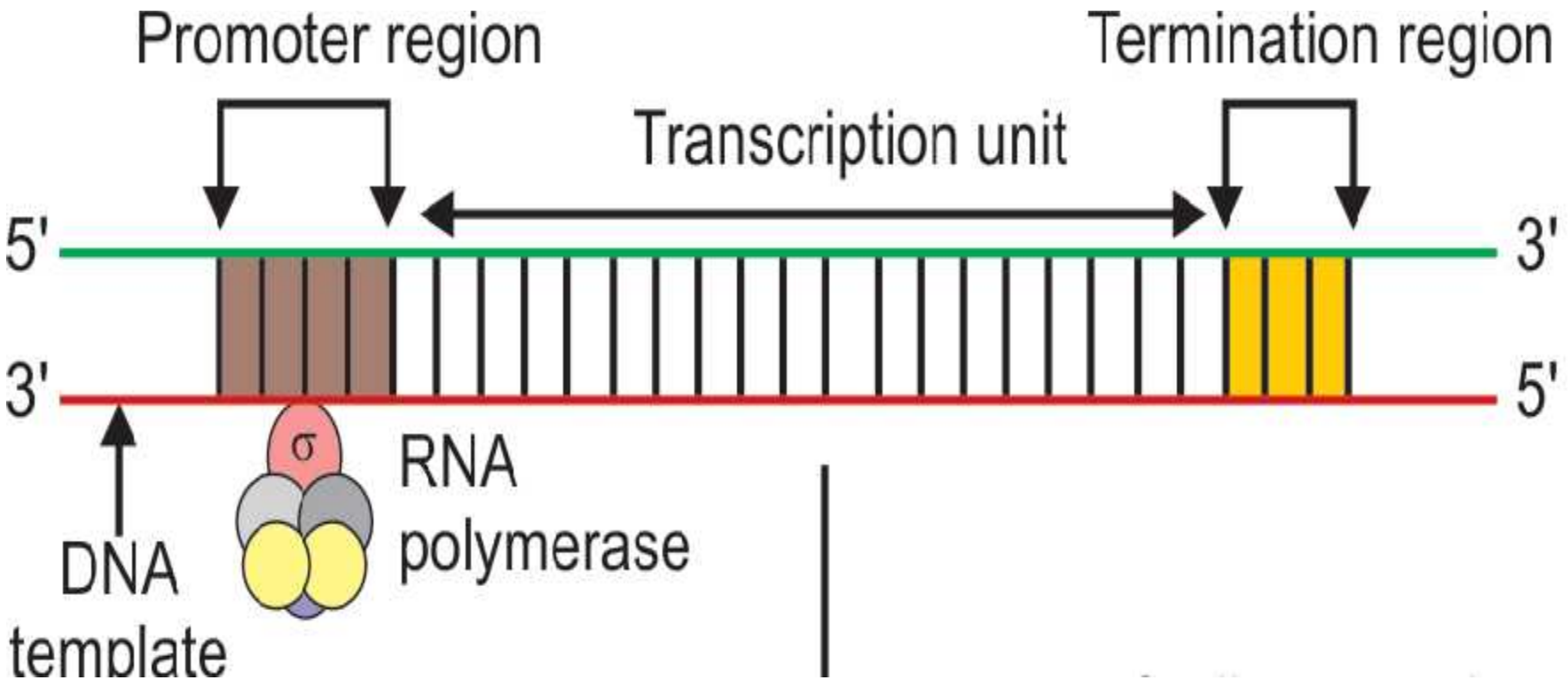
B: Binding of core enzyme and starts the synthesis of RNA

C: Elongation continues until termination region is reached

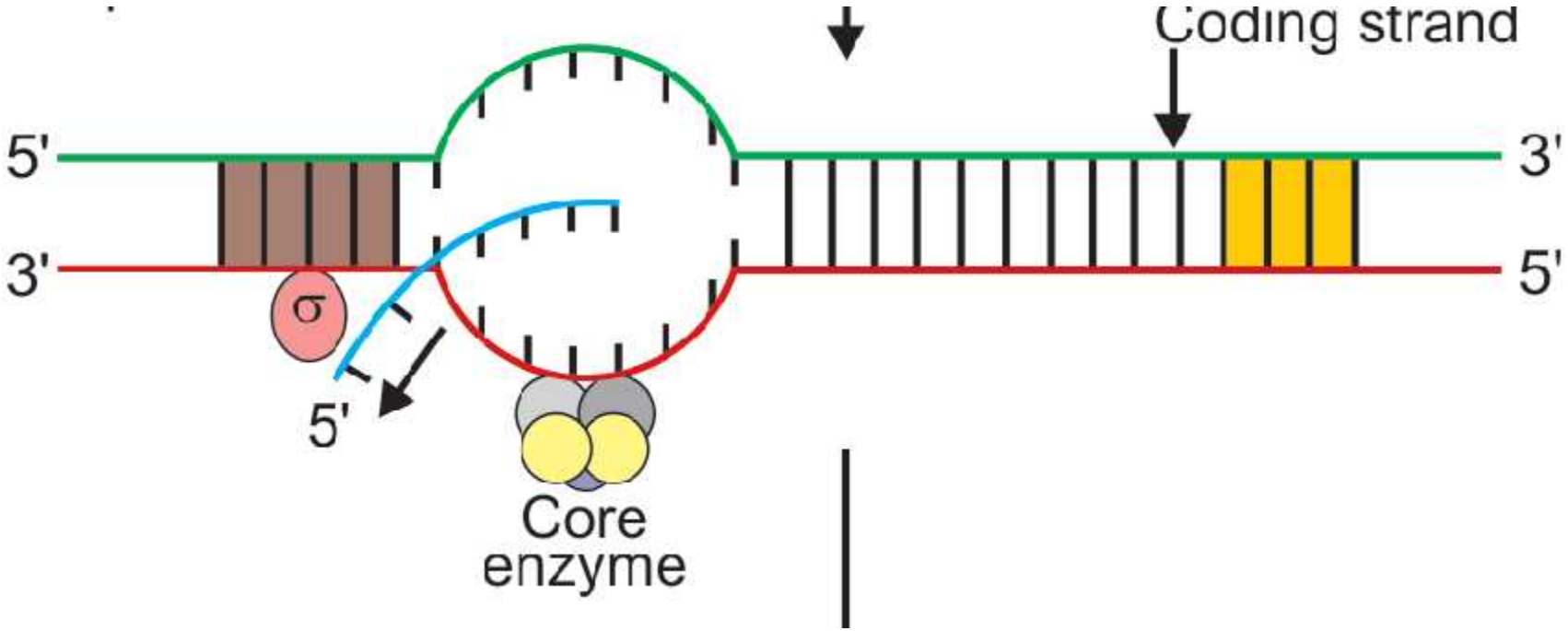
D: Termination of transcription by Rho factor

E: Newly synthesized RNA (primary transcript)

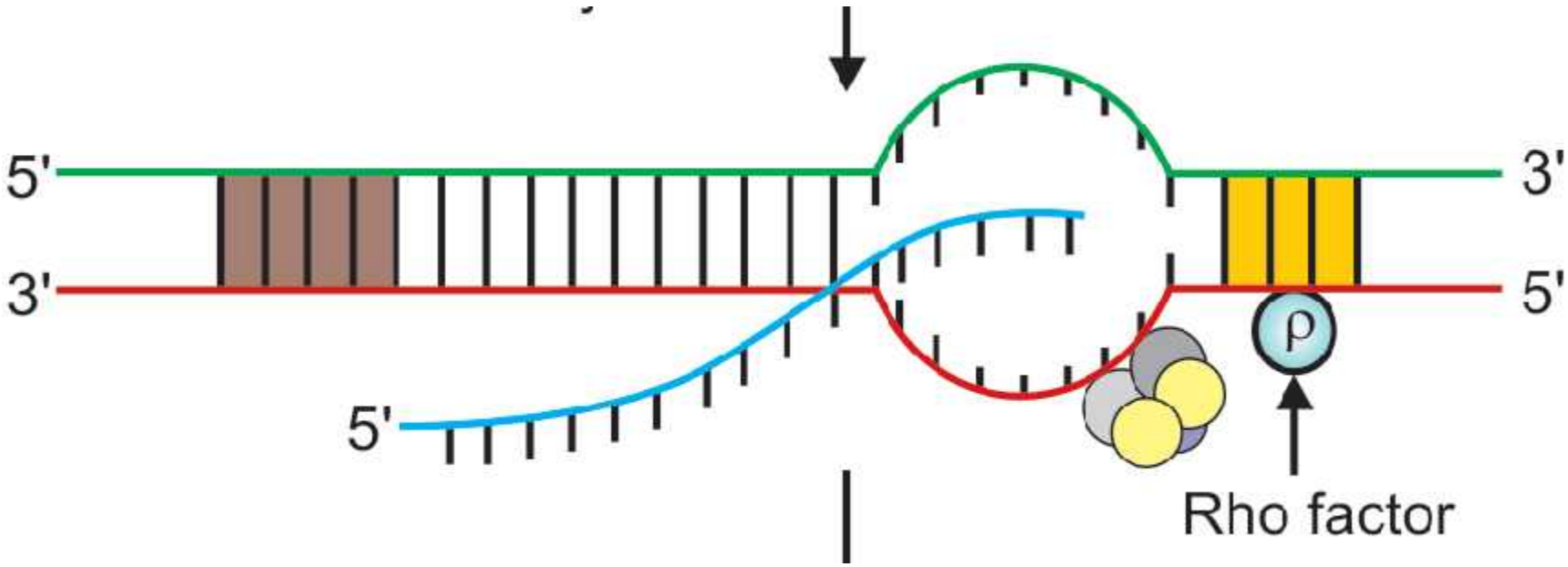
Recognition of promoter by sigma factor



Binding of core enzyme starts synthesis of RNA



Elongation continues until termination region is reached

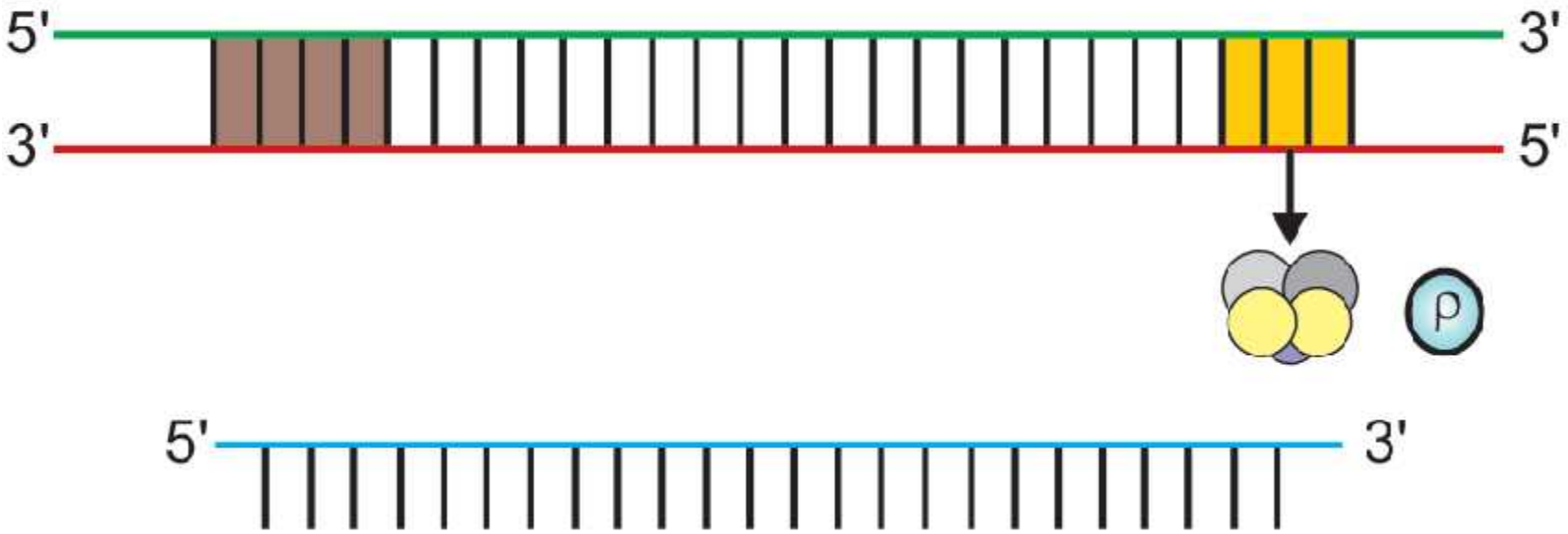


Termination

In prokaryotes, termination of transcription occurs by one of the two mechanisms:

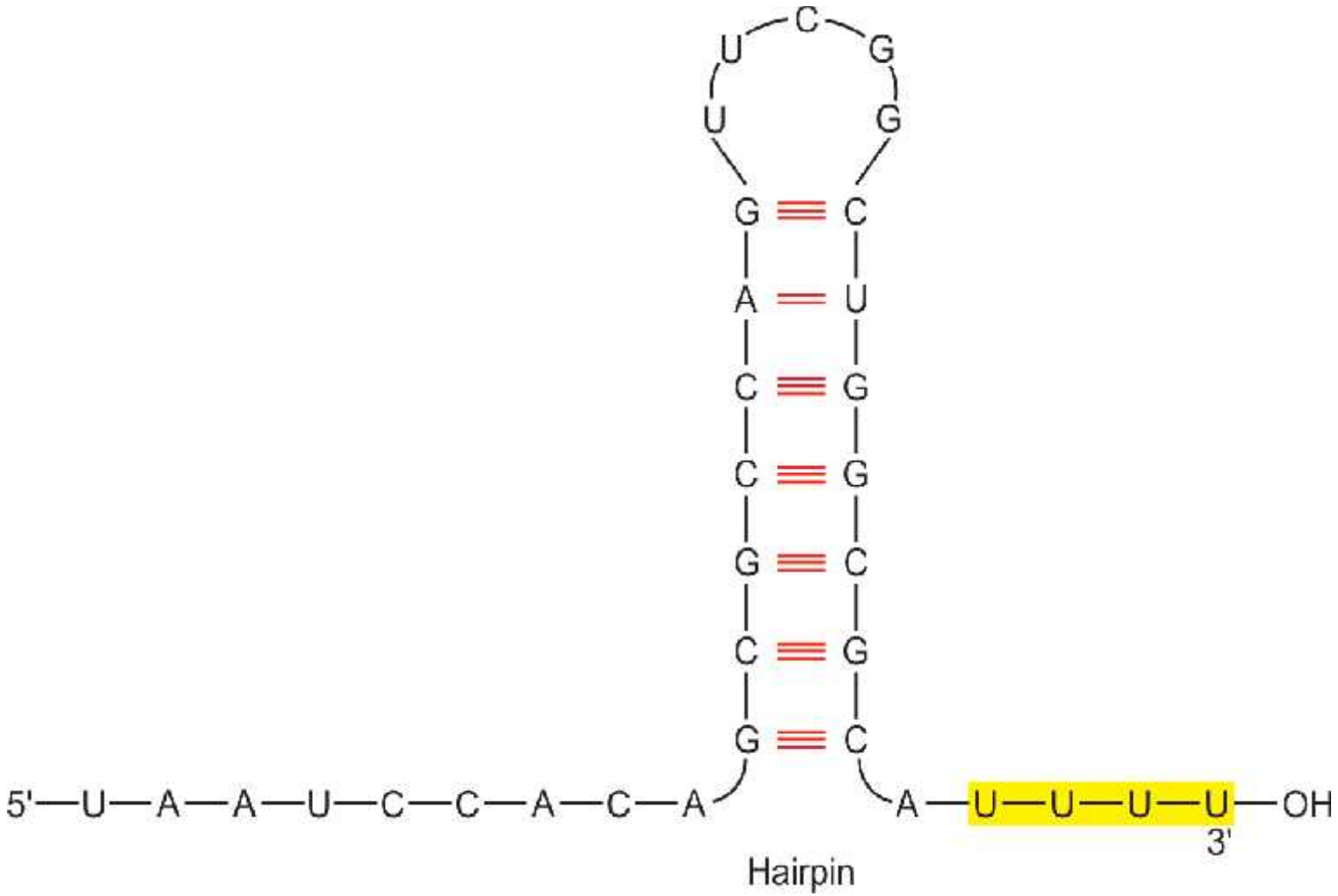
1. Rho-dependent
2. Rho-independent.

Termination of transcription by Rho factor



Newly synthesized RNA

A hairpin loop structure followed by a sequence of uridine residues at the 3’ end of an mRNA transcript



Hairpin

Post-transcriptional Processing

- The RNAs formed during transcription are called primary transcript. The primary transcript normally undergo further enzymatic alteration, called post- transcriptional processing.
- Post-transcriptional processing is required to convert the primary RNAs into functional or active forms.

Processing may involve either:

- Cleavage of large precursor of RNA to a smaller molecule
- Splicing
- Terminal addition of nucleotide
- Nucleoside modifications.

m-RNA processing

- In prokaryotes mRNA is not post-transcriptionally processed. Prokaryotic mRNA is functional imme- diately upon synthesis.
- In eukaryotes the primary transcript of mRNA is hnRNA (heterogeneous nuclear RNA).
- After transcription hnRNA is extensively modified to form functional mRNA.

1. 5'-capping:

The 5' end of eukaryotic mRNA is capped with 7-methylguanylate.

2. Addition of poly A tail:

The 3' end of most eukaryotic mRNAs posses a chain of 200–300 adenine nucleotides and called Poly A tail. Poly A tail is not transcribed by DNA but rather is added after transcription.

3. Removal of introns

- Introns are nucleotide sequences on mRNA that do not code for proteins. Exons are the coding sequences that code for proteins.
- The process by which introns are excised and exons are linked to form functional mRNA is called splicing.

Inhibitors of Transcription

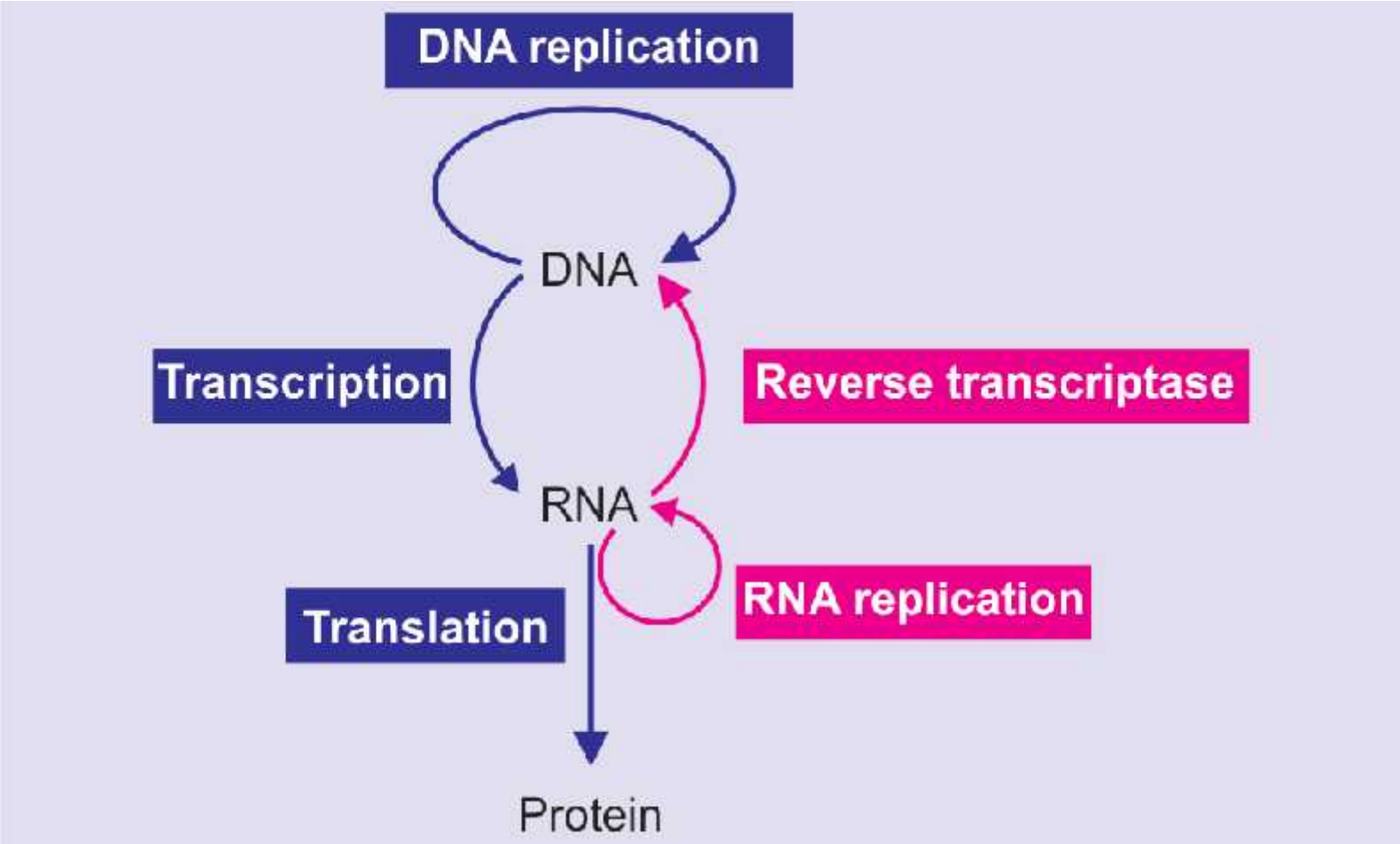
- Rifampin: It is an anti-tuberculosis drug, which inhibits the initiation of transcription by binding b-subunit of prokaryotic RNA polymerase. Rifampin has no effect on eukaryotic nuclear RNA poly- merases

- Dactinomycin (Actinomycin D): Dactinomycin is a therapeutic agent in the treatment of some cancer. It binds tightly and specifically to double helical DNA and thereby prevents the movement of the RNA polymerase.

REVERSE TRANSCRIPTION

- Certain RNA viruses that infect animal cells carry within the viral particle an **RNA-dependent DNA polymerase** called **reverse transcriptase**.
- The RNA viruses that contain reverse transcriptases are known as **retroviruses** (*retro* is the Latin prefix for “backward”) because the genetic information flows from RNA to DNA rather than from DNA to RNA.
- Reverse transcriptase produces DNA from viral RNA.

Extension of the central dogma to include RNA dependent synthesis of RNA and DNA.



- Some retroviruses, classified as **RNA tumor viruses**, contain an **oncogene** that can cause the cell to grow abnormally.
- The first retrovirus of this type to be studied was the **Rous sarcoma virus.**
- Another important class of retroviruses includes **human immunodeficiency virus** (HIV), identified in 1983, which causes **acquired immunodeficiency syndrome (AIDS)**.

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Thank You