Cell Biology, Genetics and Evolution

Transcription

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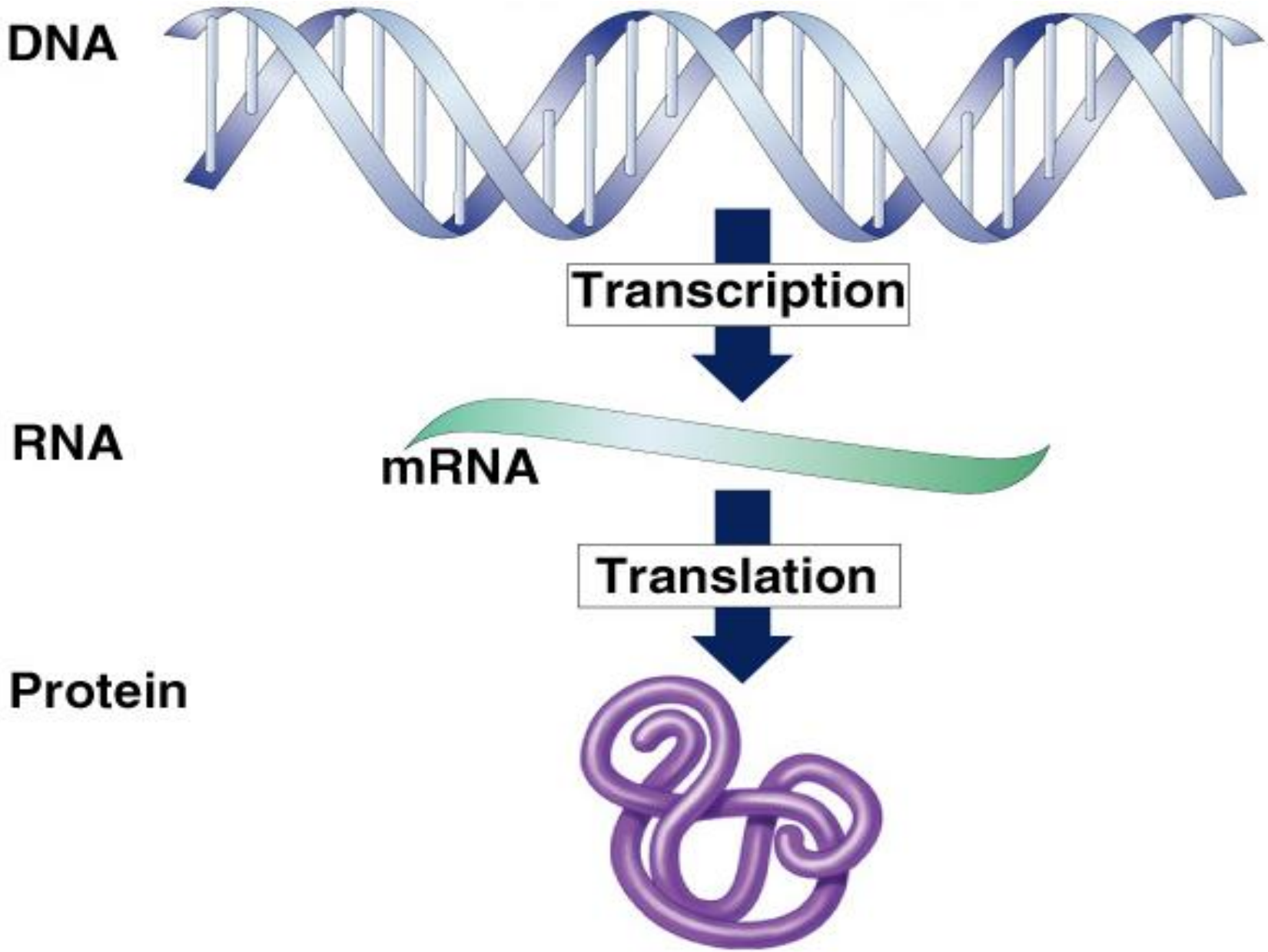
Processes of Protein Synthesis

- **a. Transcription –**
- Genetic template (mRNA) for a protein is copied and carried out to the cytoplasm

- **b. Translation –**
- Template serves as a series of codes for the amino acid sequence of the protein

Cells Use RNA to Make Protein

- **The RNA Players** – mRNA, rRNA, tRNA
- During polypeptide synthesis, ribosomal RNA (rRNA) is the site of polypeptide assembly.
- Transfer RNA (tRNA) transports and positions amino acids.
- Messenger RNA (mRNA) directs which amino acids are assembled into polypeptides.
- **Central Dogma**
- DNA > RNA > Protein


DNA
Transcription
RNA
mRNA
Translation
Protein

Differences between replication and transcription

	Replication	Transcription
Template	Both strand whole genome	single strand small portion of genome
Primer	yes	no
Enzyme	DNA polymerase	RNA polymerase
Product	dsDNA	ssRNA
Base pair	A-T, G-C	A-U, T-A, G-C
Proof reading	yes	no

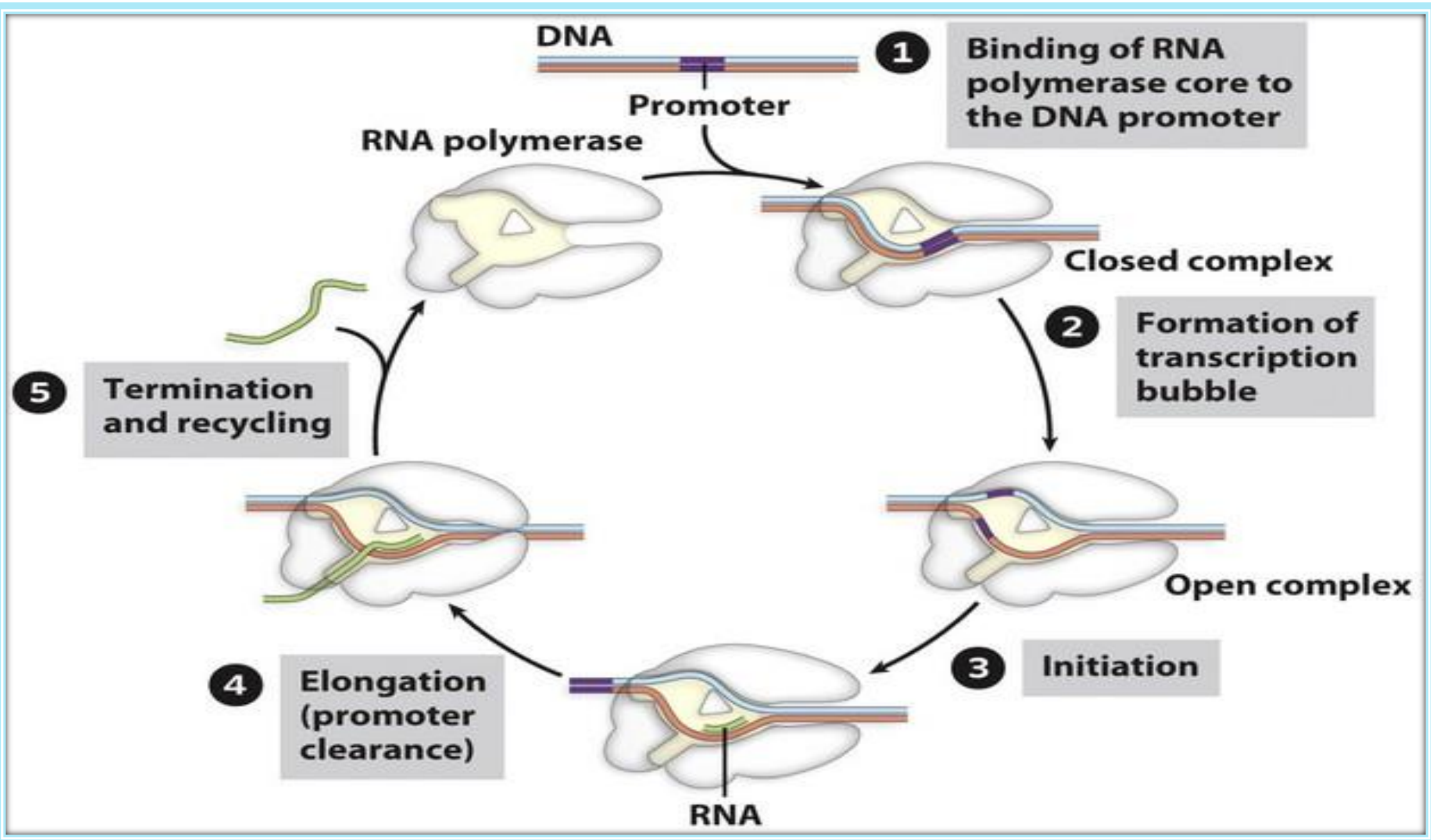
Transcription

- Transcription is the process in which the information in a DNA sequence is copied into a messenger RNA (mRNA) molecule.
- This process occurs in the nucleus of eukaryotic cells and in the cytoplasm of prokaryotic cells.
- **Important Molecules in Transcription:**
- **DNA:** The template that contains the genetic instructions.
- **RNA polymerase:** The enzyme responsible for synthesizing the RNA strand.
- **Nucleotides:** The building blocks of RNA (adenine [A], cytosine [C], guanine [G], and uracil [U]).

Stages of Transcription

- Initiation
- Elongation
- Termination

- **An overview of transcription:**
- DNA binding at the promoter leads to initiation of transcription by the polymerase holoenzyme, followed by elongation and termination.

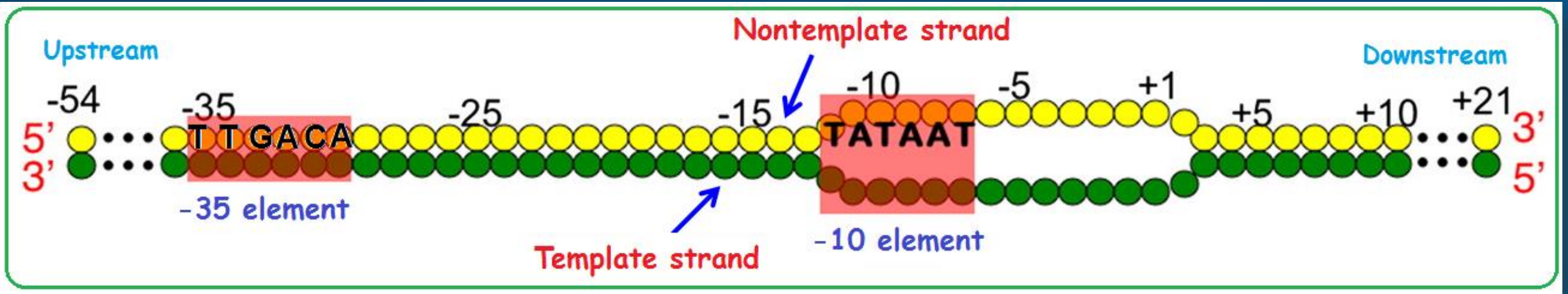

DNA
Promoter
1
Binding of RNA polymerase core to the DNA promoter
RNA polymerase
Closed complex
2
Formation of transcription bubble
Open complex
3
Initiation
4
Elongation (promoter clearance)
RNA
5
Termination and recycling

Initiation

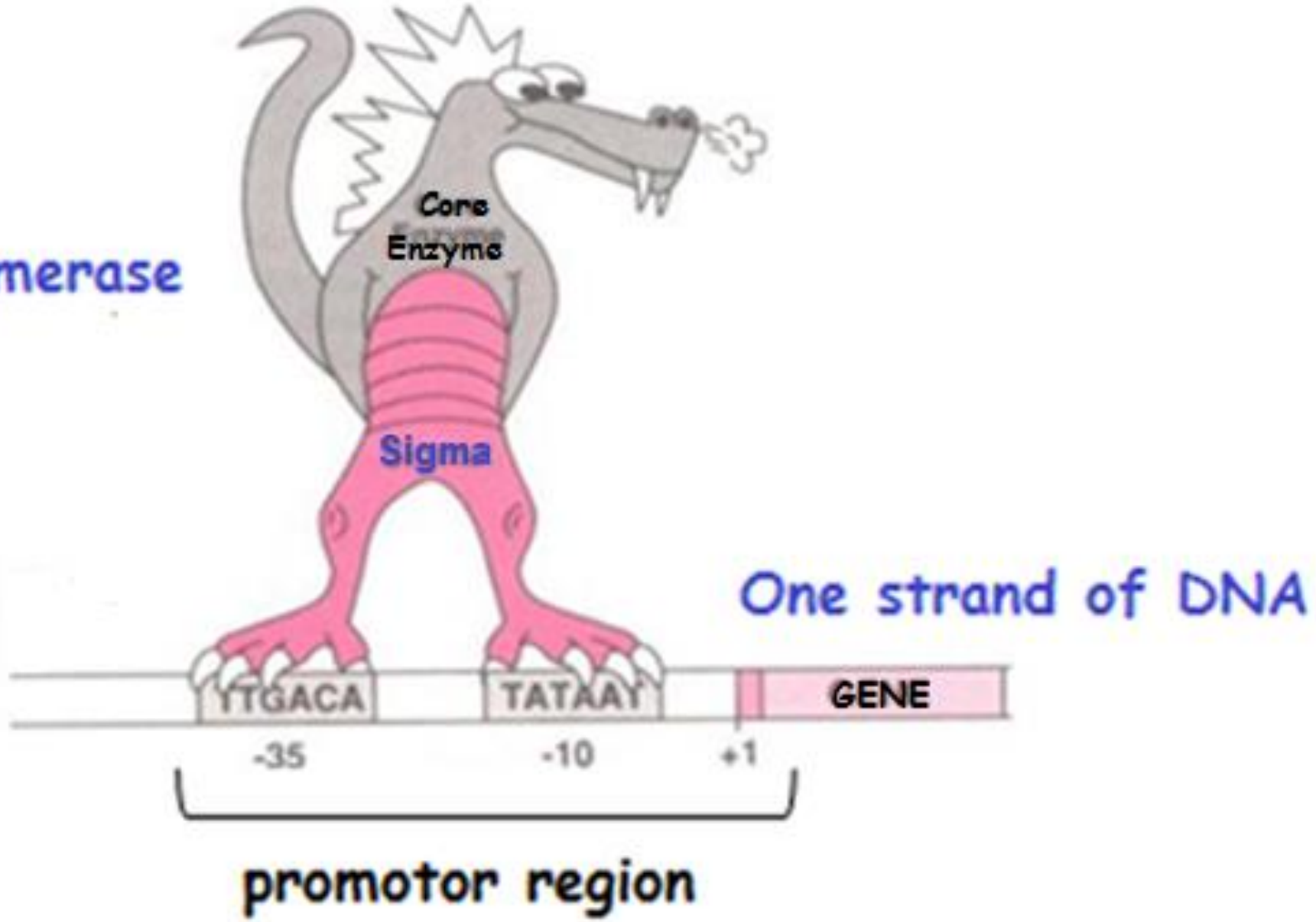
- **Promoter Region Binding:** The transcription process begins when RNA polymerase binds to a specific sequence of DNA known as the promoter. The promoter is usually located upstream (before) the gene that needs to be transcribed.
- **Unwinding the DNA:** Once RNA polymerase binds to the promoter, it causes the DNA double helix to unwind, exposing the template strand of DNA. One of the two strands of DNA serves as the template strand for RNA synthesis.
- **Transcription Factors (in eukaryotes):** These are proteins (sigma factors) that help position RNA polymerase on the promoter region and regulate the beginning of transcription.

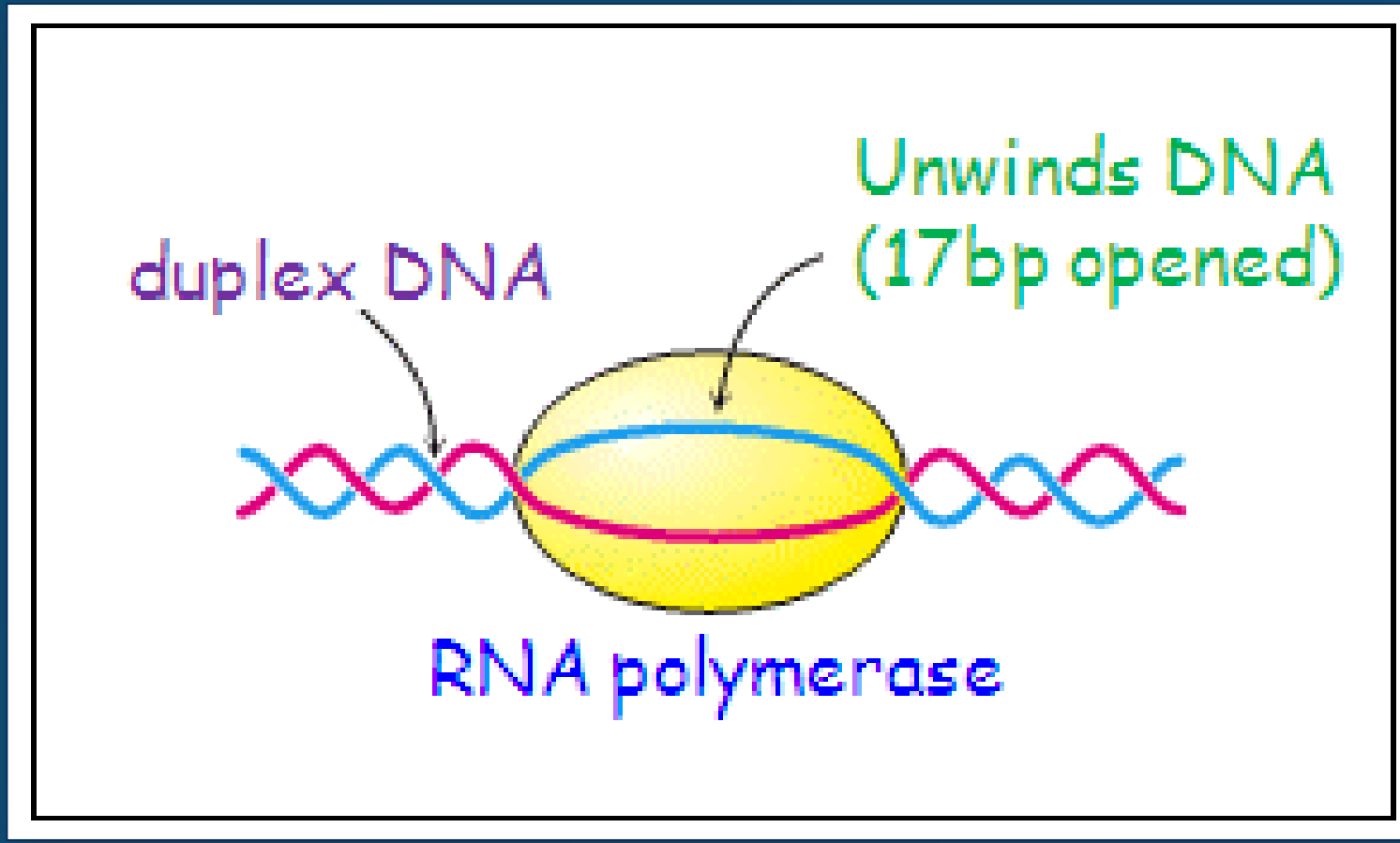
Promoter Sites

- Transcription starts at the RNA polymerase binding site called promoter on the DNA template strand. In prokaryotes within promoter there are two binding sites TTGACA also called -35 sequence and TAT A AT sequence also called -10 sequence, which have affinity for the RNA polymerase. In eukaryotes these sites are at -75 and -25 sites, respectively



RNA Polymerase



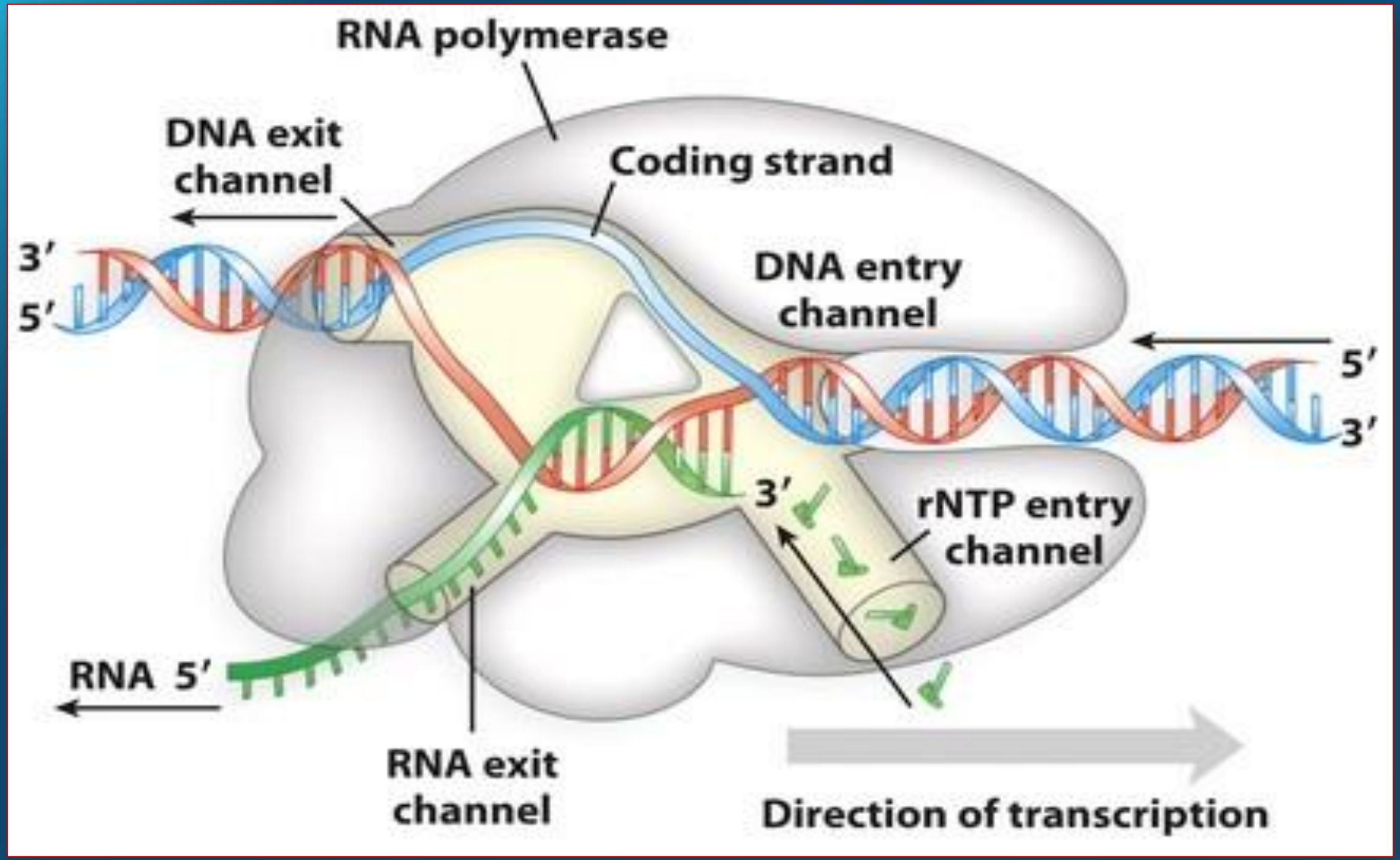

Unwinds DNA
(17bp opened)
duplex DNA
RNA polymerase

Role of Sigma Factor

- Recognizes promoter sequences and guides RNA polymerase to the right starting point for transcription.
- Unwinds DNA at the promoter region to create the transcription bubble.
- Facilitates transcription initiation by helping RNA polymerase begin RNA synthesis.
- Dissociates after initiation, allowing elongation to proceed.
- Regulates gene expression by using different sigma factors for different environmental or cellular conditions.
- In essence, the sigma factor ensures that transcription begins at the correct location and in response to specific signals, making it a key regulator of gene expression in prokaryotes.

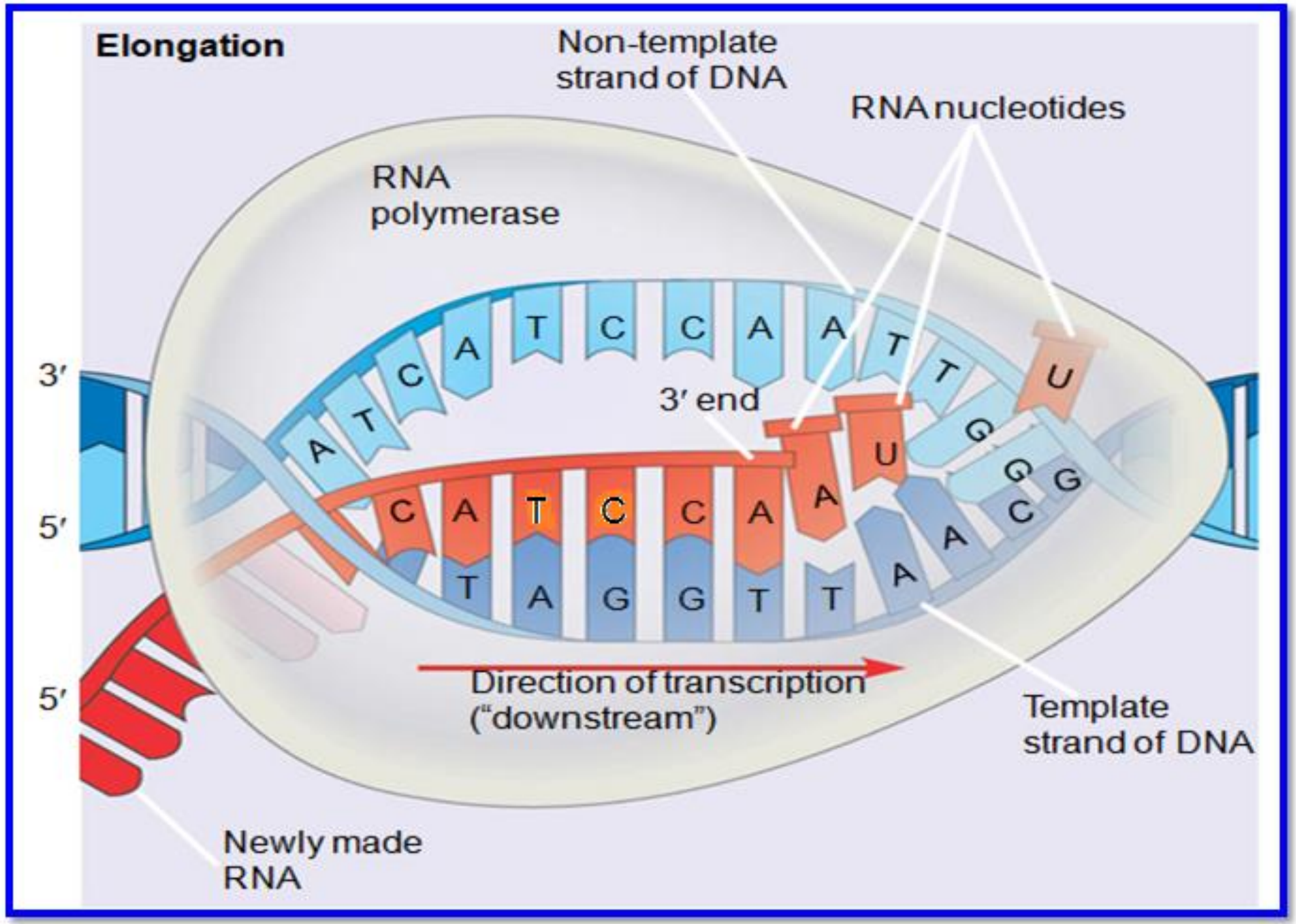
Elongation

- **RNA Polymerase Movement:**
- After RNA polymerase is bound and the DNA is unwound, the enzyme starts to "read" the template strand of DNA in the 3' to 5' direction.
- The RNA strand is synthesized in the 5' to 3' direction, meaning new nucleotides are added to the 3' end of the growing RNA molecule.


RNA polymerase
DNA exit channel
Coding strand
3′
5′
DNA entry channel
5′
3′
3′
rNTP entry channel
RNA 5′
RNA exit channel
Direction of transcription

Elongation

- **Nucleotide Pairing:** RNA polymerase adds RNA nucleotides that are complementary to the DNA template strand:
- Adenine (A) pairs with Uracil (U) in RNA (instead of thymine [T] as in DNA).
- Cytosine (C) pairs with Guanine (G).
- Guanine (G) pairs with Cytosine (C).
- Thymine (T) pairs with Adenine (A).
- **RNA Strand Growth:** As RNA polymerase moves along the DNA, the RNA strand elongates. The RNA strand will be complementary to the DNA template but with uracil (U) instead of thymine (T).


Elongation
Non-template strand of DNA
RNA nucleotides
RNA polymerase
3′
5′
3′ end
5′
Direction of transcription ("downstream")
Template strand of DNA
Newly made RNA

Termination

- **Termination Signal:** Eventually, RNA polymerase reaches a specific sequence on the DNA known as the terminator sequence. This signals the end of transcription.
- **Release of mRNA:** The newly formed mRNA (also called the pre-mRNA in eukaryotes) detaches from the DNA template.
- **DNA Rewinds:** After the RNA strand is synthesized, the DNA double helix reforms as the RNA polymerase moves away.

Termination

- **Stop Signal:**
- The stop sequences at the end of the gene terminate the synthesis of mRNA. The simplest stop signal is a series of GC base pairs followed by a series of AT base pairs.
- The RNA formed in this region forms a GC hairpin followed by four or more U ribonucleotides. The hairpin causes RNA polymerase to stop synthesis.

Post-Transcriptional Modifications

- In bacteria the newly synthesized mRNA is directly released into the pytoplasm, when it is converted into polypeptide chain.
- In eukaryotes however, it has to travel long distance from inside the nucleus to ribosomes outside in the cytoplasm. The eukaryotic mRNA is therefore modified in several ways to aid this journey.

Post-Transcriptional Modifications

- A cap and a tail is added so that the molecule may remain stable during long journey to ribosome.
- The cap is in the form of 7 methyl GTP, which is linked 5’ to 5’ with the first nucleotide
- Tail is in the form of many A nucleotides linked to 3’ end of the RNA.
- These caps and tails save the mRNA from variety of nucleases and phosphatases.

Final Product: mRNA

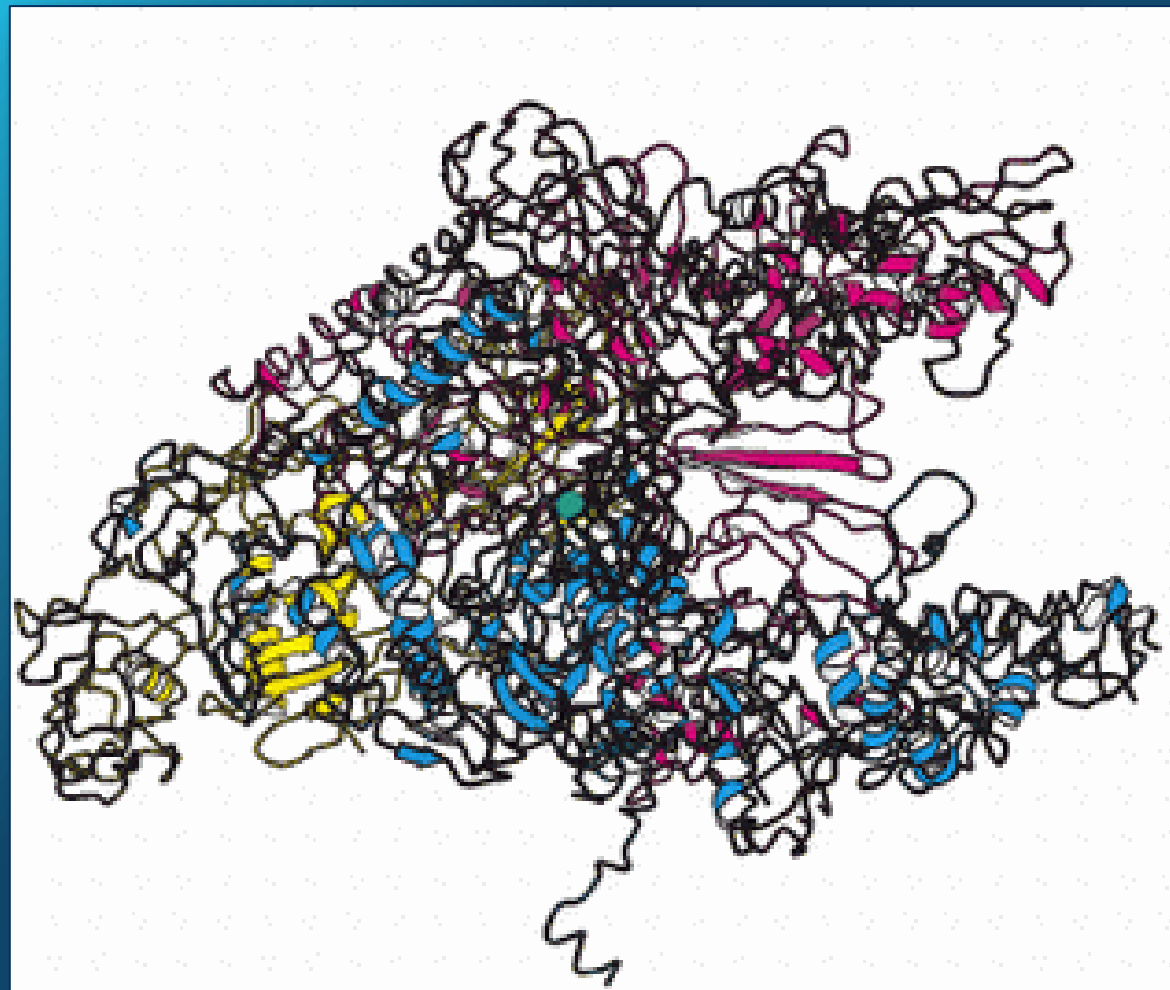
- The mature mRNA molecule is now a copy of the gene's instructions.
- In eukaryotic cells, it leaves the nucleus through the nuclear pore and enters the cytoplasm, where it will be used in translation to make proteins.

Summary

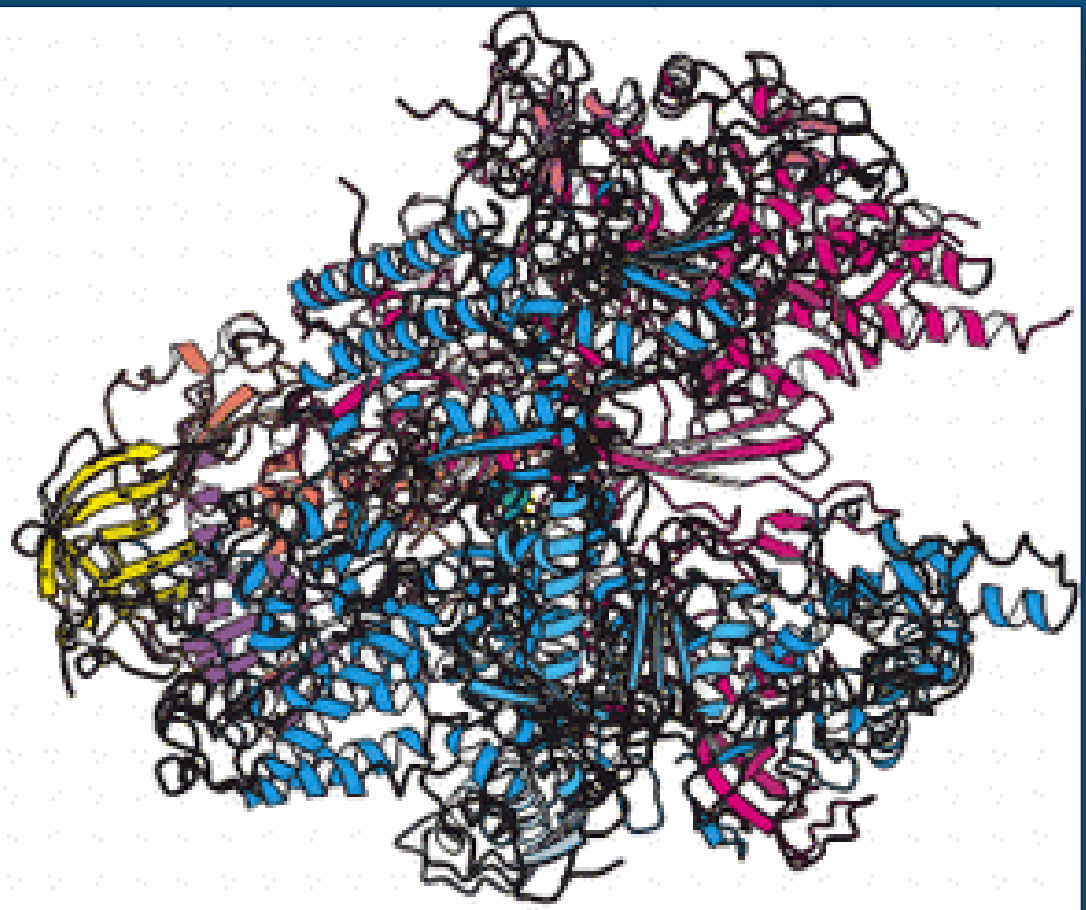
- **Initiation:** RNA polymerase binds to DNA at the promoter and unwinds the DNA.
- **Elongation:** RNA polymerase reads the DNA template strand, adding complementary RNA nucleotides.
- **Termination:** Transcription ends at the terminator sequence, releasing the RNA.
- **Post-transcriptional modifications:** In eukaryotes, the RNA is processed into mature mRNA.

Functions of RNA Polymerase

- It searches DNA for initiation sites, called promoter sites.
- It unwinds a short stretch of double-helical DNA to produce a single-stranded DNA template from which it takes instructions.
- It selects the correct ribonucleoside triphosphate and catalyzes the formation of a phosphodiester bond. This process is repeated many times as the enzyme moves unidirectionally along the DNA template.
- It detects termination signals that specify where a transcript ends.
- It interacts with activator and repressor proteins that modulate the rate of transcription initiation. These proteins, which play a more prominent role in eukaryotes than in prokaryotes, are called transcription factors.
- Gene expression is controlled mainly at the level of transcription



Prokaryotic RNA polymerase



Eukaryotic RNA polymerase

- RNA Polymerase Structures: The similarity of these structures reveals that these enzymes have the same evolutionary origin and have many mechanistic features in common.


Thank
You!