

Quadrant II – Transcript and Related Materials

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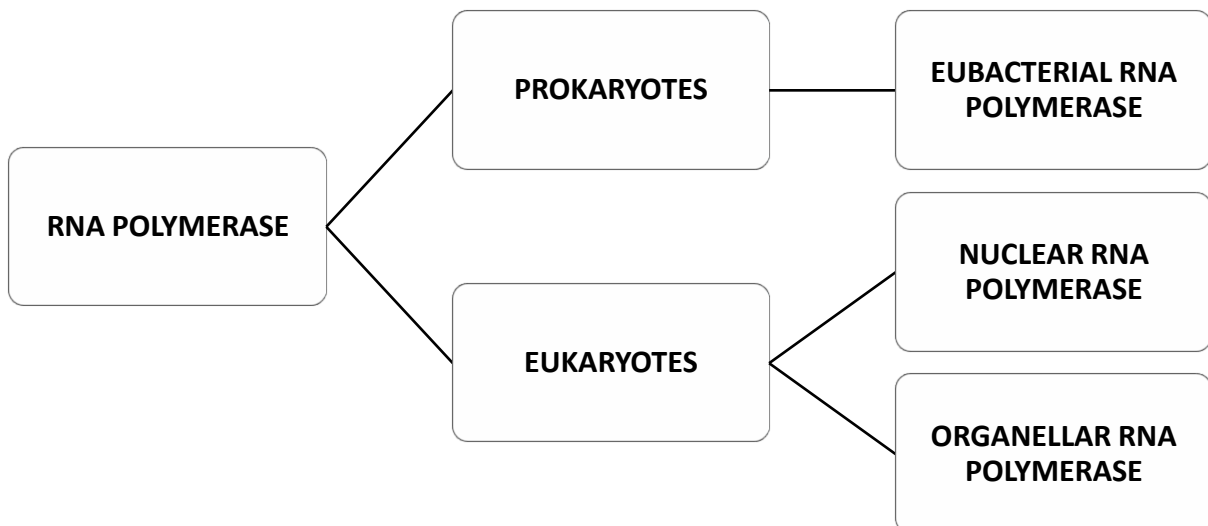
Module Name: Enzymes in transcription, Transcription-Initiation, Elongation and Termination

Module No: 13

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Notes:

Enzymes in Transcription



Eubacterial RNA polymerase

RNA polymerase enzyme was discovered by Samuel B. Weiss & Jerard Hurwitz in 1960. In eubacteria, single type of RNA polymerase is responsible for the synthesis of all different types of RNA such as mRNA, rRNA & tRNA. In *E.coli*, RNA polymerase is a multi subunit enzyme made up of 5 different polypeptides – α , β , β' , ω , σ . The holoenzyme ($\alpha\beta\beta'\omega\sigma$) can be separated into 2 components: the core enzyme ($\alpha\beta\beta'\omega$) & the sigma factor (σ).

SUBUNITS	FUNCTION
α	Assembly of the core enzyme Promoter recognition
β	Catalytic centre Involved in chain elongation
β'	Catalytic centre
ω	Assembly of RNA polymerase Stabilizes assembled RNA polymerase
σ	Promoter recognition Transcription initiation

Basic Features of transcription

- The new strand is made from ribonucleotides (NTPs) rather than deoxyribonucleotides (dNTPs).
- It is primer independent. RNA polymerase can initiate a new RNA chain on a DNA template without the need for a primer.
- It is a selective process.
- Transcription selectively copies only certain parts of the genome called a transcription unit. In contrast, during replication entire genome replicates.
- Only one of the DNA strands acts as a template on which the RNA strand is built.
- It is unidirectional.

Transcription unit

- Transcription is a selective process.
- Each transcribed segment of DNA is called a TRANSCRIPTION UNIT.
- It starts from transcription start site & terminates at transcription termination site.
- Eukaryotes: a transcription unit carries the information of just one gene called monocistronic transcription unit.

- Prokaryotes: a set of adjacent genes is transcribed as a unit called polycistronic transcription unit.
- The immediate product of transcription is called PRIMARY TRANSCRIPT.
- Transcription starts from the first base pair – TRANSCRIPTION START SITE.
- From this site, RNA polymerase moves along the template, synthesizing RNA, until it reaches TERMINATION SITE.
- Sequences prior to the transcription start site are described as UPSTREAM of it.
- Sequences after the start site are DOWNSTREAM of it.
- Upstream sequences are allotted negative values.
- Downstream sequences are allotted positive values.
- During transcription, only one strand of the transcription unit is transcribed.
- The transcript is identical in sequence with 1 strand of the DNA – CODING STRAND.
- The transcript is complementary to the other strand – TEMPLATE STRAND.
- The coding strand is also known as sense (+) strand.
- The template strand is known as antisense (-) strand.
- In principle, any region of the DNA double helix could be copied into 2 different RNA molecules – one from each of the two DNA strands.
- In reality, only one DNA strand is used as a template for each region.

Transcription in *E.coli*

- Transcription can be divided into 3 stages:
 - ✓ Initiation
 - ✓ Elongation
 - ✓ Termination
- Transcription begins when RNA polymerase molecule binds to a Promoter sequence on the DNA double helix.
- The RNA polymerase then moves along the template strand.
- The growing chain is extended in the 5' to 3' direction by stepwise addition of ribonucleotides.
- At the termination signal, the newly synthesized RNA chain & RNA polymerase are released from DNA.

Initiation

- RNA polymerase as a holoenzyme binds at the promoter & starts the initiation reaction by forming a CLOSED BINARY COMPLEX.
- Closed – DNA remains a duplex
- Sigma factor confers the ability to recognize consensus sequence within the promoter.
- Core enzyme does not distinguish between promoters & other sequences of DNA.
- Sigma factor changes the DNA binding properties of RNA polymerase so that its affinity for promoters is increased & reduced for other sequences of DNA.
- RNA polymerase holoenzyme binds to DNA and it covers 75-80 bp which extends from -55 to +20 bp.
- Closed complex is converted to OPEN COMPLEX by melting of a short region of DNA within the sequence bound by the enzyme.
- TRANSCRIPTION BUBBLE – is created by a local unwinding that begins at the site bound by RNA polymerase.
- Length of transcription bubble is 12-14 bp
- TERNARY COMPLEX - contains RNA, DNA & enzyme. The incorporation of first two nucleotides and phosphodiester bond forms between them.
- Only the holoenzyme can initiate transcription.
- Core enzyme can synthesize RNA on DNA template, but cannot initiate transcription at the proper sites.
- Sigma factor is released when RNA chain reaches 8-9 nucleotides and leaves core enzyme to undertake elongation.
- Holoenzyme remains at the promoter, incorporates the 1st 9 nucleotides.
- ABORTIVE INITIATION – RNA synthesis is frequently aborted after 2/3/10 nucleotides have been joined.
- Once a polymerase manages to make RNA longer than 10 bp, a stable ternary complex is formed.
- Initiation phase ends when the enzyme succeeds in extending the chain & clears the promoter.
- RNA synthesis can start de novo.
- No primer is required.
- First nucleotide with purine nitrogenous base present in the transcript at 5' end is nucleoside phosphate (pppG/ pppA).

Elongation

- Elongation involves the movement of the transcription bubble.
- The enzyme moves along the DNA & extends the growing chain.
- As the enzyme moves, it unwinds DNA helix to expose a new segment of the template in a single-stranded condition.
- Nucleotides are covalently added to the 3' end of the growing RNA chain forming a RNA-DNA hybrid.
- Length of RNA-DNA hybrid within open complex is 8-9 bp.
- During each nucleotide addition, the β and γ phosphates are removed from the incoming nucleotide.
- Hydroxyl group is removed from the 3' carbon of the nucleotide present at the end of the chain.
- The overall reaction rate is ~40 nucleotides/second at 37°C.
- During elongation, RNA polymerase transcribes DNA unwinding & rewinding occurs.
- As RNA polymerase moves forward along the double helix it generates positive supercoils ahead and leaves negative supercoils behind.
- Gyrase : removes positive supercoil & introduces negative supercoil.
- Topoisomerase I removes negative supercoils.
- Cordycepin inhibits elongation by addition to 3' end of RNA.

Termination

- It involves recognition of the point at which no further bases should be added to the chain.
- TERMINATOR: a sequence of DNA required for the termination.
- Enzyme stops adding nucleotides to the growing RNA chain.
- Completed product is released and enzyme dissociates from the DNA template.

Intrinsic termination

- Promote dissociation of polymerase by destabilizing the attachment of growing transcript to the template.
- Transcript forms a hairpin structure by forming a complementary base pairing.
- GC rich inverted repeats form the hairpin structure followed by U rich region.
- Distance between U rich region & hairpin is 7-9 nucleotides.

Rho dependent

- Rho protein is ATP dependent RNA stimulated helicase which disrupts nascent RNA-DNA complex
- It is a ~275 kDa hexamer of identical subunits
- It binds to RNA at rut site (rho utilization site) – rich in C, poor in G and translocates along RNA in 5' to 3' direction until it reaches RNA-DNA hybrid in RNA polymerase, releasing RNA from DNA.

Inhibitors:

- Antibiotics:
 - ✓ Rifampicin – binds in a pocket of β subunit and blocks the path of elongating RNA, thereby blocking transcription.
 - ✓ Actinomycin D – binds tightly & specifically to dsDNA and prevents it from being an effective template for RNA synthesis.