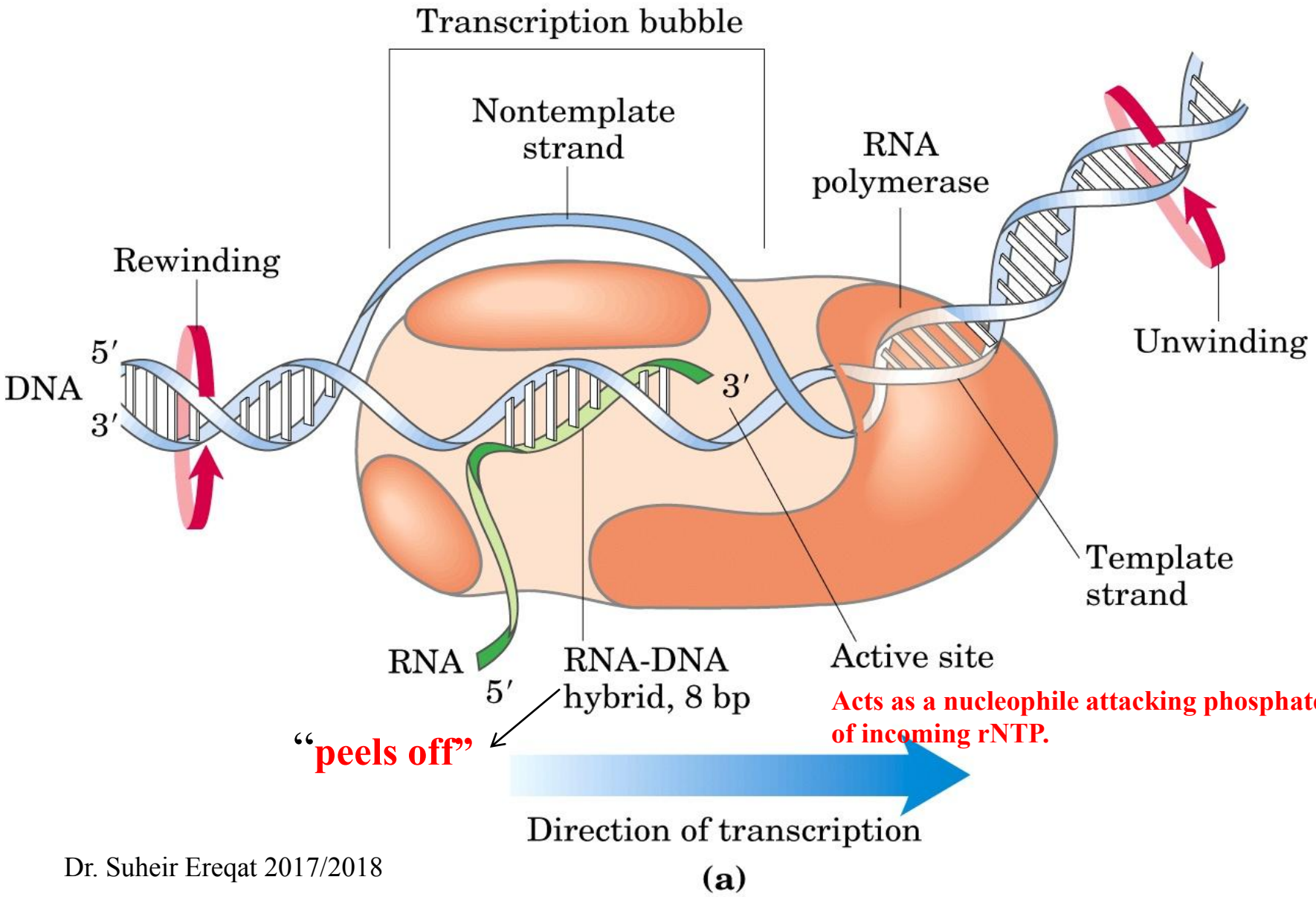
RNA Metabolism

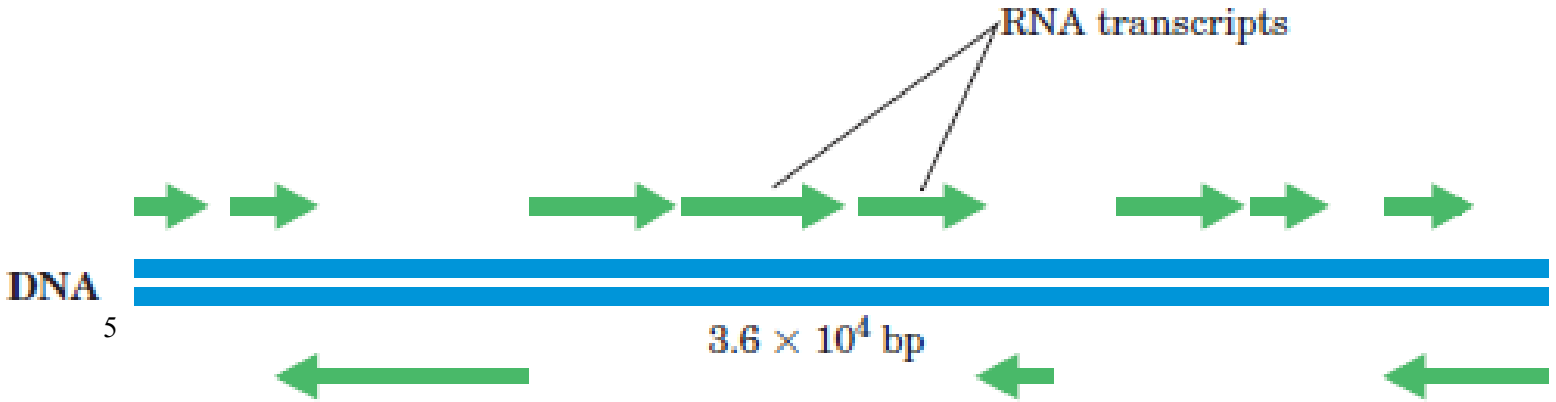
RNA synthesis

Dr. Suheir Ereqat

RNA Is Synthesized by DNA-dependent RNA Polymerase



Adenovirus genome



- Only particular genes or groups of genes are transcribed at any one time, and some portions of the DNA genome are never transcribed.

- **Initiation**

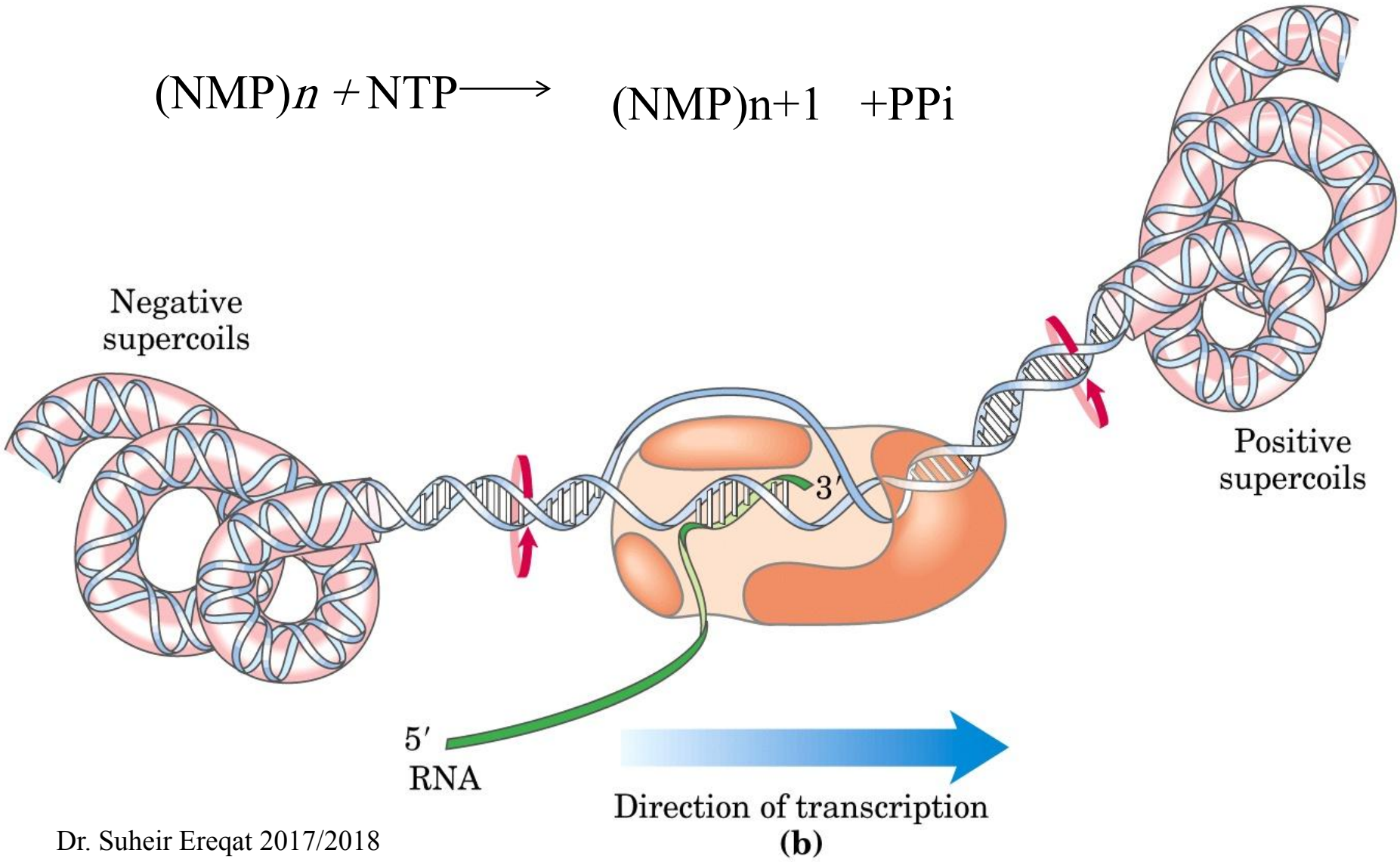
starts at a promoter.

Elongation

Termination

Changes in DNA supercoiling :

$$(NMP)n + NTP \longrightarrow (NMP)n+1 + PPi$$



RNA transcription

1-The DNA duplex must unwind over a short distance, forming a transcription "bubble." (about 17 bp unwound)

2- The 8 bp RNA-DNA hybrid occurs in this unwound region

3-Elongation of a transcript by *E. coli* RNA polymerase proceeds at a rate of 50 to 90 nucleotides/s

3-movement of a transcription bubble requires considerable strand rotation of the nucleic acid molecules.

4- moving RNA polymerase generates waves of positive supercoils ahead of the transcription bubble and negative supercoils behind

5- the topological problems caused by transcription are relieved by topoisomerases

Sense strand

(5′) CGCTATAGCGTTT(3′) DNA nontemplate (coding) strand

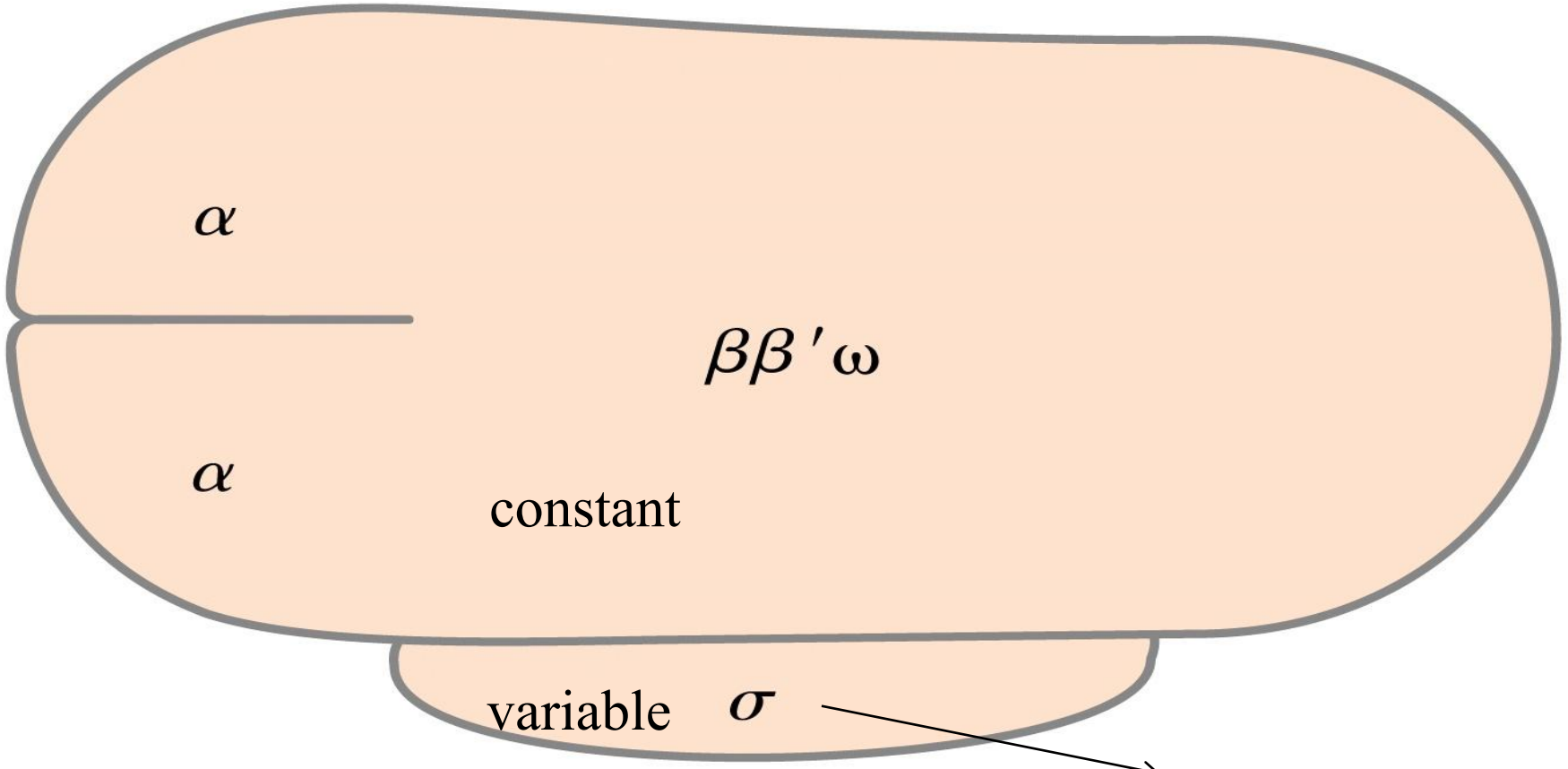
(3′) GCGATATCGCAAA(5′) DNA template strand :antisense

(5′) CGCUAUAGCGUUU(3′) RNA transcript

The RNA transcript is synthesized on the template strand and is identical in sequence (with U in place of T) to the nontemplate strand, or coding strand.

RNA polymerase holoenzyme = five core subunits+sigma factor

Lack 3>5 proof reading



70=housekeeping genes
32=heatshock protein
etc

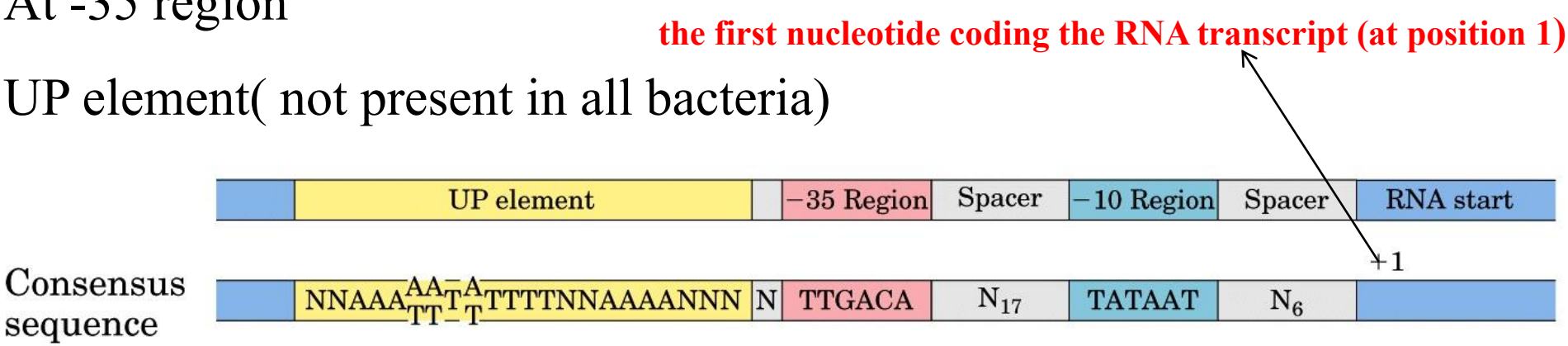
E. coli promoters

Several recognition sequences:

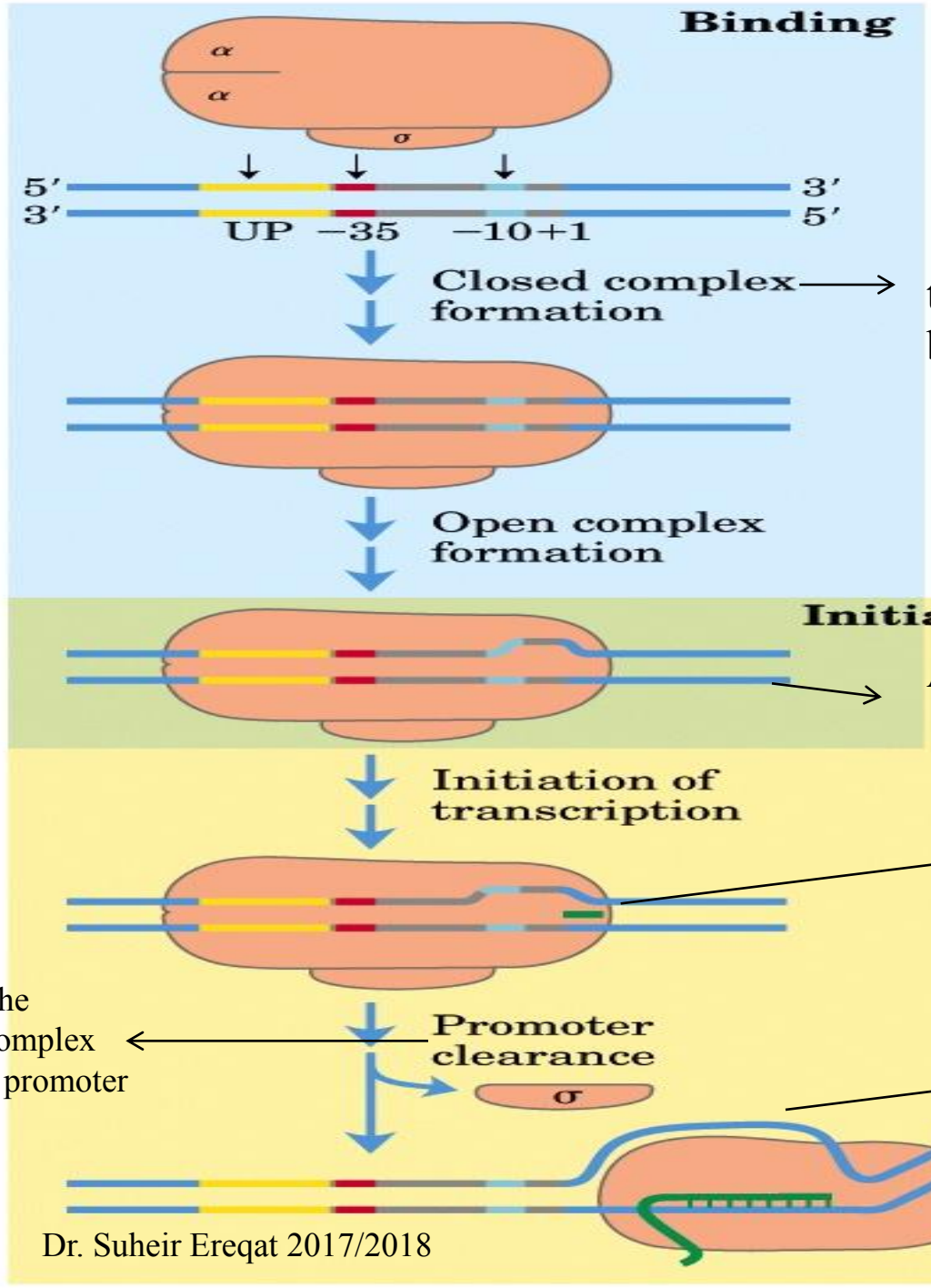
At -10 region

At -35 region

UP element(not present in all bacteria)



Variations in the consensus sequence affect the efficiency of RNA polymerase binding and transcription initiation= The promoter sequence establishes a basal level of expression that can vary greatly from one gene to the next



the promoter DNA is stably bound but not unwound.

A 12 to 15 bp region of DNA within the -10 region to position 2+ or 3+is then unwound

transcription is initiated=Hybrid

movement of the transcription complex away from the promoter

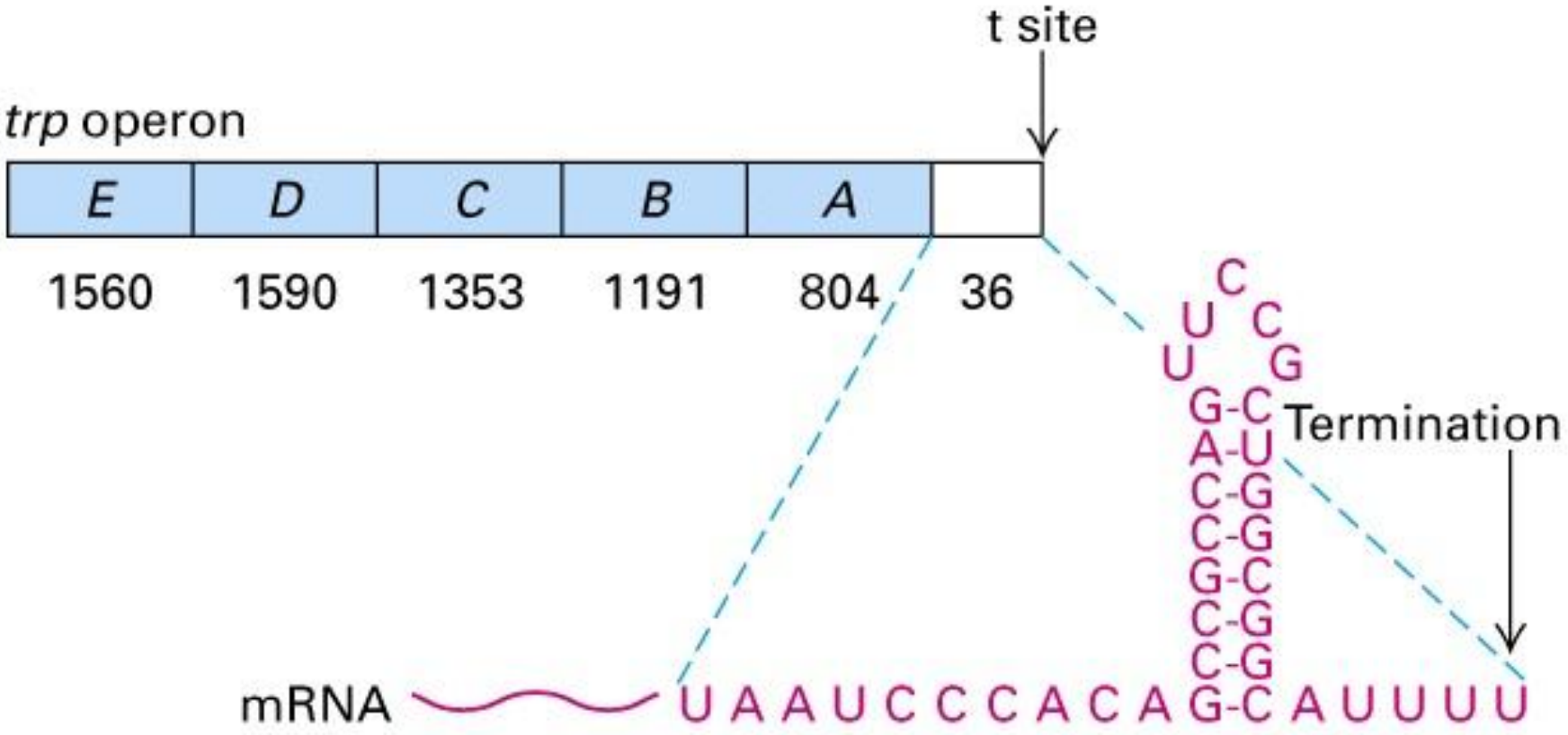
Once the *subunit is released and the polymerase leaves the* promoter = becomes committed to elongation of the RNA

Termination of Transcription in E coli:

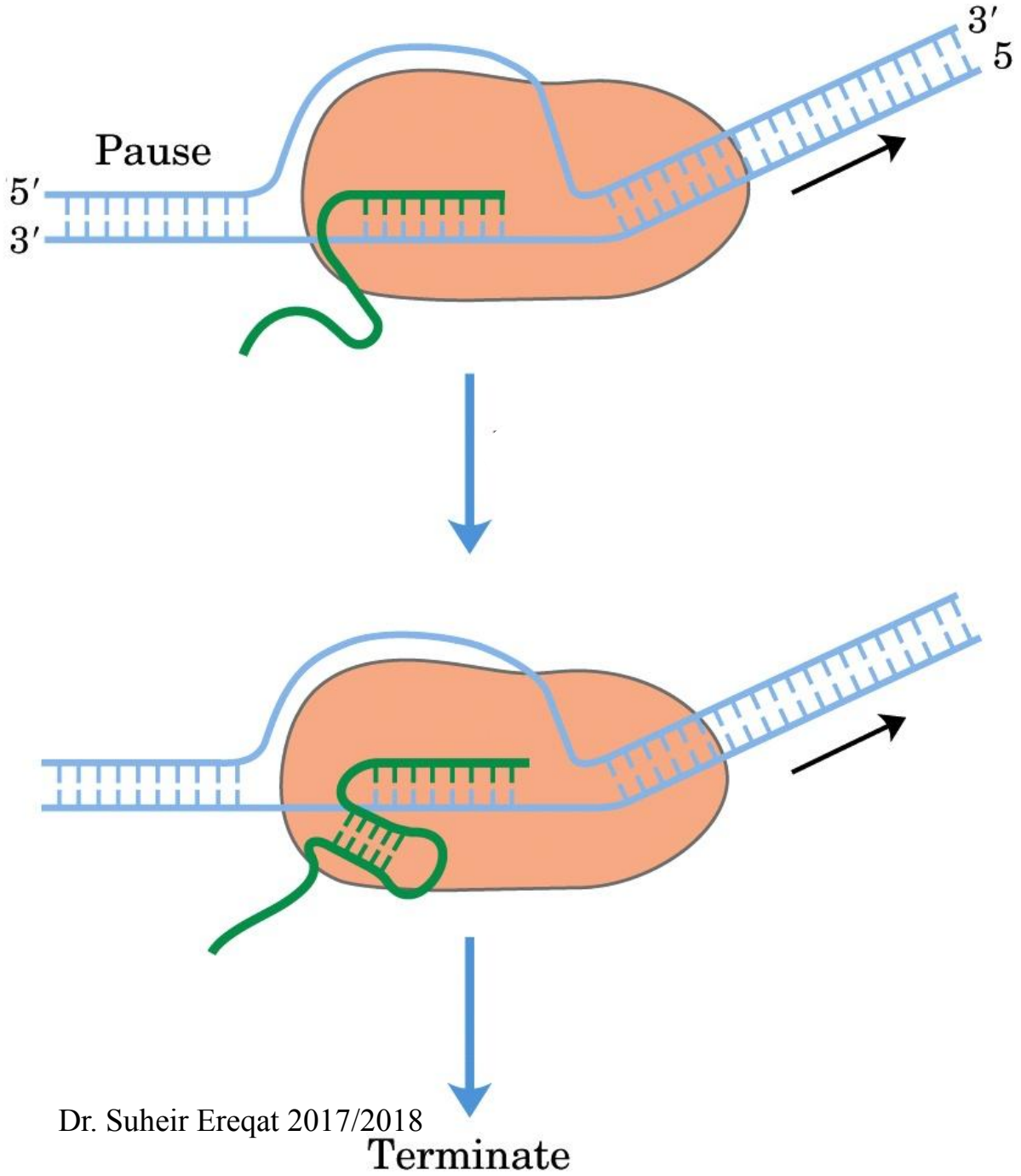
- **(1) RhoFactor independent** produces an RNA transcript with self-complementary sequences, permitting the formation of a stem and loop structure(hairpin structure)

(2) Rho (ρ) Factor Dependent The protein associates with the RNA at specific binding sites and migrates in the 5>3 direction until it reaches the transcription complex that is paused at a termination site.

Rho-independent termination occurs at characteristic sequences



Termination:



Rho-Dependent Termination

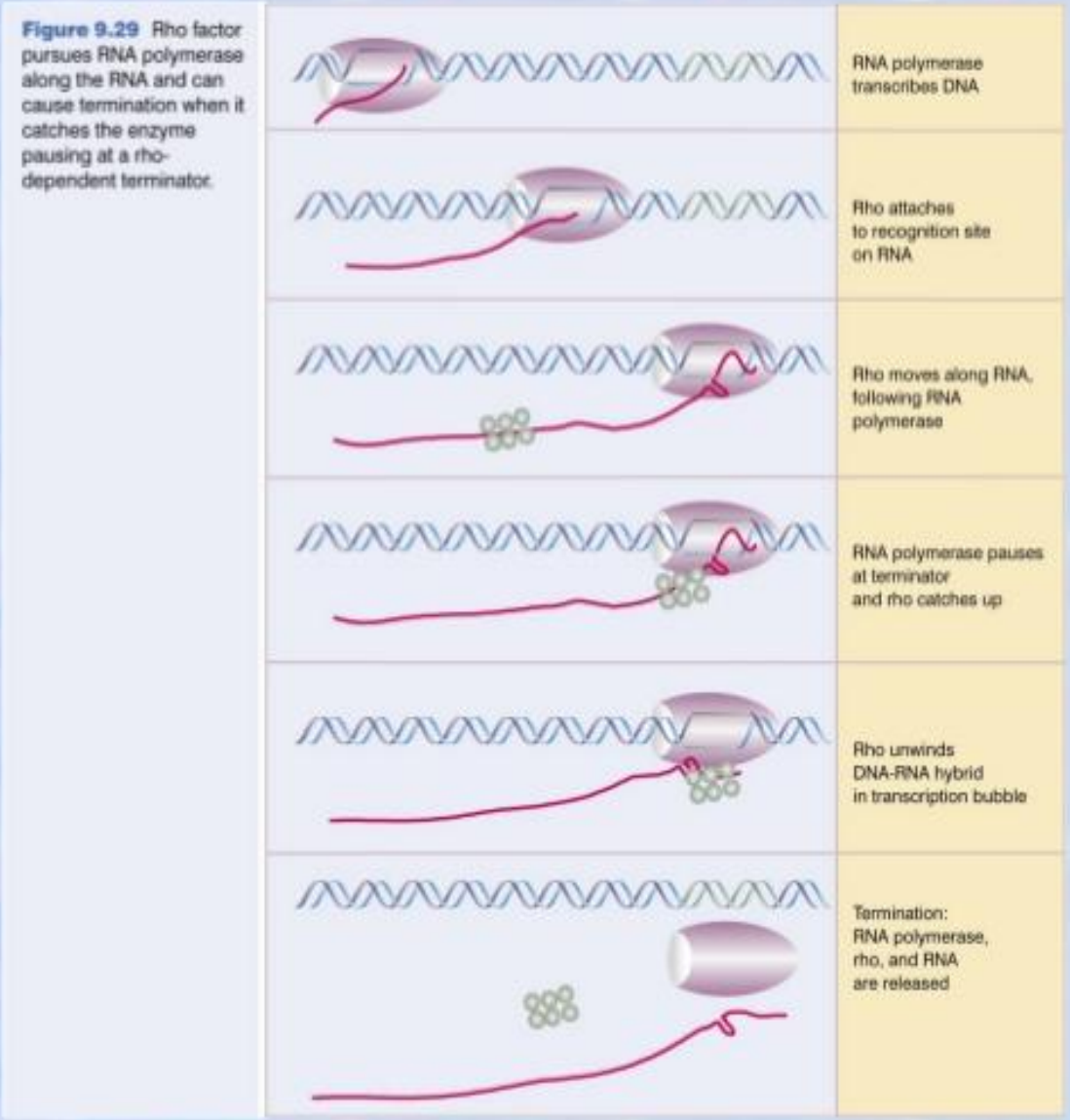


Figure 9.29 Rho factor pursues RNA polymerase along the RNA and can cause termination when it catches the enzyme pausing at a rho-dependent terminator.

Three eukaryotic RNA polymerases

- RNA pol I: synthesis pre-rRNA, which contains the precursor for the 18S, 5.8S, and 28S rRNAs

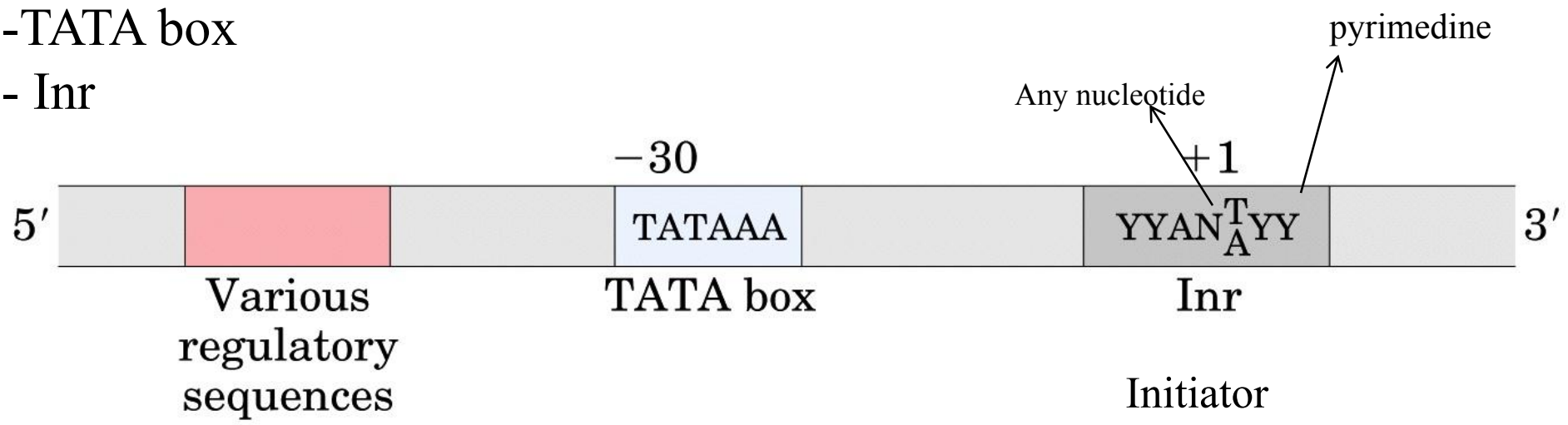
- RNA pol II: synthesis of mRNAs, requires an array of other proteins, called **transcription factors,** in order to form the active transcription complex

- RNA pol III: makes tRNAs, the 5S rRNA, and some other small specialized RNAs

Promoter in Eukaryotes

-TATA box

- Inr



RNA pol II huge enzyme 12 subunits:

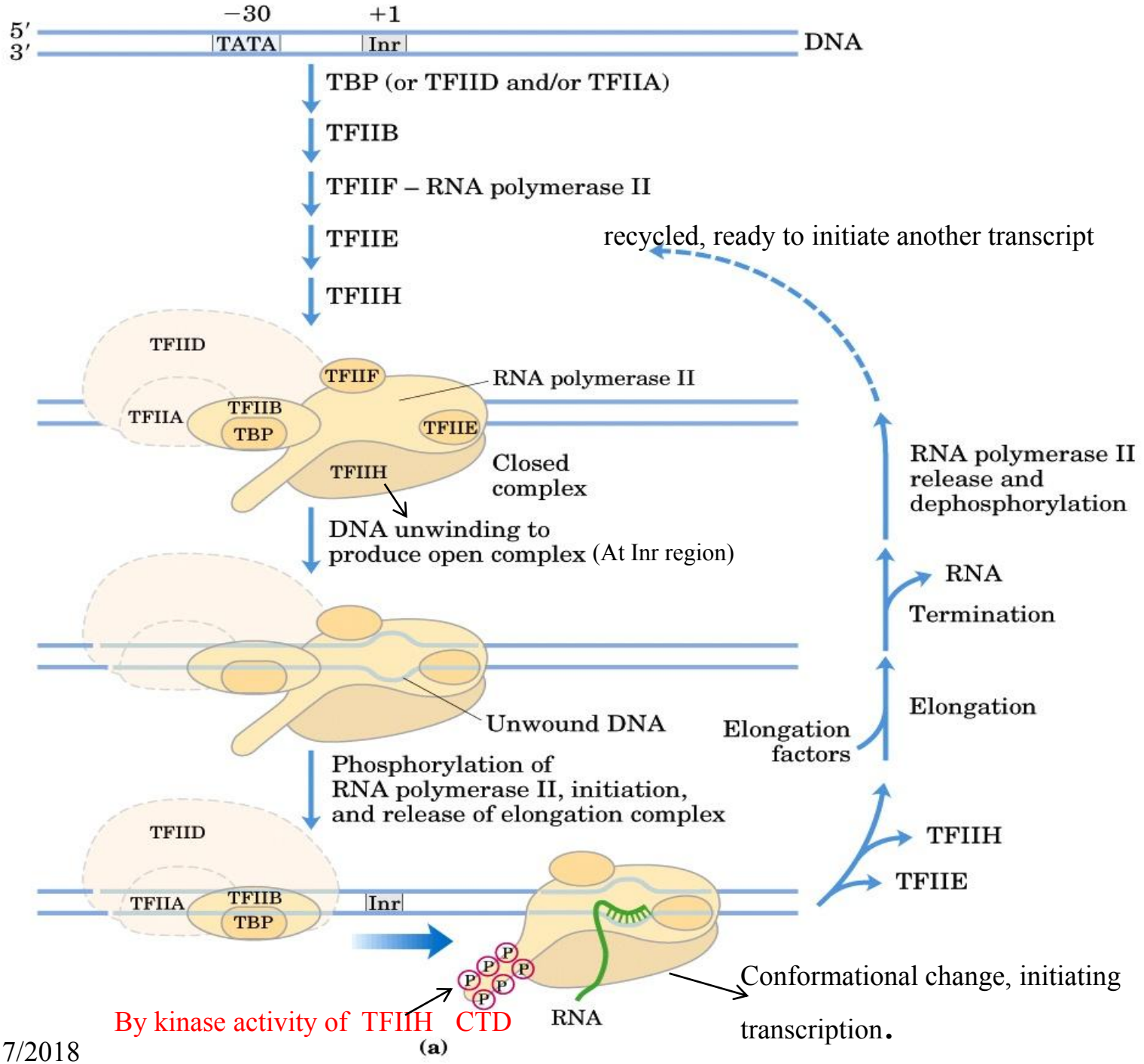
RBP1

RBP2

RBP3…… and RBP11

carboxyl-terminal domain (CTD)

Transcription at RNA polymerase II promoters.



Transcription at RNA polymerase II promoters.

1-sequential assembly of TBP (often with TFIIA), TFIIB, TFIIF plus Pol II, TFIIE, and TFIIH = a closed complex.

2-Within the complex, the DNA is unwound at the Inr region by the helicase activity of TFIIH and perhaps of TFIIE= open complex.

3-The carboxyl-terminal domain of the Pol II subunit is phosphorylated by TFIIH

4. the polymerase then escapes the promoter and begins elongation which accompanied by the release of many transcription factors and is also enhanced by elongation factors

5-After termination, Pol II is released, dephosphorylated, and recycled.

TABLE 26–2 Proteins Required for Initiation of Transcription at the RNA Polymerase II (Pol II) Promoters of Eukaryotes

Transcription protein	Number of subunits	Subunit(s) M_r	Function(s)
Initiation			
Pol II	12	10,000–220,000	Catalyzes RNA synthesis
TBP (TATA-binding protein)	1	38,000	Specifically recognizes the TATA box
TFIIA	3	12,000, 19,000, 35,000	Stabilizes binding of TFIIB and TBP to the promoter
TFIIB	1	35,000	Binds to TBP; recruits Pol II–TFIIF complex
TFIIE	2	34,000, 57,000	Recruits TFIIH; has ATPase and helicase activities
TFIIF	2	30,000, 74,000	Binds tightly to Pol II; binds to TFIIB and prevents binding of Pol II to nonspecific DNA sequences
TFIIH → Genetic loss of certain TFIIH=XP disease	12	35,000–89,000	Unwinds DNA at promoter (helicase activity); phosphorylates Pol II (within the CTD); recruits nucleotide-excision repair proteins
Elongation*			
ELL†	1	80,000	
pTEFb	2	43,000, 124,000	Phosphorylates Pol II (within the CTD)
SII (TFIIS)	1	38,000	
Elongin (SIII)	3	15,000, 18,000, 110,000	

When RNA pol II halts at the site of a DNA lesion:

TFII H (not only involved in open complex formation). can interact with the lesion and recruit the entire nucleotide-excision repair complex.

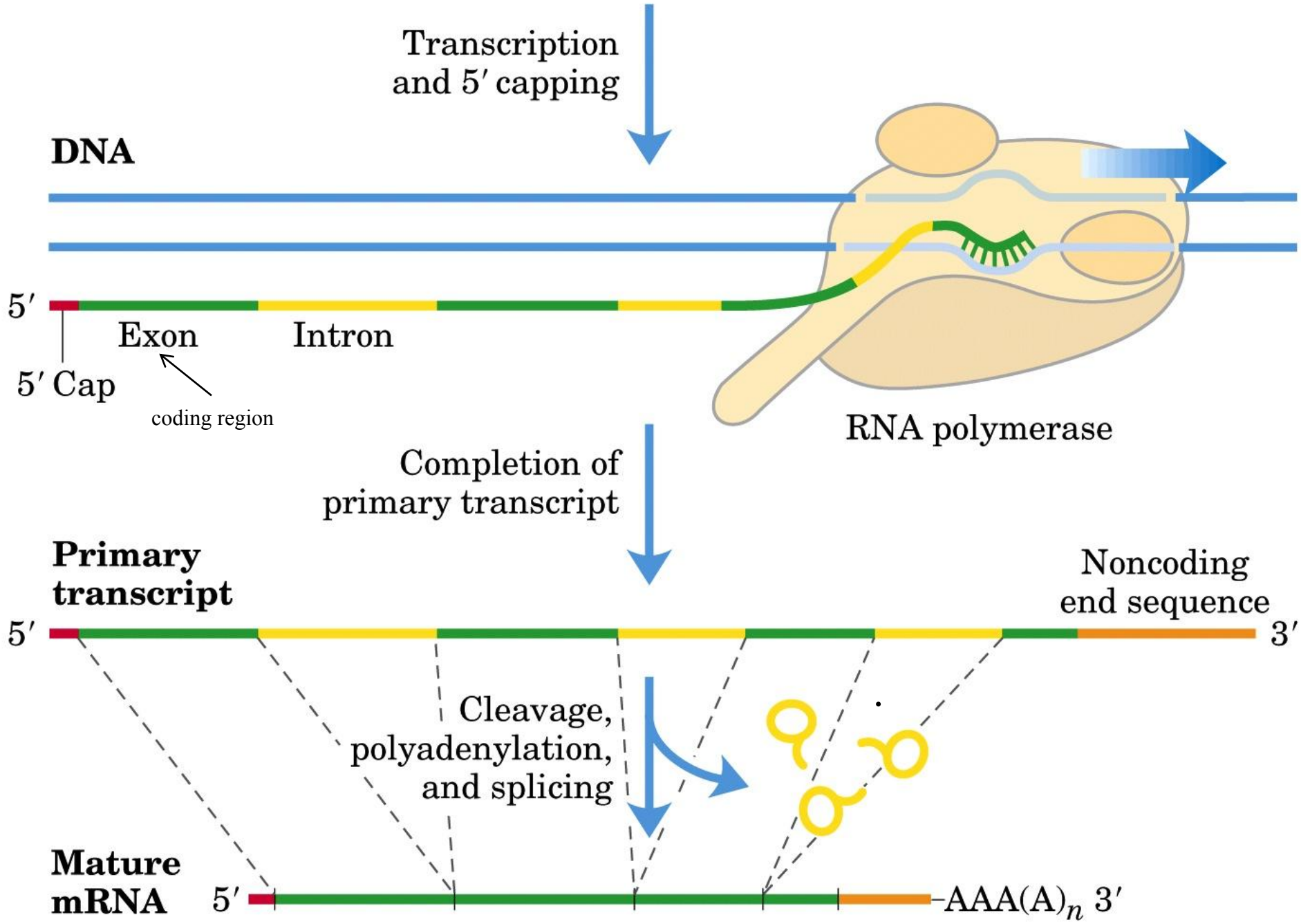
mRNA modification in eukaryotes

1) Capping The 5' cap is added before synthesis of the primary transcript is complete

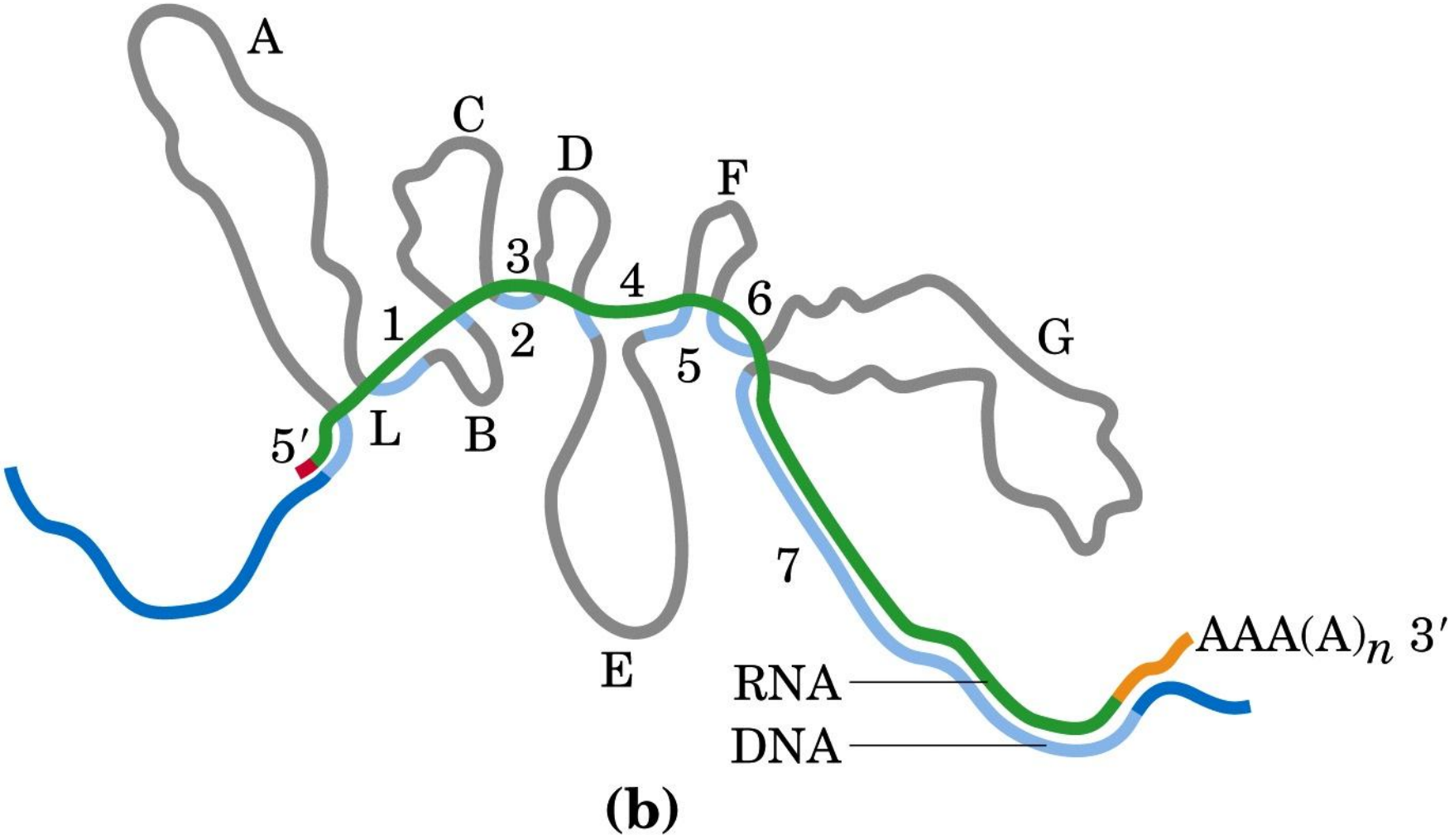
2) **Splicing:** the introns are removed from the primary transcript and the exons are joined to form a continuous sequence =functional polypeptide

Alternative splicing has regulatory significance

3) **Polyadenylation:** The 3' end of mRNA is cleaved, and 80 to 250 A residues are added to create a poly(A) "tail."


Transcription
and 5′ capping
DNA
5′
Exon
Intron
5′ Cap
coding region
RNA polymerase
Completion of
primary transcript
Primary
transcript
Noncoding
end sequence
5′
3′
Cleavage,
polyadenylation,
and splicing
Mature
mRNA
5′
–AAA(A)n 3′

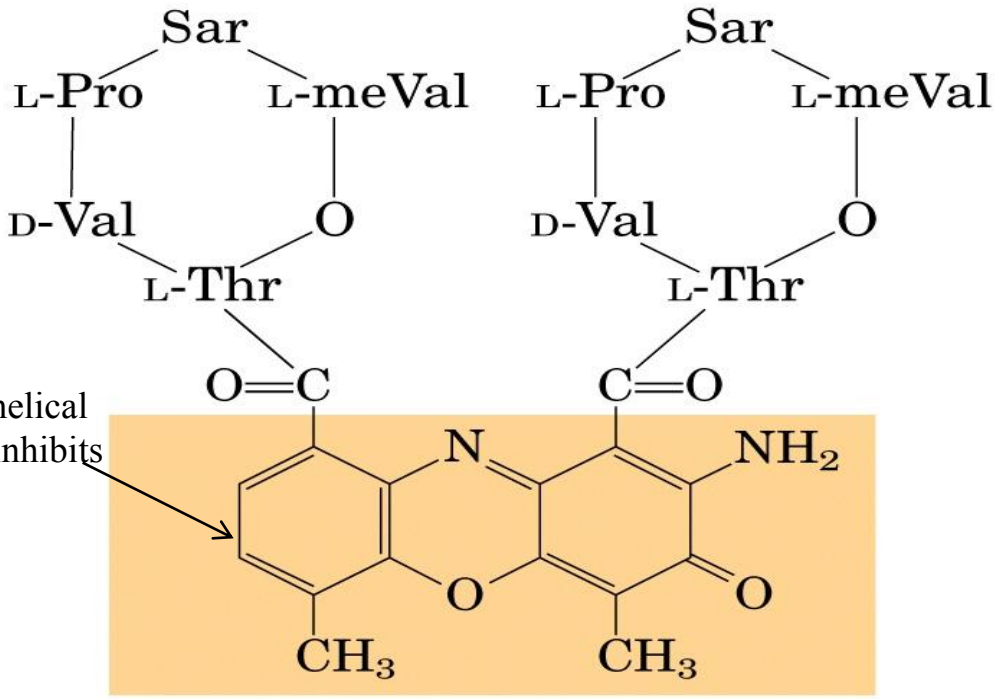
Modified RNA :



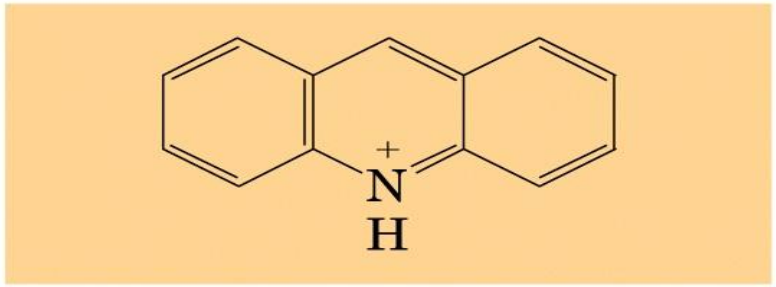
RNA Polymerase Undergoes Selective Inhibition

Intercalating agents:

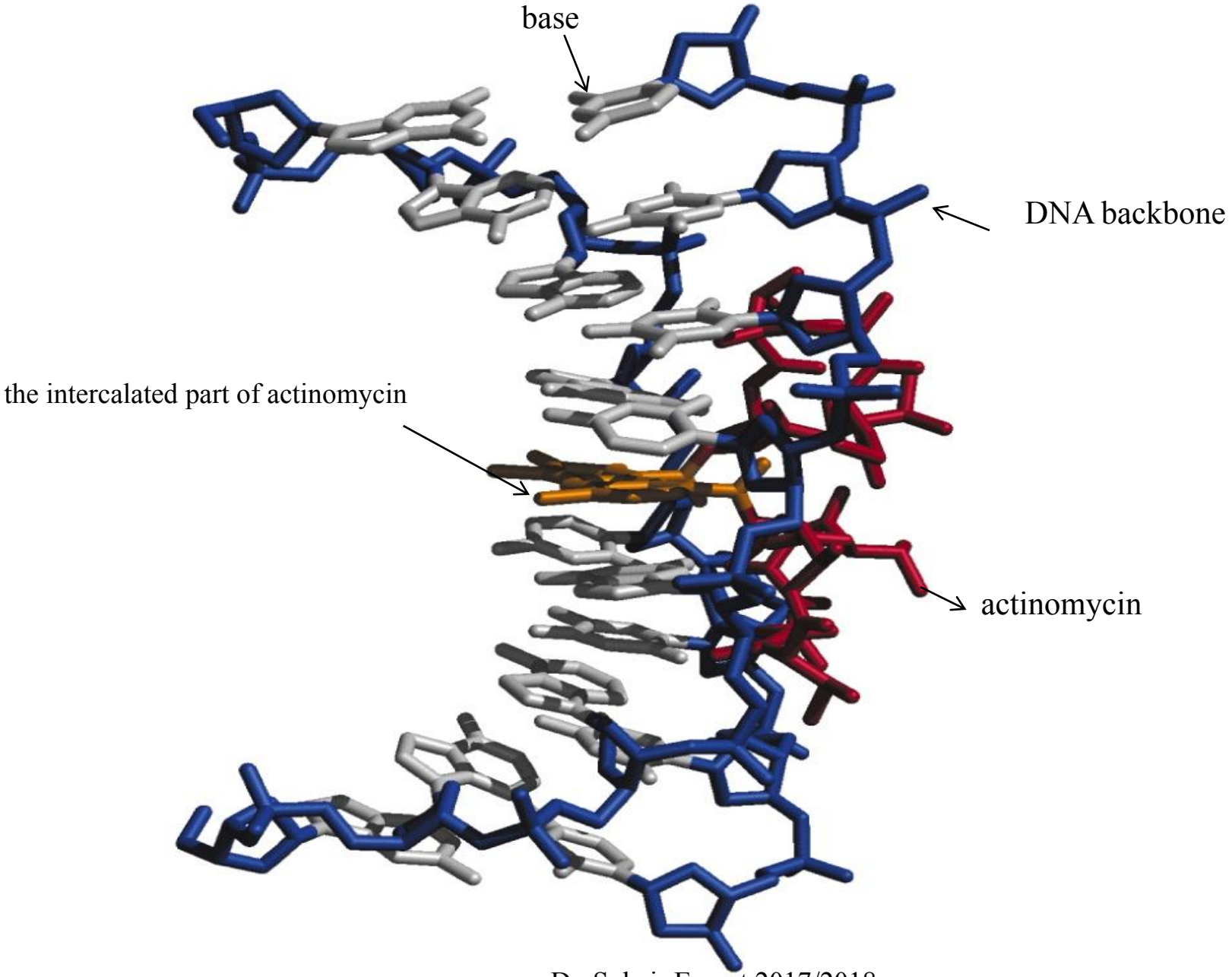
intercalates into the double-helical DNA ,deforming the DNA=inhibits RNA elongation



Actinomycin D



Acridine



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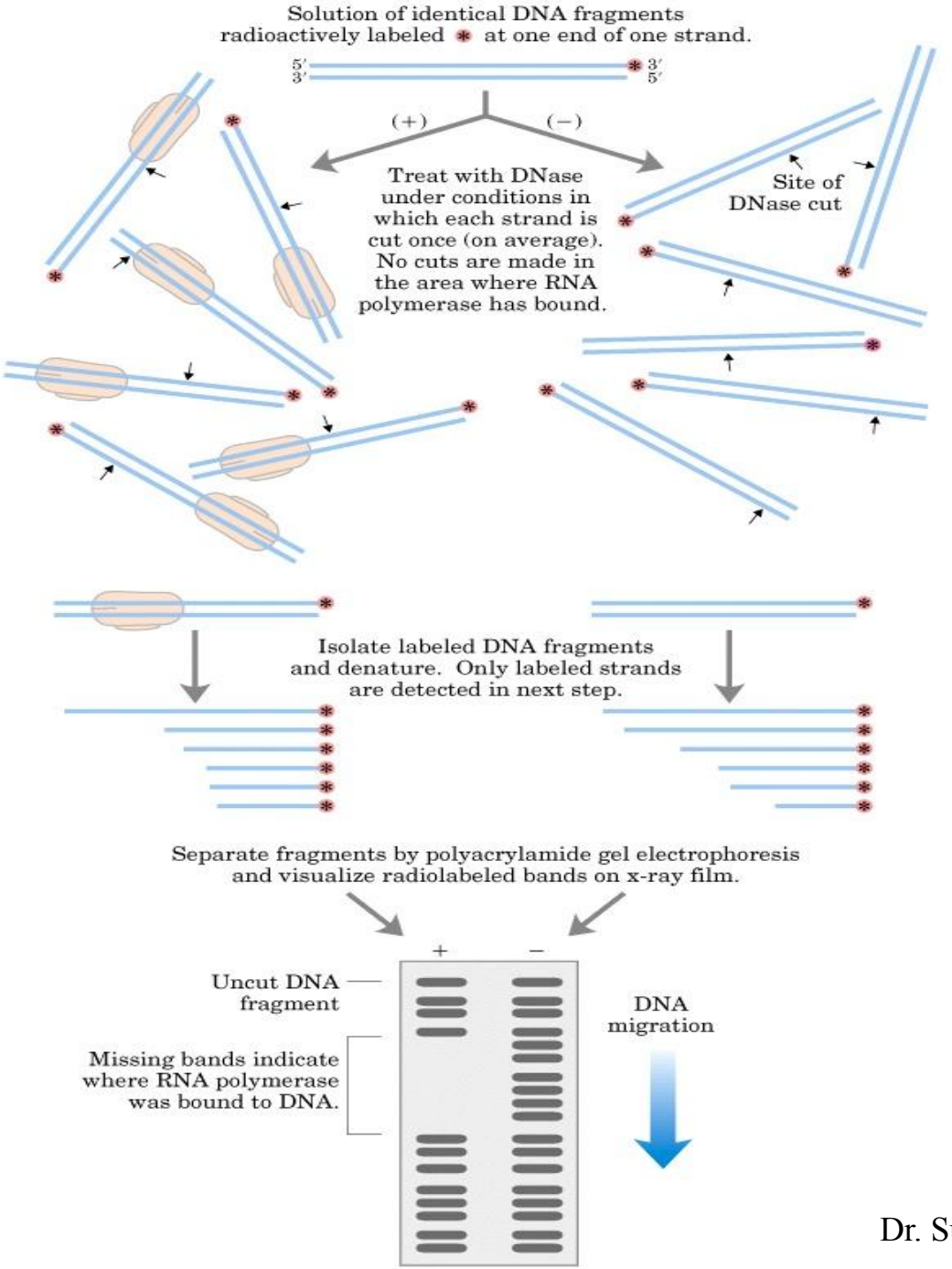
(b)

Rifampicin inhibits bacterial RNA synthesis by:
binding to the B subunit of bacterial RNA polymerases, preventing the promoter clearance step of transcription

DNA Footprinting:

identifies the DNA sequences bound by a particular protein.



RNA polymerase leaves its footprint on a promotoer

DNA footprinting: identifies the DNA sequences bound by a particular protein