RNA

- RNA the only macromolecule known to have a storage ,transmission of information & catalysis.
- Catalytic RNA:

  ribozyme changed the definition of enzyme.
- **Transcription:**

  conversion of genetic information from DNA into RNA with a base sequence complementary to one of the DNA strands.

The three roles of RNA in protein synthesis

- Three types of RNA molecules perform different but complementary roles in protein synthesis (translation)
- **mRNA** : carries information copied from DNA in the form of a series of three base “words” termed codons
- **tRNA** : transmits the code and delivers the specified amino acid
- **rRNA** : associates with a set of proteins to form ribosomes, structures that function as protein-synthesizing machines

In replication , the entire chromosome copied, but transcription is selective. Only particular genes are transcribed at a time, some regions never transcribed.

- Specific regulatory sequences mark the beginning and end of the DNA transcribed + which strand is in DNA duplex is the template to be transcribed.

DNA-dependent Synthesis of RNA

Transcription vs. Replication:

1- Similar chemical reaction/ mechanism: synthesis is driven forward by hydrolysis of PPi

2- Stages: initiation, elongation , termination.

3- need for a template.

4- Polarity direction of synthesis (5'→3')

5- Transcription no need for a primer.

6- Transcription for a limited segment of DNA.

7- Transcription no 3'→5' proofreading activity. cannot excise mismatched nucleotides.

Transcription:

Requirements

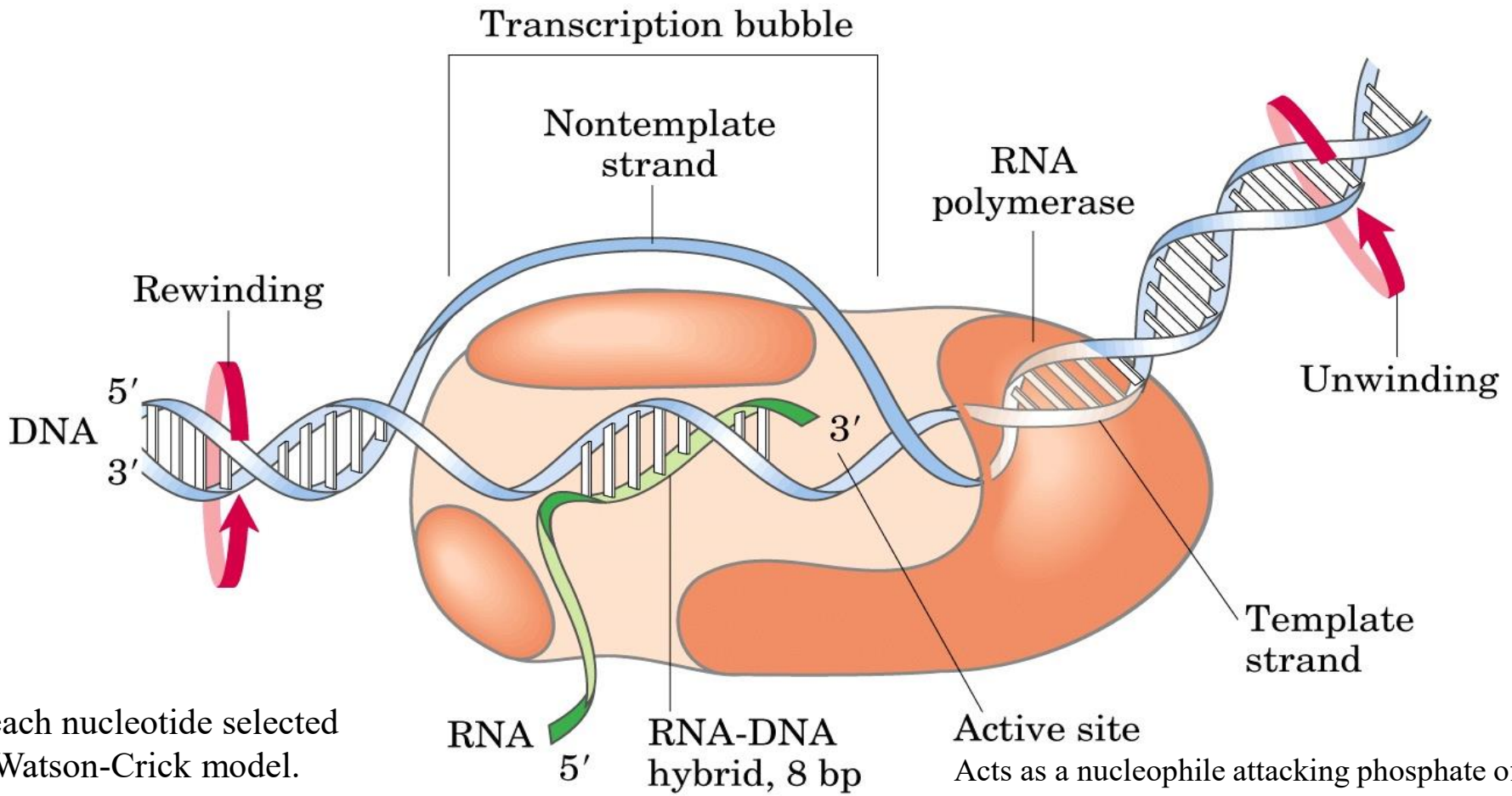
1. Enzyme: RNA Polymerase
2. DNA Template (3'→ 5' strand)
3. No primer required
4. Nucleoside triphosphates: ATP, GTP, CTP, UTP
5. Synthesis is 5'→ 3'
6. Mg2+, Zn2+

Overall Reaction:

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

RNA lengthened RNA

RNA polymerase elongates an RNA strand by adding ribonucleotide units to 3'-hydroxyl end, building RNA in 5'→3' direction.



1- each nucleotide selected by Watson-Crick model.

2- Base pair geometry contribute to base selection.

Dr. rula

Left →→→ right

Initiation

starts at a promoter.

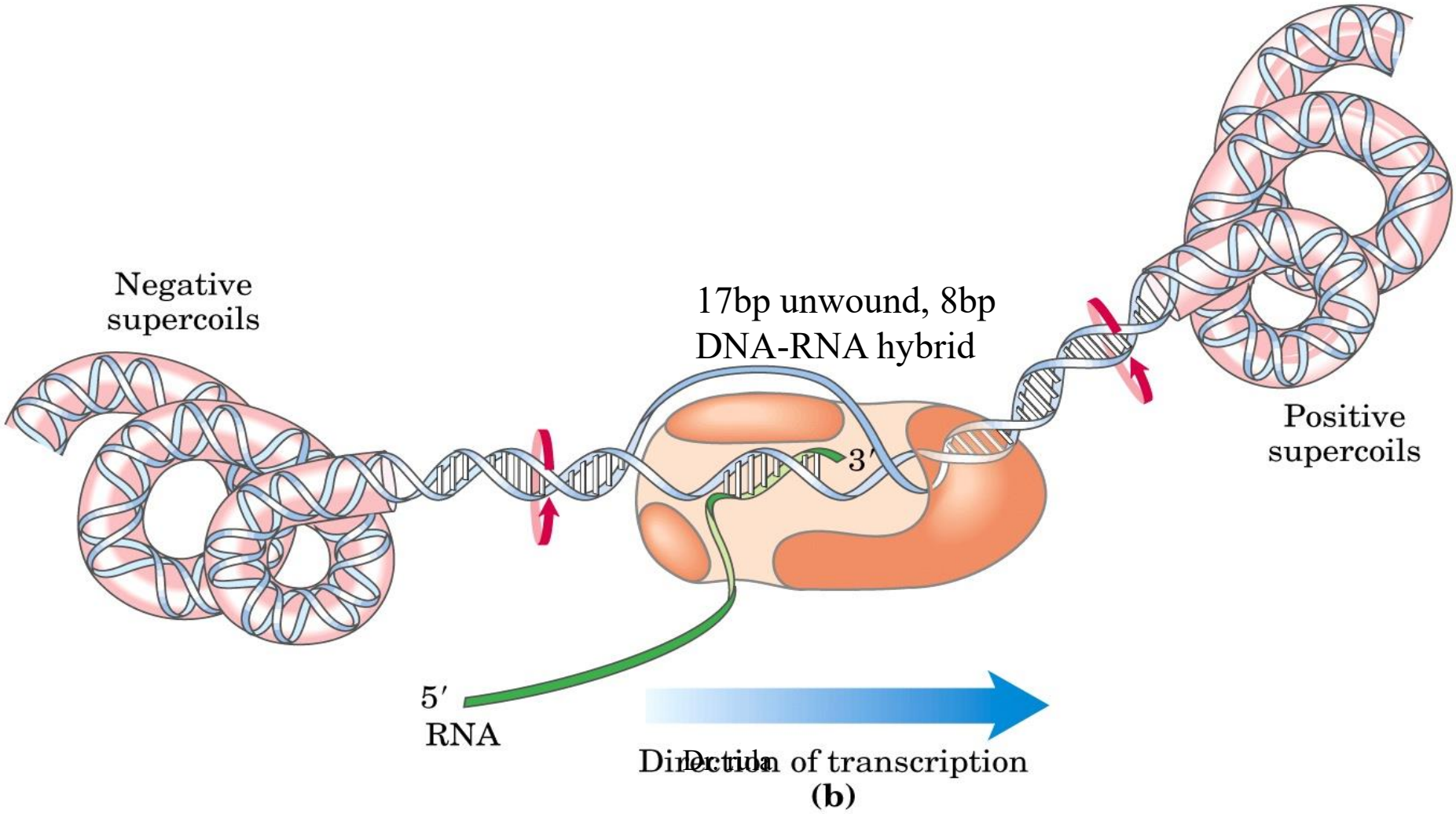
Binding of RNA pol to specific DNA sequences.

Elongation

- The growing end of new RNA strand base pairs temporally with DNA template forming a short RNA-DNA hybrid = 8bp .
- Hybrid peels off shortly and DNA duplex reforms.
- Elongation in the rate of 50-90 nucleotide/s

Changes in DNA supercoiling :

Since DNA is a helix movement of the Transcription bubble requires a strand rotation. → Supercoiling (in vitro + in vivo) relieved by topoisomerase.



The DNA strands have different roles in transcription:

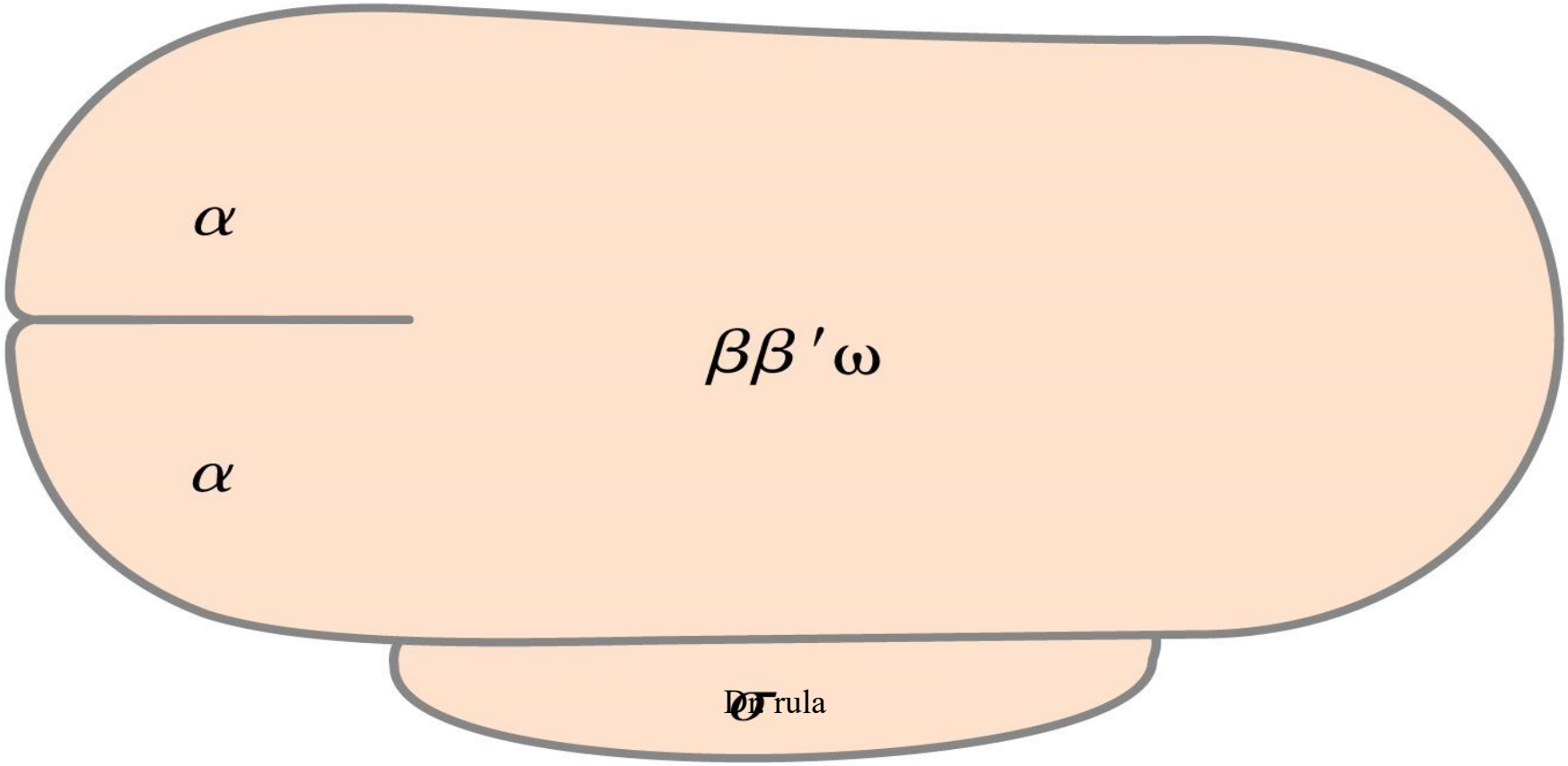
Coding strand =

Identical in sequence to RNA transcript (U instead of T)

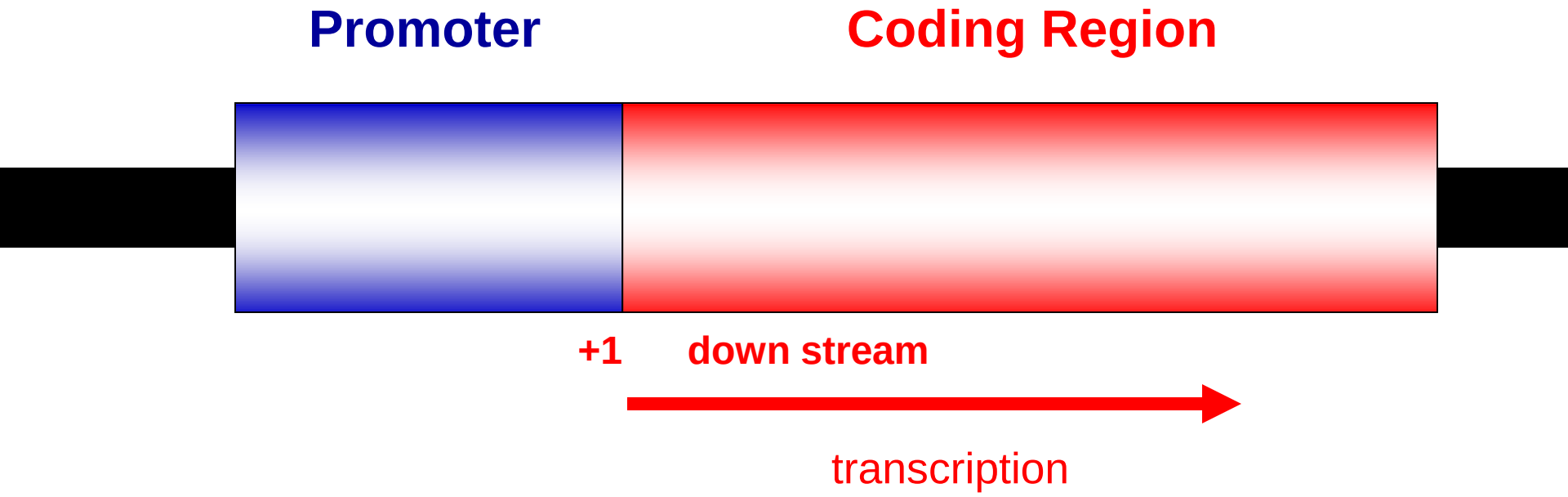
(5′) CGCTATAGCGTTT(3′)	DNA nontemplate (coding) strand
(3′) GCGATATCGCAAA(5′)	DNA template strand
(5′) CGCUAUAGCGUUU(3′)	RNA transcript

DNA dependent RNA pol in E. coli:

- Large complex enzyme, 5 core subunits + a sixth sigma σ subunit, binds transiently to core , directs the enzyme to specific binding sequences of DNA.
- No proofreading activity → one error/ 104-105



Typical Gene Structure



Initiation:

RNA pol binding occurs within 70bp before and 30bp after it.

Promoter -70 to +30

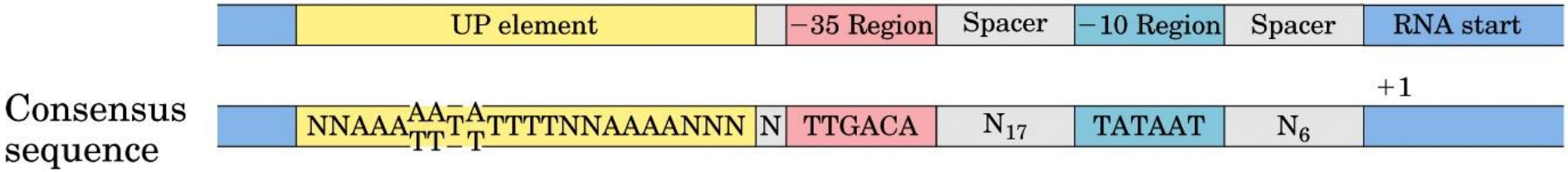
In E coli -10 to -35 similar sequence important for sigma binding & interaction.

Common sequences= consensus sequences. Similar to Ori C in replication

Several recognition sequences:

At -10 region 5'- TATAAT-3'

At -35 region 5'-TTGACA-3'



UP ELEMENT= upstream promoter

Mutations at -10 or -35 affect pol efficiency binding & initiation of transcription.

Transcription:

1) Binding:

Pol bind promoter forming

A- closed complex , DNA intact

B- open complex DNA intact +

unwound at -10

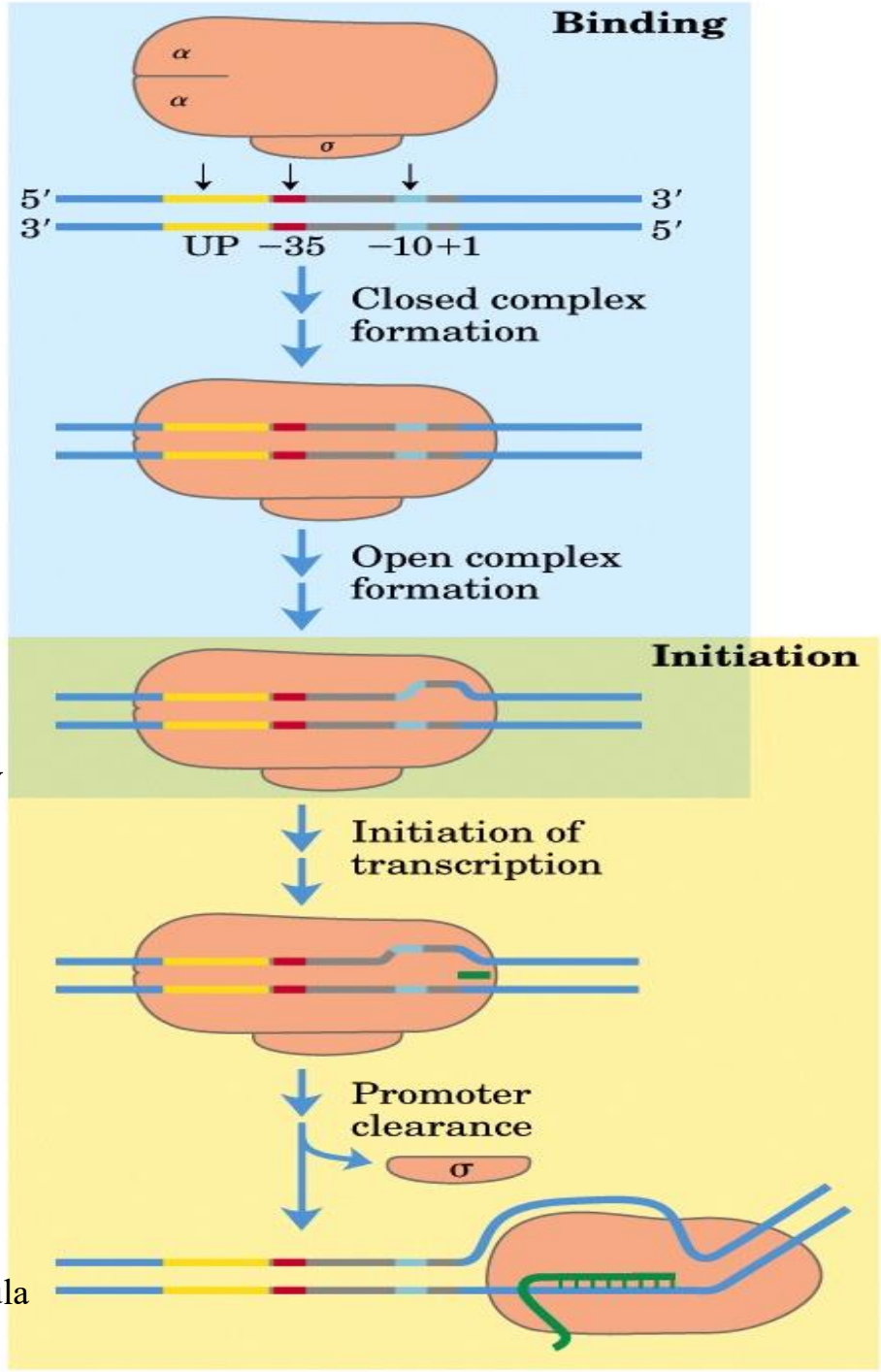
2) Initiation:

Start of transcription

Movement of the transcription complex away from promoter(promoter clearance).

3)Elongation:

Sigma dissociates



Regulation can occur at any stage of transcription.

Mainly at transcription initiation:

Binding of proteins at promoter activate / inhibit transcription
→ control gene expression.

1) Activators:

e.g cAMP receptor protein (CRP) activate transcription of genes coding for enzymes that metabolize sugars.

2) Inhibitors:

e.g. Lac repressor, inhibits transcription of the enzymes lactose metabolism.

Termination of Transcription:

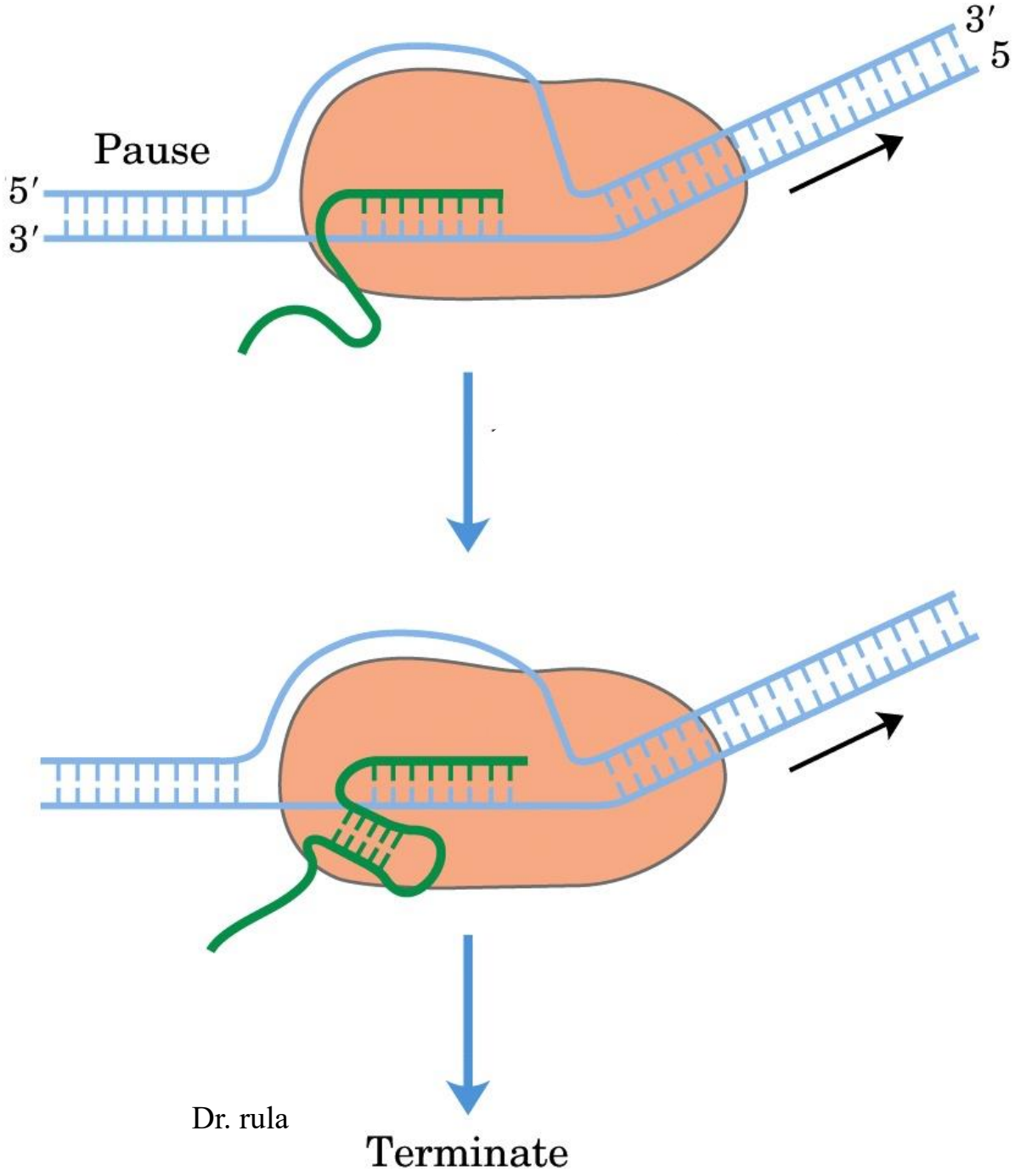
(1) Factor independent

- GC rich segment in the transcript . GC base pairs make the template hard to unwind→ slows down RNA pol
- The series of A=U bonds (relatively weak) leads to further weakening and dissociation – results in the collapse of the transcription bubble

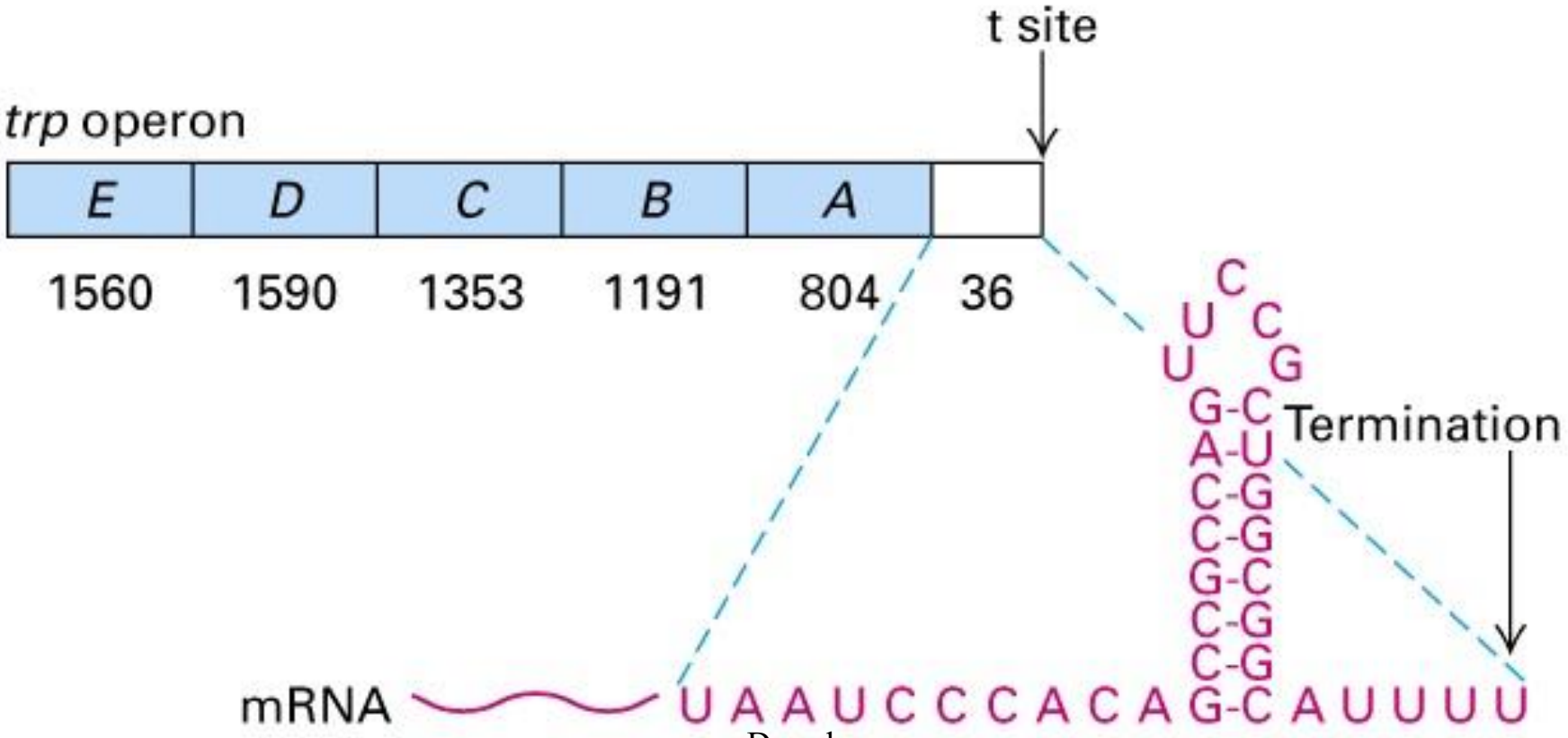
(2) Rho (ρ) Factor Dependent

- ρ protein loads onto mRNA at specific binding sites and migrates 5'→ 3' until it reaches the transcription complex.
- after binding, Rho proceeds along mRNA and probably 'outraces' or "catches up" RNA pol
- Rho has an ATP-dependent helicase activity disrupts RNA-DNA hybrid
- Termination is completed by the release of Rho and RNA polymerase from the nucleic acids

Termination:



Rho-independent termination occurs at characteristic sequences



Three eukaryotic RNA polymerases employ different termination mechanisms

- RNA pol I terminated by a termination factor.

- RNA pol II termination is coupled to the process that cleaves and polyadenylates the 3′ end of a transcript.

- RNA pol III terminated after polymerizing a series of U residues.

Transcription:

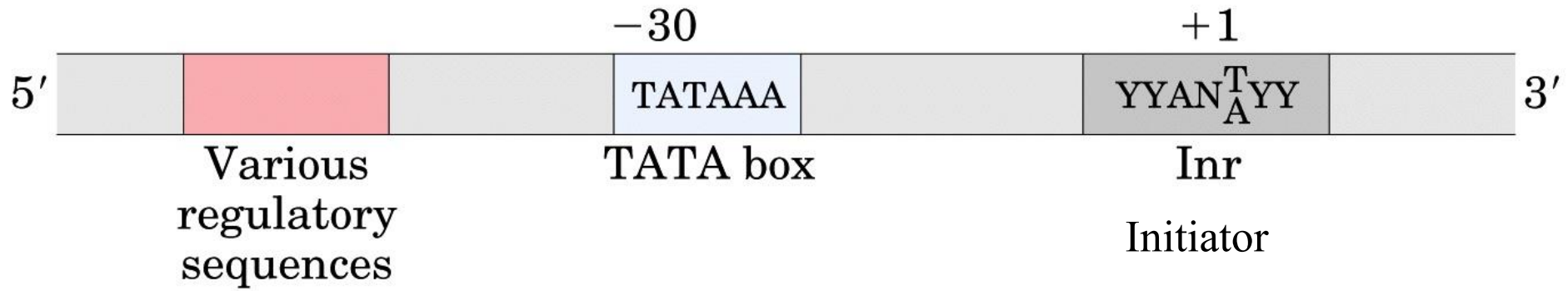
RNA polymerases in eukaryotes:

- **RNA polymerase I** → Transcribes rRNA.
- **RNA polymerase II** → Transcribes mRNAs.
- **RNA polymerase III** → Transcribes tRNAs.

- All three are multimeric enzymes (> than 10 subunits)

Common sequences in promoters recognized by eukaryotic RNA pol II

- TATA box (eukaryotic consensus sequence TATAAA) near base -30



RNA pol II huge enzyme 12 subunits:

BRB1 : High degree homology to **β'** subunits of bacterial RNA pol.

BRB2 : High degree homology to **β** subunits of bacterial RNA pol.

BRB3 and **BRB11** : homolog to bacterial **α** subunit.

carboxyl-terminal tail (CTD)

RNA pol II requires transcription factors (TFII) highly conserved in all eukaryotes :

Assembly

Initiation

Elongation

Termination

RNA pol …'switch on the engine' but 'cant leave the station' then "takes off"

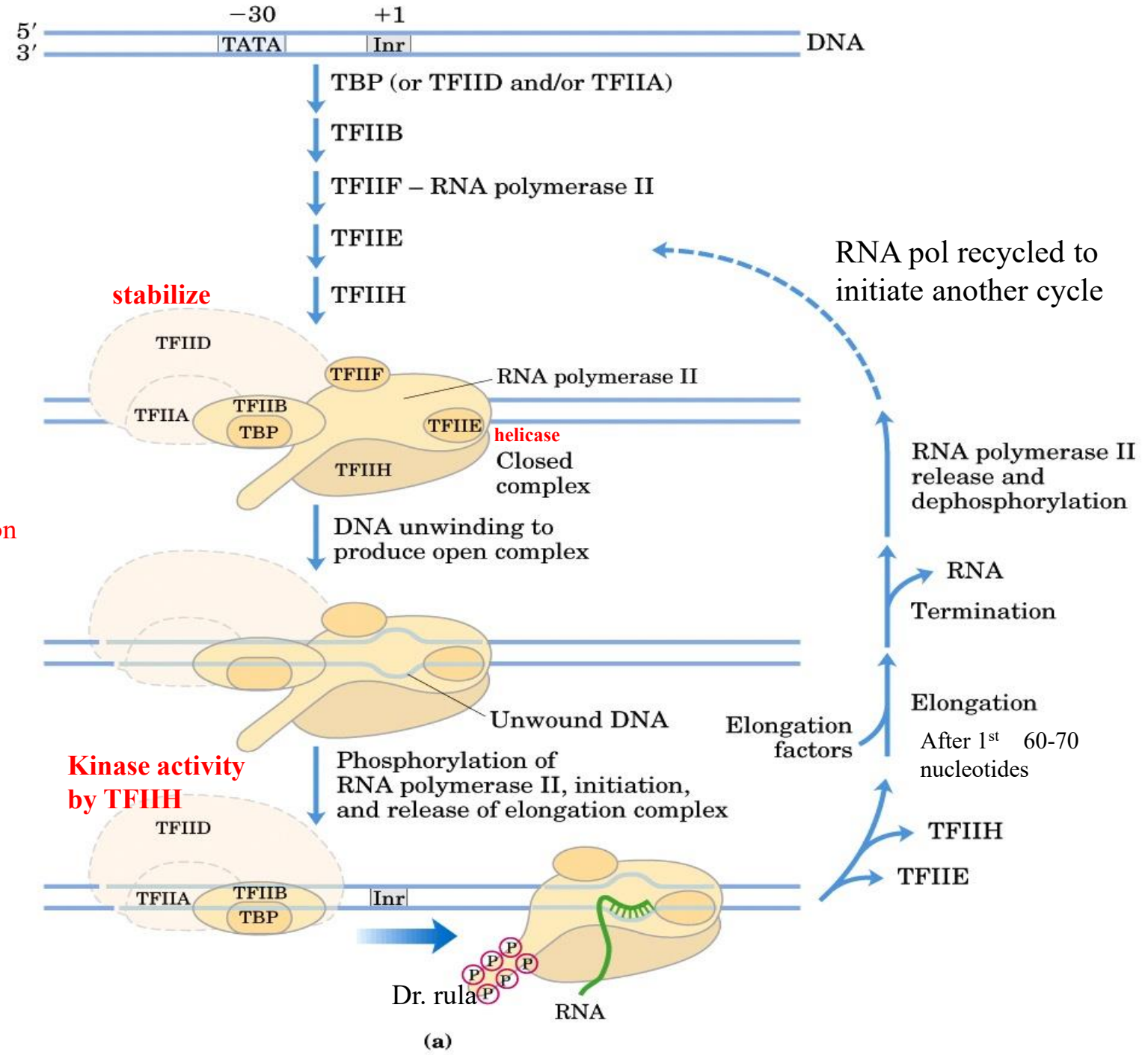


table 26–1

Proteins Required for Transcription at the RNA Polymerase II Promoters of Eukaryotes

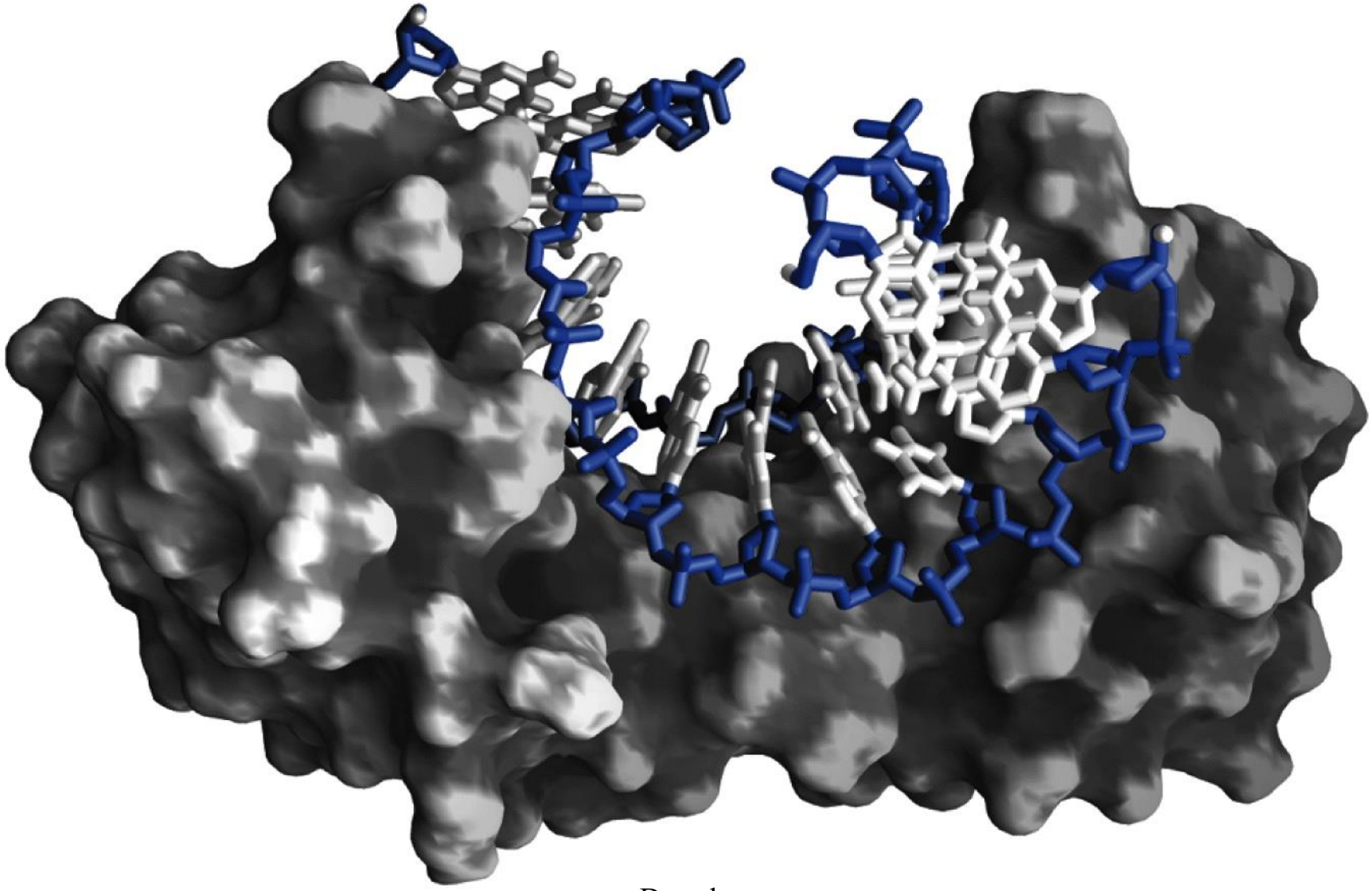
Transcription factor	Number of subunits	Subunit M_r	Functions
Initiation			
RNA polymerase II	12	10,000–220,000	Catalyzes RNA synthesis
TBP (TATA-binding protein)	1	38,000	Specifically recognizes the TATA box
TFIIA	3	12,000, 19,000, 35,000	Stabilizes binding of TFIIB and TBP to the promoter
TFIIB	1	35,000	Binds to TBP; recruits RNA polymerase–TFIIF complex
TFIID	12	15,000–250,000	Interacts with positive and negative regulatory proteins
TFIIE	2	34,000, 57,000	Recruits TFIIH; ATPase and helicase activities
TFIIF	2	30,000, 74,000	Binds tightly to RNA polymerase II; binds to TFIIB and prevents binding of RNA polymerase to nonspecific DNA sequences
TFIIH	12	35,000–89,000	Unwinds DNA at promoter; phosphorylates RNA polymerase; recruits nucleotide-excision repair complex
Elongation*			
ELL†	1	80,000	
P-TEFb	2	43,000, 124,000	
SII (TFIIS)	1	38,000	
Elongin (SIII)	3	15,000, 18,000, 110,000	

More than 30 polypeptide

*All elongation factors suppress the pausing or arrest of transcription by the RNA polymerase II – TFIIF complex.

†The name is derived from the term *e*leven-nineteen *l*ysine-rich *l*eukemia. The gene for the factor ELL is the site of chromosomal recombination events frequently associated with the cancerous condition known as acute myeloid leukemia.

Human TATA-binding protein (TBP) bound to DNA



Dr. rula

(b)

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

TFII H (not only involved in closed complex formation).

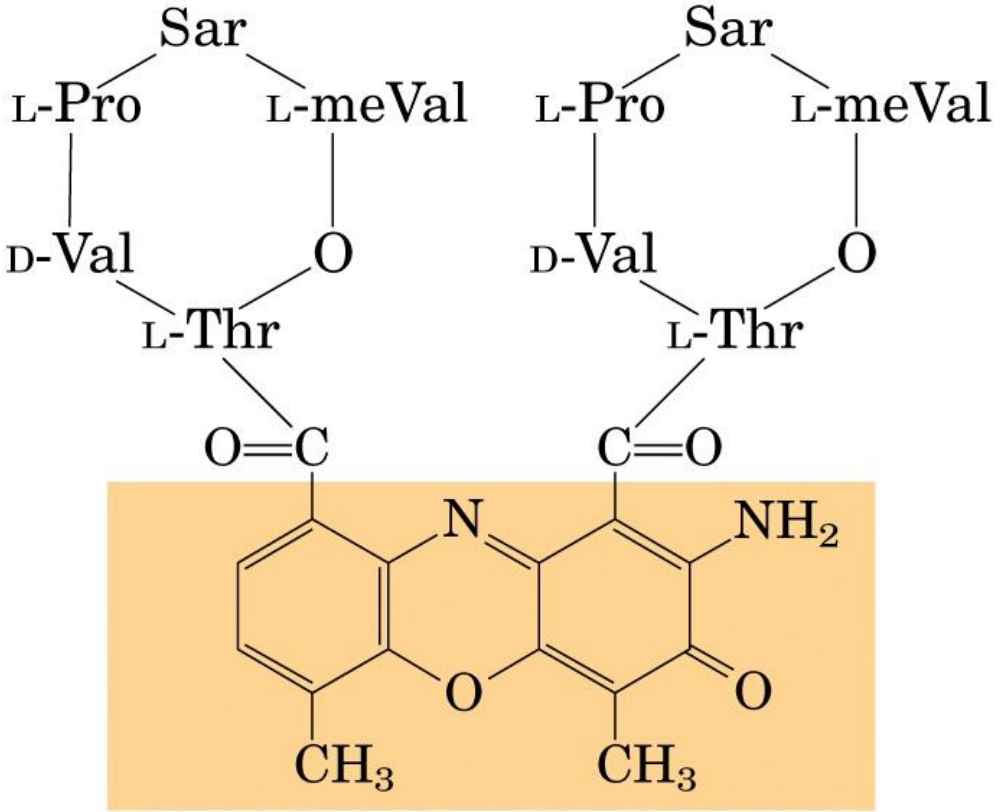
1) Interacts with the lesion.
2) Recruits the entire nucleotide excision repair.

Template strand repaired >> efficiently than nontemplate.

Intercalating agents:

inserts= intercalates into the double helical DNA.

Deforming DNA . Preventing RNA pol movement along template.



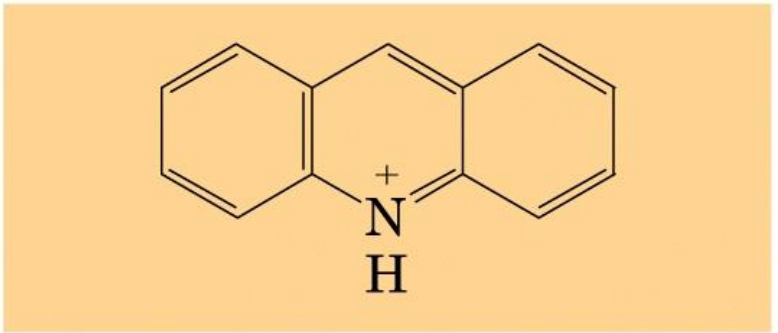
Actinomycin D

Actinomycin

inhibits elongation.

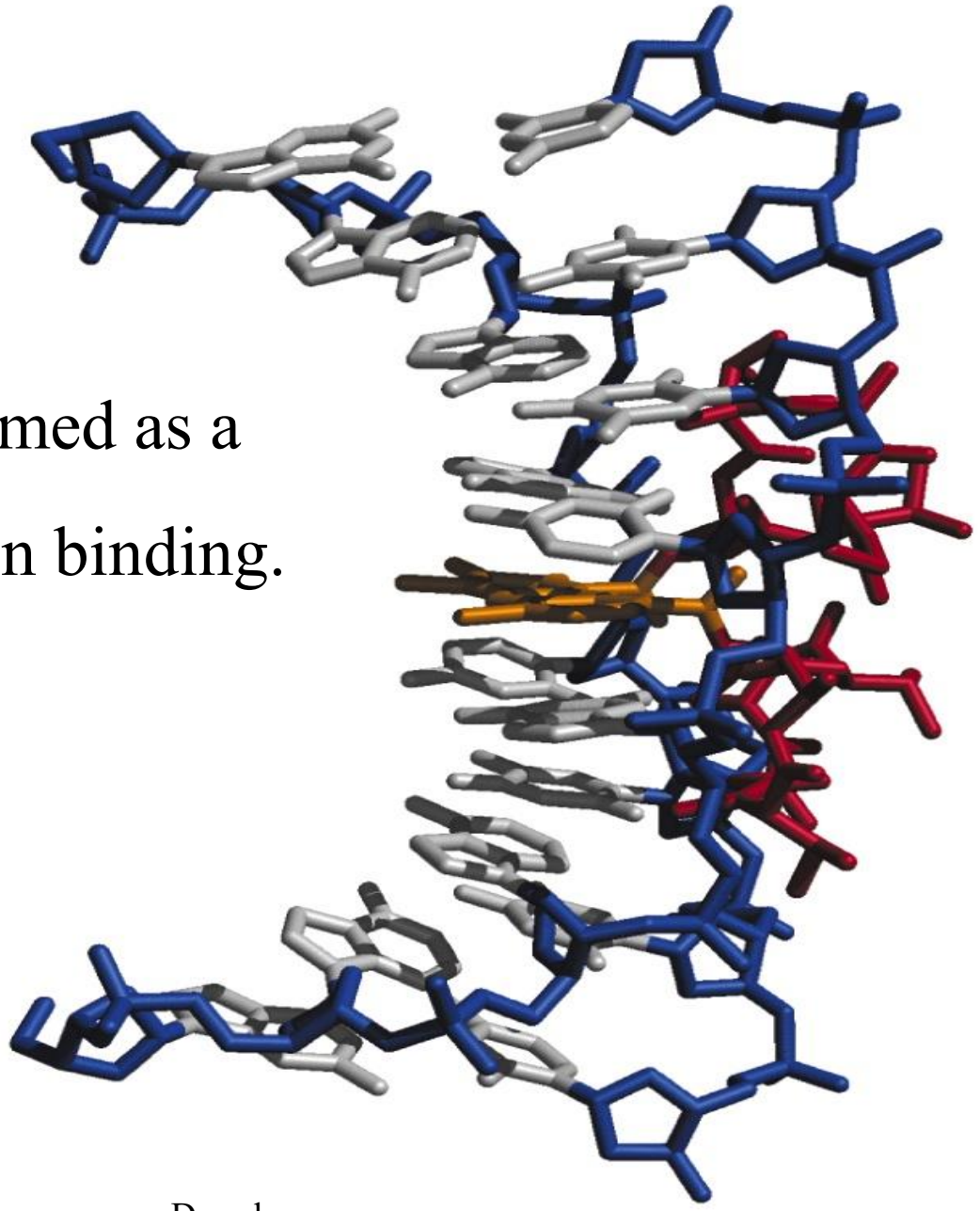
Rifampicin

inhibits promoter clearance.



Acridine

DNA bent and deformed as a result of Actinomycin binding.



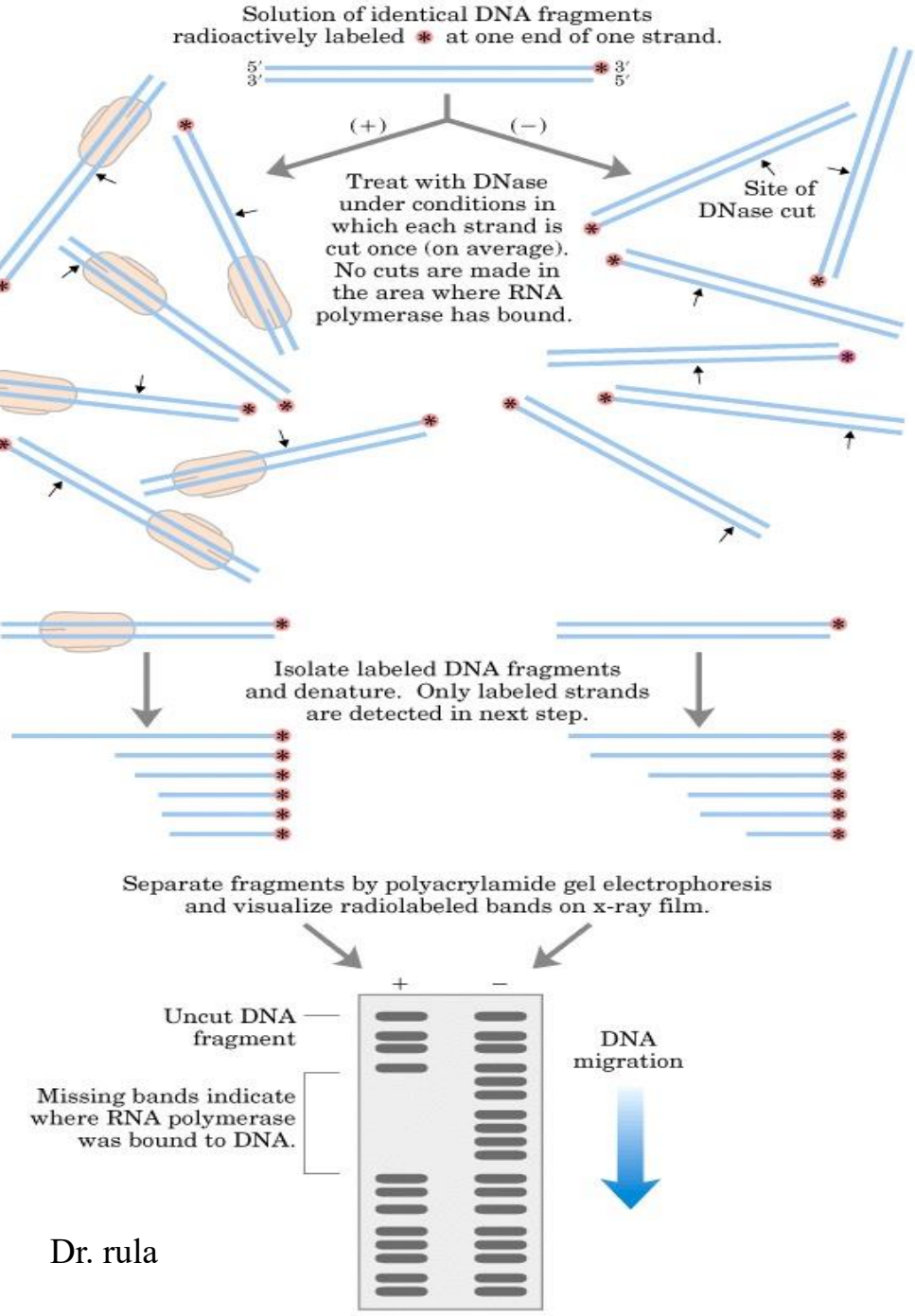
Dr. rula

(b)

DNA Footprinting:

- For identifying specific sequence of DNA / **binding site** for particular protein.
- Performed on proteins thought to play functional role such as **gene regulation**.
- Uses a **damaging agent** (chemical reagent / nuclease).
-In this technique, DNA <u>first</u> in the presence of DNA-binding proteins .
-then exposed to a damaging agent, compared to DNA never exposed to binding protein.

- The DNA sequence that is protected from cleaving identified as the **binding site.**



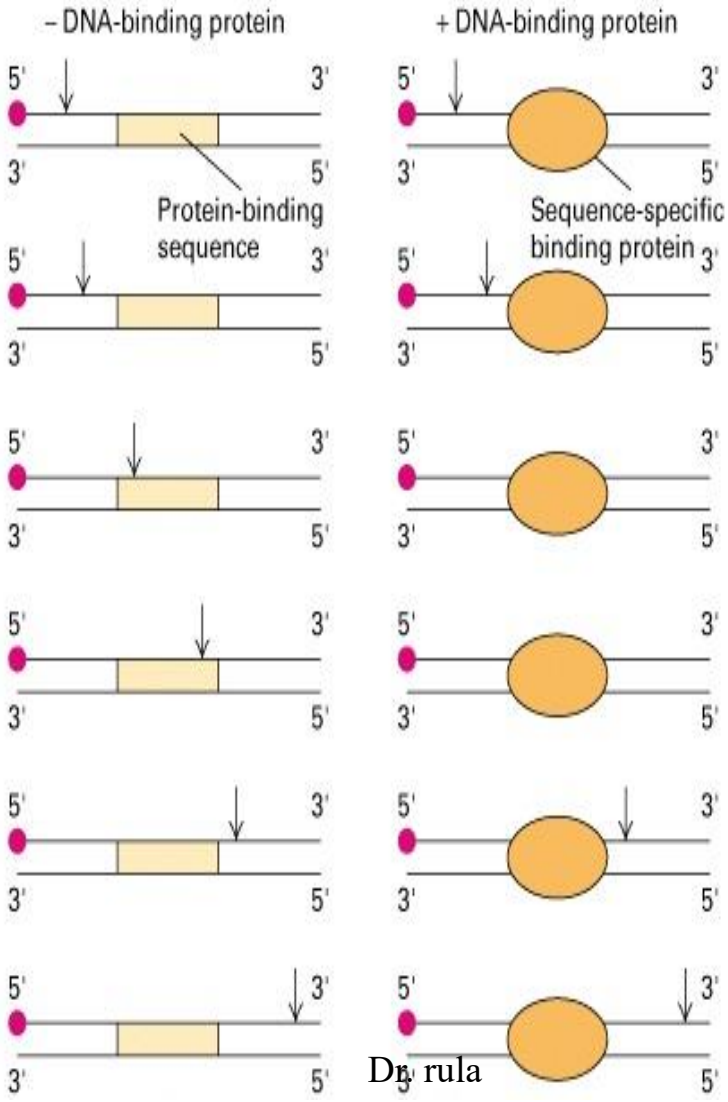
Dr. rula

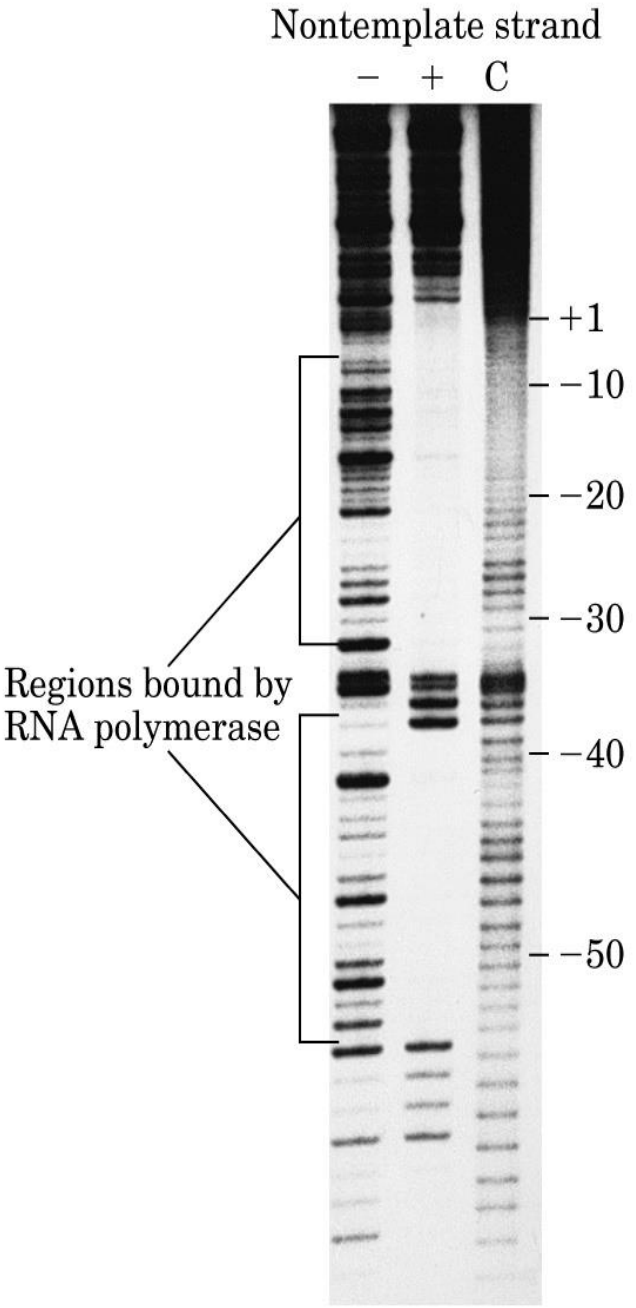
DNase I footprinting assays identify specific regions of protein-DNA interactions.

METHOD

- Add DNA-binding protein
- Partially digest with DNaseI
- Separate by polyacrylamide gel electrophoresis

Controls: no protein





- What's Next ? after the primary transcript ?

- In prokaryotes:

  RNA translated directly into protein.

- In eukaryotes:

  RNA modified, exported to cytoplasm and translated.

Transcription and RNA Processing in eukaryotes

- Genes are transcribed in the nucleus → RNA
- In the nucleus, mRNAs are:
  - **Spliced**
  - modified at each end:
  - **5'capped**
  - **3'polyadenylated**

- mRNAs are **transported to cytoplasm for** translation.

mRNA modification in eukaryotes:

1) Splicing:

Non-coding segments of *primary transcript* (introns) are removed i.e. *spliced out* by the spliceosome. The exons joined together to make the mature *mRNA*.

Alternative splicing has regulatory significance:

Some transcripts can be spliced in more than one way (alternative splicing).

This way, more than one protein can be made from a single gene.

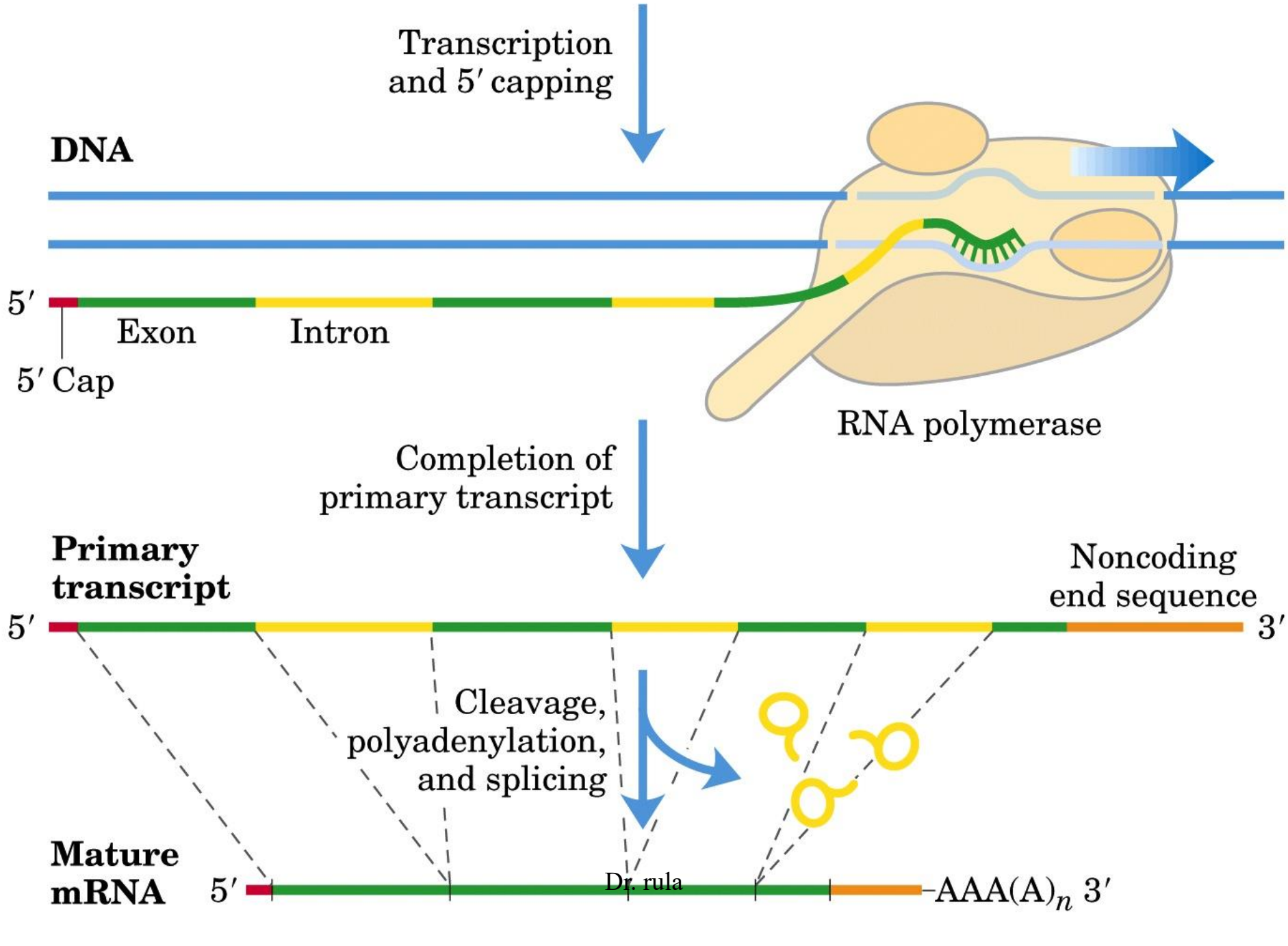
2) **Capping:**

Before synthesis of primary transcript is completed. A modified residue called 5'-cap is added at 5' end.

3) **Polyadenylation:**

The 3' end is cleaved and 80-250 A residues added to create a poly A tail.

These 3 modifications elaborate together with phosphorylated CTD and RNA pol II.


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