Transcription

DNA → **RNA**

PHYA- Sem-IV

Molecular Biology

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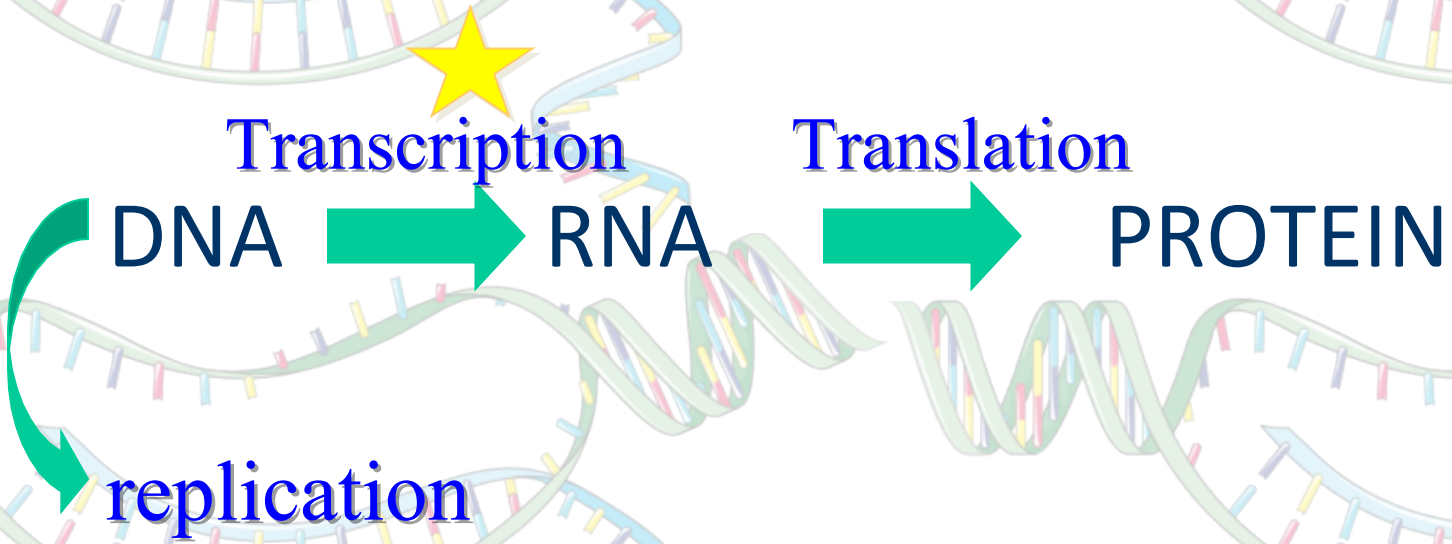
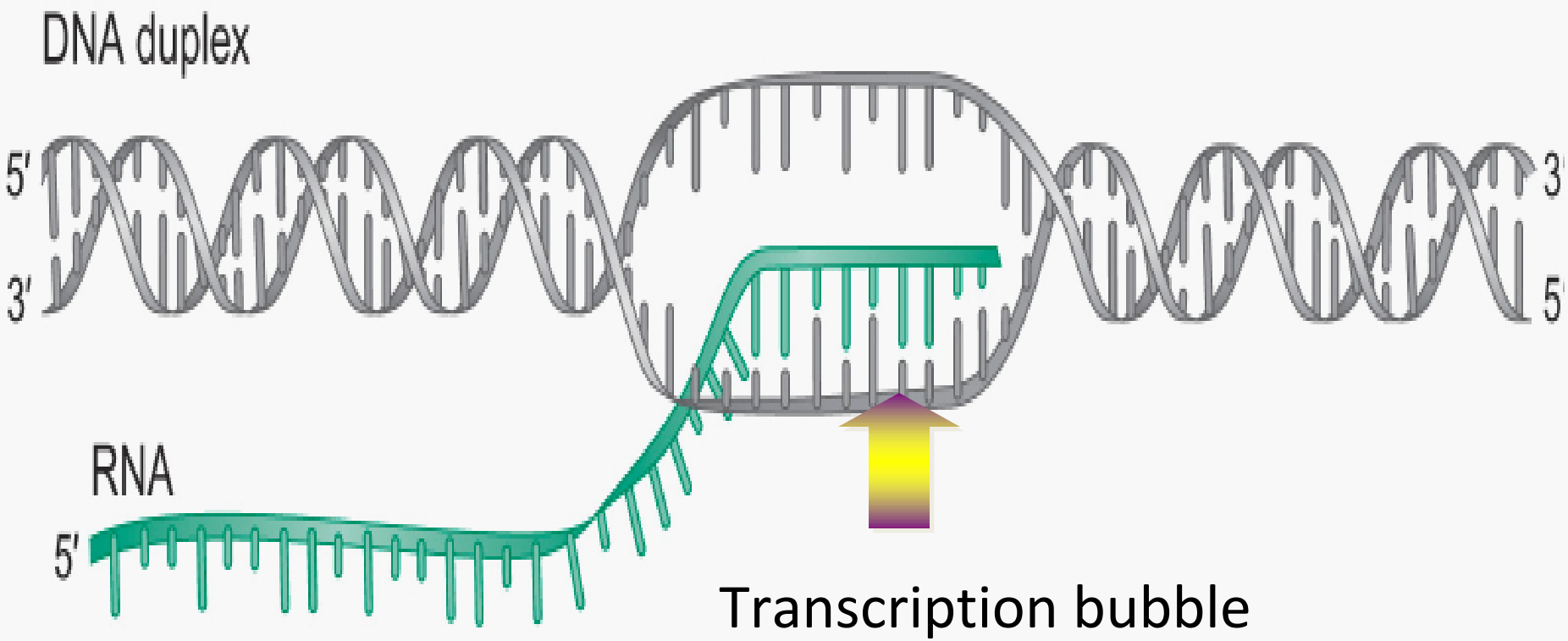
The Central Dogma





Transcription of DNA into RNA

Transcription is very similar to DNA replication but there are some important differences:

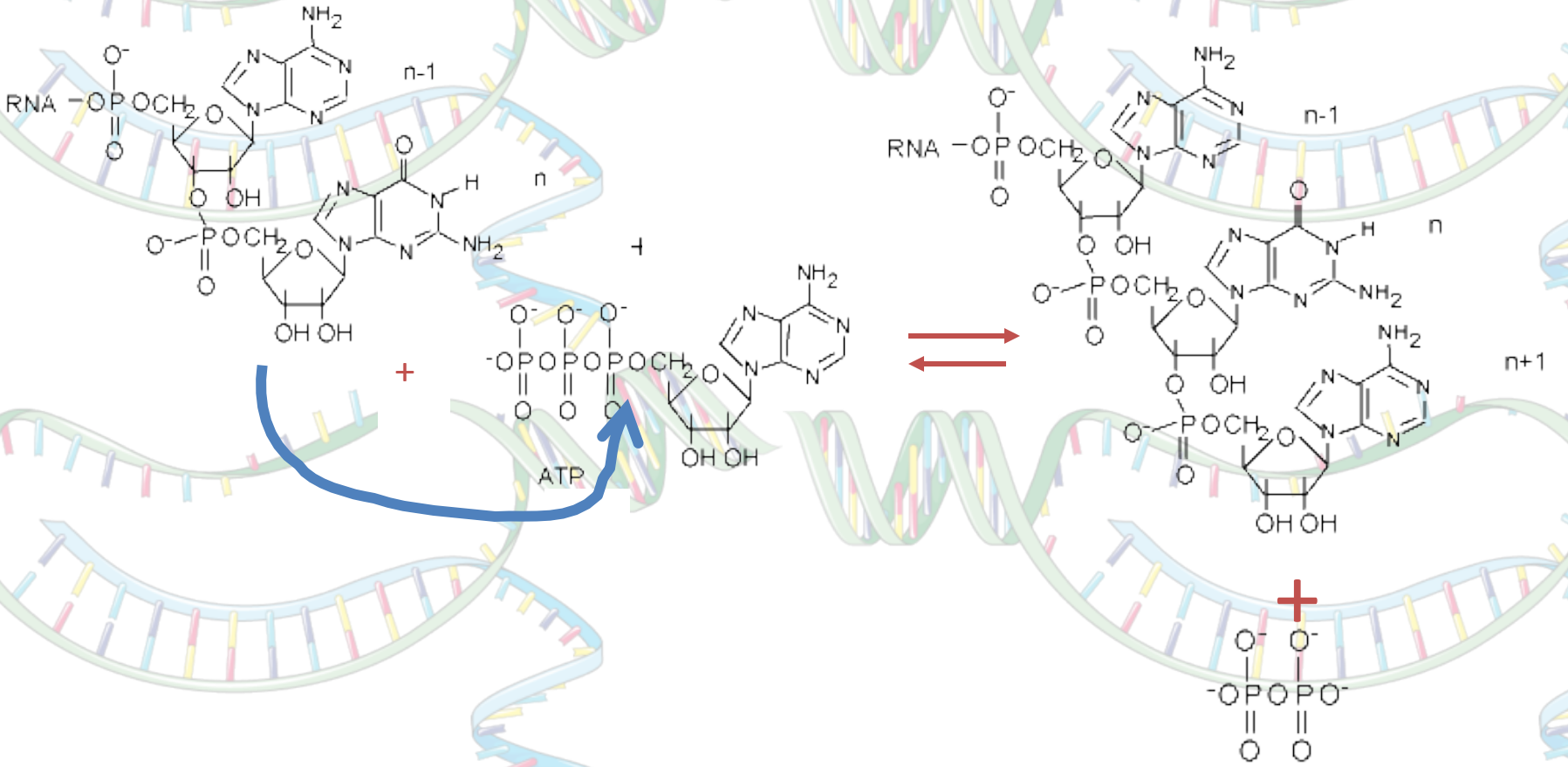
1. RNA is made of ribonucleotides
2. RNA polymerase catalyzes the reaction
3. The synthesized RNA does not remain base-paired to the template DNA strand
4. Less accurate (error rate: 10^{-4})

5. Transcription selectively copies only certain parts of the genome and makes one to several hundred, or even thousand, copies of any given section of the genome.

Reaction catalyzed by RNA polymerase

- Catalyzes the synthesis of RNA directed by DNA as a template = **transcription**
- Makes an RNA chain with a sequence complementary to the template strand of DNA
- Does NOT require a primer; can start RNA synthesis at a site on the DNA template

Sequential addition of ribonucleotides



Pyrophosphate = PPi

RNA polymerase and the transcription cycle

RNA polymerases come in different forms, but share many features

- RNA polymerases performs essentially the same reaction in all cells
- Bacteria have only a single RNA polymerases while in eukaryotic cells there are three: RNA Pol I, II and III

- RNA Pol III is the focus of eukaryotic transcription, because it is the most studied polymerase, and is also responsible for transcribing most genes-indeed, essentially all protein-encoding genes
- RNA Pol I transcribe the large ribosomal RNA precursor gene
- RNA Pol II transcribe tRNA gene, some small nuclear RNA genes and the 5S rRNA genes

Table : The subunits of RNA polymerases

Prokaryotic		Eukaryotic		
Bacterial	**Archaeal**	**RNAP I**	**RNAP II**	**RNAP III**
Core	**Core**	**(Pol I)**	**(Pol II)**	**(Pol III)**
β'	A′/A″	RPA1	RPB1	RPC1
β	B	RPA2	RPB2	RPC2
α^{I}	D	RPC5	RPB3	RPC5
α^{II}	L	RPC9	RPB11	RPC9
ω	K	RPB6	RPB6	RPB6
	[+6 others]	[+9 others]	[+7 others]	[+11 others]

RNA polymerase and the transcription cycle

Role of a subunit in assembly of RNA polymerase and other functions

α–NTD

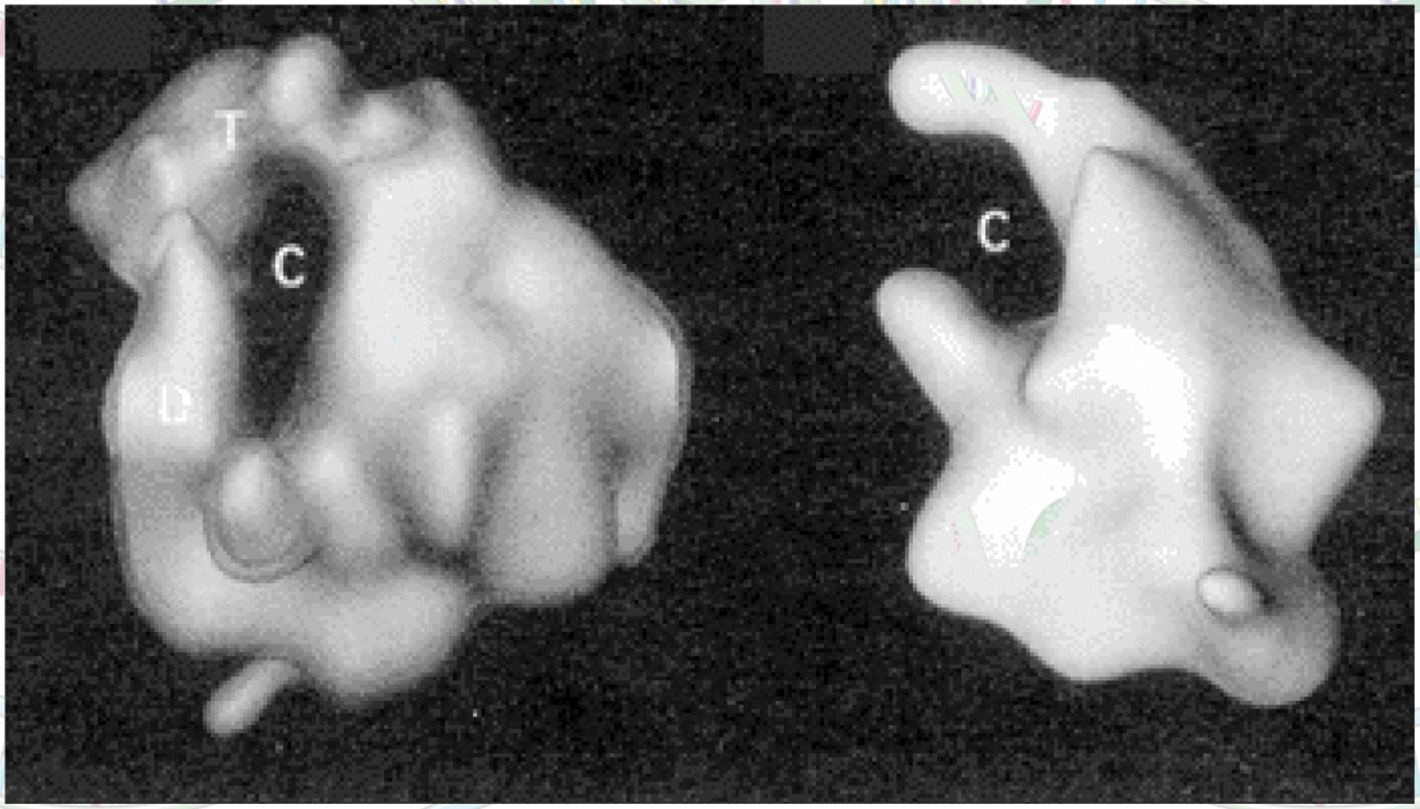
- DIMERIZATION
- ASSEMBLY

$$2\alpha \rightarrow \alpha_2$$
$$\alpha_2 + \beta \rightarrow \alpha_2\beta$$
$$\alpha_2\beta + \beta' \rightarrow \alpha_2\beta\beta'$$
$$\alpha_2\beta\beta' + \sigma \rightarrow \alpha_2\beta\beta'\sigma$$

α–CTD

- DIMERIZATION (weak)
- BIND DNA
- BIND ACTIVATORS
- POST TRANSLATIONAL MODIFICATION SITE

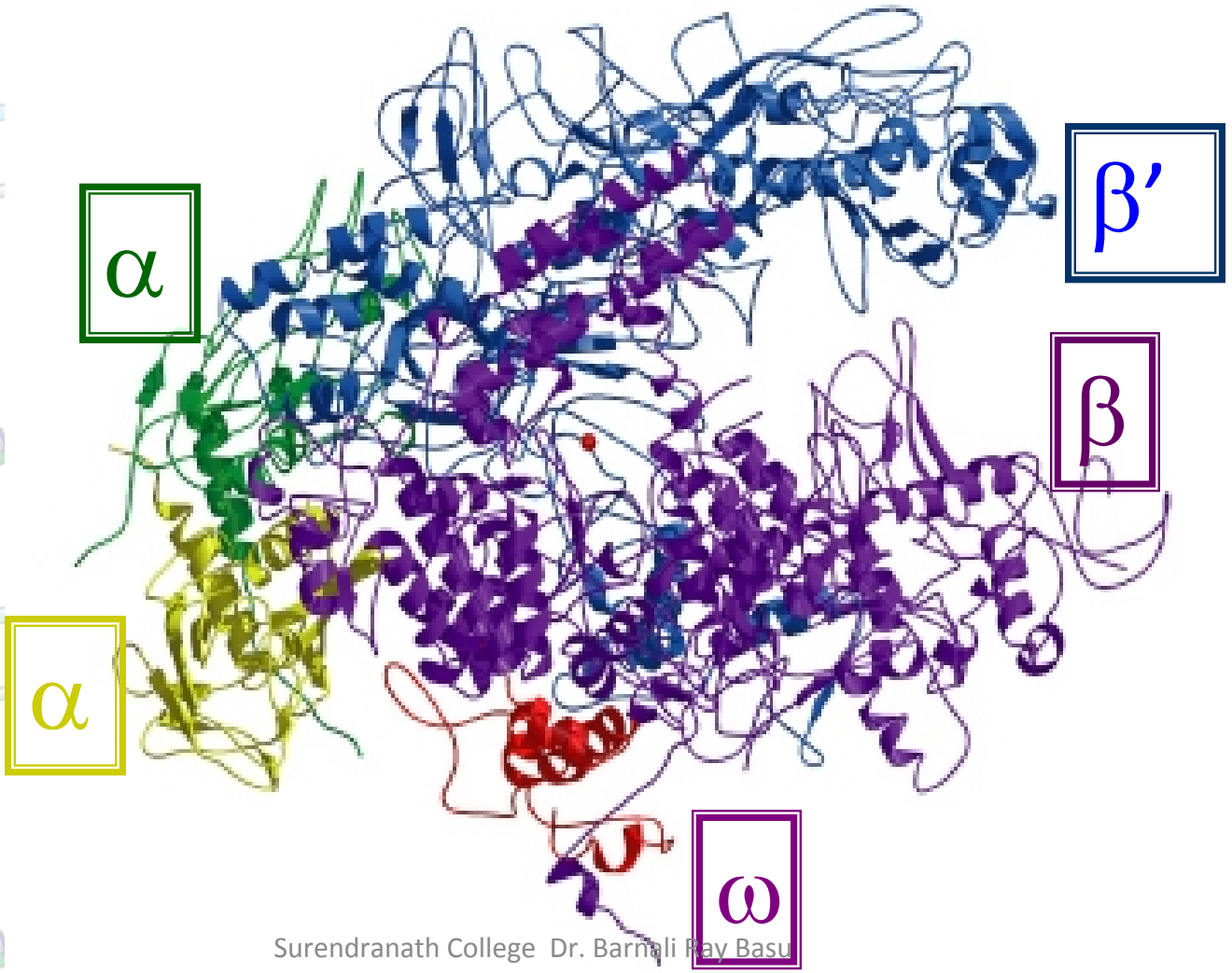
3-D images of core and holoenzyme



Core Holoenzyme

The bacterial RNA polymerase

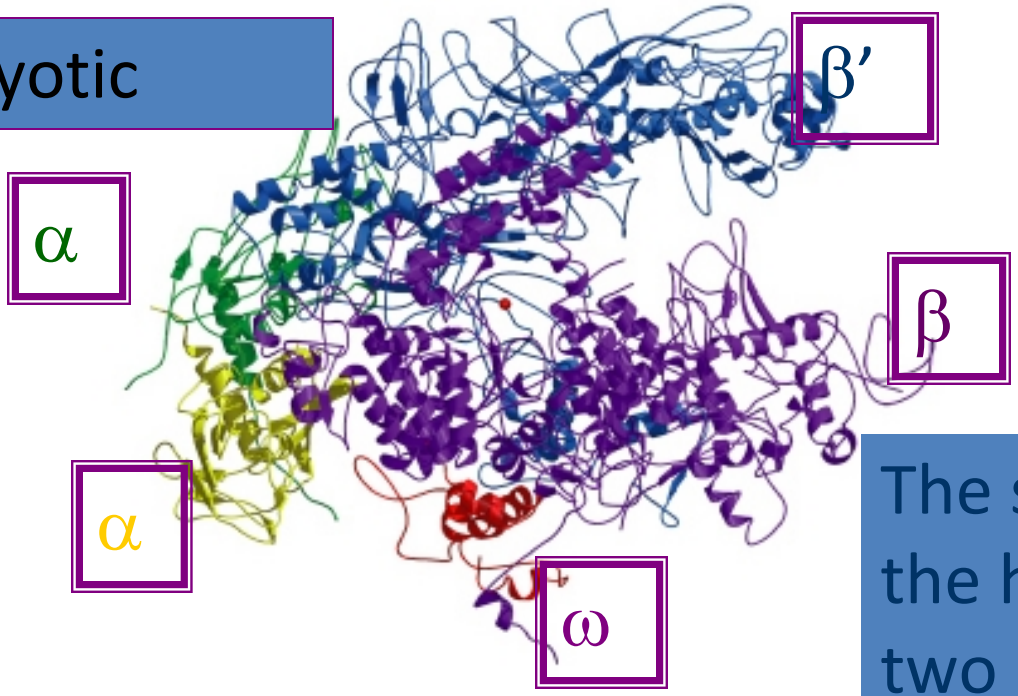
The core enzyme alone synthesizes RNA



RNA polymerase and the transcription cycle

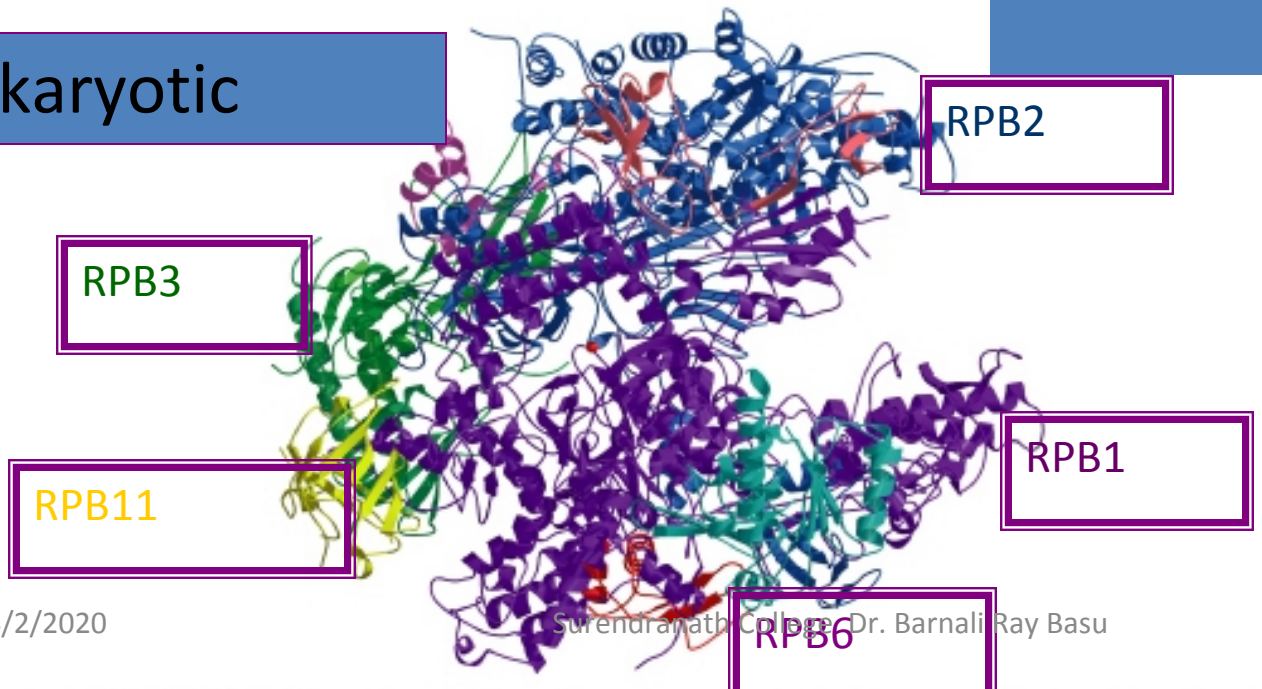
Fig : RNA Polymerase Comparison

prokaryotic

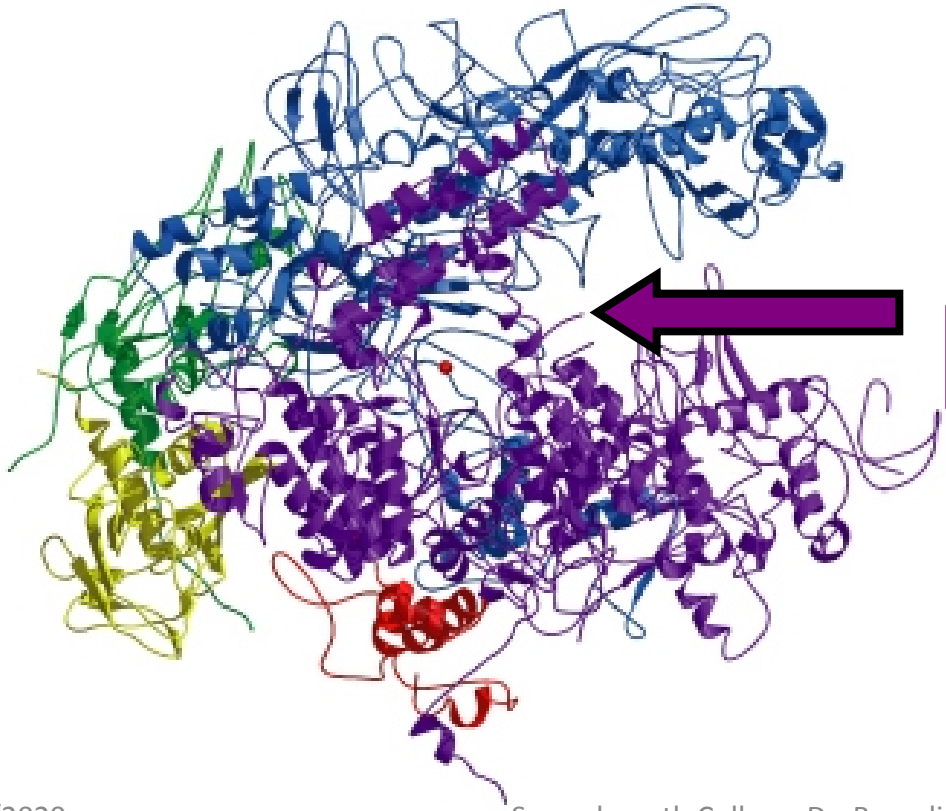


The same color indicate the homologous of the two enzymes

eukaryotic



“Crab claw” shape of RNAP



Active center cleft

There are various channels allowing DNA, RNA and ribonucleotides (rNTPs) into and out of the enzyme's active center cleft

NTP uptake channel is in the back

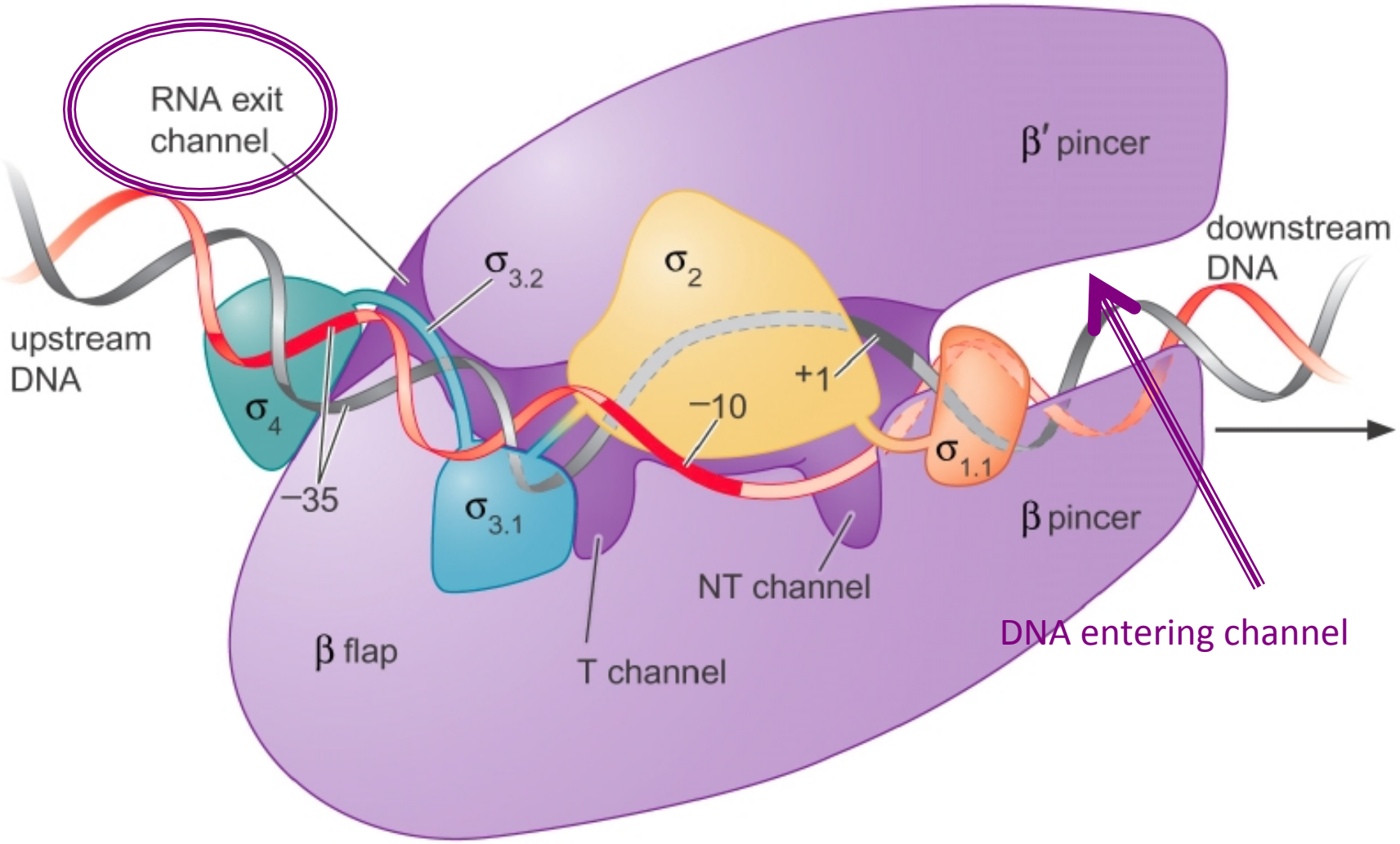


Fig - channels into and out of the open complex

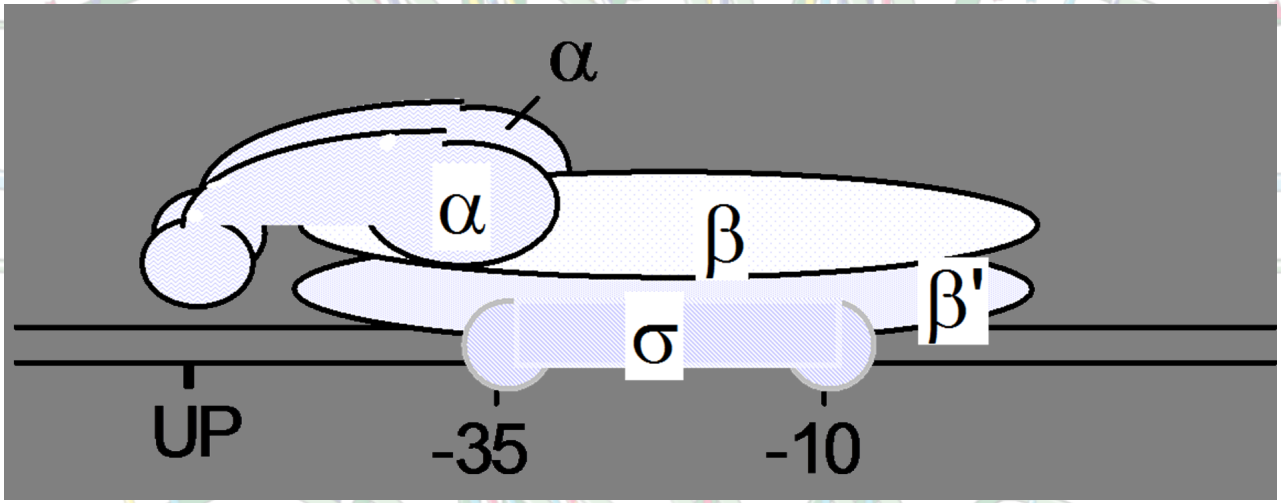
Transcription by RNA polymerase proceeds in a series of steps

- Initiation
- Elongation
- Termination

Initiation

- Promoter: the DNA sequence that initially binds the RNA polymerase
- The structure of promoter-polymerase complex undergoes structural changes to proceed transcription
- DNA at the transcription site unwinds and a "bubble" forms
- Direction of RNA synthesis occurs in a 5'-3' direction (3'-end growing)

RNA polymerase at a promoter

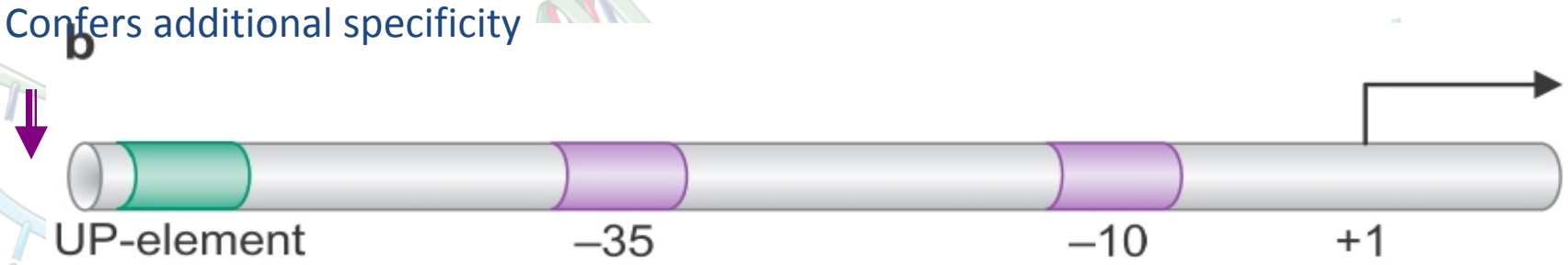


α: assembly and binds to UP

β+β': form catalytic center

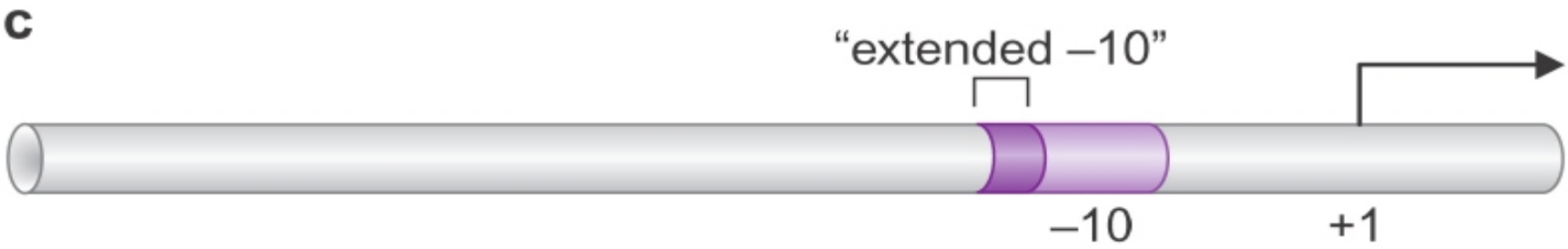
σ: binds -10 and -35 of promoter to confer specificity during initiation

Fig - bacterial promoter



UP-element is an additional DNA elements that increases polymerase binding by providing the additional interaction site for RNA polymerase

Fig -bacterial promoter



Another class of σ^{70} promoter lacks a –35 region and has an "extended –10 element" compensating for the absence of –35 region

Transcription initiation involves 3 defined steps

RNA polymerase and the transcription cycle

1. Forming closed complex

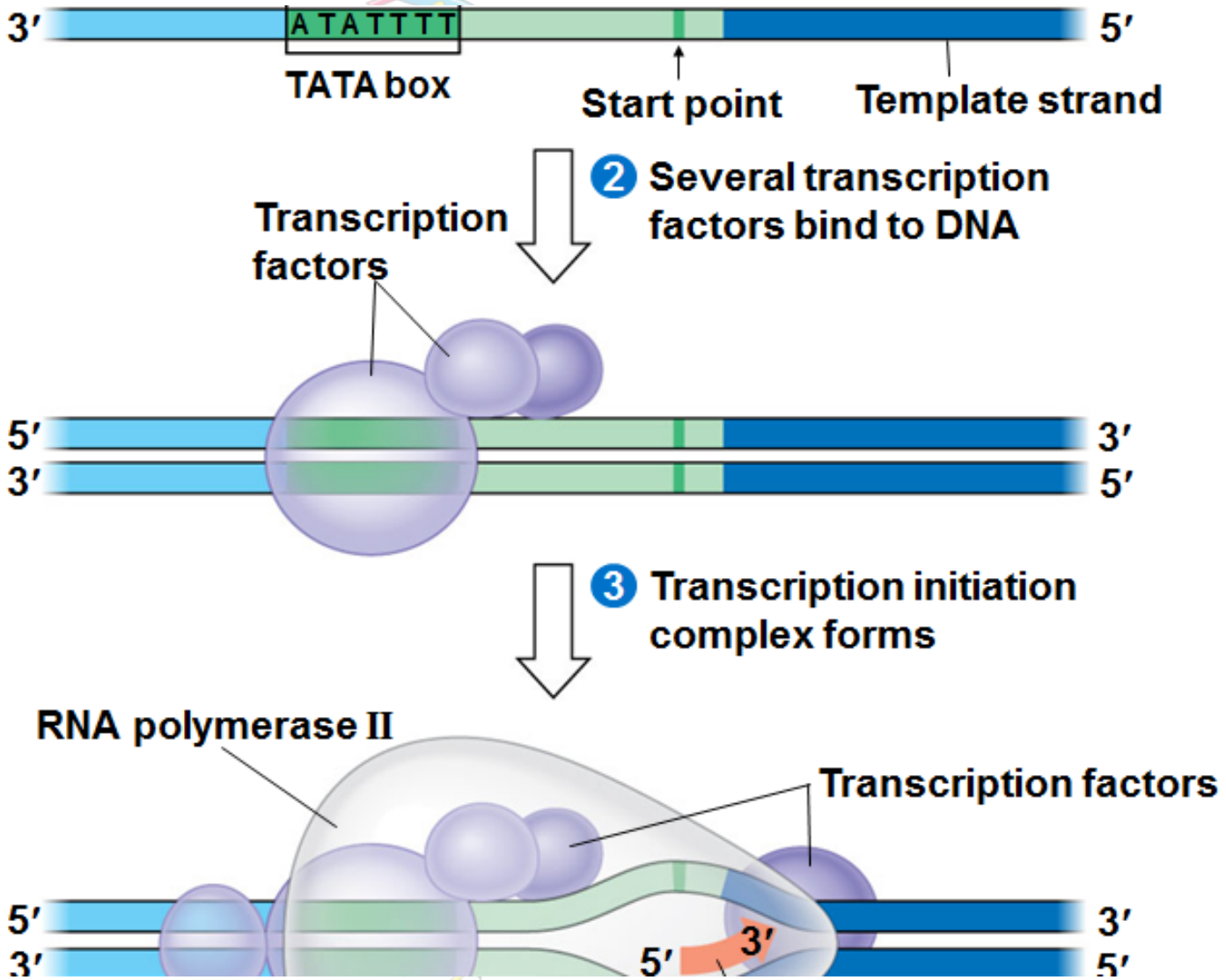
Closed complex means DNA remains double stranded

2. Forming open complex

Open complex means DNA become unwind and RNA Polymerase can sit and starts elongation

3. Promoter escape

RNA polymerase and the transcription cycle



RNA polymerase and the transcription cycle

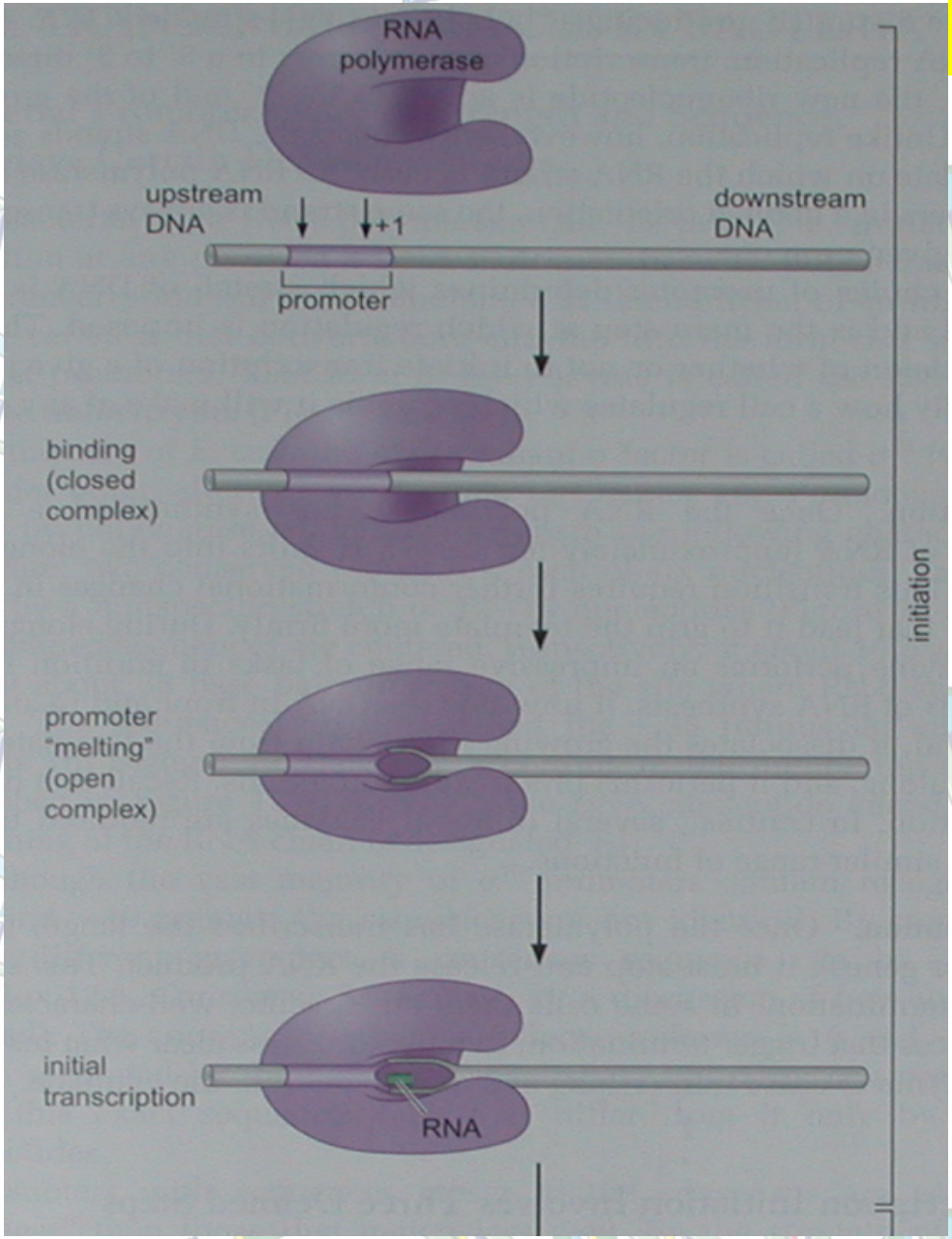


Fig -initiation

Binding (closed complex)

Promoter "melting" (open complex)

Initial transcription

Closed complex

- The initial binding of polymerase to a promoter
- DNA remains double stranded
- The enzyme is bound to one face of the helix

Open complex

- the DNA strand separate over a distance of ~14 bp (-11 to +3) around the start site (+1 site)
- Replication bubble forms

Stable ternary complex

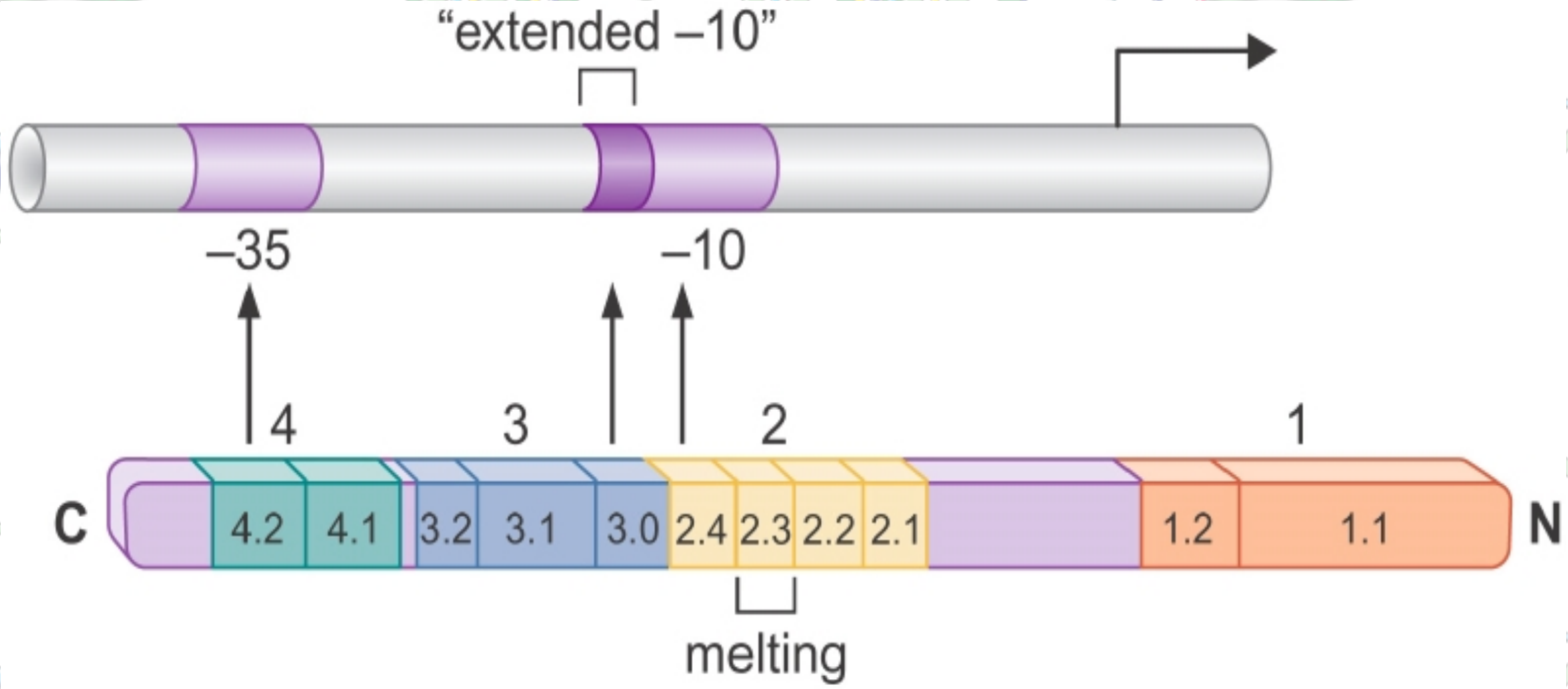
- The enzyme escapes from the promoter
- The transition to the elongation phase
- Stable ternary complex

  =DNA +RNA + enzyme

The σ factor mediates binding of polymerase to the promoter

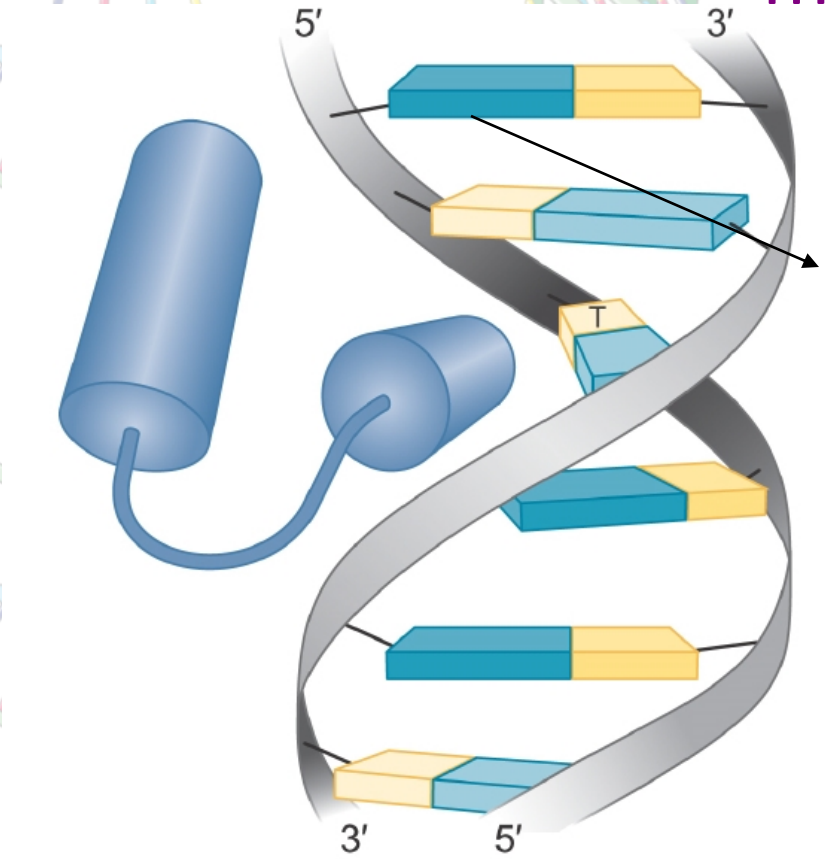
- The σ^{70} factor comprises four regions called σ region 1 to σ region 4.

Fig : regions of σ



Region 4 recognizes -35 element Region 2 recognizes -10 element, Region 3 recognizes the extended –10 element

Binding of –35 Two helices within region 4 form a common DNA-binding motif, called a helix-turn-helix motif



One helix inserts into the DNA major groove interacting with the bases at the –35 region. The other helix lies across the top of the groove, contacting the DNA backbone

Fig -* Helix-turn-helix DNA-binding motif

Interaction with –10 is more elaborate and less understood

- The -10 region is within DNA melting region
- The α helix recognizing –10 can interacts with bases on the non-template strand to stabilize the melted DNA.

UP-element is recognized by a carboxyl terminal domain of the α-subunit (αCTD), but not by σ factor

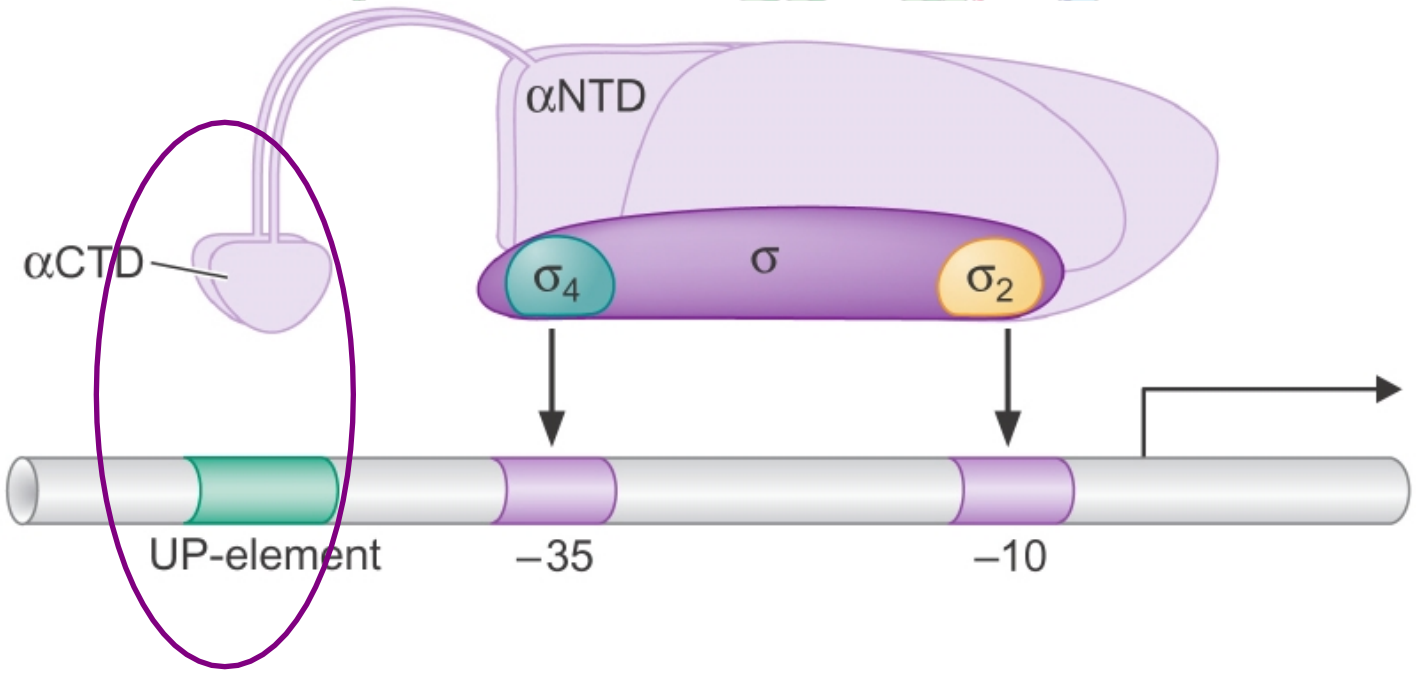


Fig - σ and α subunits recruit RNA pol core enzyme to the promoter

Transition to the open complex involves structural changes in RNA polymerase and in the promoter DNA

This transition is called

Isomerization

- For σ^{70} –containing RNA polymerase, isomerization is a spontaneous conformational change in the DNA-enzyme complex to a more energetically favorable form. (No extra energy requirement)

Change of the promoter DNA

- the opening of the DNA double helix, called "melting", at positions -11 and +3.

The striking structural change in the polymerase

- 1. the β and β' pincers down tightly on the downstream DNA
- 2. A major shift occurs in the N-terminal region of σ (region 1.1) shifts. In the closed complex, σ region 1.1 is in the active center; in the open complex, the region 1.1 shift to the outside of the center, allowing DNA access to the cleft

Transcription is initiated by RNA polymerase without the need for a primer

Initiation requires:

- The initiating NTP (usually an A) is placed in the active site
- The initiating ATP is held tightly in the correct orientation by extensive interactions with the holoenzyme

RNA polymerase synthesizes several short RNAs before entering the elongation phase

Abortive initiation: the enzyme synthesizes and releases short RNA molecules less than 10 nt.

Structural barrier for the abortive initiation

- The 3.2 region of σ factor lies in the middle of the RNA exit channel in the open complex.
- Ejection of this region from the channel (1) is necessary for further RNA elongation, (2) takes the enzyme several attempts

Synthesizing by RNA polymerase

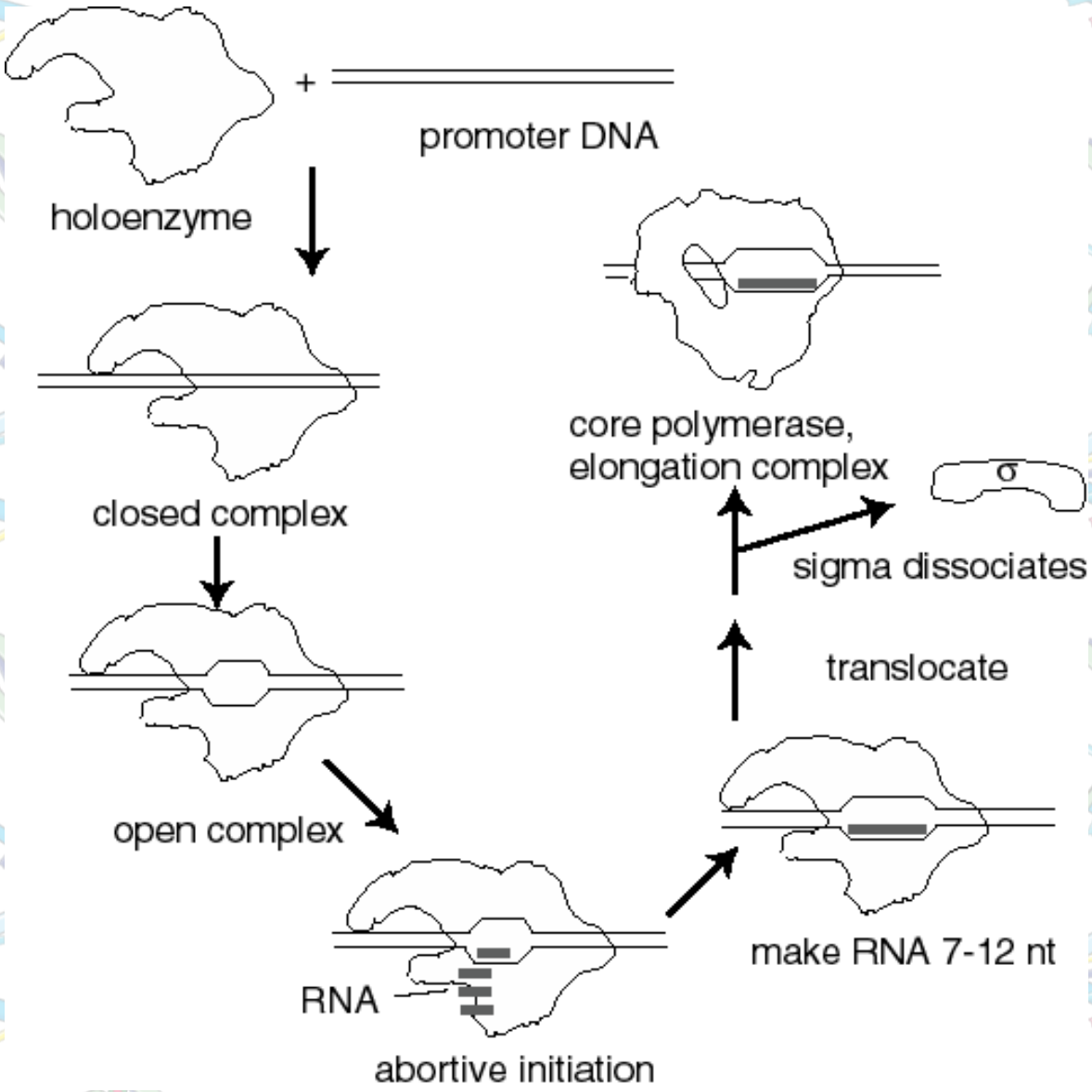
1. DNA enters the polymerase between the pincers
2. Strand separation in the catalytic cleft
3. NTP addition
4. RNA product spooling out (Only 8-9 nts of the growing RNA remain base-paired with the DNA template at any given time)
5. DNA strand annealing in behind

Events at initiation of transcription

RNA polymerase and the transcription cycle

- **Closed complex** means DNA remains double stranded

- **Open complex** means DNA become unwind and RNA Polymerase can sit and starts elongation



Transcription cycle

- Initiation
  - Holoenzyme binds to the promoter, unwinds DNA, and forms phosphodiester bonds between 7 to 12 nucleotides
  - Need σ
- Elongation
  - σ dissociates
  - Core elongates RNA with high processivity
  - May use NusA
- Termination
  - Polymerase dissociates from template DNA and releases new RNA
  - Often use ρ.

Elongation

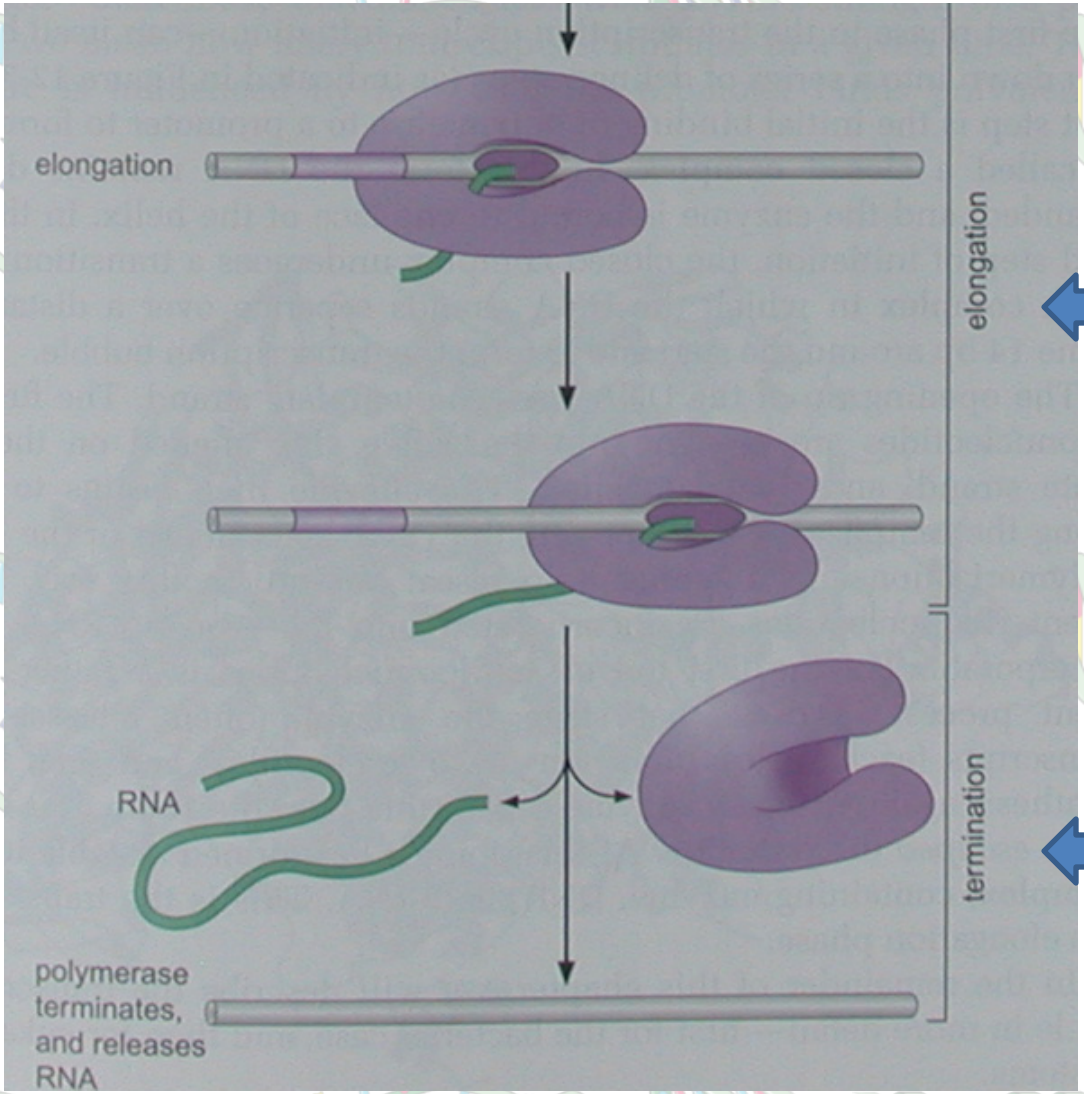
- Once the RNA polymerase has synthesized a short stretch of RNA (~ 10 nt), transcription shifts into the elongation phase.
- This transition requires further conformational change in polymerase that leads it to grip the template more firmly.
- Functions: synthesis RNA, unwinds the DNA in front, re-anneals it behind, dissociates the growing RNA chain

Termination

- After the polymerase transcribes the length of the gene (or genes), it will stop and release the RNA transcript.
- In some cells, termination occurs at the specific and well-defined DNA sequences called terminators. Some cells lack such termination sequences.

Fig : Elongation and termination

RNA polymerase and the transcription cycle



Elongation

Termination

Transcription is terminated by signals within the RNA sequence

Terminators: the sequences that trigger the elongation polymerase to dissociate from the DNA

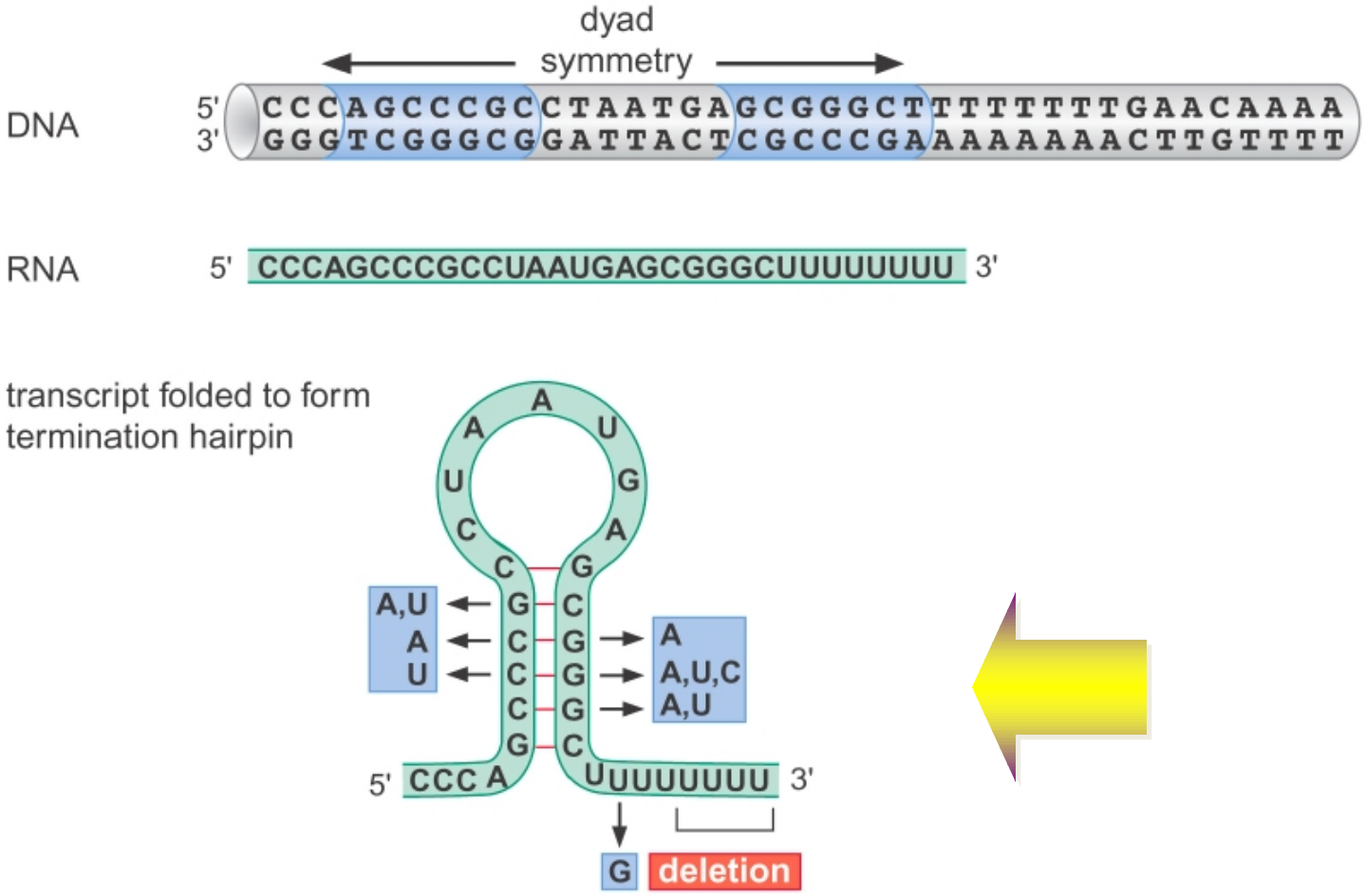
- Rho-dependent (requires Rho protein)
- Rho-independent, also called intrinsic terminator

Rho-independent termination

- In Rho-independent termination, the template inverted repeat of DNA is followed by a series of adenines. This series produces a run of perhaps half a dozen Uracils in the m-RNA. So, the m-RNA in rho-independent termination has the following structure.

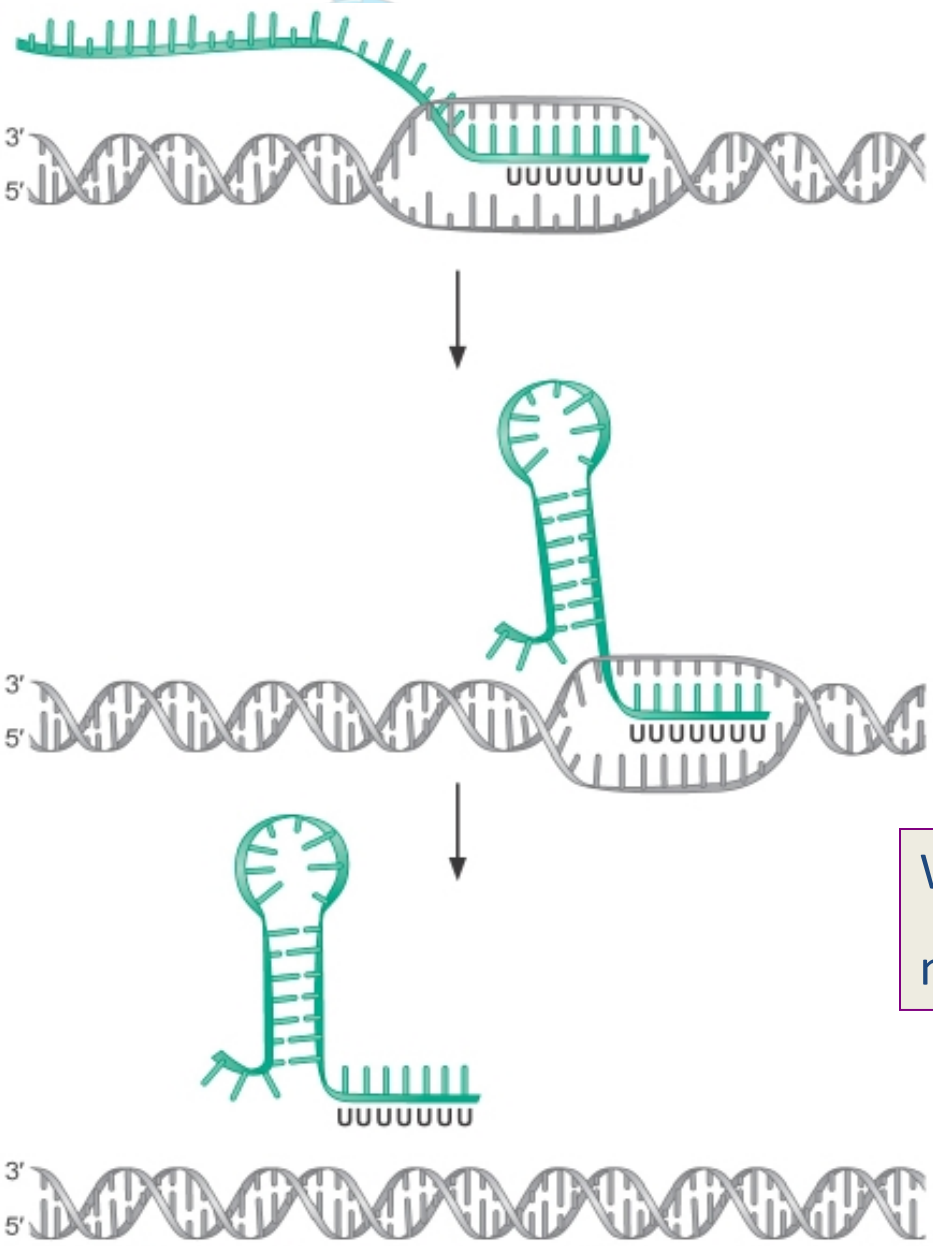
Rho-independent terminator contains a short inverted repeat (~20 bp) and a stretch of ~8 A:T base pairs.

RNA polymerase and the transcription cycle



- At the point where the poly "U" sequence is attached to the DNA sequence, the hybrid DNA-RNA is unusually weak (A-U bonds are weak) and it requires very little energy to break the hydrogen bonds holding the two strands together. When separation occurs, m-RNA synthesis, transcription stops. This type of termination is rho-independent; no termination factor is required.

RNA polymerase and the transcription cycle



transcription termination

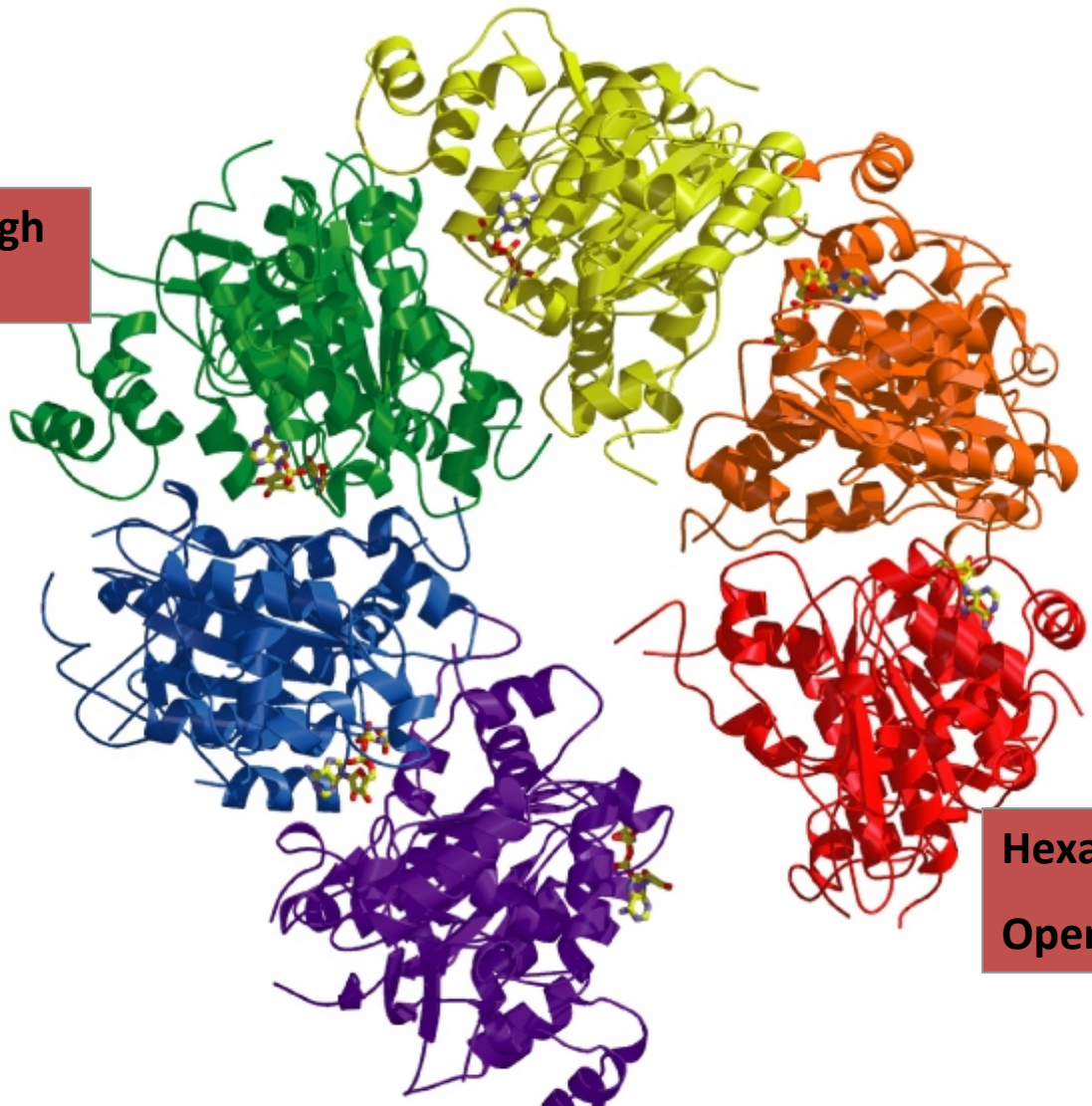
Weakest base pairing: A:U

make the dissociation easier

Rho (r) -dependent terminators

- Have less well-characterized RNA elements, and requires Rho protein for termination
- Rho is a ring-shaped single-stranded RNA binding protein, like SSB
- Rho binding can wrest the RNA from the polymerase-template complex using the energy from ATP hydrolysis
- Rho binds to *rut* (r utilization) RNA sites
- Rho does not bind the translating RNA

Fig - the ρ transcription terminator



RNA tread trough the "ring"

Hexamer,

Open ring

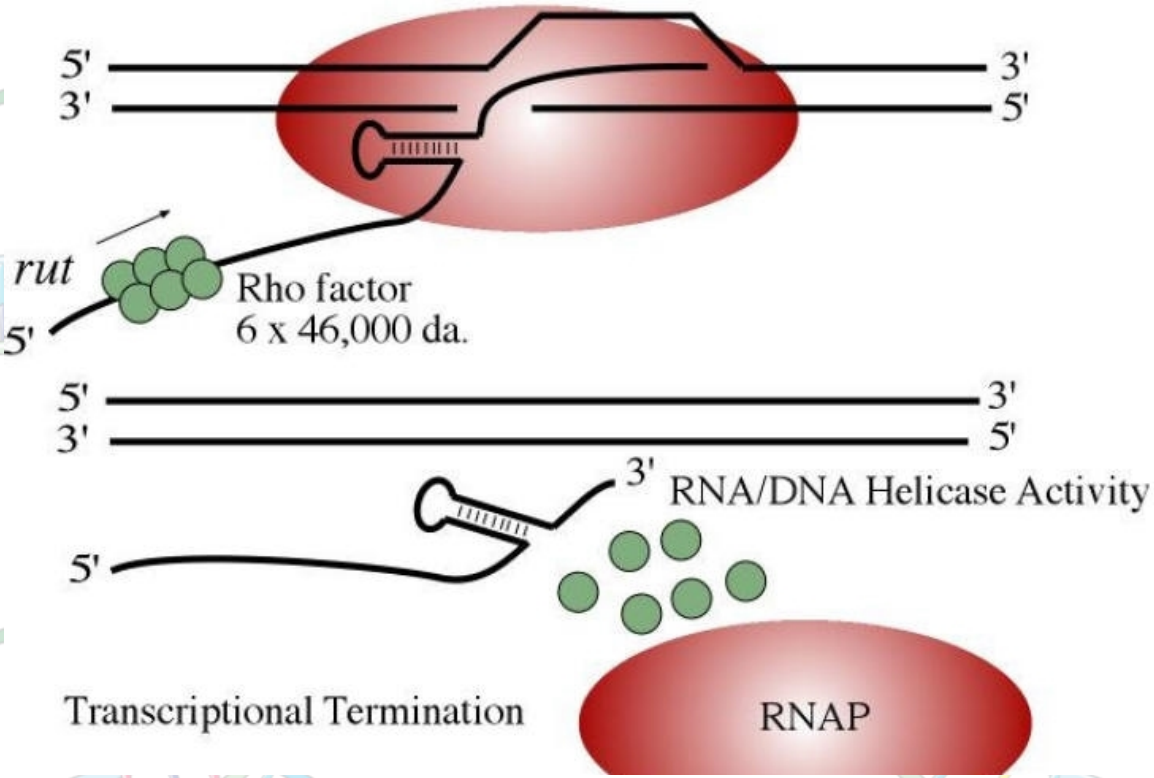


Fig : Rho- dependent Termination

- Rho dependent termination also uses a hairpin m-RNA formation but dissociation of the DNA-RNA hybrid needs the assistance of the protein rho and no poly “U” follows the hairpin.
- Rho, a tetramer of about 5 kdaltons binds to RNA Polymerase and brings about the excision of RNA transcript by a mechanism which is not fully understood.
- It is proposed that first rho protein binds to the 5’-end of a nascent RNA chain and then moves along the RNA, using the hydrolysis of ATPs to provide the necessary energy.

- Then, when RNA Polymerase pauses at certain sites having high GC sequences or stem-loop structure it catches up and binds with RNA Polymerase.

- When rho binds with RNA Polymerase it assume ATPase and brings about the hydrolysis of RNA chain after which the rho factor and the enzyme dissociates from the template.

Reference Books

- **Lehninger Principles of Biochemistry *by* David L. Nelson and Michael M. Cox**
- **Molecular Biology of the Cell, 4th edition *by*** Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter.