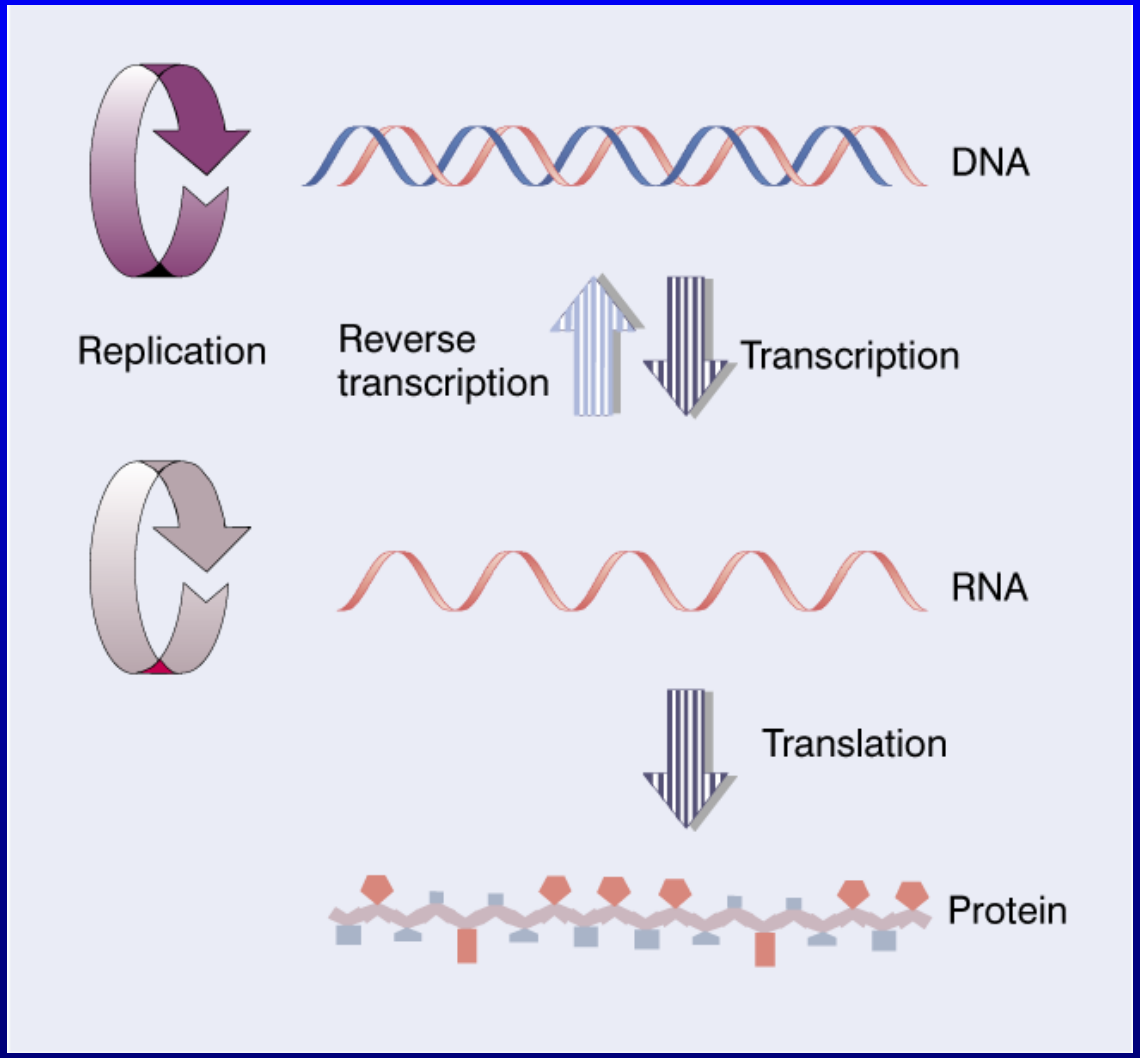
Chapter 17

RNA synthesis

Central dogma



WHICH CAME FIRST, THE chicken or the egg? The biological silences have a variation: which came first, DNA or protein? You see, among the many tasks performed by proteins is assembling DNA molecules. But DNA contains the information needed to make proteins. So which came first?

RNA and RNA world

Walter Gilbert

1980 Nobel Prize

Origin-of-Life Theories

RNA has the ability to act as both genes and enzymes

RNA synthesis

- Transcription
  - The synthesis of RNA molecules using DNA strands as the templates so that the genetic information can be transferred from DNA to RNA.
  - Four stages: Initiation, Elongation, Termination, Post-transcriptional modification
  - Products: mRNA, tRNA, rRNA, sRNA
- RNA replication

Asymmetric transcription

- Only the template strand is used for the transcription, but the coding strand is not.
- Both strands can be used as the templates.
- The transcription direction on different strands is opposite.
- This feature is referred to as the asymmetric transcription.

Template

- The template strand is the strand from which the RNA is actually transcribed. It is also termed as antisense strand.
- The coding strand is the strand whose base sequence specifies the amino acid sequence of the encoded protein. Therefore, it is also called as sense strand.

Similarity between replication and transcription

- Both processes use DNA as the template.
- Phosphodiester bonds are formed in both cases.
- Both synthesis directions are from 5′ to 3′.

Differences between replication and transcription

	Replication	Transcription
Template	Double strands	Single strand
Substrate	dNTP	NTP
Primer	yes	no
Enzyme	DNA polymerase	RNA polymerase
Product	dsDNA	ssRNA
Base pair	A-T, G-C	A-U, T-A, G-C

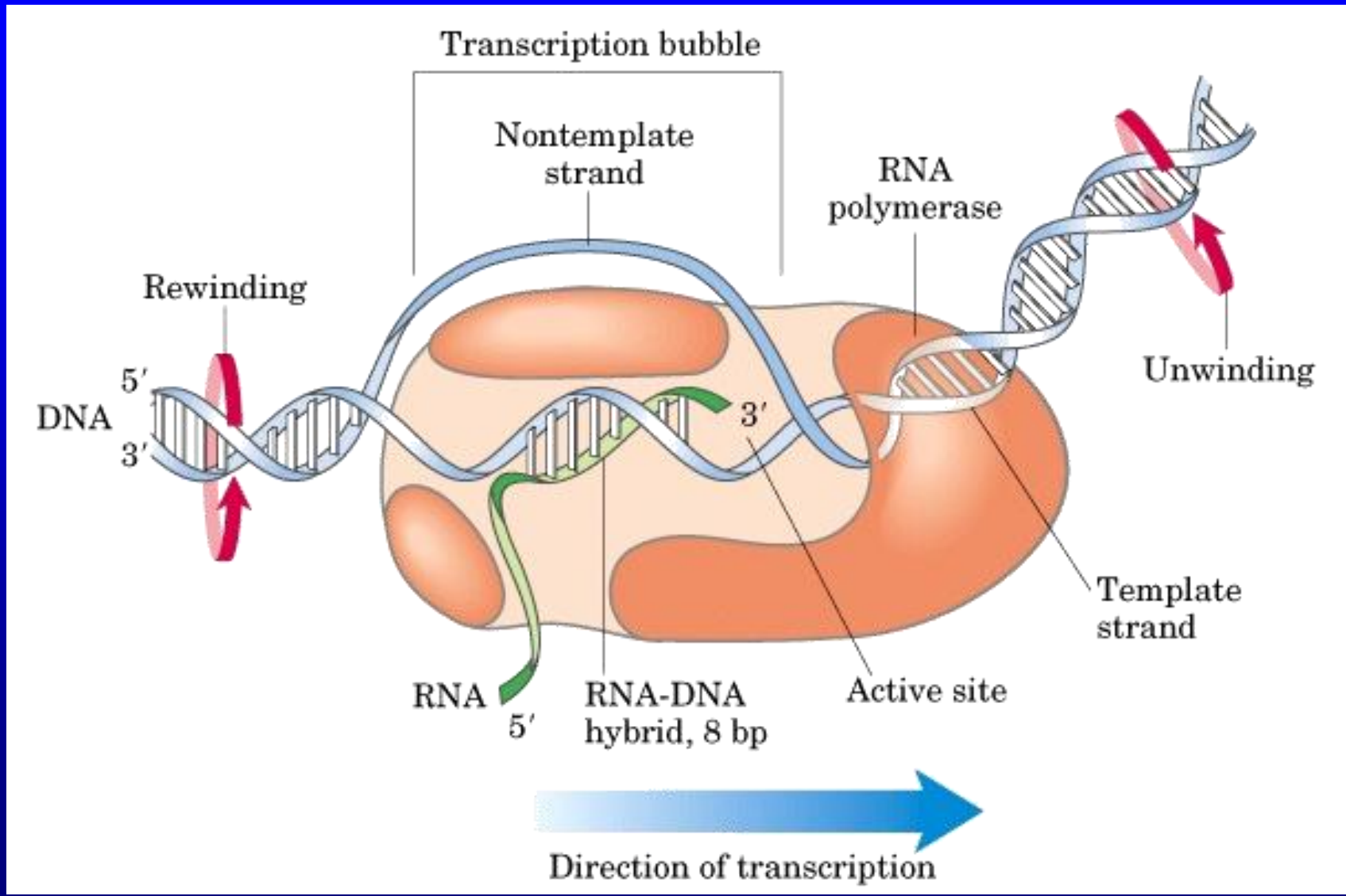
- The whole genome of DNA needs to be replicated, but only small portion of genome is transcribed in response to the development requirement, physiological need and environmental changes.
- DNA regions that can be transcribed into RNA are called structural genes.

What do the most DNA do in deed?

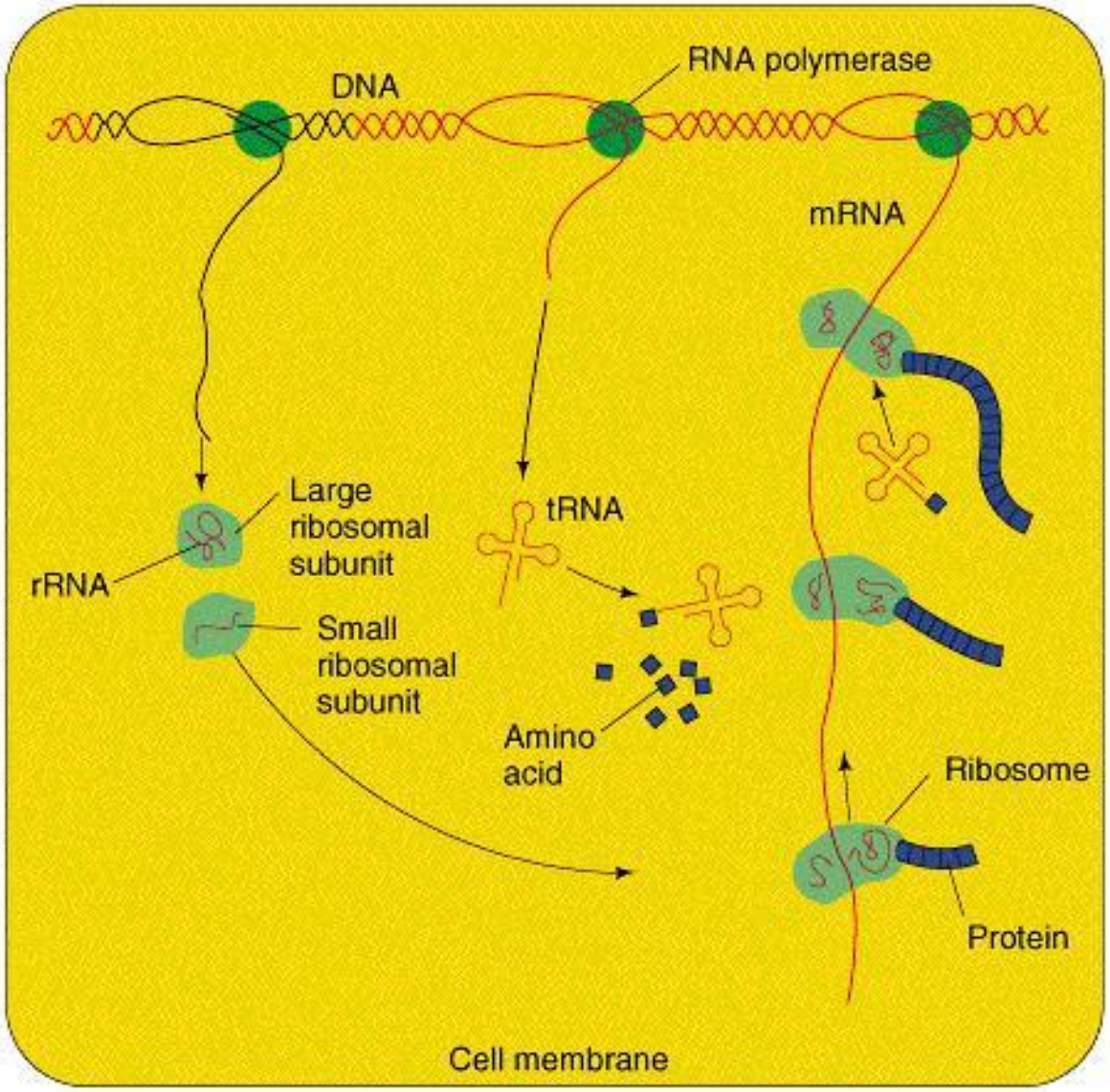
General concepts of Transcription Process

- Three phases: initiation, elongation, and termination.
- The prokaryotic RNA-pol can bind to the DNA template directly in the transcription process.
- The eukaryotic RNA-pol requires co-factors to bind to the DNA template together in the transcription process.

Transcription bubble



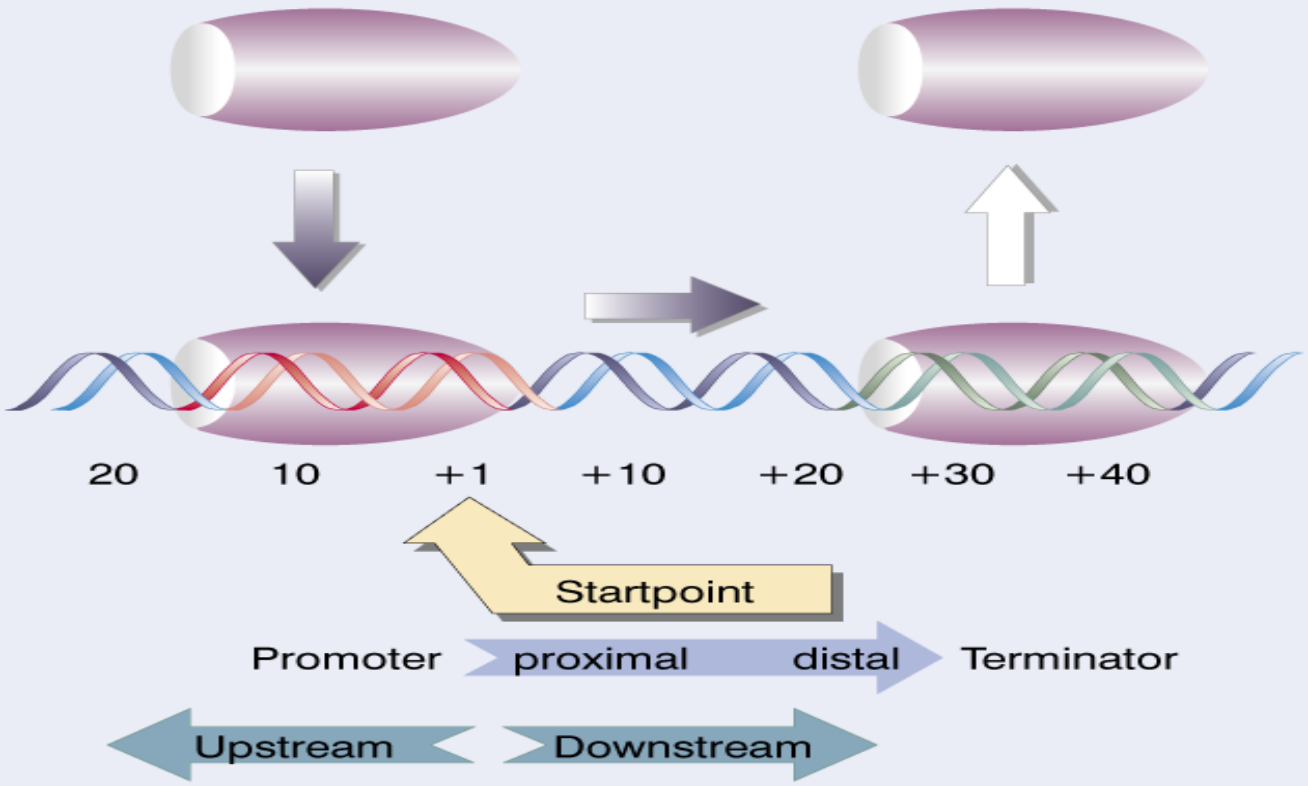
Transcription in prokaryotes



(a) Prokaryote

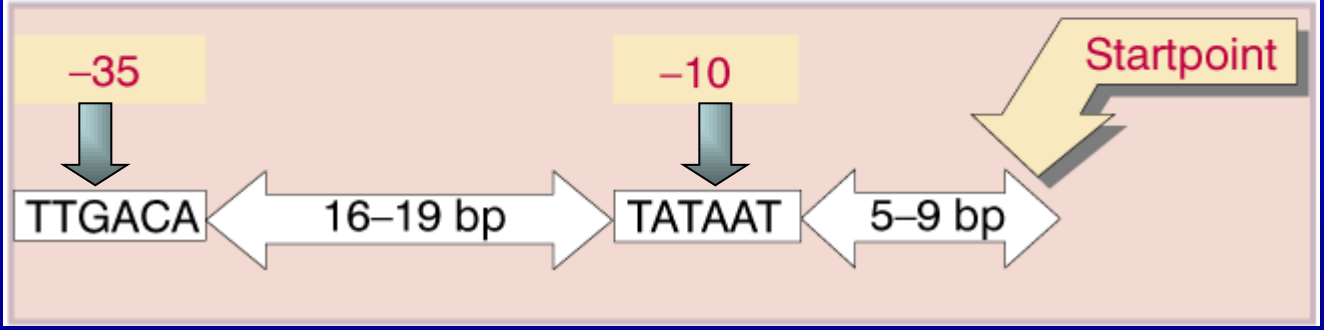
Transcription Unit

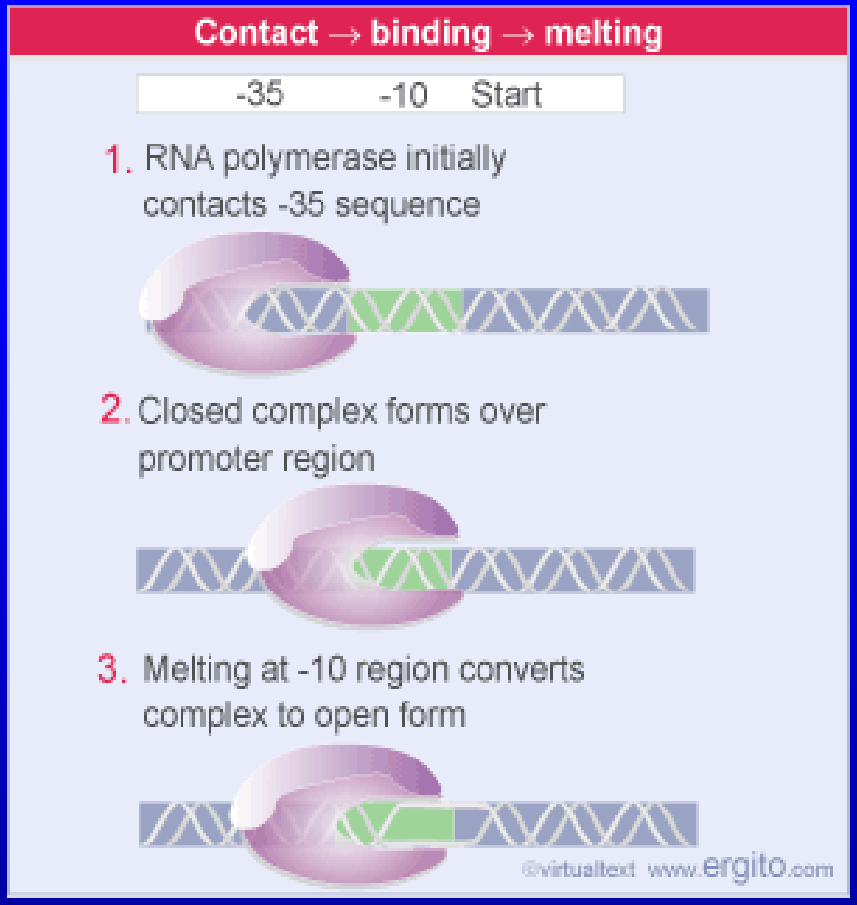
Figure 9.2 *Overview*: a transcription unit is a sequence of DNA transcribed into a single RNA, starting at the promoter and ending at the terminator.



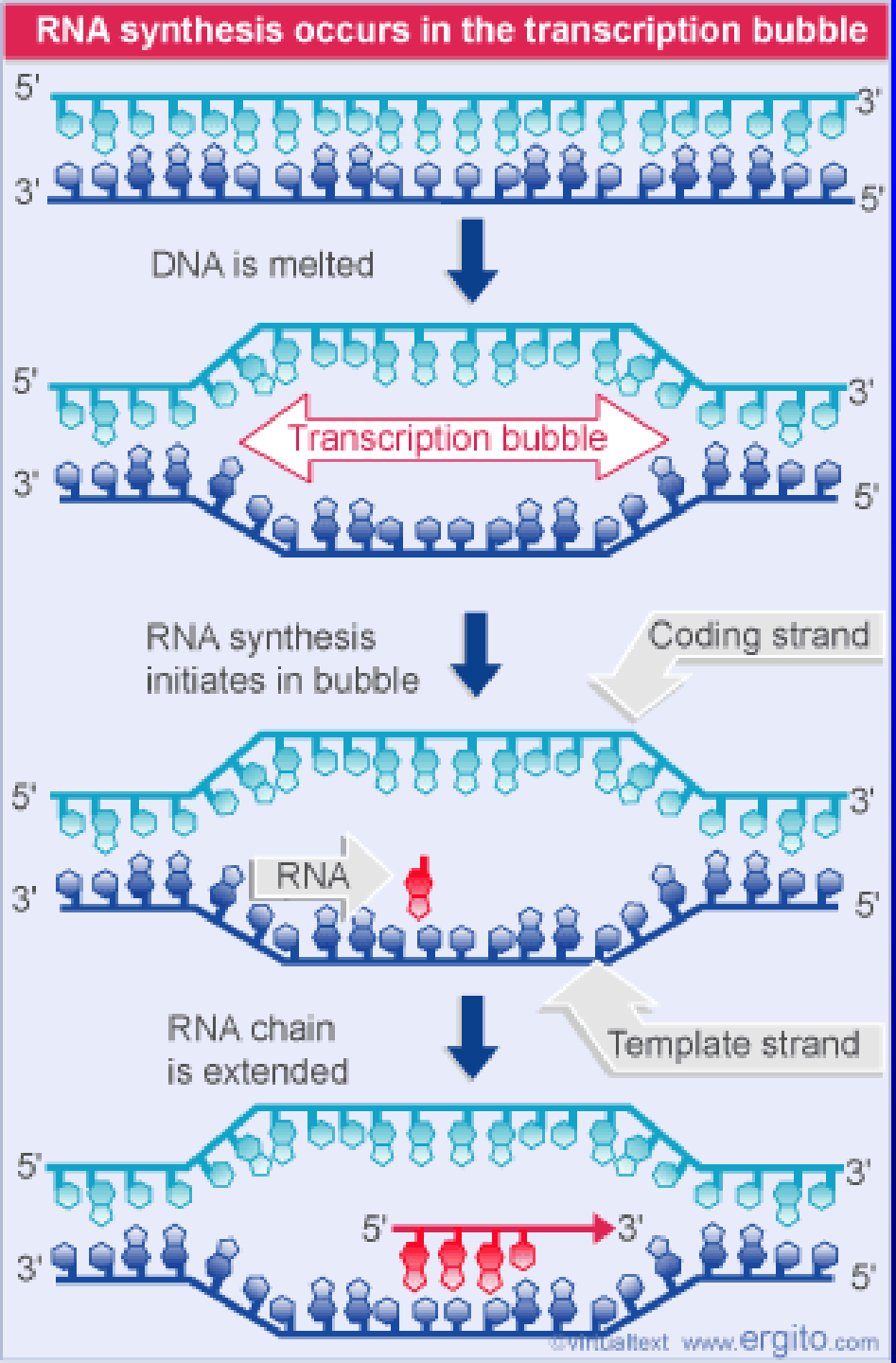
Optimal Promoter

Figure 9.15 A typical promoter has three components, consisting of consensus sequences at –35 and –10, and the startpoint.





The –35 sequence is used for initial recognition, and the –10 sequence is used for the melting reaction that converts a closed complex to an open complex.


RNA synthesis occurs in the transcription bubble
5'
3'
3'
5'
DNA is melted
5'
3'
3'
5'
Transcription bubble
RNA synthesis
initiates in bubble
Coding strand
5'
3'
3'
5'
RNA
RNA chain
is extended
Template strand
5'
3'
3'
5'
5'
3'
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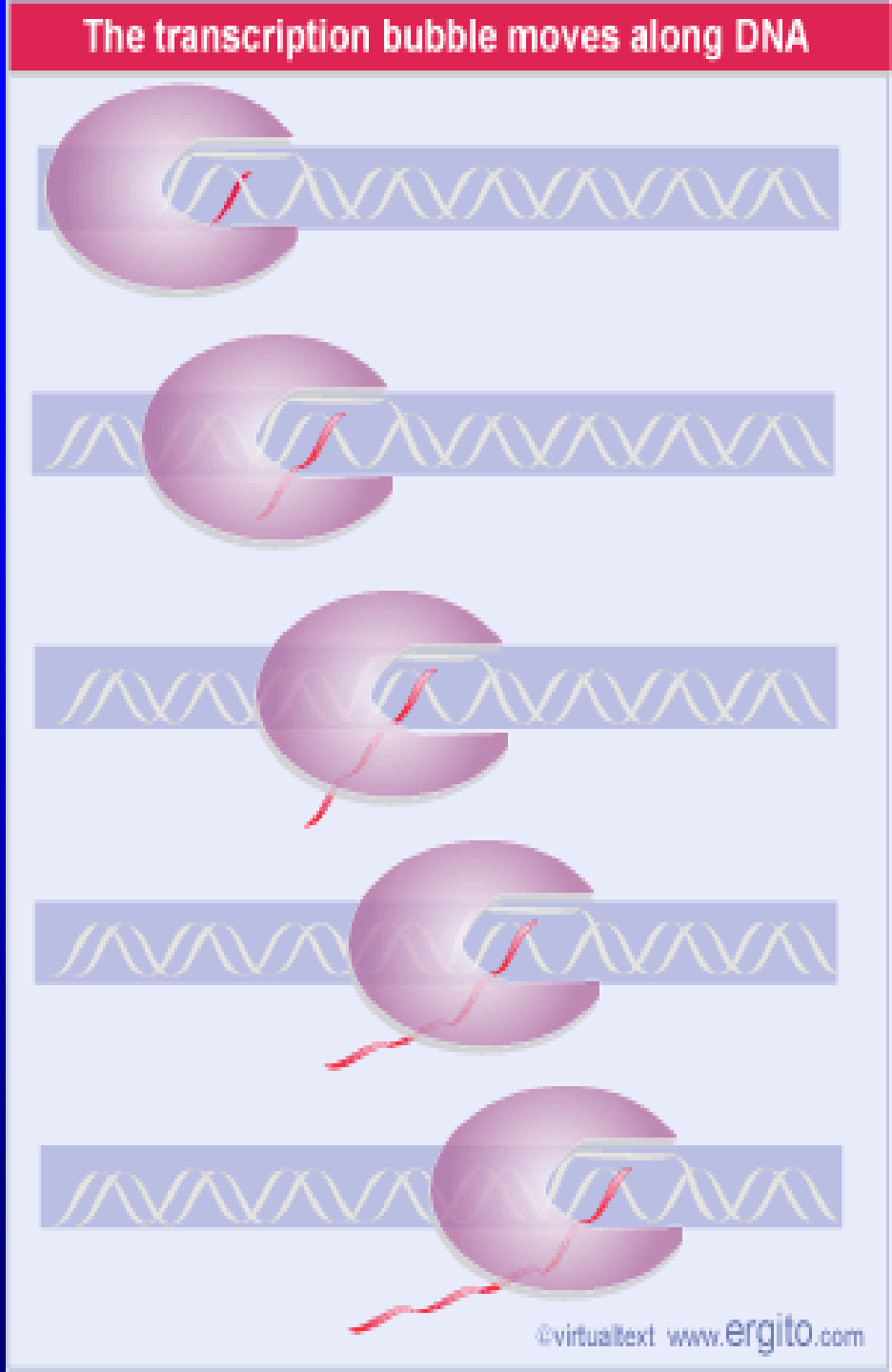
The transcription bubble moves along DNA
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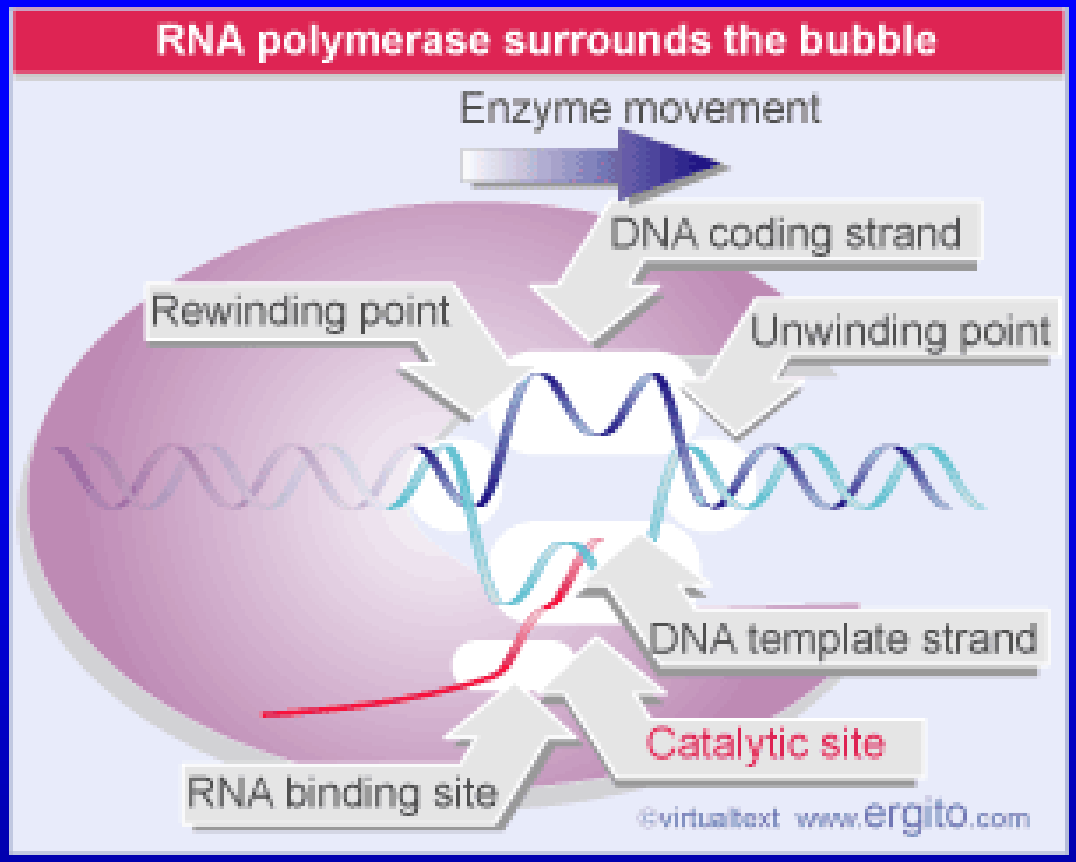
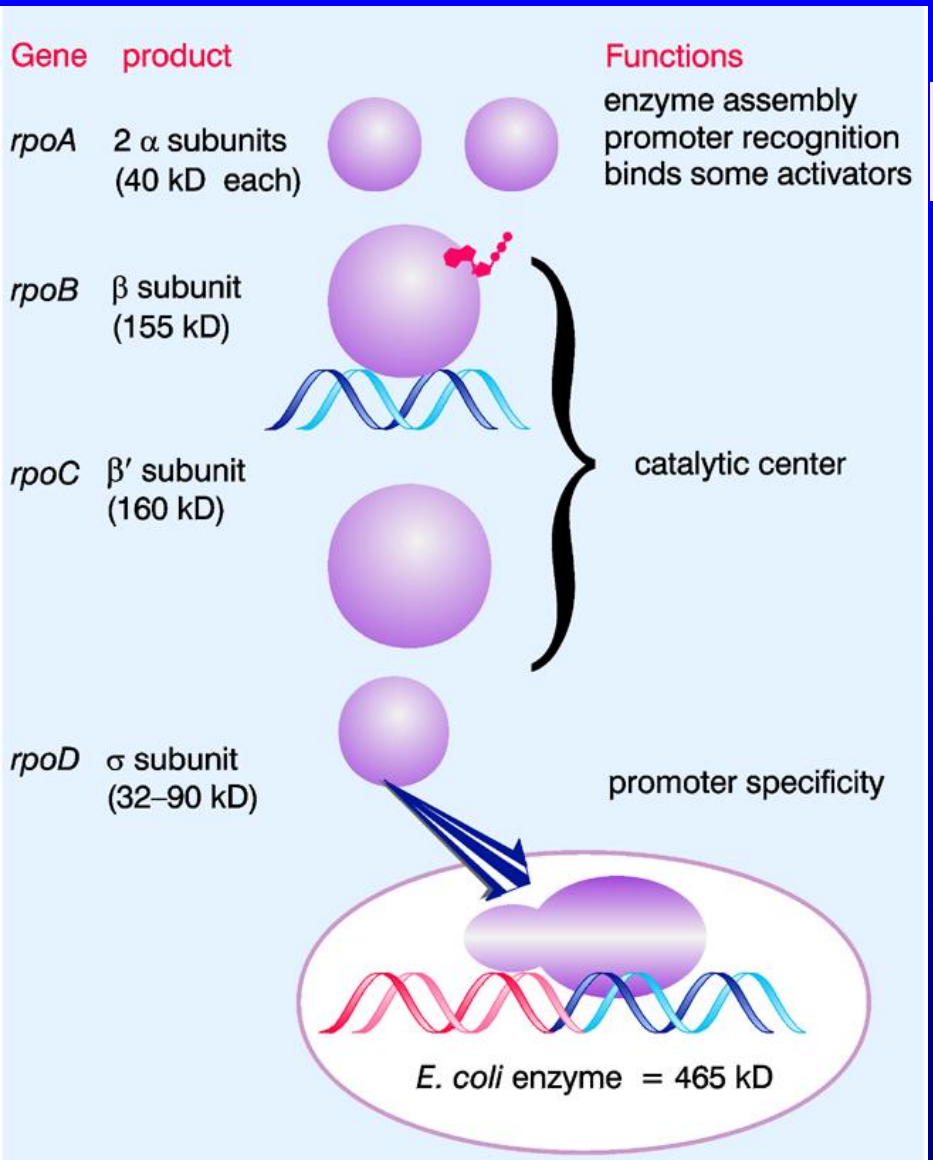


- During transcription, the bubble is maintained within bacterial RNA polymerase, which unwinds and rewinds DNA, maintains the conditions of the partner and template DNA strands, and synthesizes RNA.

Bacterial RNA Polymerases

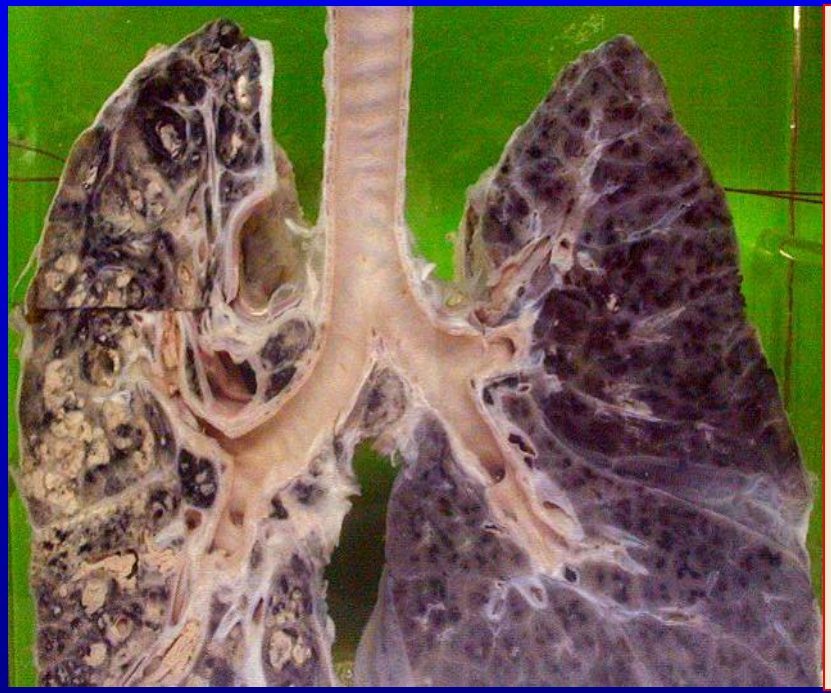


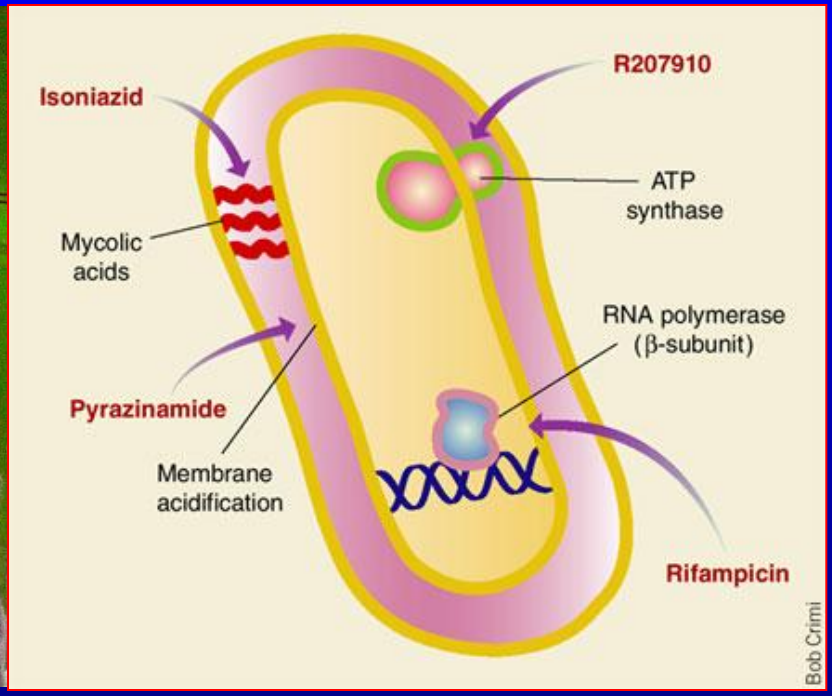
How does RNA polymerase work?

A single type of RNA polymerase is responsible for almost all synthesis of mRNA, rRNA and tRNA in a eubacterium.

About 7,000 RNA polymerase molecules are present in an ***E. coli*** cell. Probably 2,000~5,000 enzymes are synthesizing RNA at any one time, the number depending on the growth conditions.

RNA pol β subunit is the target of rifamycin

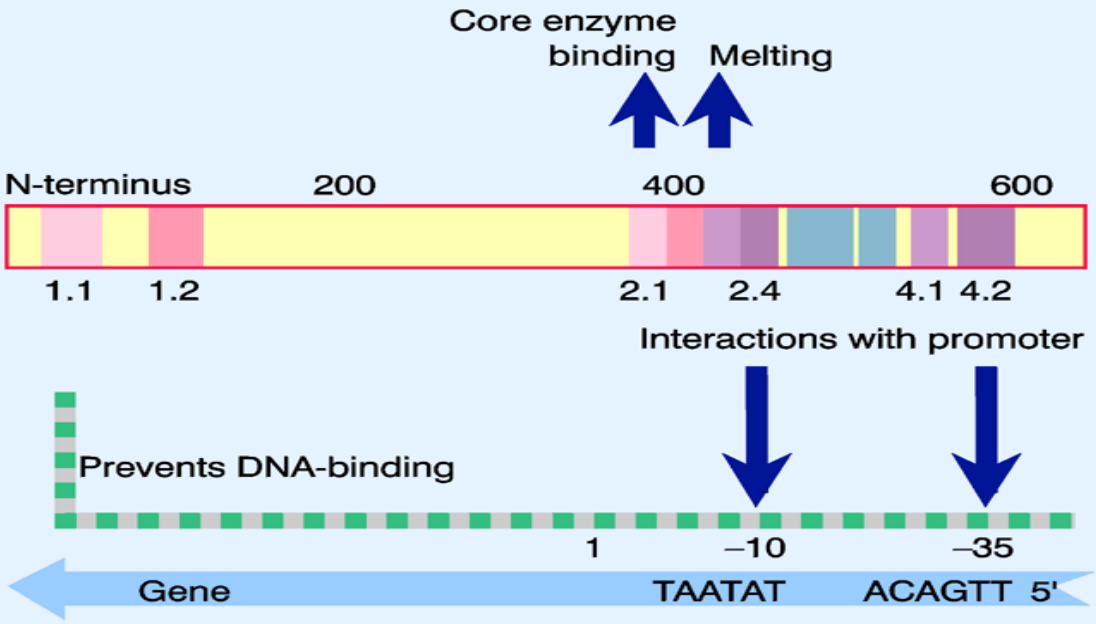


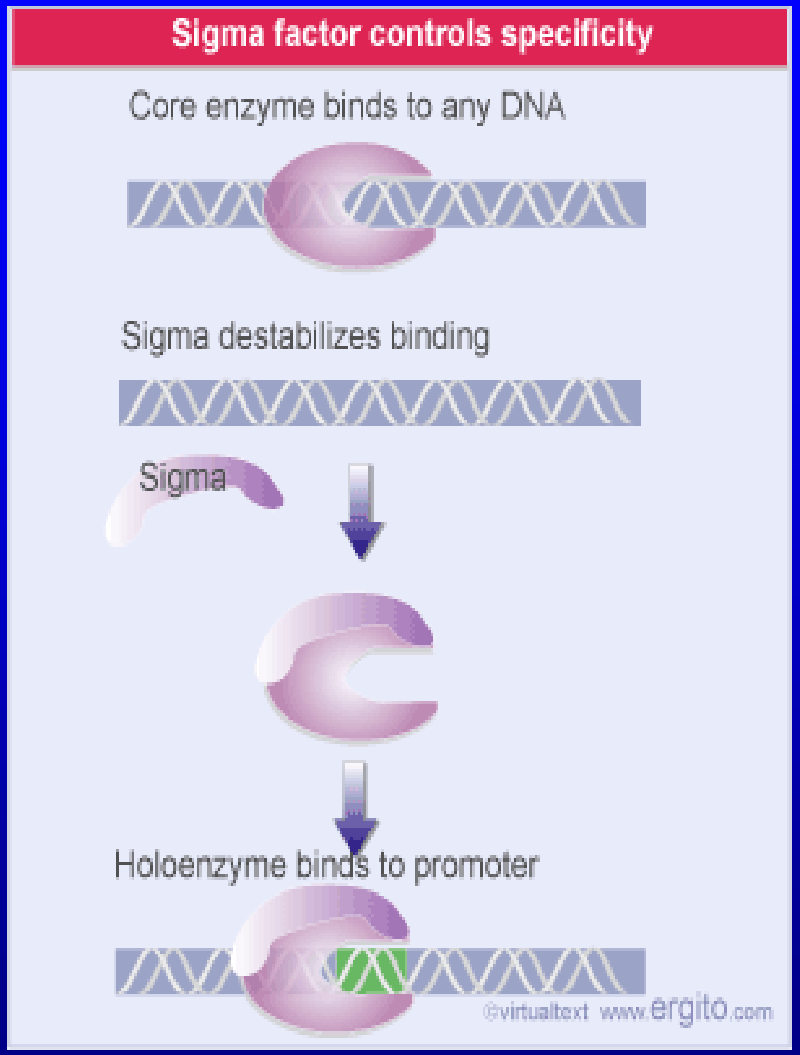


How many sigma factors exist in E. coli ?

Gene	Factor	Use	–35 Sequence	Separation	–10 Sequence
rpoD	σ^{70}	general	TTGACA	16–18 bp	TATAAT
rpoH	σ^{32}	heat shock	CCCTTGAA	13–15 bp	CCCGATNT
rpoE	σ_E	heat shock	not known	not known	not known
rpoN	σ^{54}	nitrogen	CTGGNA	6 bp	TTGCA
fliA	σ_F	flagellar	CTAAA	15 bp	GCCGATAA

Figure 9.20 A map of the *E. coli* σ^{70} factor identifies conserved regions. Regions 2.1 and 2.2 contact core polymerase, 2.3 is required for melting, and 2.4 and 4.2 contact the –10 and –35 promoter elements. The N-terminal region prevents 2.4 and 4.2 from binding to DNA in the absence of core enzyme.




Sigma factor controls specificity
Core enzyme binds to any DNA
Sigma destabilizes binding
Sigma
Holoenzyme binds to promoter
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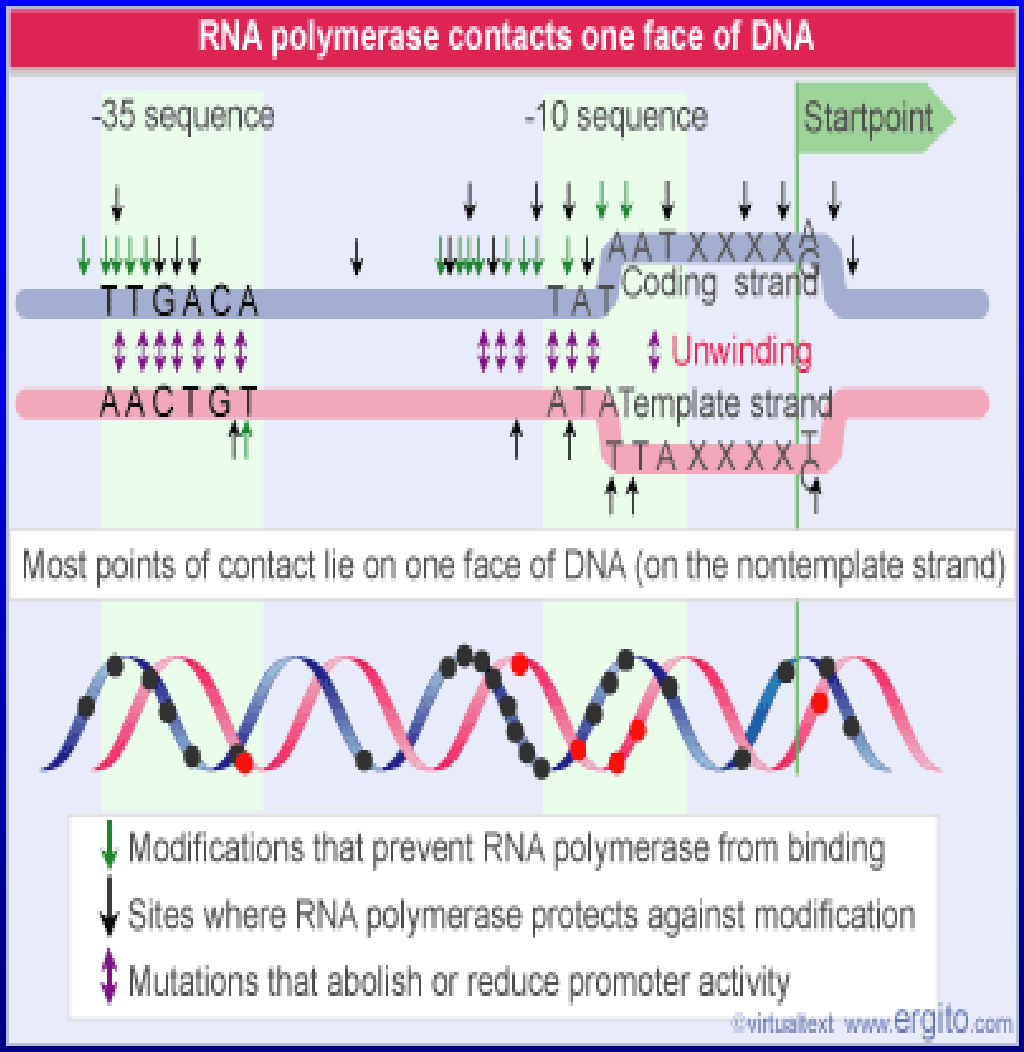
RNA polymerase contacts one face of DNA
-35 sequence
-10 sequence
Startpoint
TTGACA
TAT
AATXXXXA
Coding strand
Unwinding
AACTGT
ATA
Template strand
TTAXXXXT
Most points of contact lie on one face of DNA (on the nontemplate strand)
Modifications that prevent RNA polymerase from binding
Sites where RNA polymerase protects against modification
Mutations that abolish or reduce promoter activity
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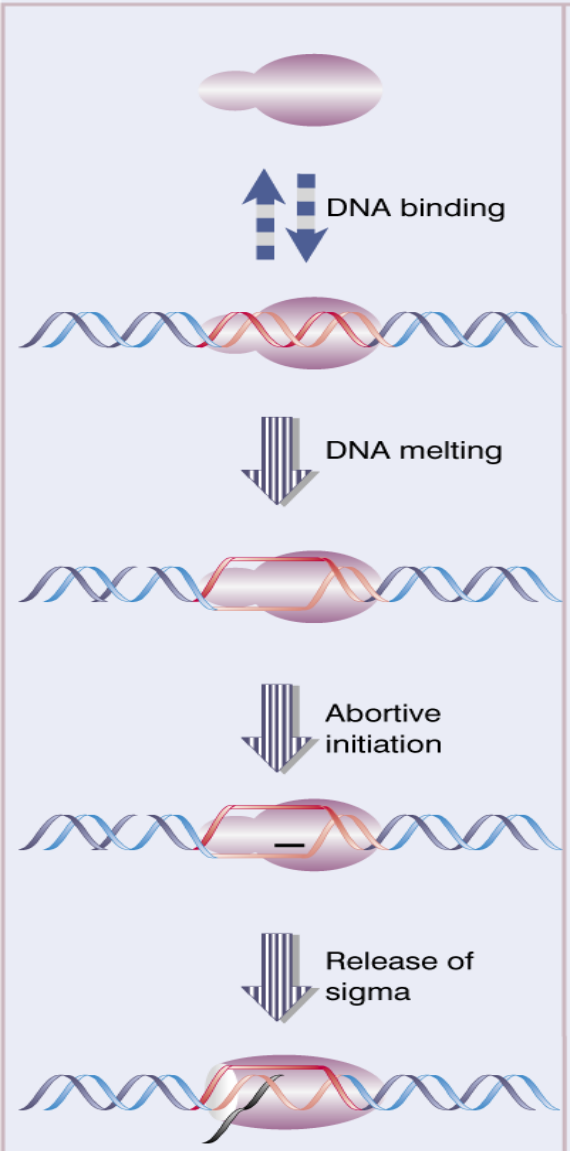
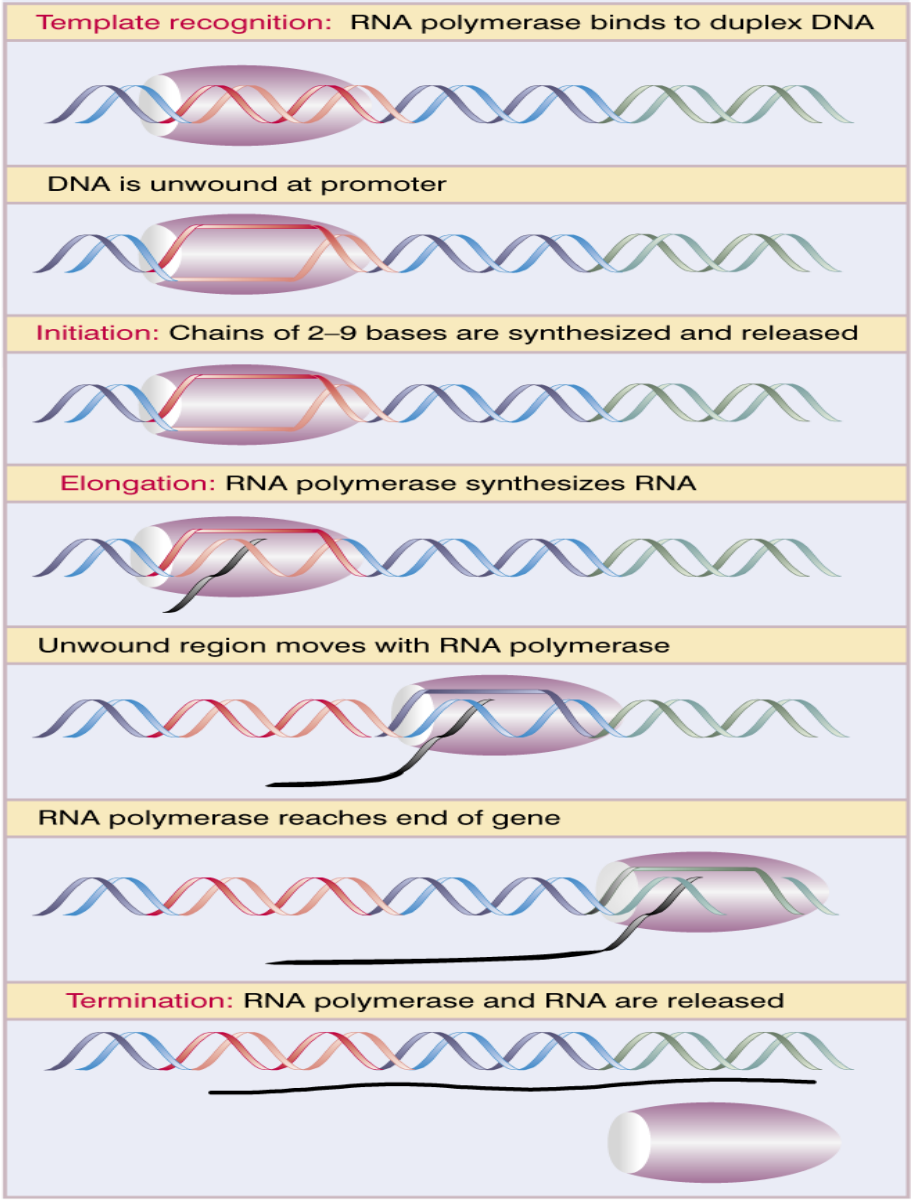
How does transcription initiate?

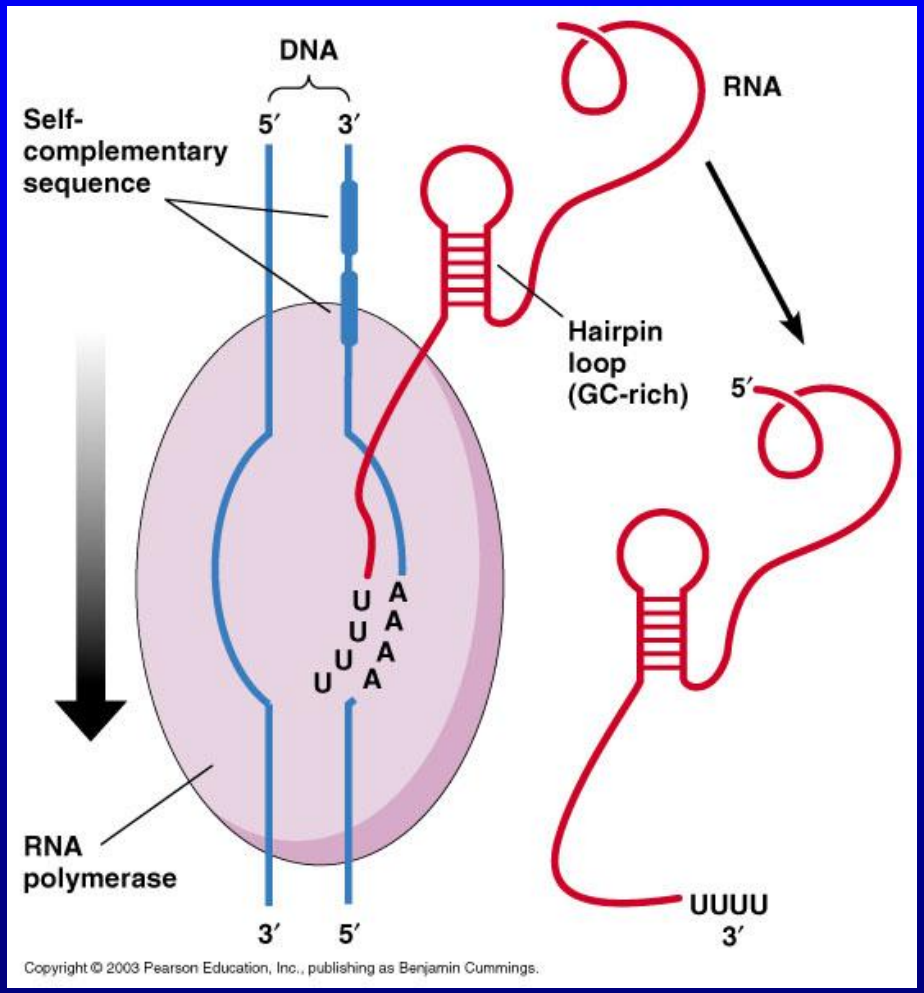


Four stages of Transcription

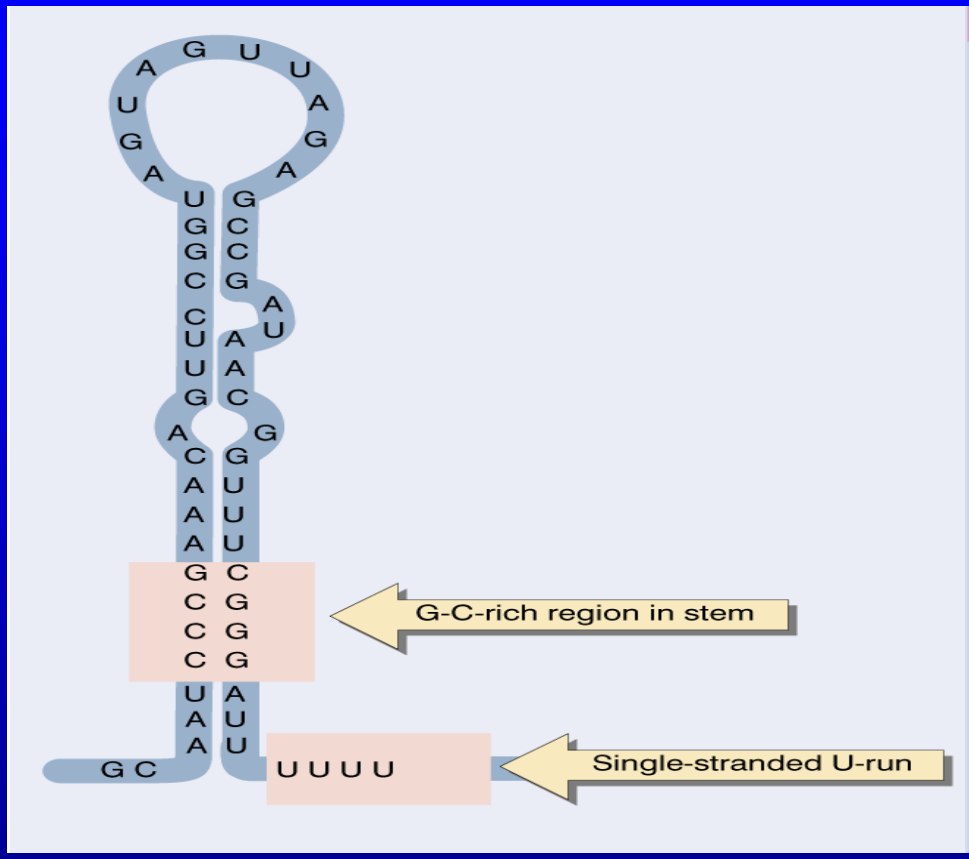


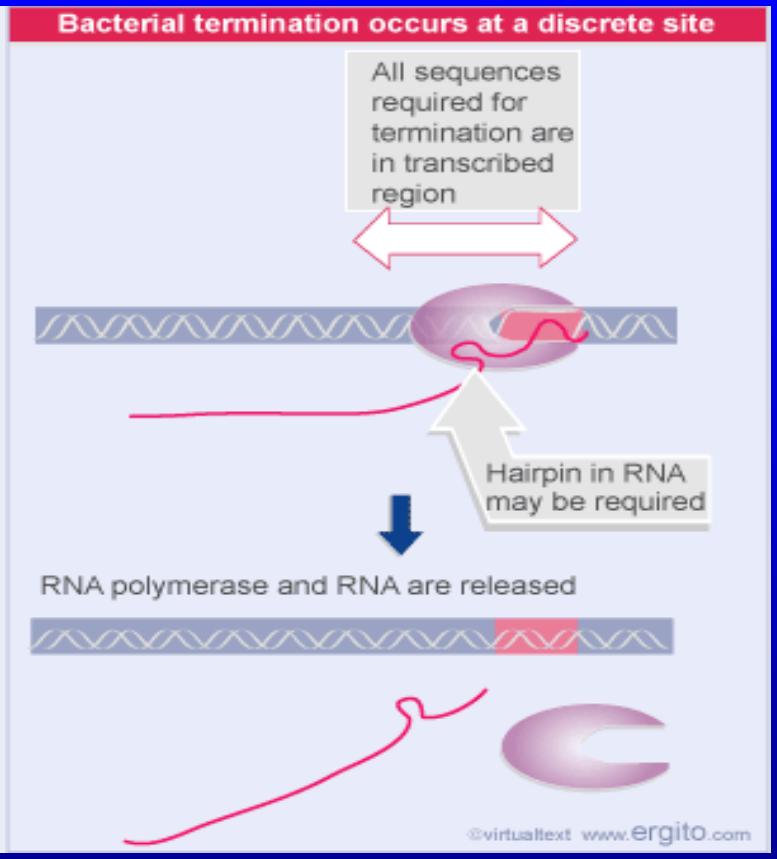
Termination

- The terminator is in the *transcript*, not the DNA
- Forms a hairpin
- Self-complementary
- The hairpin structure is the signal for termination
- Rho (ρ)-dependent vs. ρ-independent



Intrinsic terminators ρ-independent

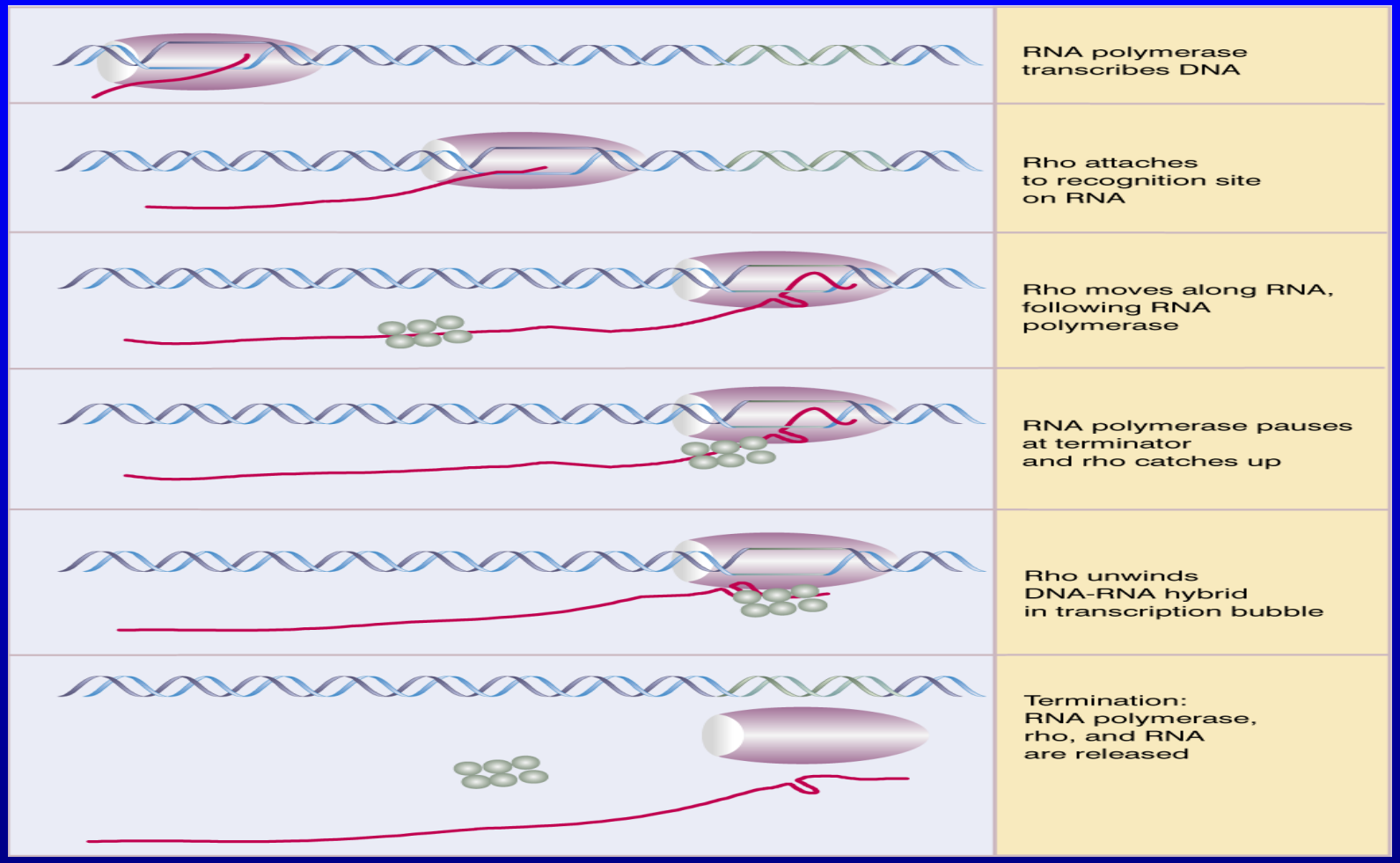




An inverted repeat that allows a hairpin to form at the end of the transcripts

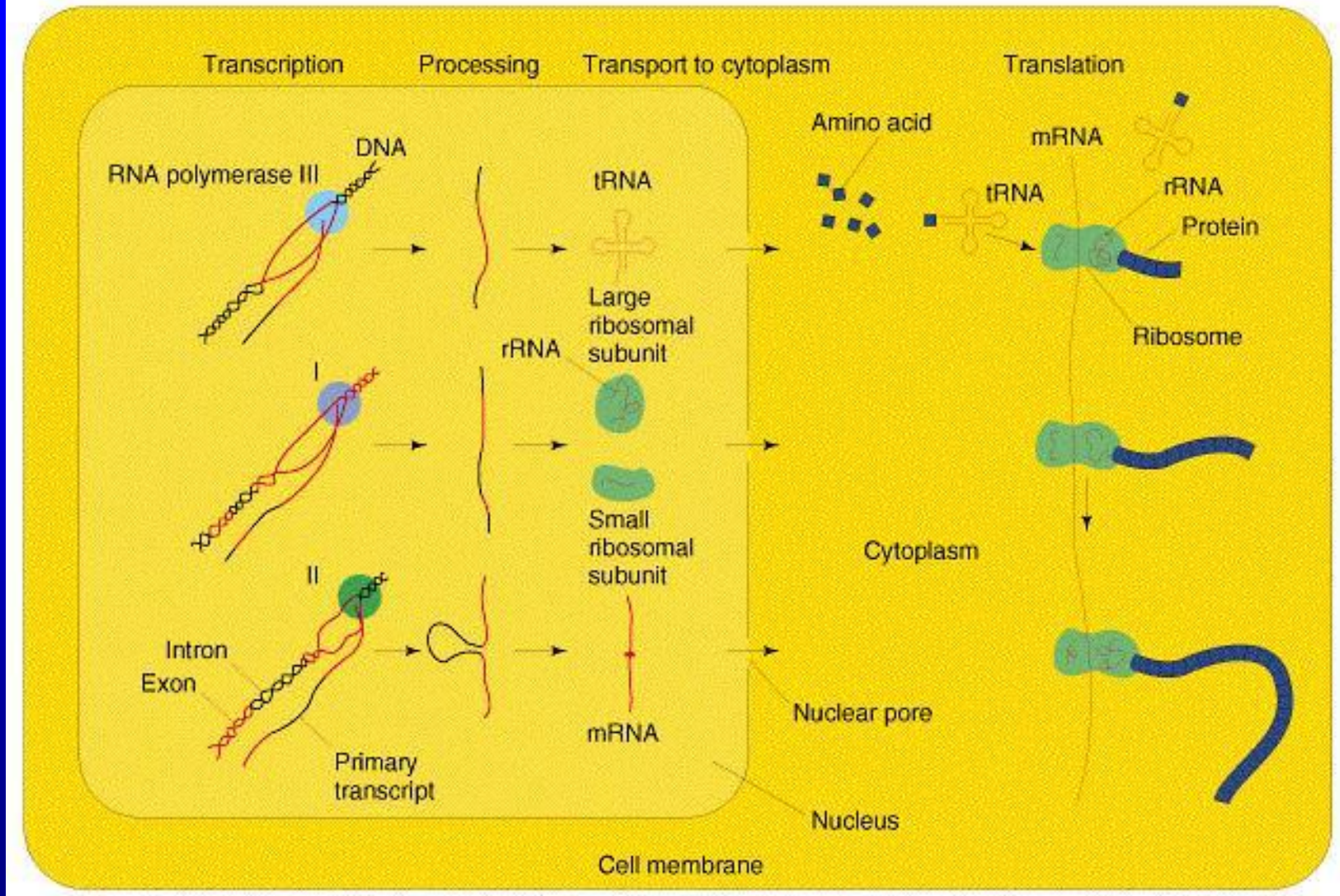
A string of T's in the nontemplate strand that results in a string of weak rU-dA base pairs holding the transcript to the template strand

Termination of ρ-dependent



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

Transcription in Eukaryotes

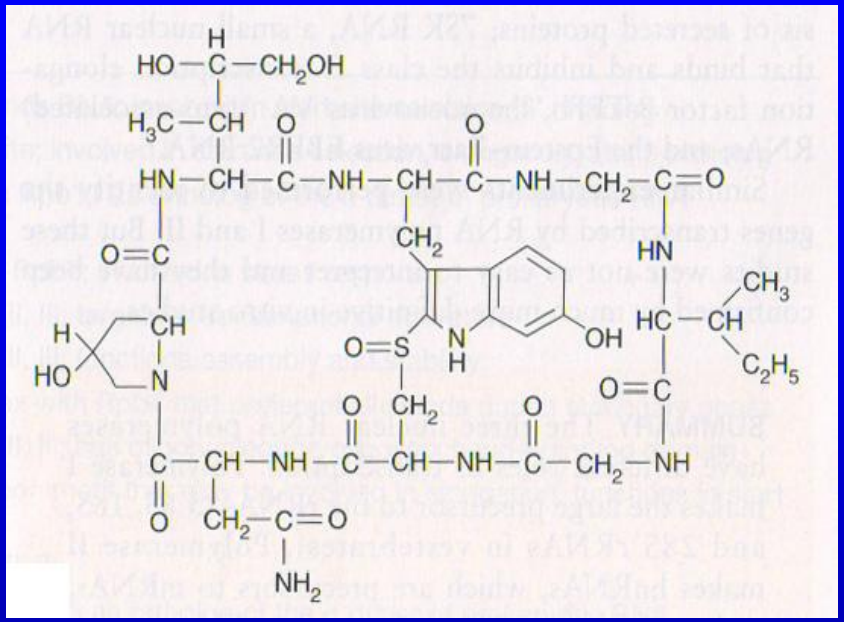

Transcription
Processing
Transport to cytoplasm
Translation
DNA
RNA polymerase III
tRNA
Amino acid
mRNA
tRNA
rRNA
Protein
Large ribosomal subunit
Ribosome
rRNA
I
Small ribosomal subunit
Cytoplasm
II
Intron
Exon
mRNA
Nuclear pore
Primary transcript
Nucleus
Cell membrane

(b) Eukaryote

RNA polymerases in Eukaryotes

- RNA polymerase I transcribes rRNA
- RNA polymerase II transcribes hnRNA
- RNA polymerase III transcribes tRNA and other small RNAs.

Amanita phalloides **(the death cap)**



Structure of α-amanitin

Animal RNA Polymerases

Animal DNA-dependent RNA Polymerases

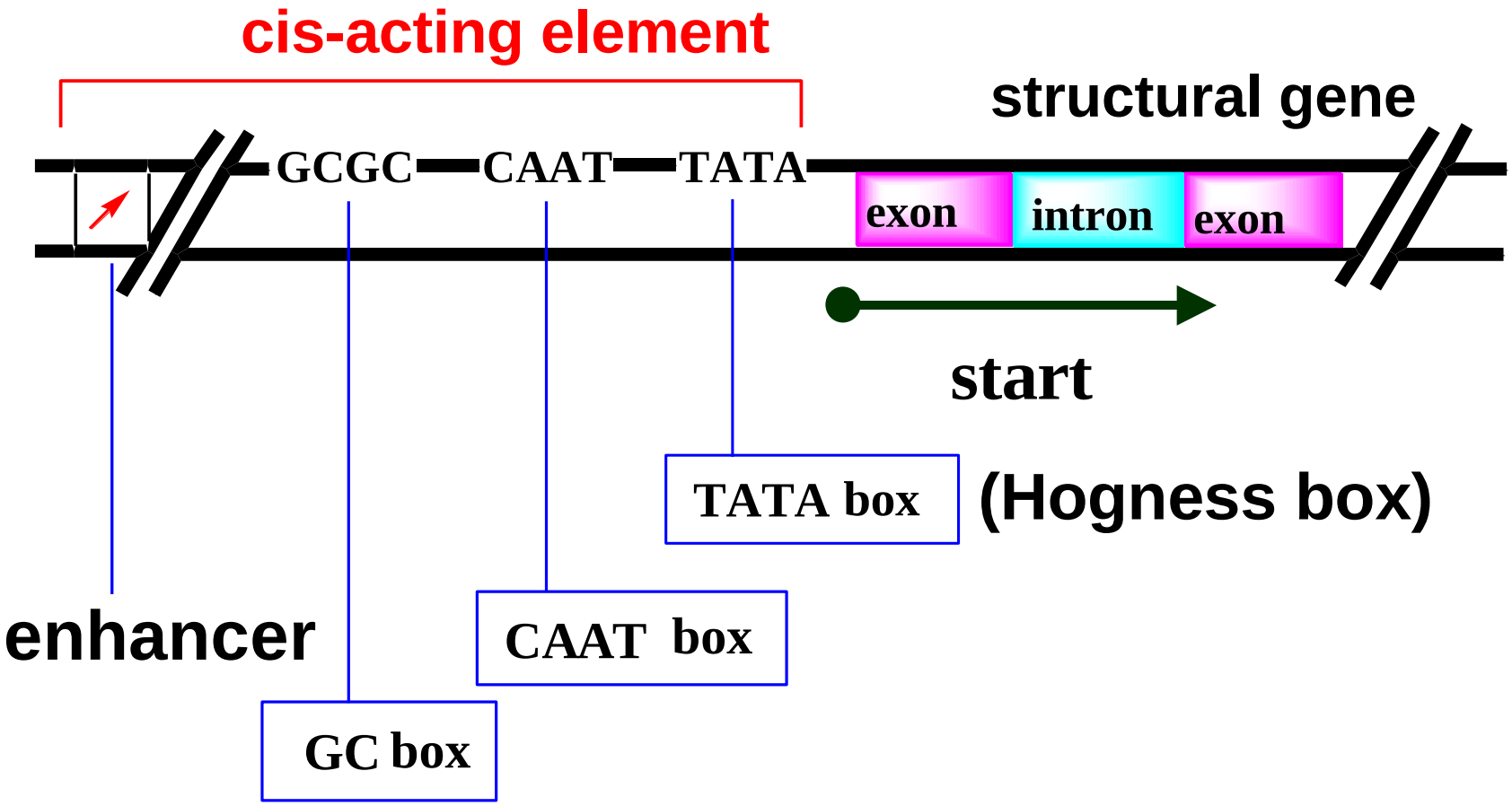
Class	α-amanitin sensitivity	Major Products
I	Insensitive	rRNA
II	Low Conc. (1-10 nM)	hnRNA
III	High conc.	tRNA, 5S RNA and small RNAs

All have in common 2 large subunits and a number of smaller subunits, as well as being zinc metalloenzymes.

Eukaryotic Transcription Initiation

- Transcription initiation needs promoter and upstream regulatory regions.
- The cis-acting elements are the specific sequences on the DNA template that regulate the transcription of one or more genes.

Cis-acting element

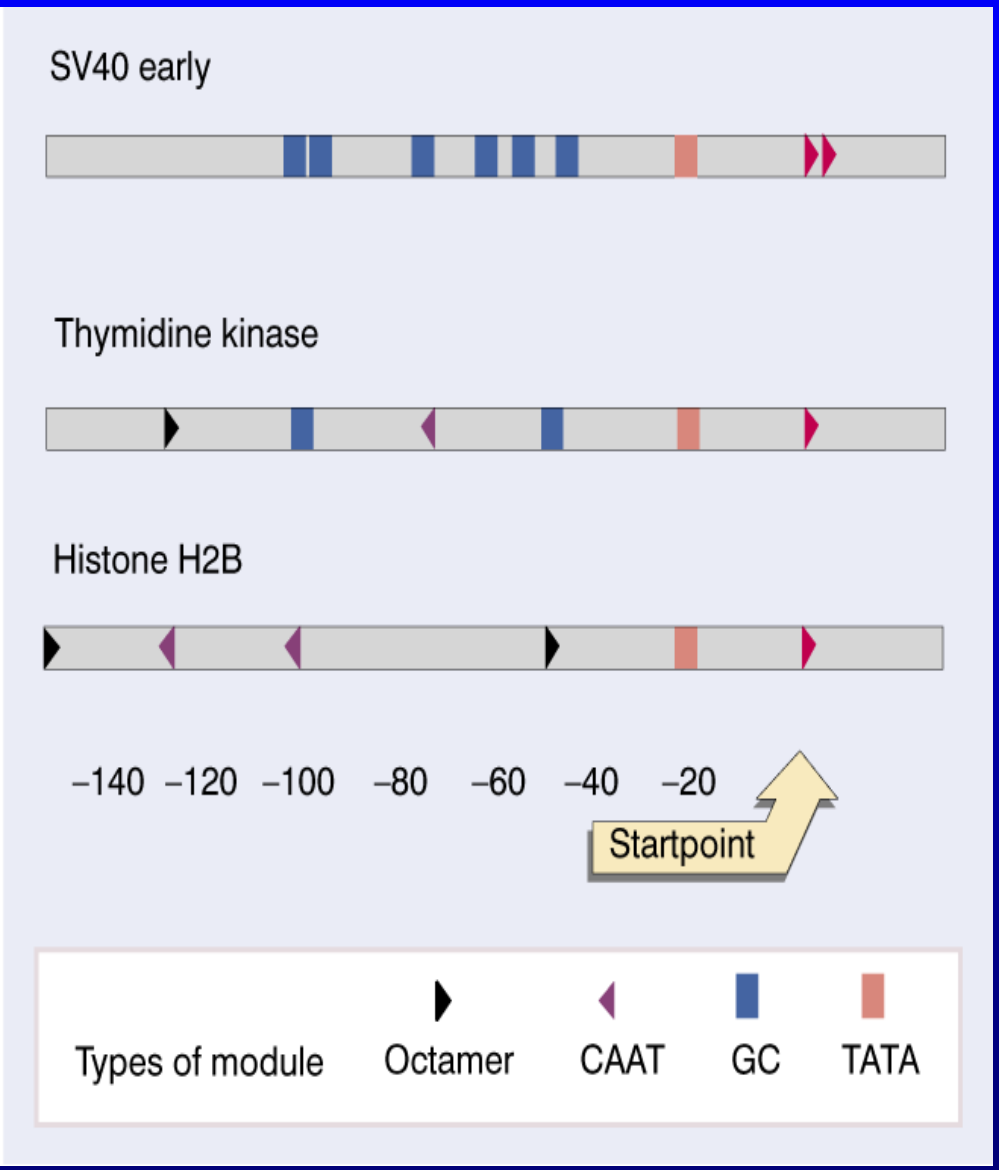


Transcription factors

- RNA-pol does not bind the promoter directly.
- RNA-pol II associates with six transcription factors, TFII A - TFII H.
- The trans-acting factors are the proteins that recognize and bind directly or indirectly cis-acting elements and regulate its activity.

Initiation of RNA polymerase II

Elements combination in type II Promoters



TATA box is a septamer (TATAAAA) at -25 and is involved in positioning the enzyme for correct initiation.

GC box is at -90 contains the sequence GGGCGG and is recognized by the factor SP1.

CAAT box (CCAATCT) is at –75 and is recognized by a large group of transcription factors and plays a strong role in determining the efficiency of the promoter.

Enhancer; Dehancer; Silencer; Upstream Activating Sequences (UAS)

Enhancer

- Enhancers Work Upstream, Downstream or in the Middle of a Gene
- They also work forwards or backwards
- Possible ways of working

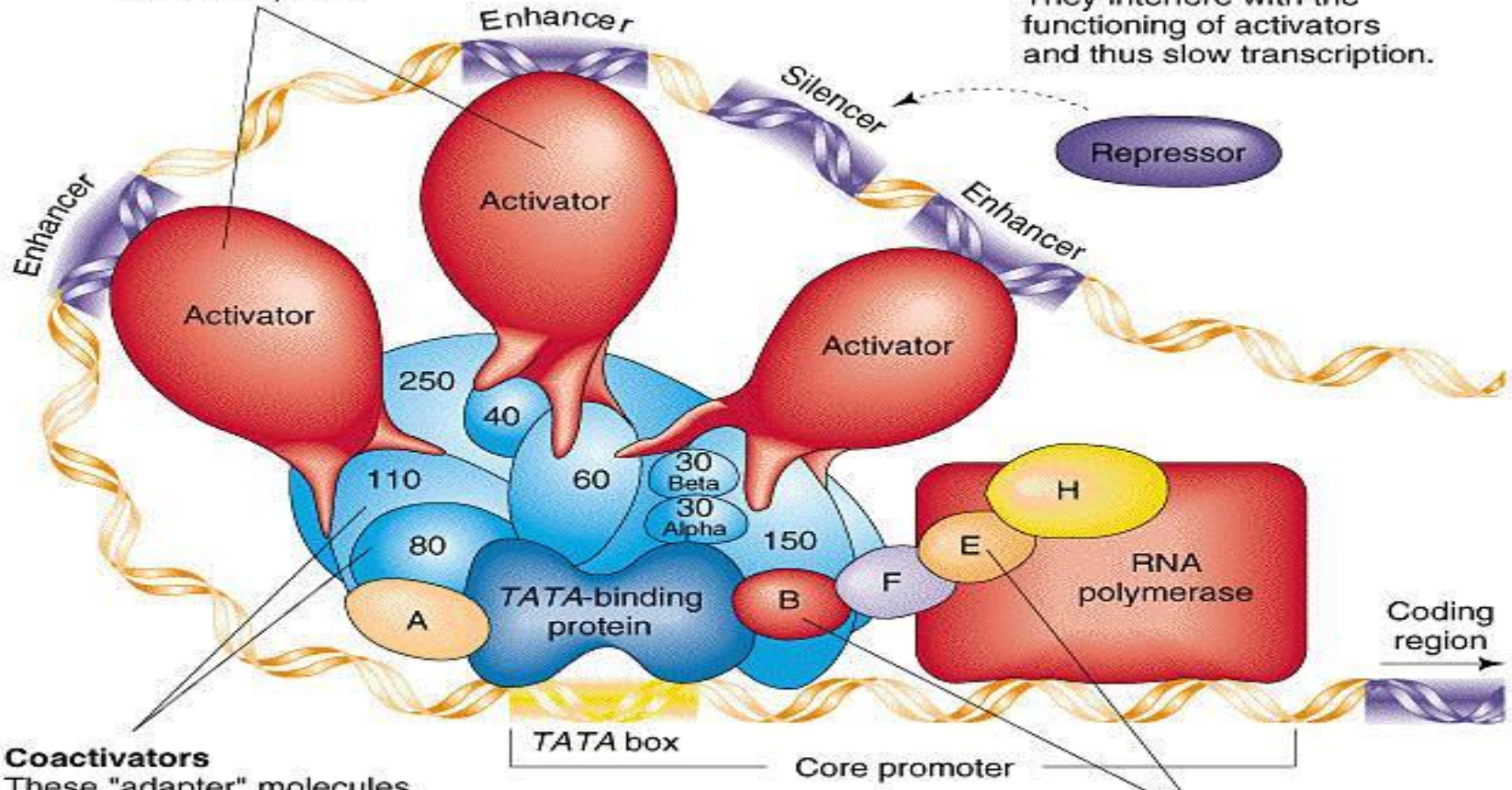
  Different transcription factors

  Order of binding (differing concentrations)

  Affinity of transcription factors

Activators
These proteins bind to genes at sites known as *enhancers.* Activators help determine which genes will be switched on, and they speed the rate of transcription.

Repressors
These proteins bind to selected sets of genes at sites known as *silencers.* They interfere with the functioning of activators and thus slow transcription.

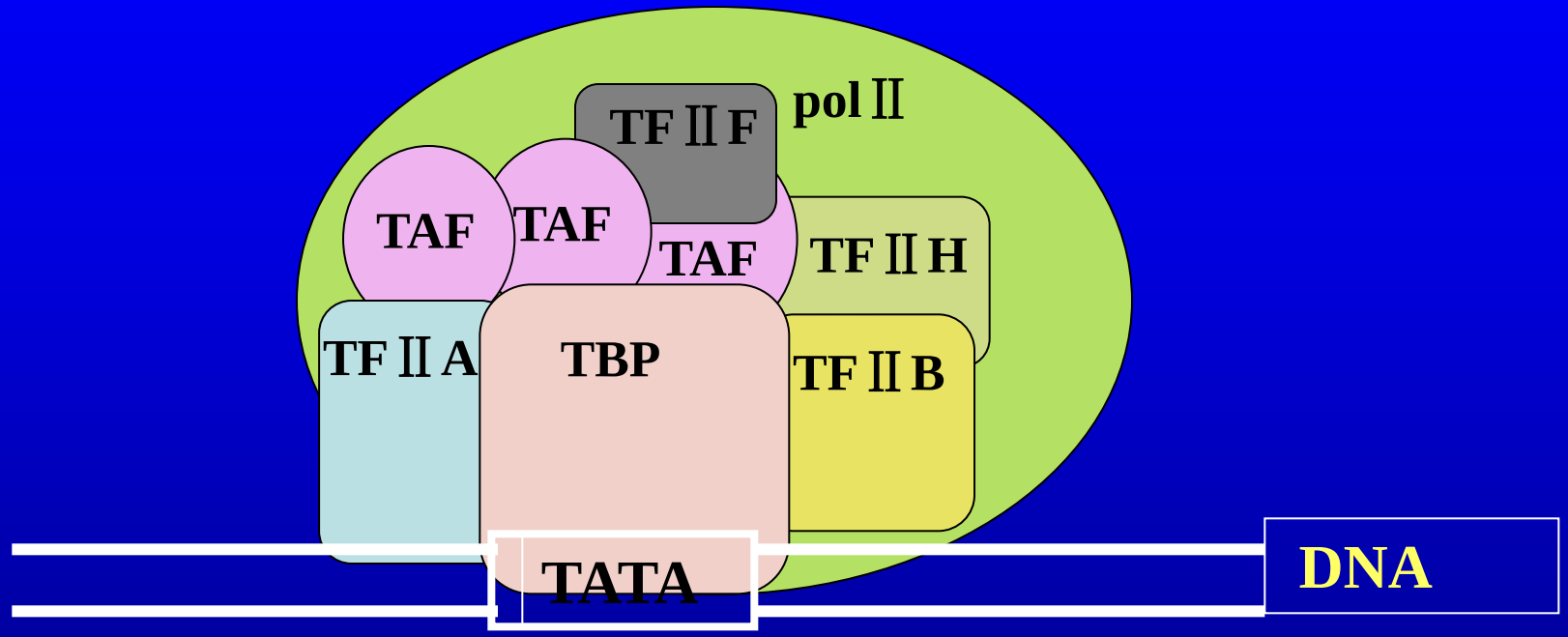


Coactivators
These "adapter" molecules integrate signals from activators and perhaps repressors and relay the results to basal factors.

Basal transcription factors
In response to injunctions from activators, these factors position RNA polymerase at the start of the protein-coding region of a gene and send the enzyme on its way.

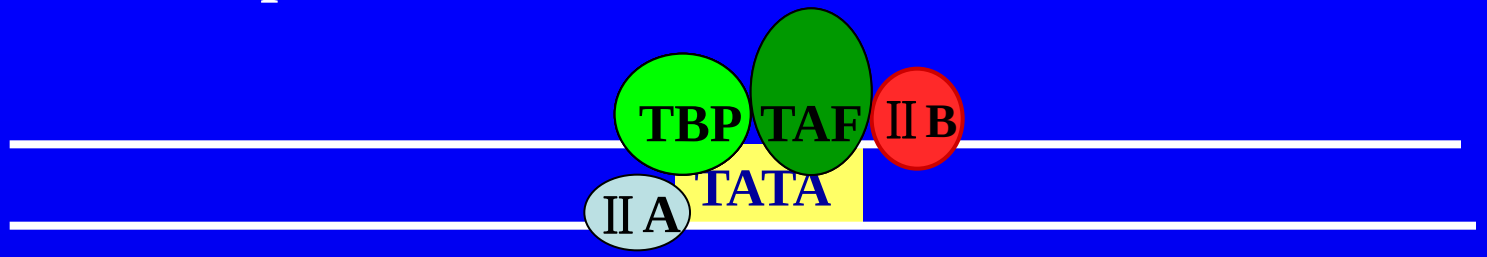
Class II transcription factors

- TFIIA activates TBP by relieving the repression that is caused by the TAFs
- TFIIB binds adjacent to TBP and TATA box
- TFIID is a complex protein containing a TATA-box binding protein and 8-10 TBP-associated factors (TAFs)
  - TBP: TATA-binding protein
  - TAFs: TBP-associated factors
- TFIIF consists of two subunits. The larger subunit has an ATP-dependent DNA helicase activity and the small one contacts the core polymerase.
- TFIIE and TFIIH are required for promoter clearance to allow RNA polymerase to commence movement away from the promoter.

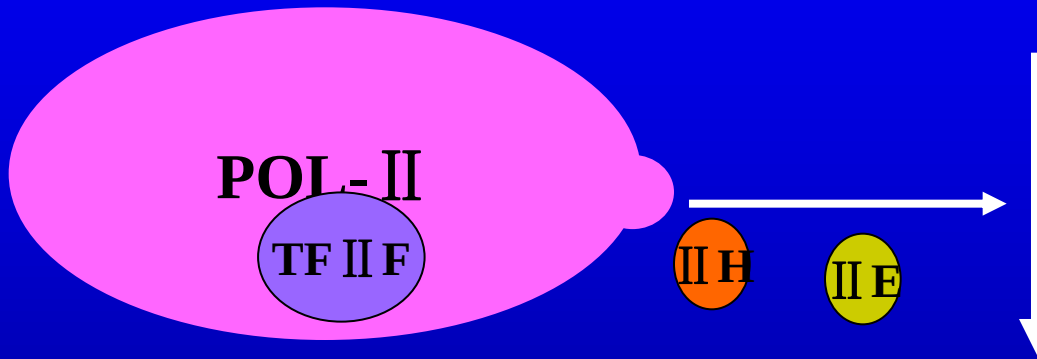


RNA pol Ⅱ with transcription factors form transcription initiation complex. TF II D is the only factor which can recognize specific sites.

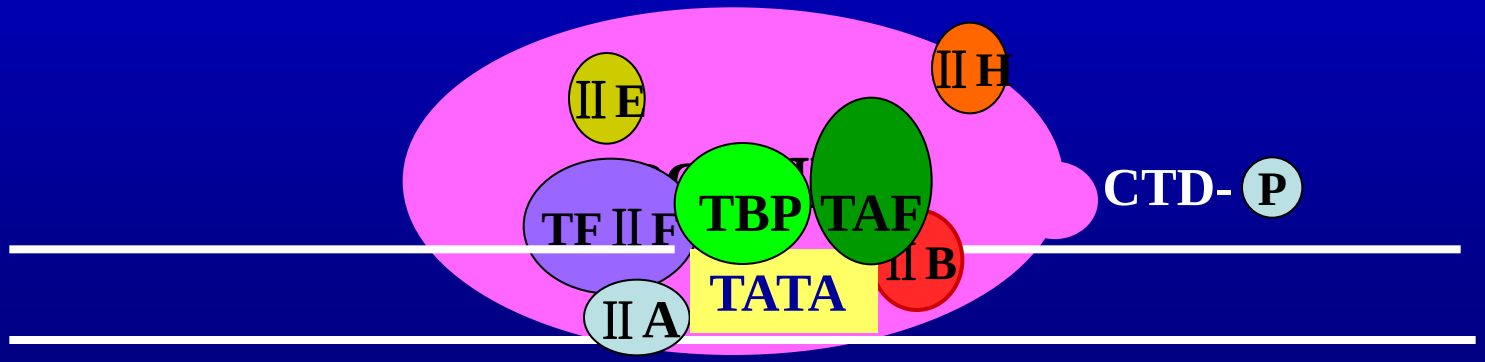
Pre initiation complex



TFⅡD-ⅡA-ⅡB-DNA complex



CTD（Carboxyl Terminal Domain）is repeated sequence of Tyr-Ser-Pro-Thr-Ser-Pro-Ser

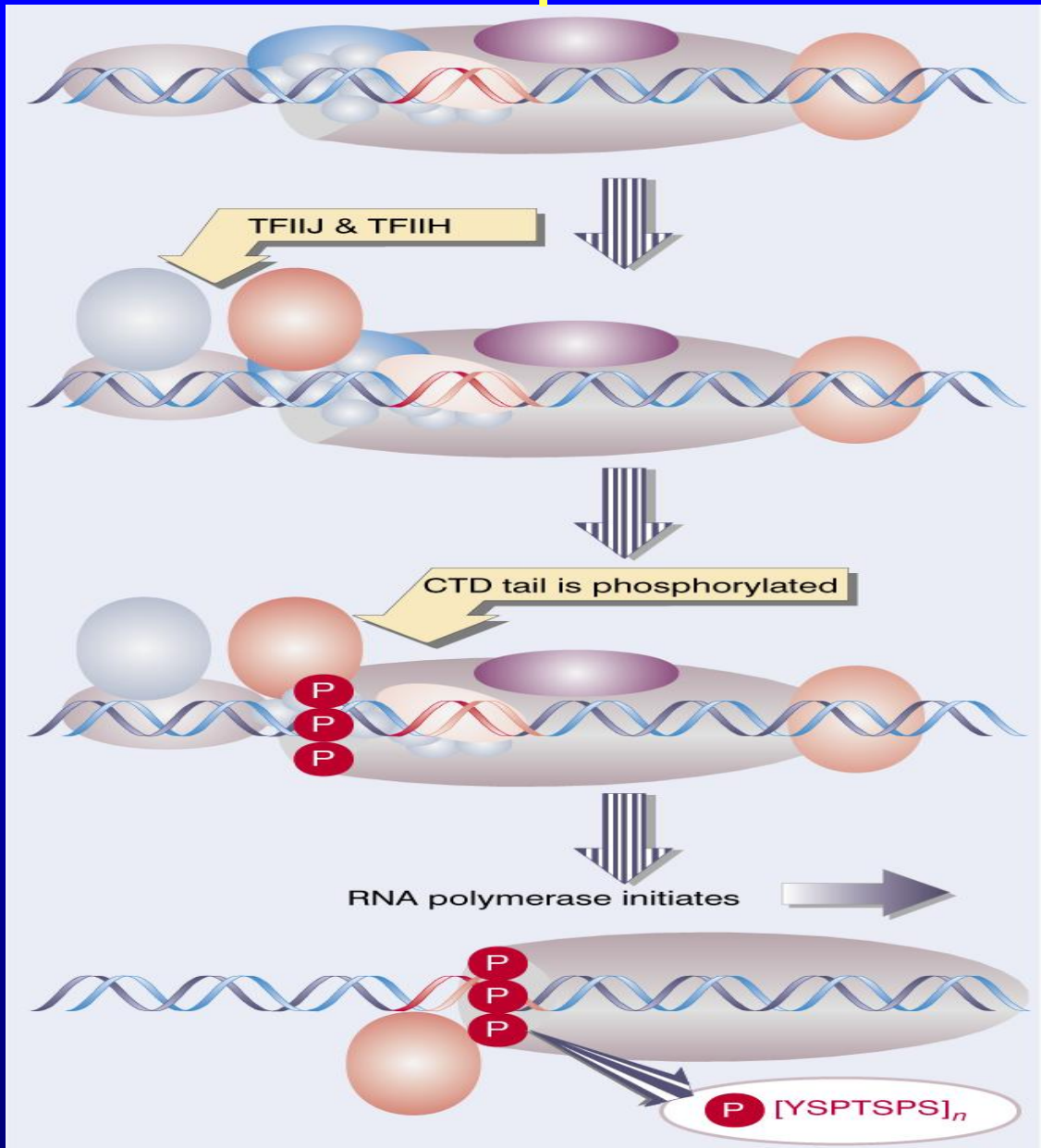


CTD tail of RNA pol II is phosphorylated by TFⅡH

Most of the TFII factors are released before RNA polymerase II leaves the promoter.

- TFIIH has several activities, including an ATPase, a helicase, and a kinase activity that can phosphorylate the CTD tail of RNA polymerase II; it is also involved in repair of damage to DNA.

Phosphorylation of the CTD by the kinase activity of TFIIH may be needed to release RNA polymerase to start transcription.



End of Initiation

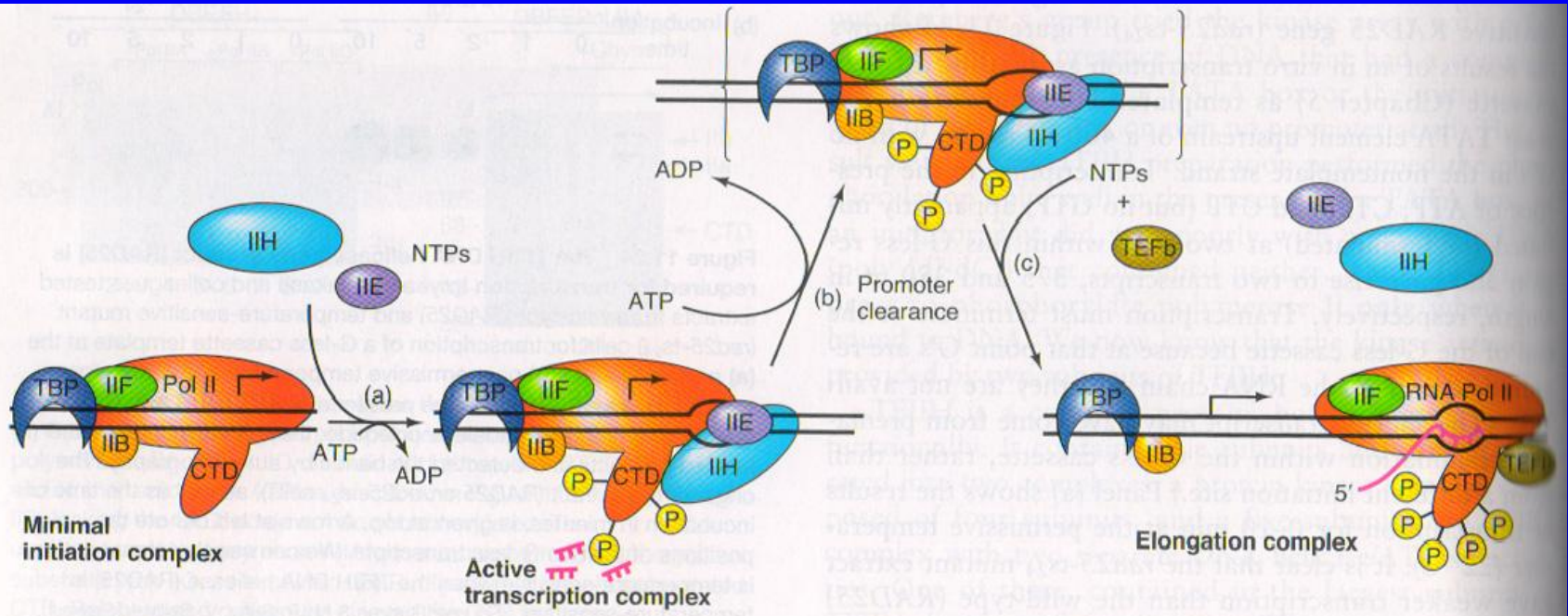
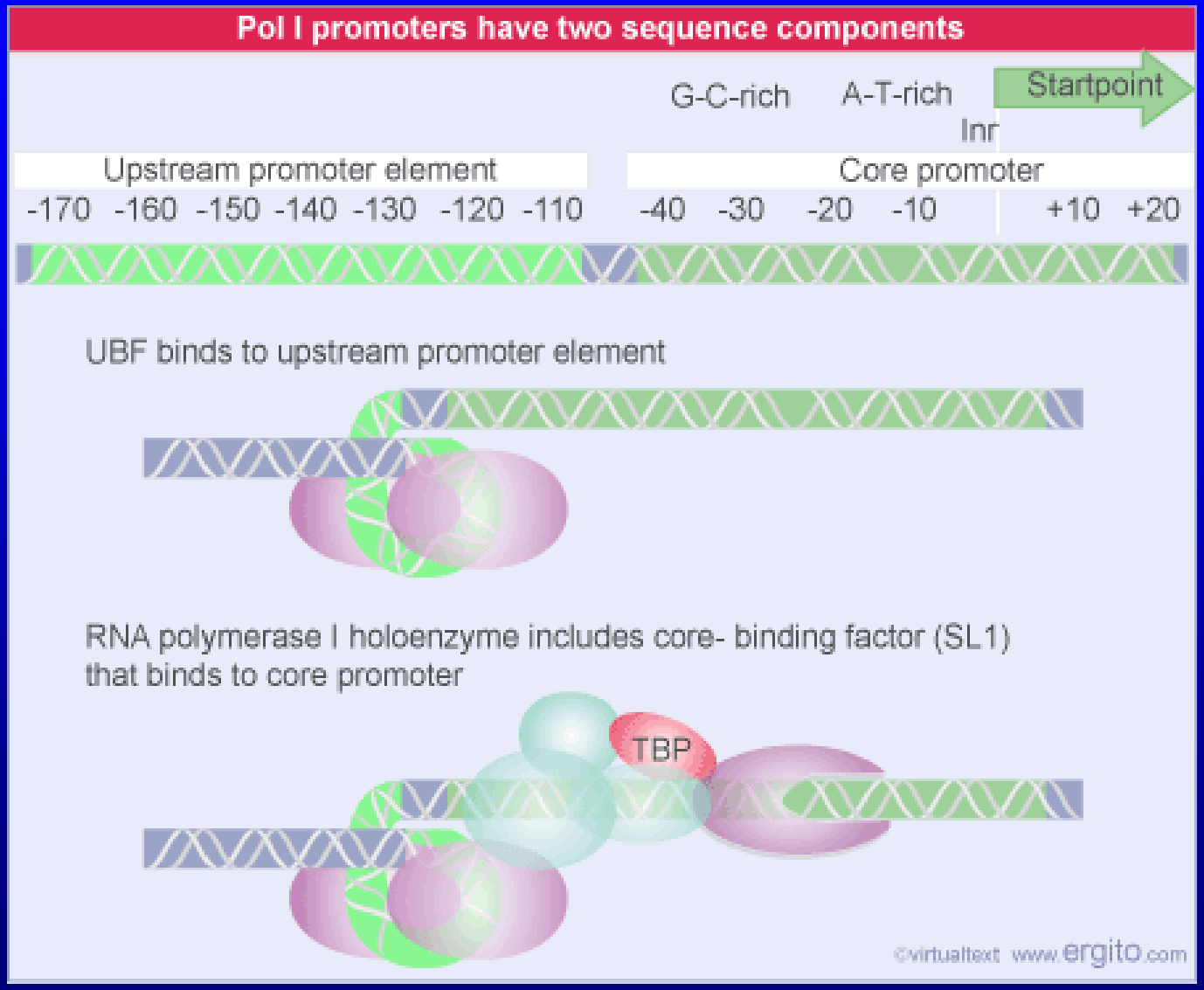


Figure 11.25 A model for the participation of general transcription factors in initiation, promoter clearance, and elongation. (a) TBP (or TFIID), along with TFIIB, TFIIF, and RNA polymerase II form a minimal initiation complex at the initiator. Addition of TFIIH, TFIIE, and ATP allows DNA melting at the initiator region and partial phosphorylation of the CTD of the largest subunit of RNA polymerase. These events allow production of abortive transcripts (magenta), but the polymerase stalls at position +10 to +12. **(b)** With energy provided by ATP, the DNA helicase of TFIIH causes further unwinding of the DNA, expanding the transcription bubble. This expansion releases the stalled polymerase and allows it to clear the promoter. **(c)** With further phosphorylation of the polymerase CTD by TEFb and with continuous addition of NTPs, the elongation complex continues elongating the RNA. TBP and TFIIB remain at the promoter. TFIIE and TFIIH are not needed for elongation and dissociate from the elongation complex. (*Source:* Modified from Goodrich, J.A. and T. Tjian. 1994. Transcription factors IIE and IIH and ATP hydrolysis direct promoter clearance by RNA polymerase II. *Cell* 77:145–56. Copyright 1994, with permission from Elsevier Science.)

Transcription unit for RNA polymerase I



Promoters in type III gene

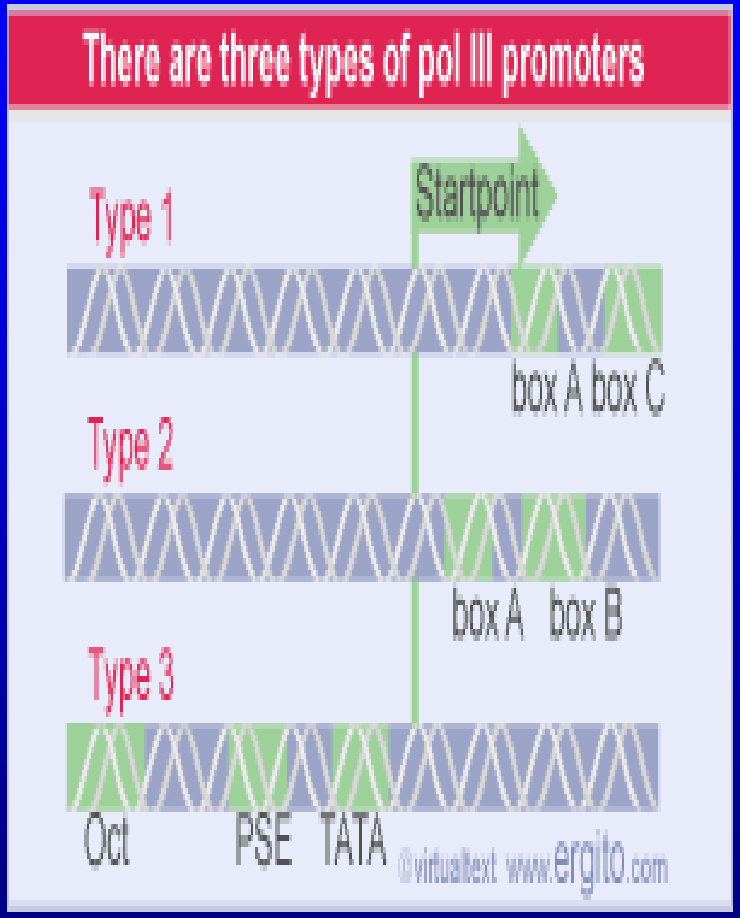
upstream promoter (type 3) and

internal promoter (type1,2)

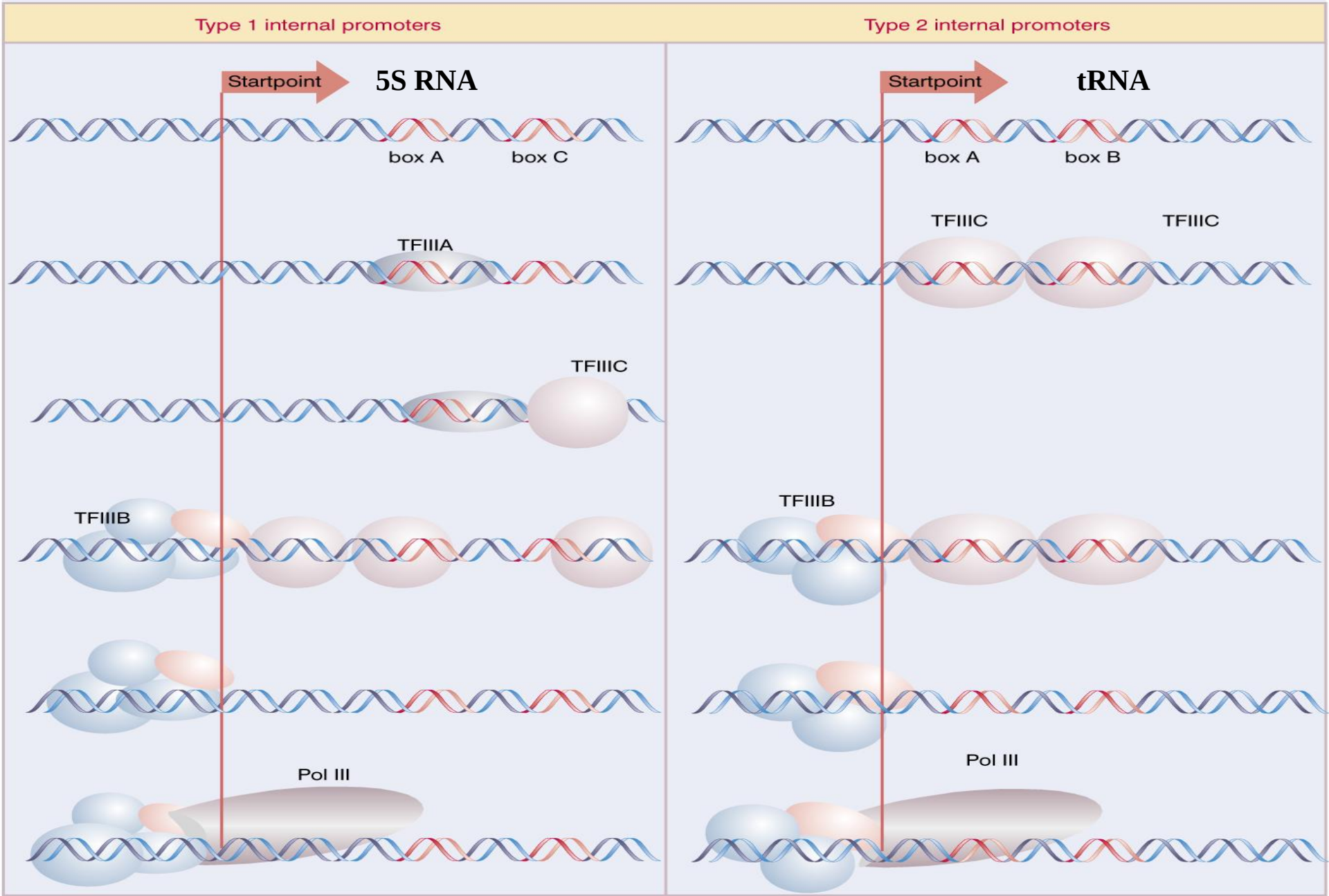
upstream promoter: U6 snRNA

Internal promoter: 5S RNA and

tRNA

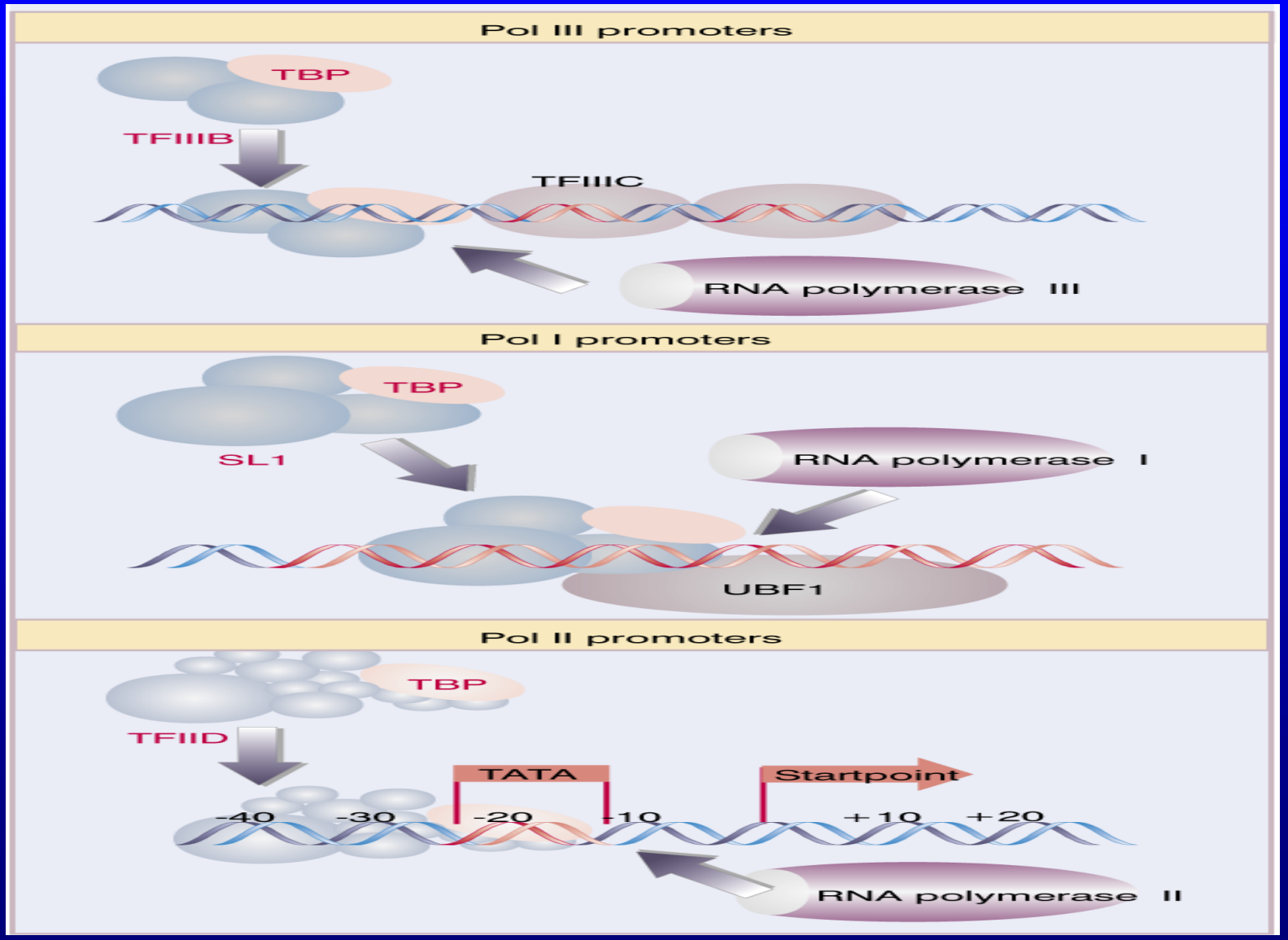


Initiation in type III gene with polymerase III



Initiation	RNA pol I	RNA pol III	RNA pol II
ATP requirement	no	no	yes
core consensus	sq. core element	A and B or C box	TATA box Inr
upstream element	UCE		CAAT box GC box etc
general TFs	SL1	TFIIIA B C	various TFIIs
upstream factors	UBF		various up-stream factors

TBP is a universal factor



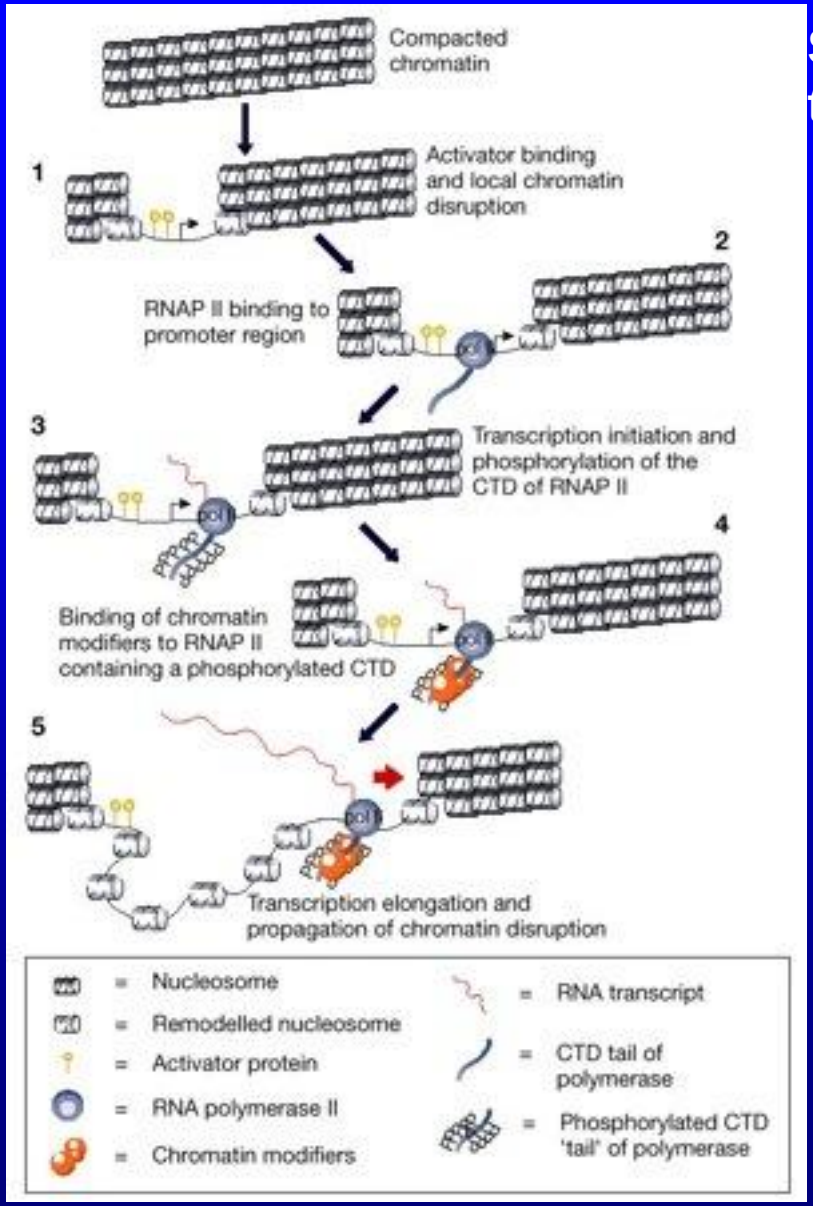
Transcriptional elongation

Steps leading to transcriptional activation

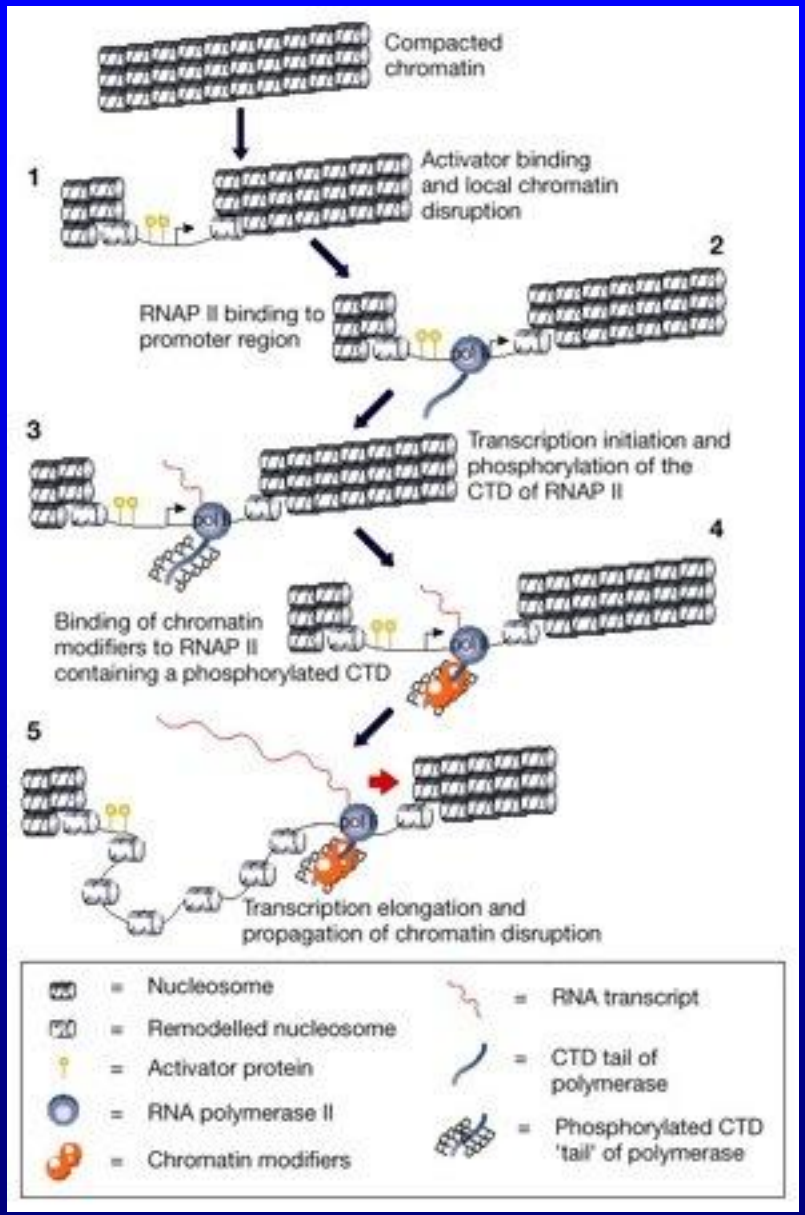
CTD phosphorylation status of RNA pol II

Promoter escape/clearance

Transition to elongation phase

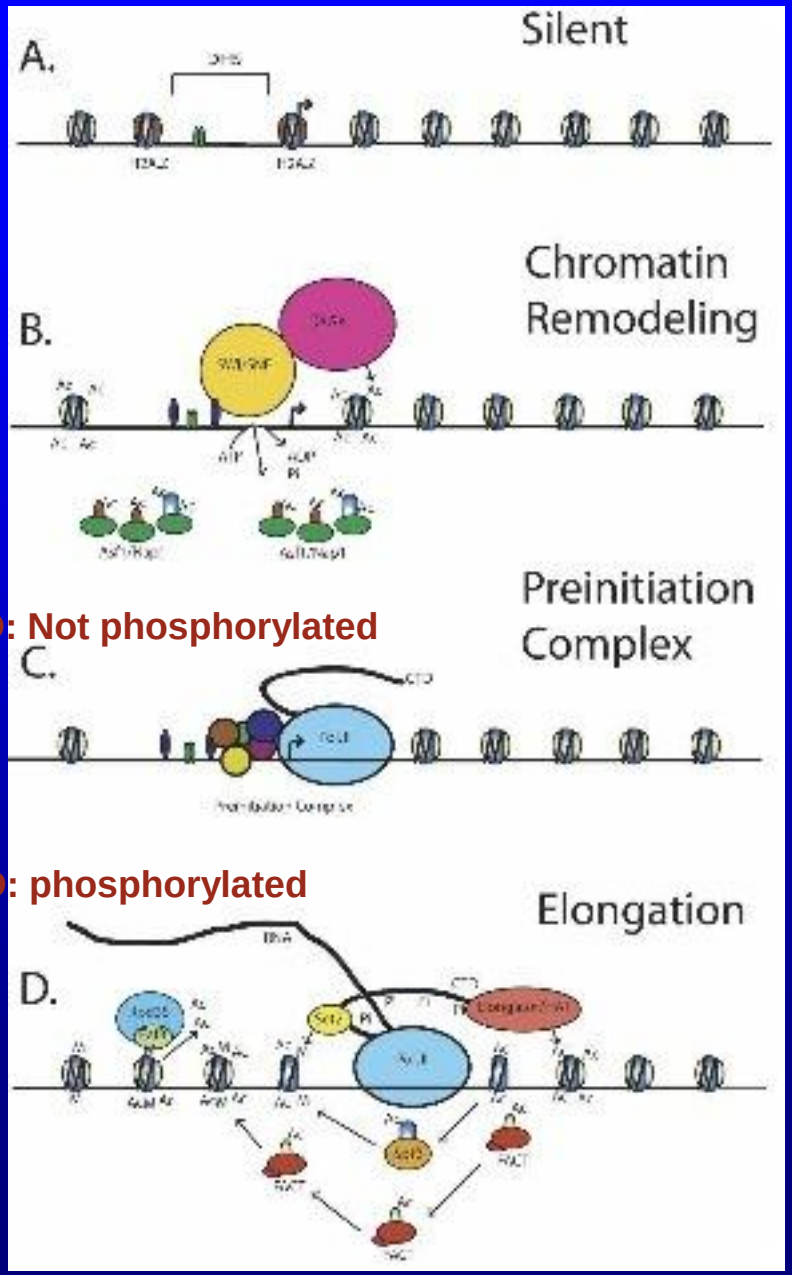


What happens during transcriptional elongation?



- Original contacts within pre-initiation complex abolished
- Formation of new contacts with elongation factors
- Change of RNA pol II to a ternary complex = high stability
- Phosphorylation of CTD

Model of nucleosome dynamics during transcription

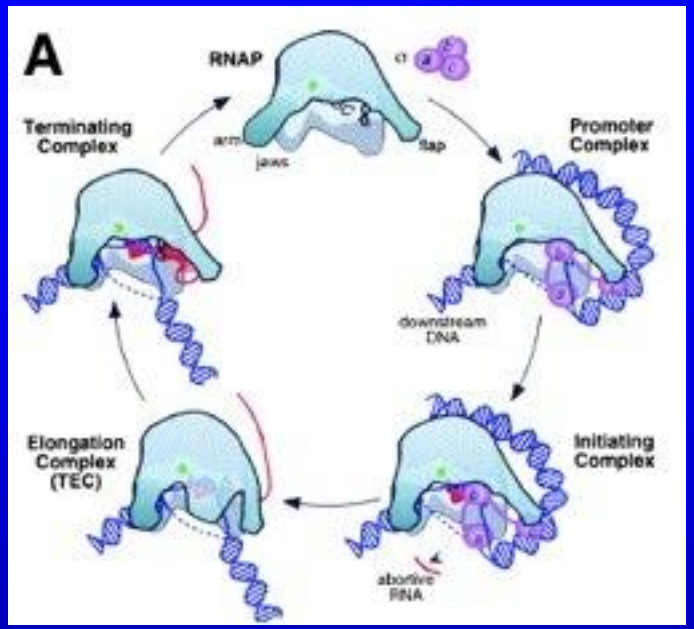


CTD: Not phosphorylated

CTD: phosphorylated

- Phosphorylation of the CTD defines the stage of transcription
- CTD consists of heptad repeats of the consensus sequence: YSPTSPS
- # of repeats differ in organisms
- Promoter clearance: Ser #5 gets phosphorylated
- Transition to elongation: Ser #2 gets phosphorylated

Experimental evidence for elongation factors



• **Comparison of RNAPII elongation rate**

• ***in vitro*: 100-300 nt/min, frequent pauses, and sometimes full arrest**

• ***in vivo*: 1200-2000 nt/min**

Why the discrepancy?

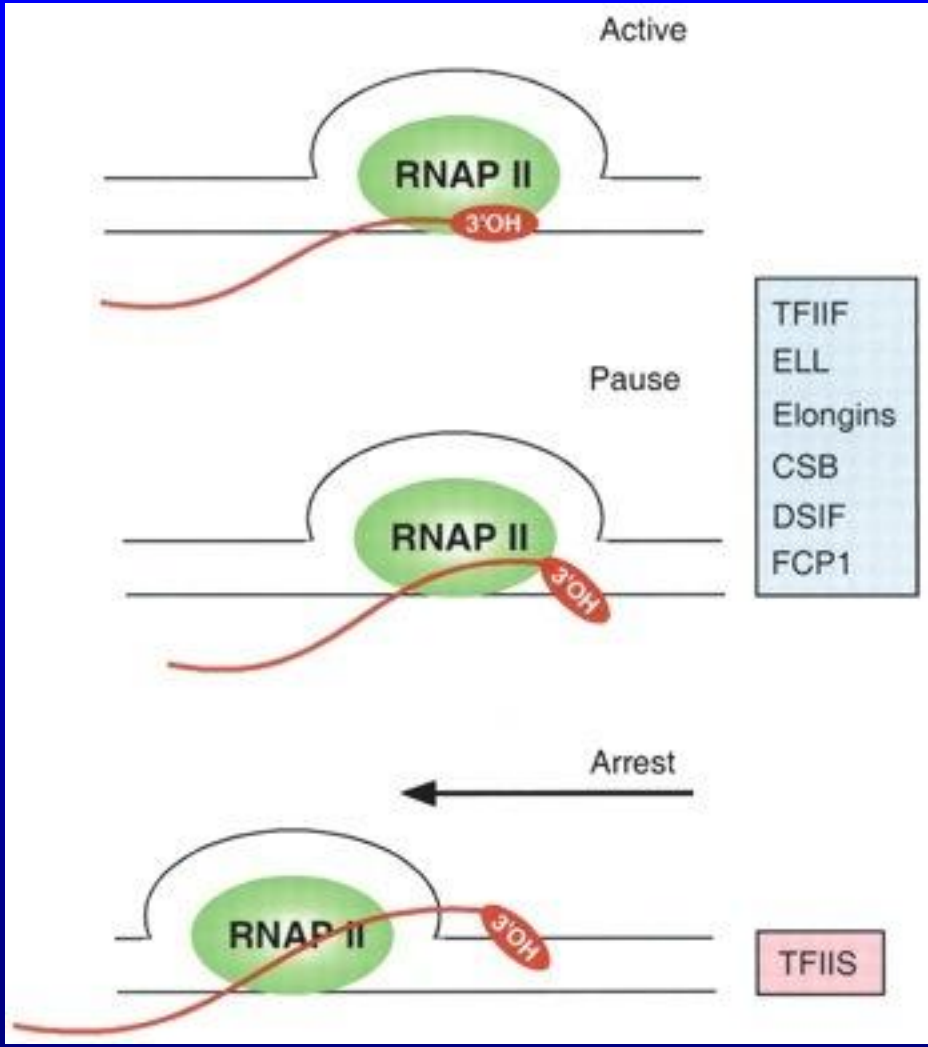
• **Use of pharmacological agents**

• **DRB(5,6-dichloro-1-ß-D-ribofuranosylbenzimidazole**

• **DRB, nucleotide-analogue, cause inhibition of hnRNA transcription by arresting RNA pol II *in vivo*, but not purified RNA pol II. Possible target?**

These evidence suggest existence of factors that facilitate transcriptional elongation

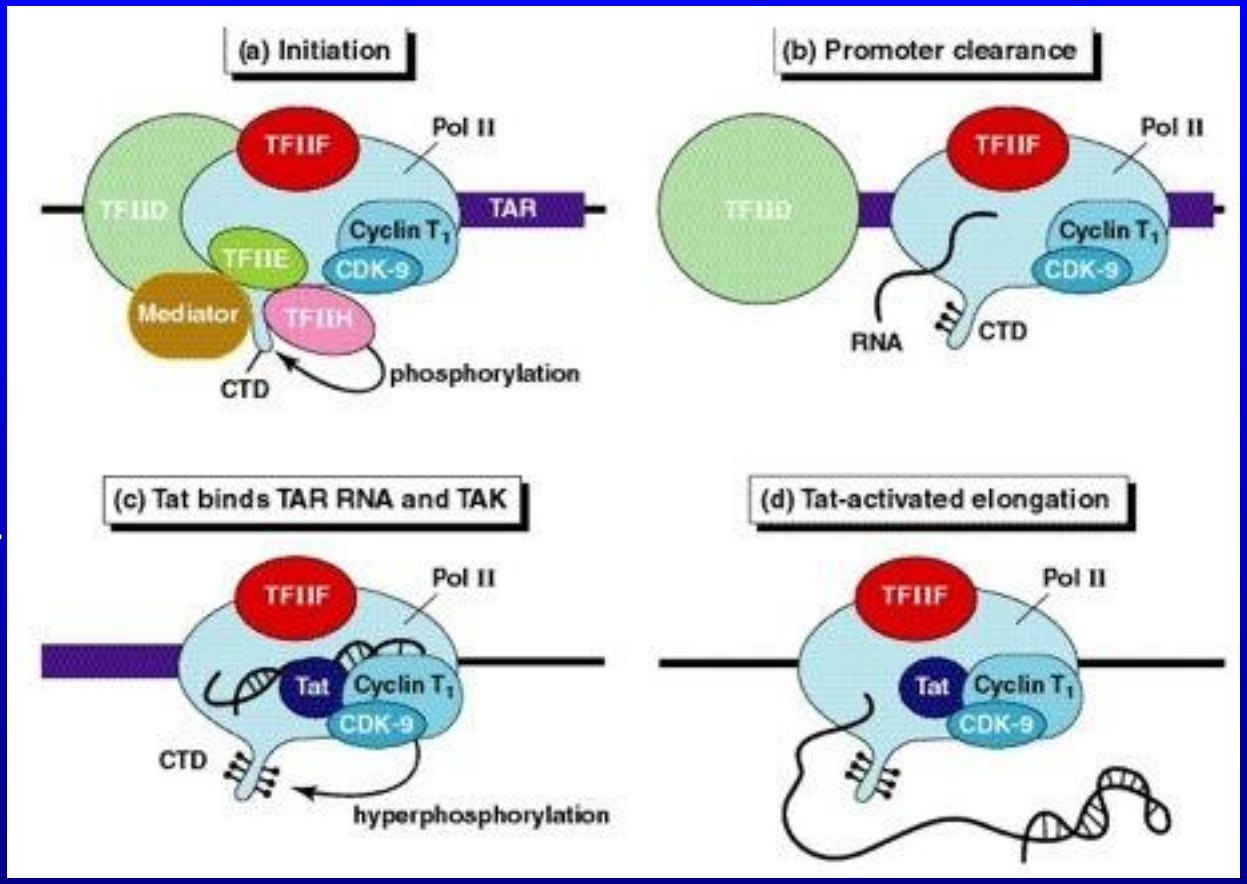
RNA polymerase II often encounters pauses & arrests



- Arrest (irreversible backsliding 7-14 nts)
- Pause (back-tracking 2-4 nts)
- Function of elongation factors: minimize these pauses & arrests

HIV virus can transactivate by hijacking elongation machinery

P-TEFb phosphorylates RNA poll CTD

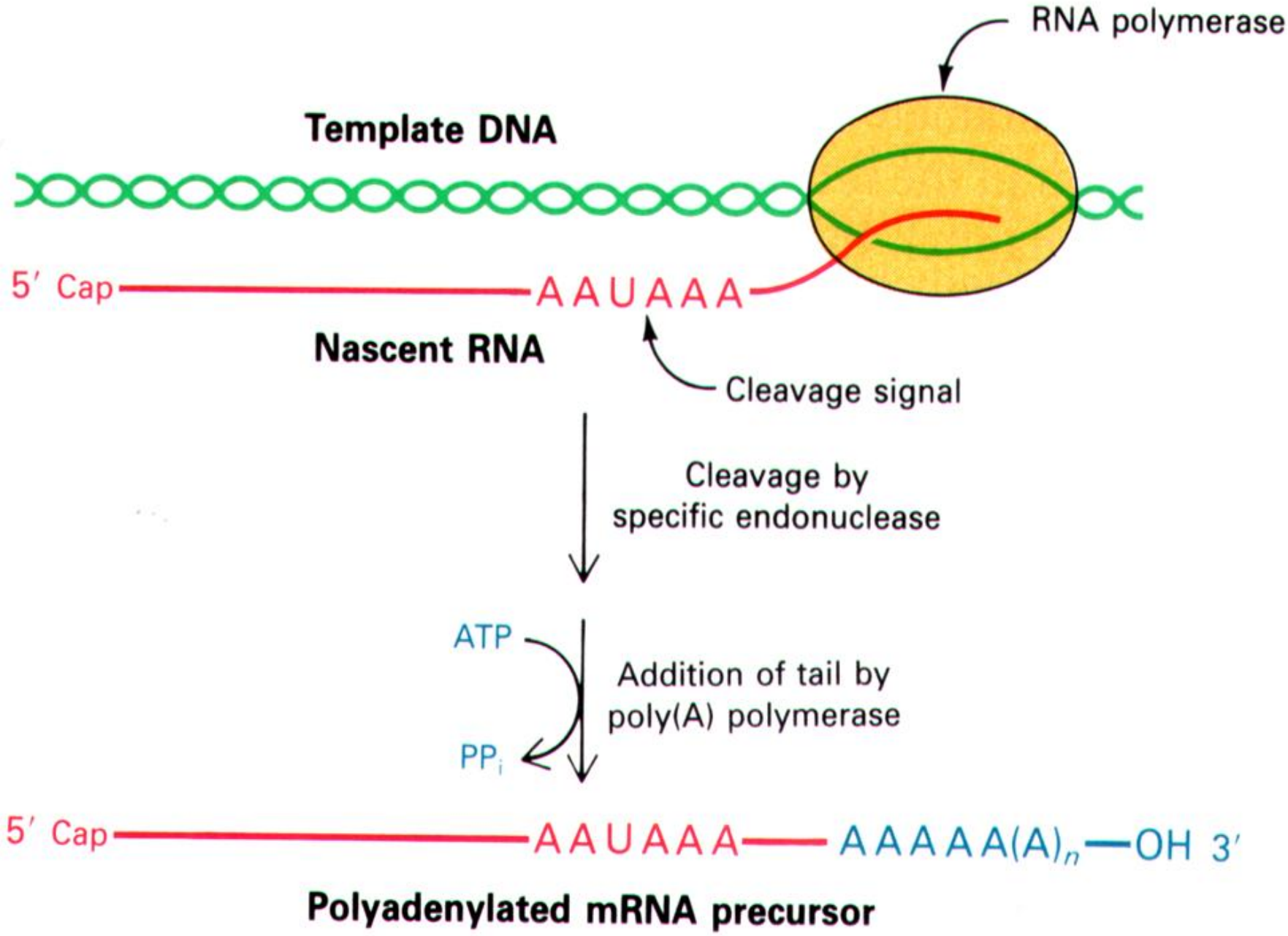


Tat: HIV's own elongation factor

HIV can bypass pre-initiation complex and head straight for elongation by hijacking RNA pol II from host

Termination of Eukaryotic Transcription

- The termination sequence is AATAAA followed by GT repeats.
- The termination is closely related to the post-transcriptional modification.


RNA polymerase
Template DNA
5′ Cap
A A U A A A
Nascent RNA
Cleavage signal
Cleavage by
specific endonuclease
ATP
PPi
Addition of tail by
poly(A) polymerase
5′ Cap
A A U A A A
A A A A A(A)n
OH 3′
Polyadenylated mRNA precursor

- Type II genes: Transcription stops after AATAAA-Polyadenylation signal.
- Type I genes:3-4 consecutive Ts
- Type III genes: Stop after synthesis of serial Us.