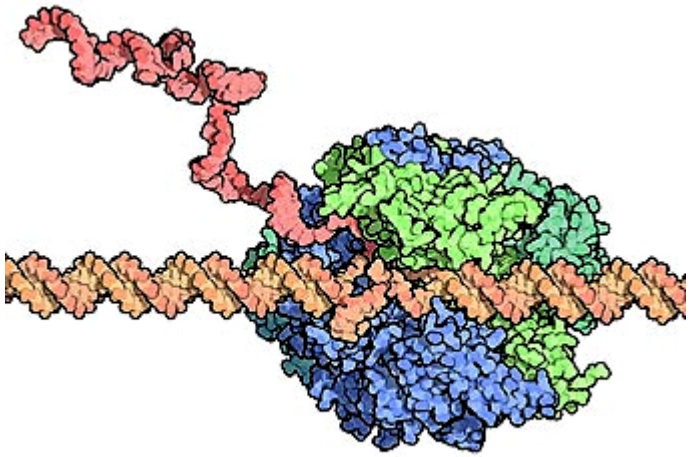
BIOSYNTHESIS
OF
Nucleic Acids and Proteins

assoc. prof. N.E.Petushok

BIOSYNTHESIS OF RNA

(transcription)



The two complementary DNA strands have different roles in transcription.

One strand serves as template for RNA synthesis (called the template strand)

The DNA strand complementary to the template, the nontemplate strand, or coding strand, is identical in base sequence to the RNA transcribed from the gene

Transcription is catalyzed by DNA-dependent RNA polymerases,

which use ribonucleoside 5-triphosphates to synthesize RNA complementary to the template strand of duplex DNA.

RNA polymerases

RNA polymerase I → rRNA

RNA polymerases II → mRNA

RNA polymerases III → tRNA & 5S rRNA

RNA polymerase structure

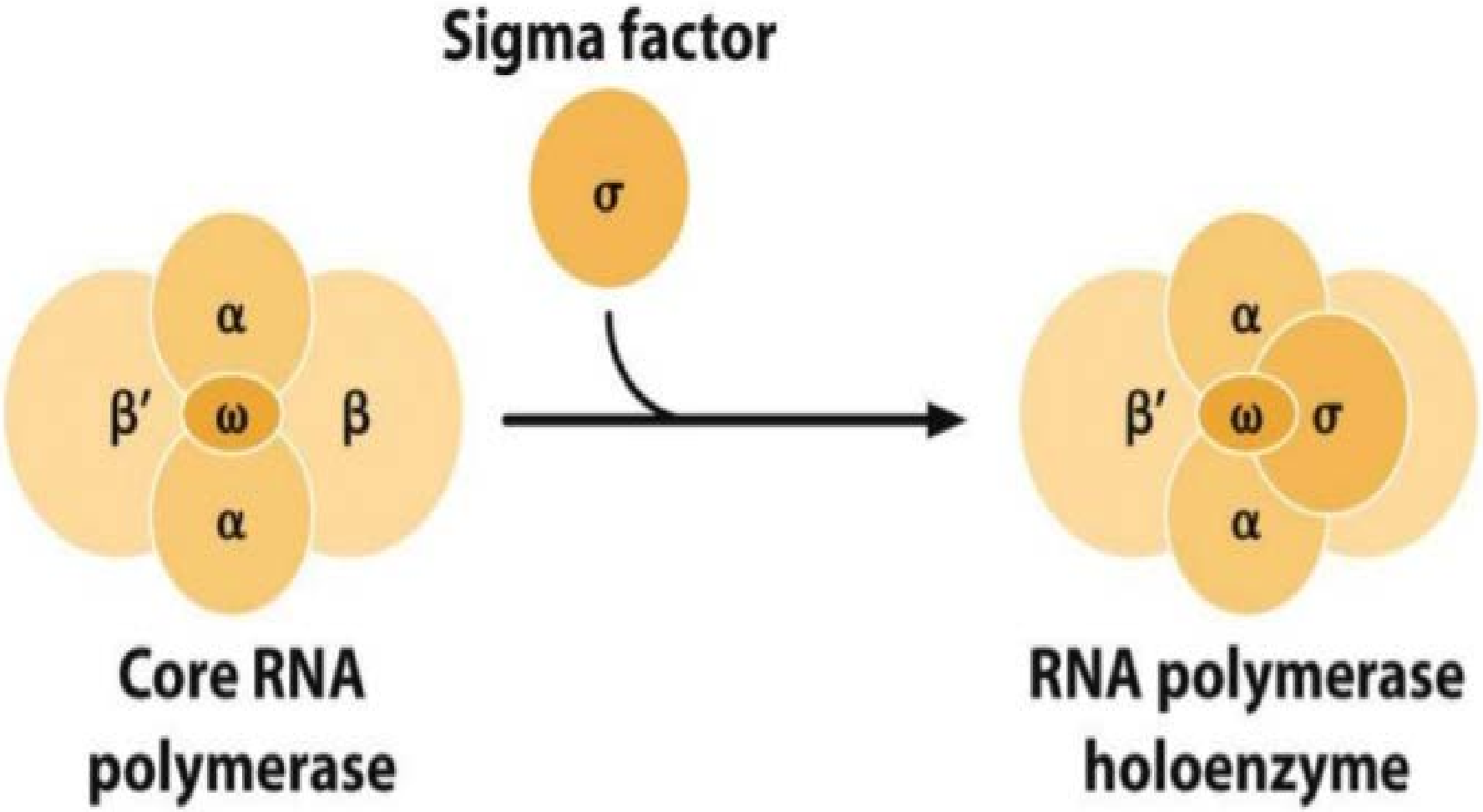


Figure 13.9a
Genetics: A Conceptual Approach, Fourth Edition

RNA polymerase structure

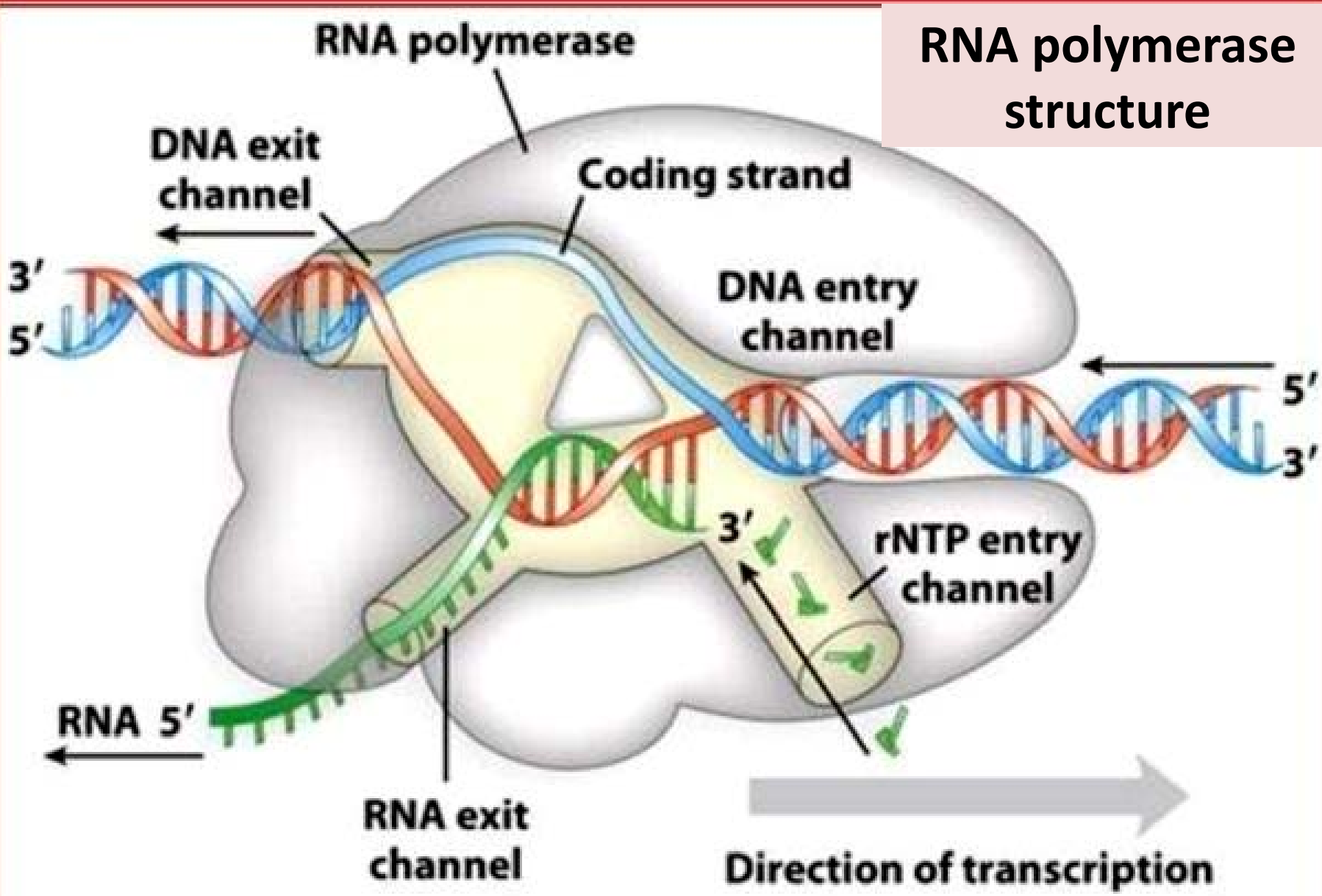


Figure 15-14
Molecular Biology: Principles and Practice

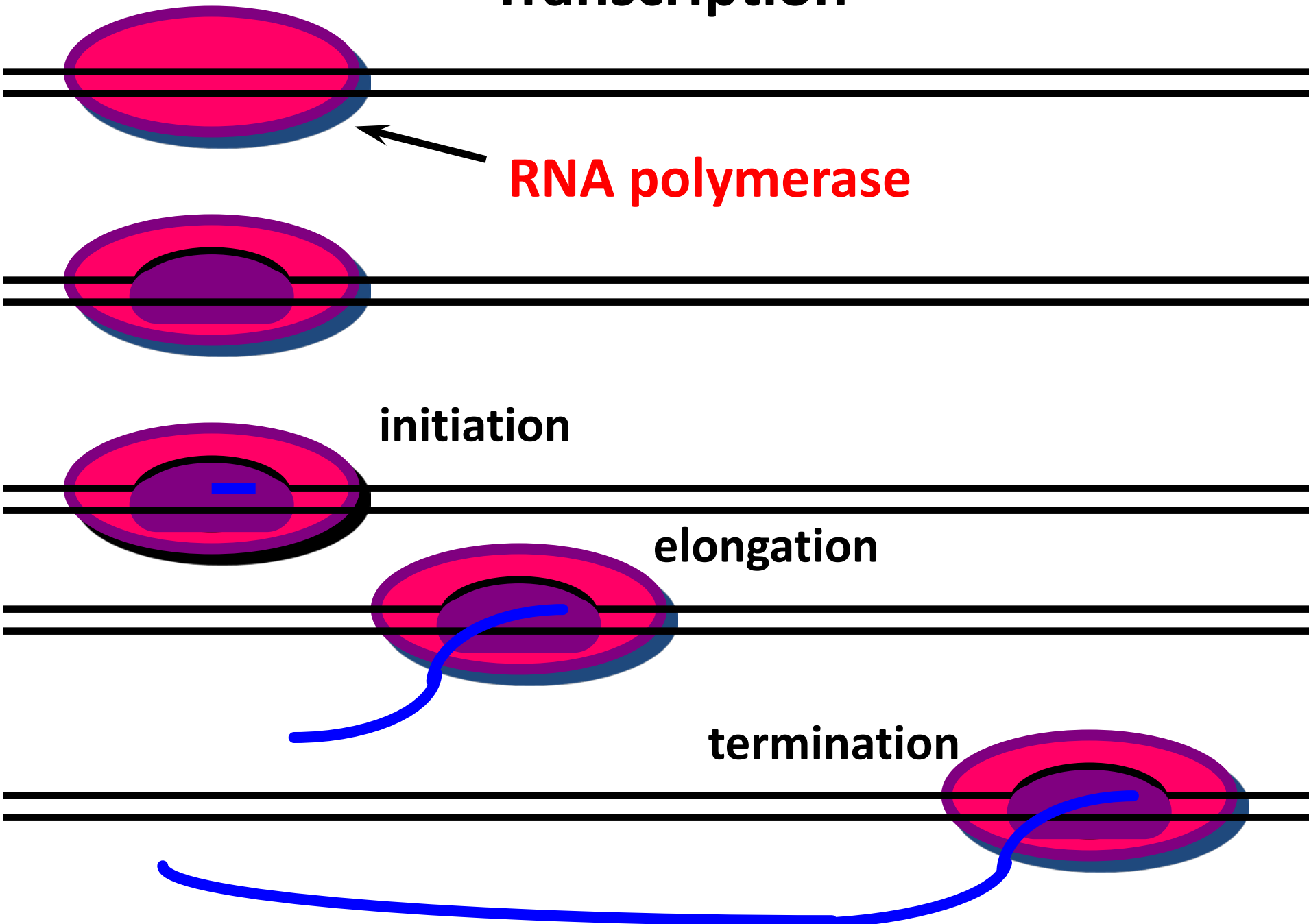
Transcription occurs in several phases:

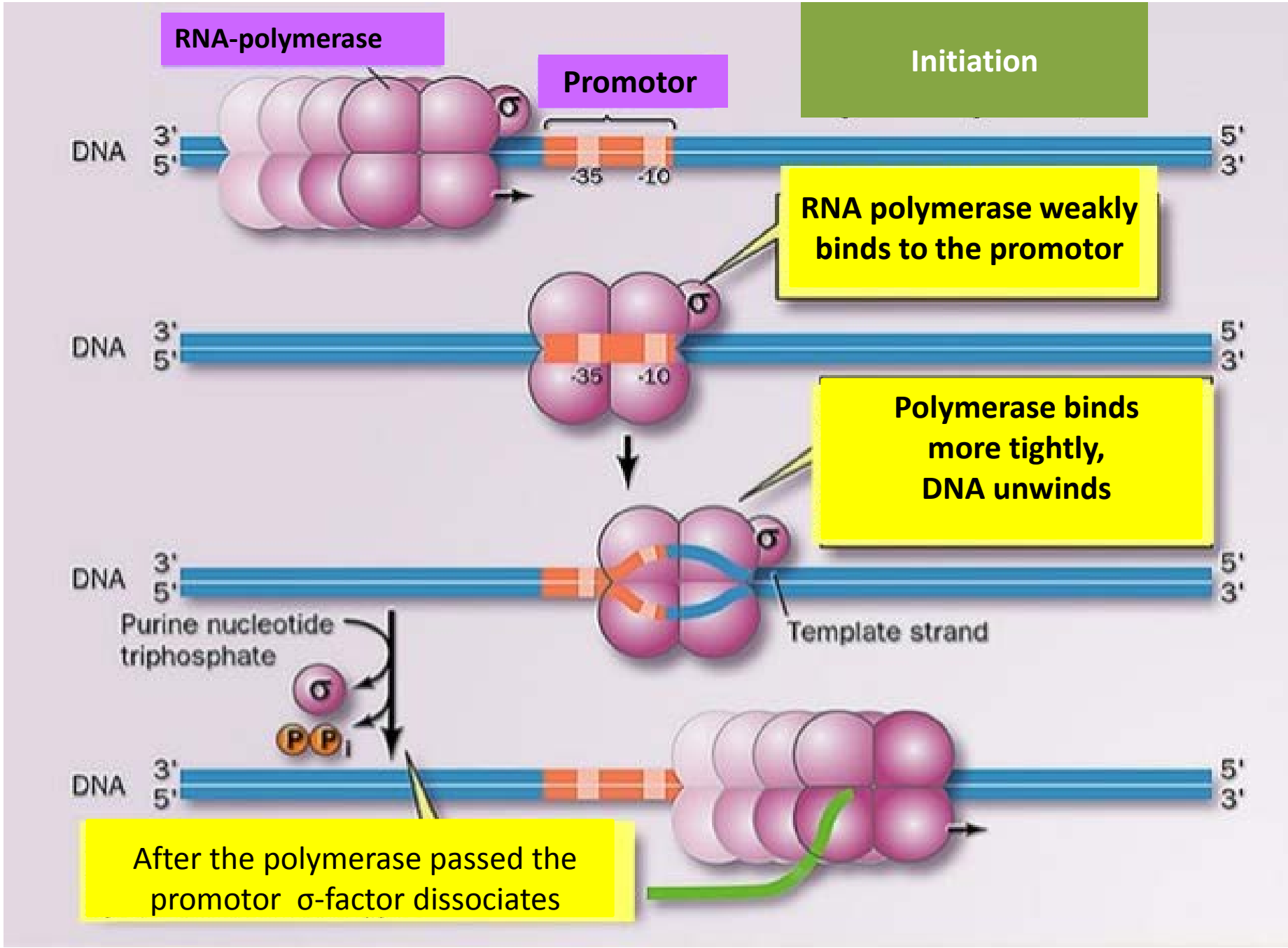
- ✓ **binding of RNA polymerase to a DNA site called a promoter and initiation of transcript synthesis**
- ✓ **elongation**
- ✓ **termination**

The first step in transcription, binding of RNA polymerase to the promoter and initiation of transcription are closely regulated.

Transcription stops at sequences called terminators.

Transcription




RNA-polymerase
Promotor
Initiation
DNA
3'
5'
5'
3'
-35
-10
σ
RNA polymerase weakly binds to the promotor
Polymerase binds more tightly, DNA unwinds
Template strand
Purine nucleotide triphosphate
P P i
After the polymerase passed the promotor σ-factor dissociates

RNA molecules in eukaryotes are processed to some degree after synthesis. A newly synthesized RNA molecule is called a primary transcript.

RNA molecules are usually processed before they become functional.

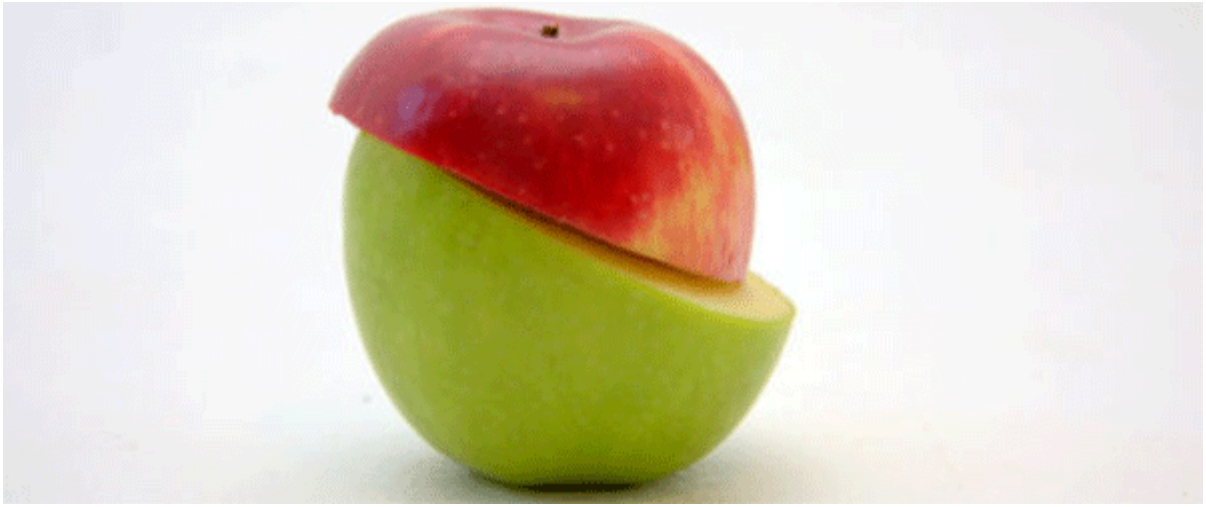
RNA processing

Eukaryotic mRNAs are modified at each end.

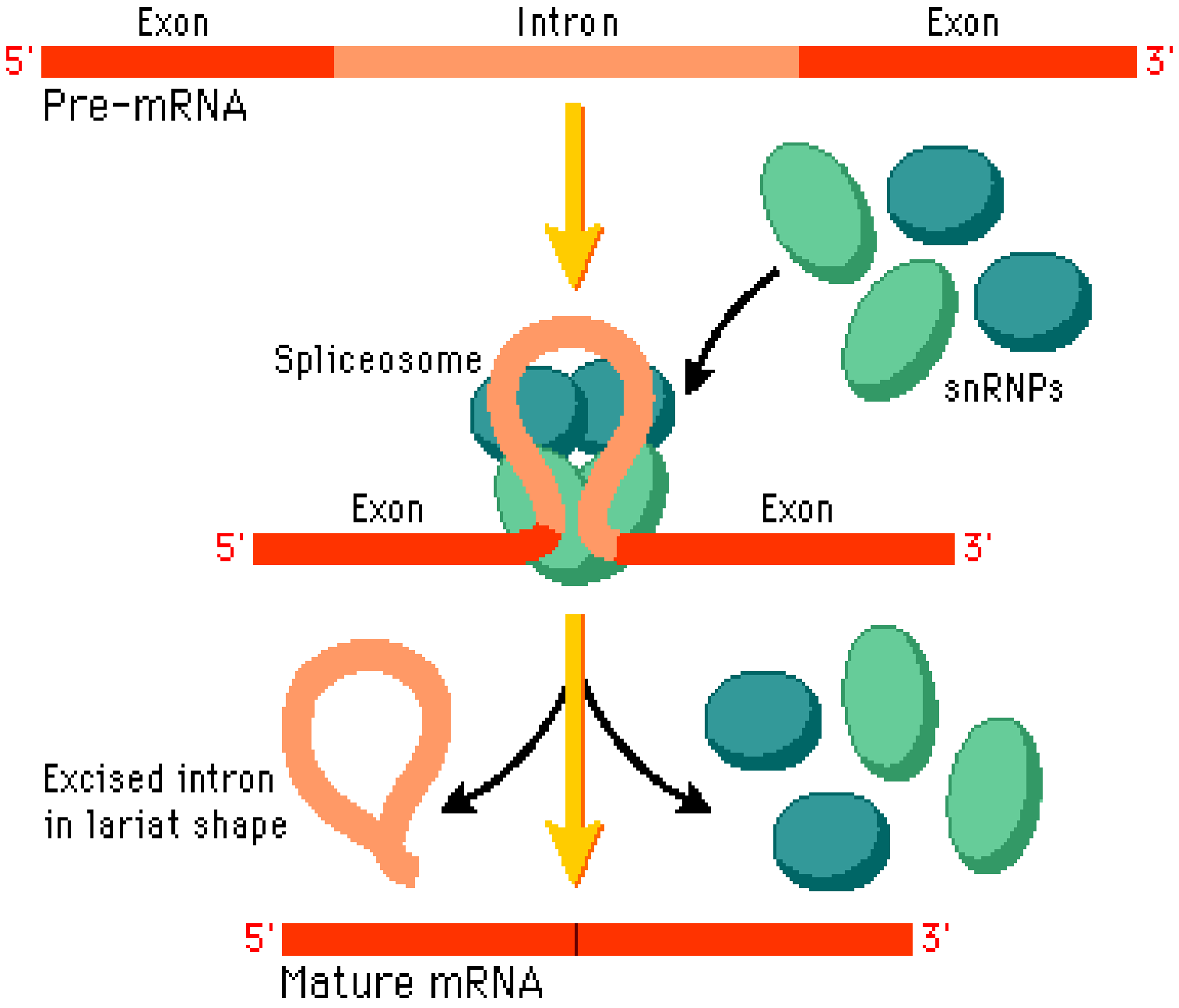
A modified residue called a 5' cap is added at the 5’ end.

The 3’ end is cleaved, and 80 to 250 A residues are added to create a poly(A) “tail.”

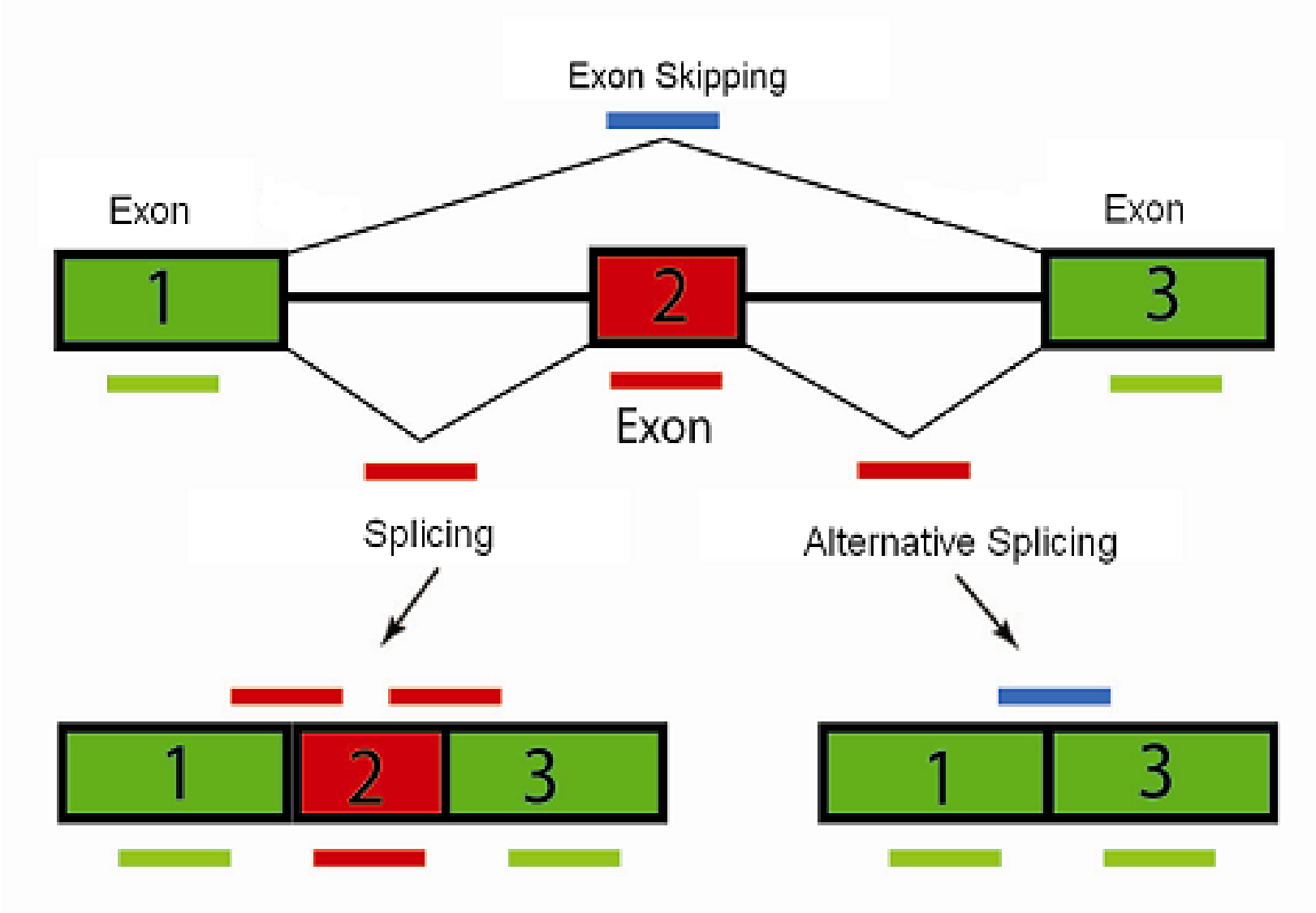
In a process called splicing, the introns are removed from the primary transcript and the exons are joined to form a continuous sequence that specifies a functional polypeptide



RNA processing



Alternative splicing



tRNA processing

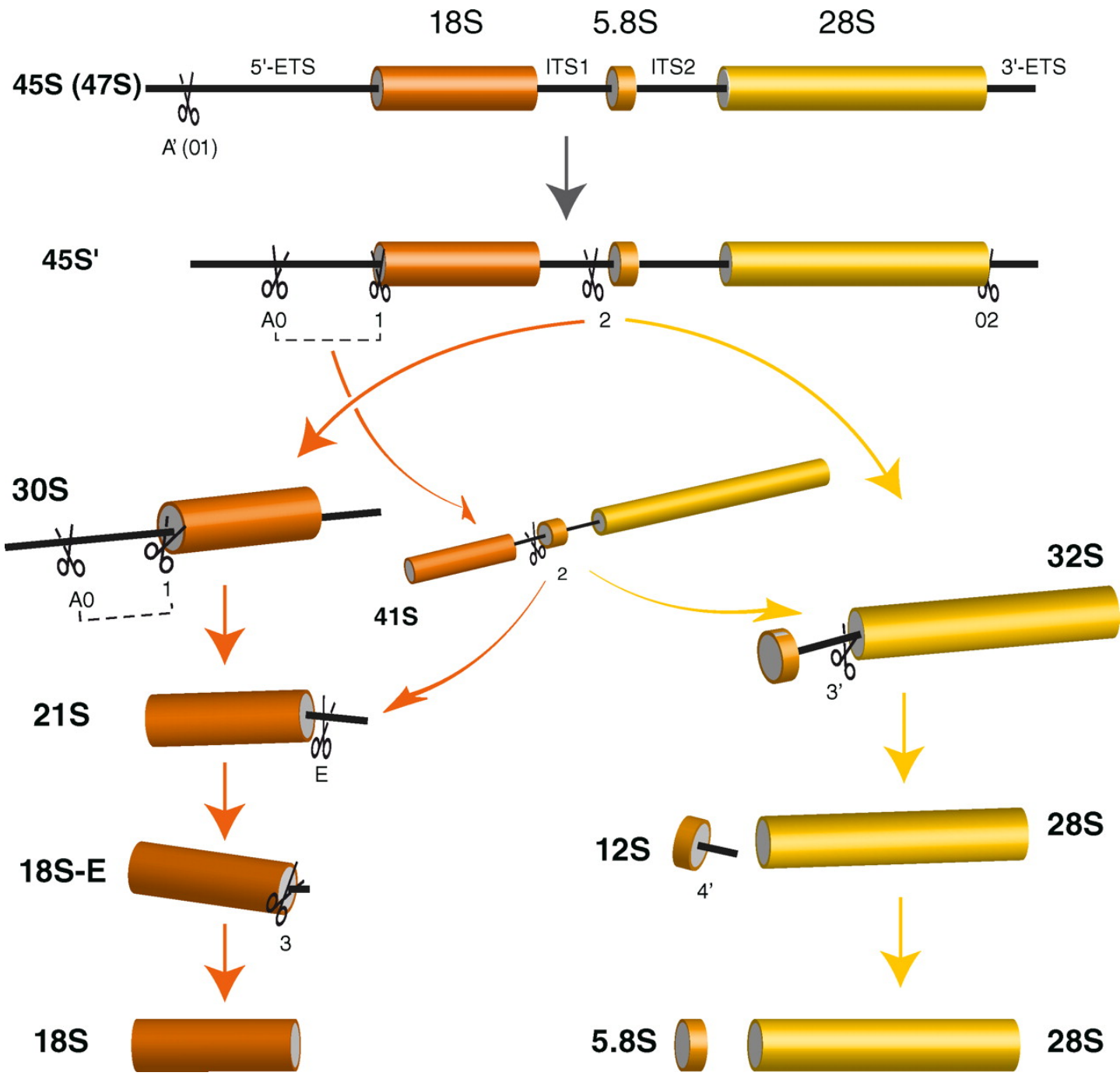
- **-…CCA at the 3' end**
- **modification of bases**

rRNA processing

Ribosomal RNAs are processed from larger precursor

45 S → **28 S**
18 S
5,8 S

rRNA processing



Regulation of transcription

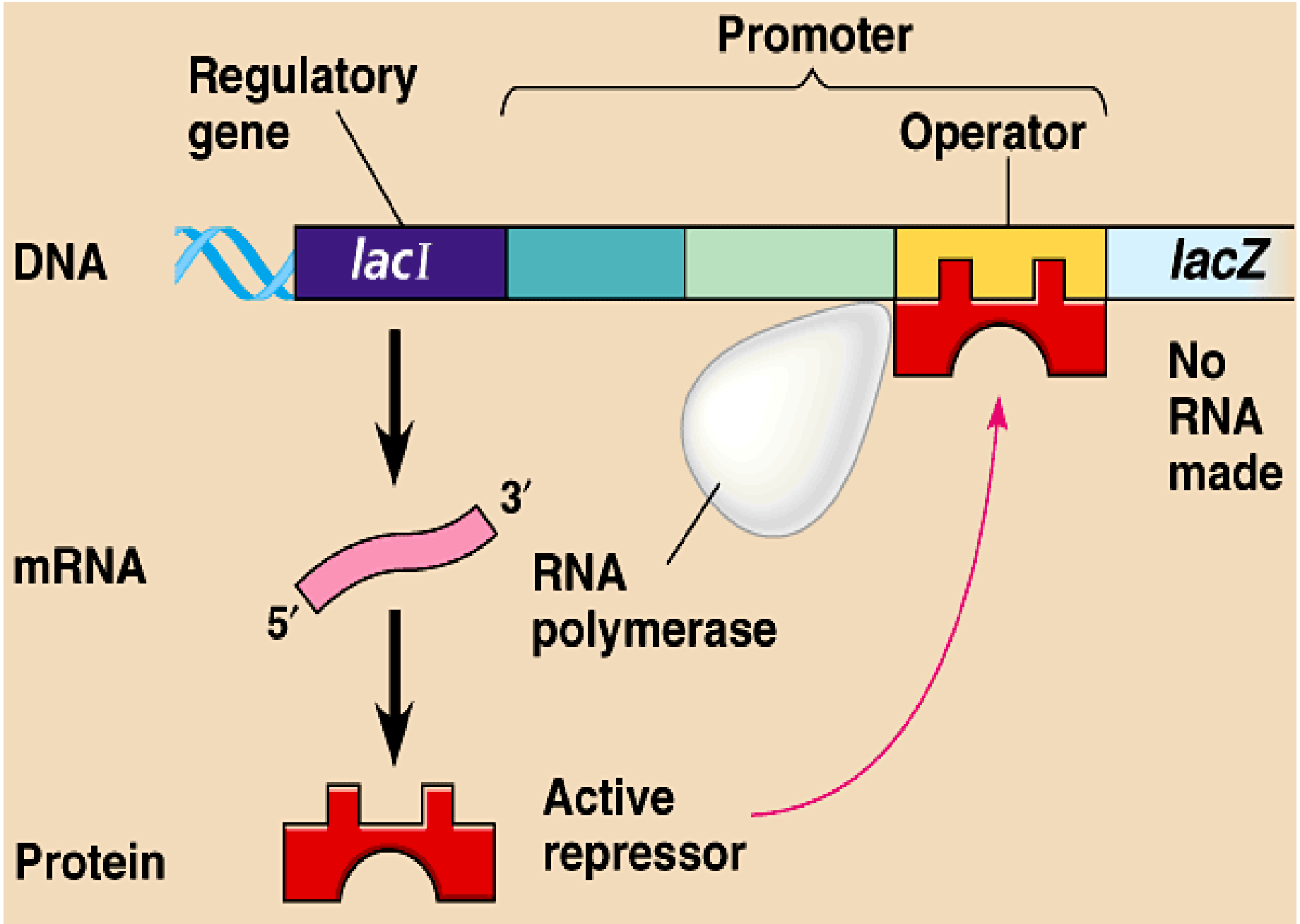
Bacteria have a simple general mechanism for coordinating the regulation of genes encoding products that participate in a set of related processes: these genes are clustered on the chromosome and are transcribed together

Many prokaryotic mRNAs are polycistronic—multiple genes on a single transcript—and the single promoter that initiates transcription of the cluster is the site of regulation for expression of all the genes in the cluster.

The gene cluster and promoter, plus additional sequences that function together in regulation, are called an operon

!!! Harper's Illustrated Biochemistry

Repression of *lac* operon



Regulation of transcription

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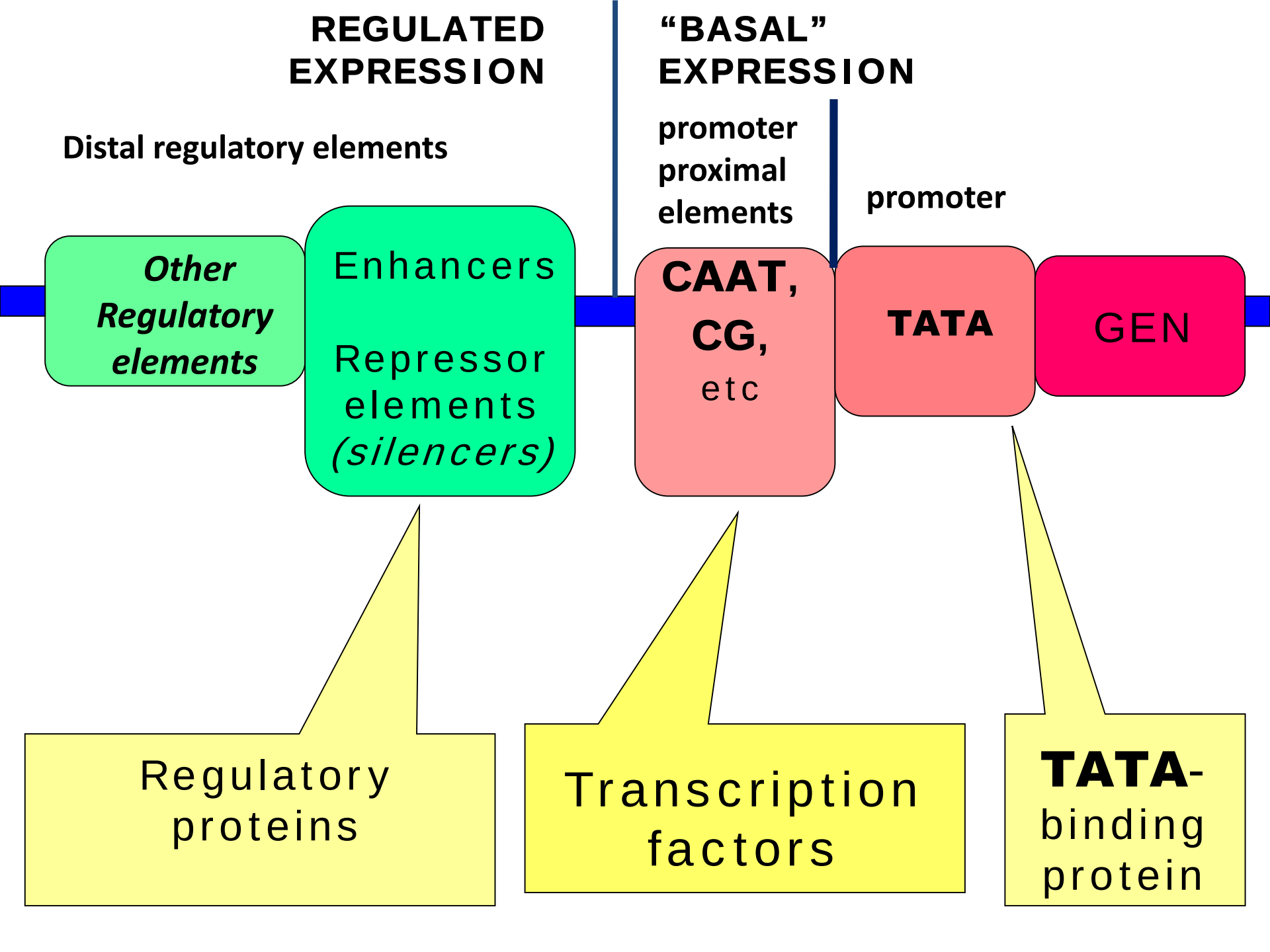
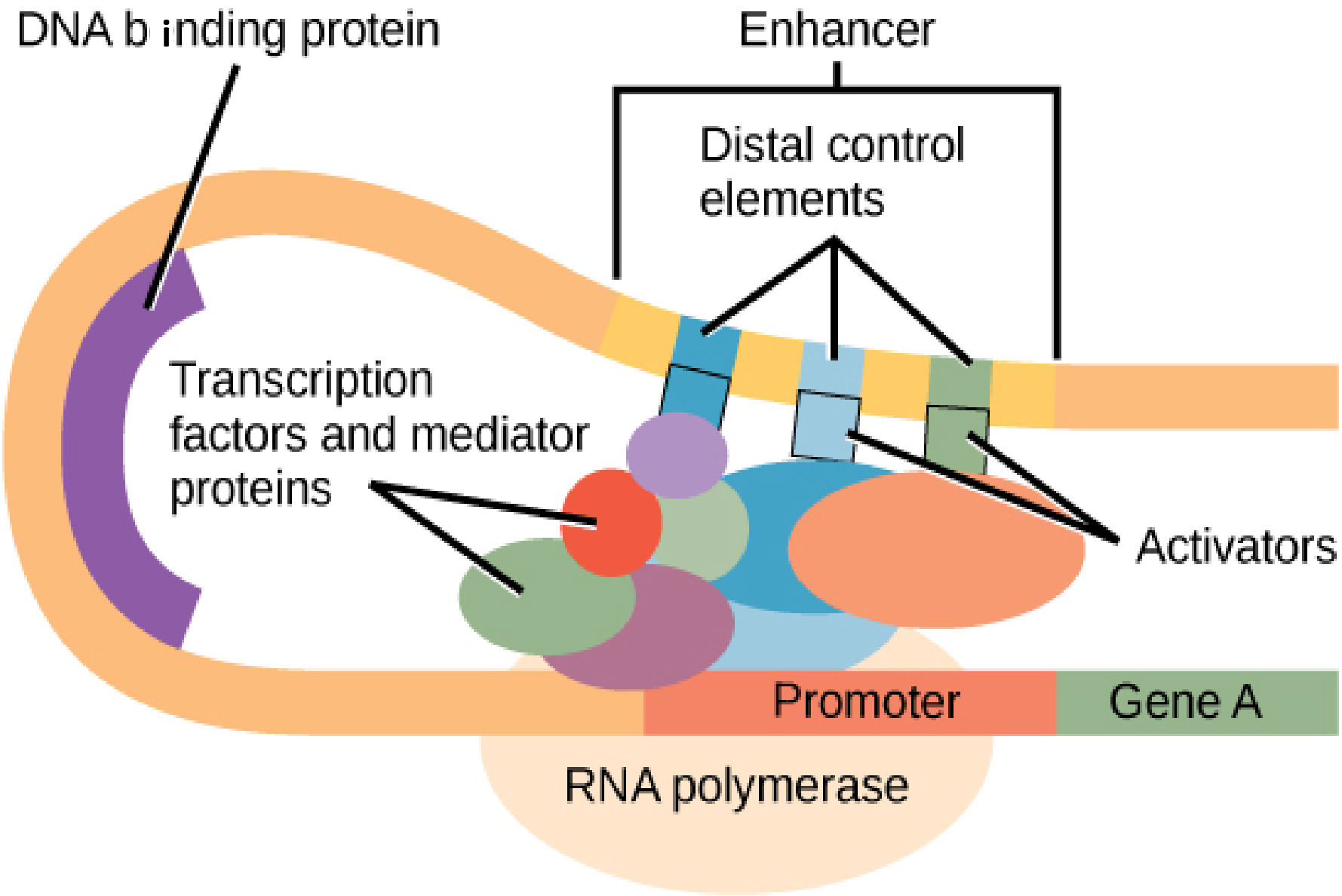
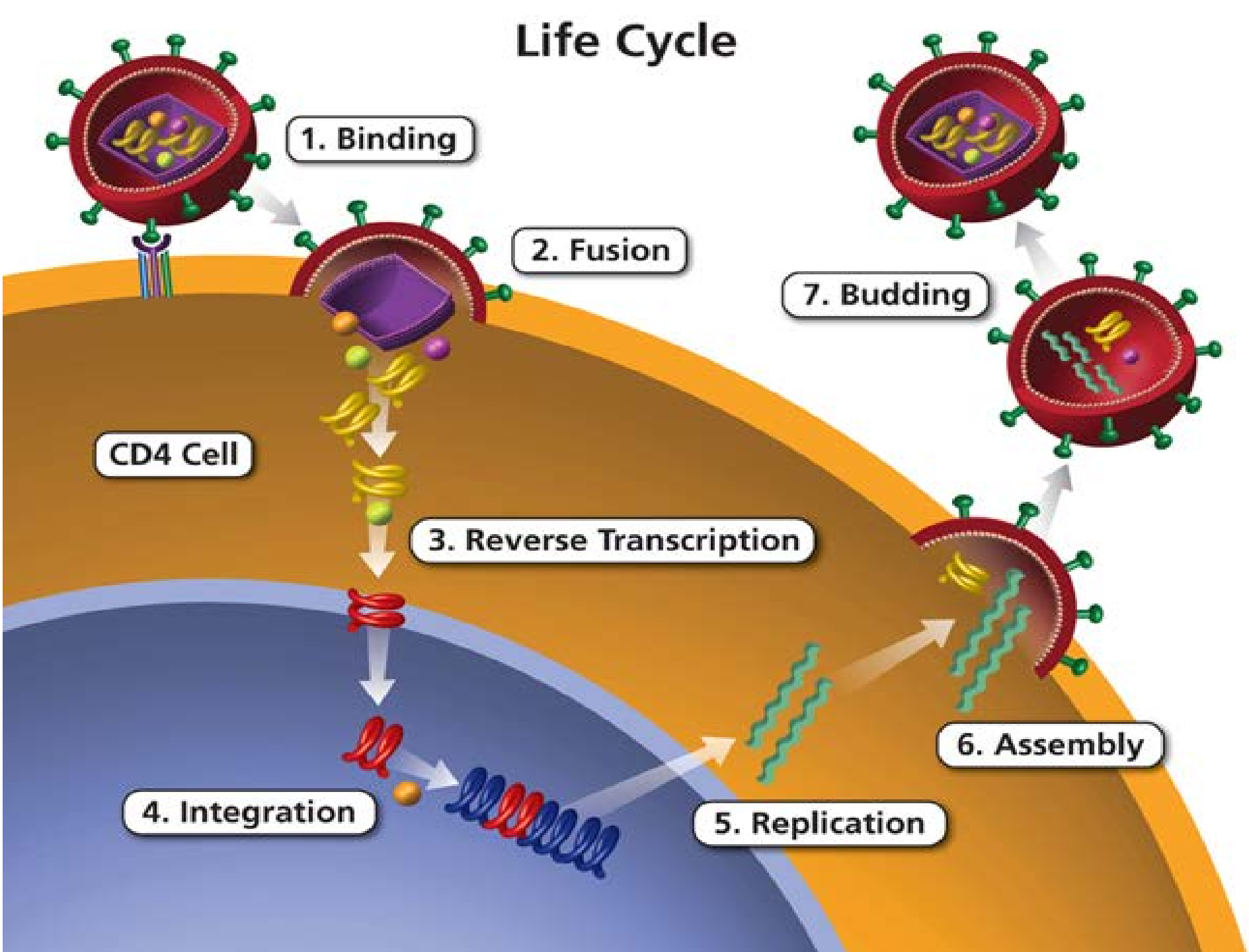

REGULATED EXPRESSION
"BASAL" EXPRESSION
Distal regulatory elements
promoter proximal elements
promoter
Other Regulatory elements
Enhancers
Repressor elements (silencers)
CAAT, CG, etc
TATA
GEN
Regulatory proteins
Transcription factors
TATA-binding protein


DNA b inding protein
Enhancer
Distal control elements
Transcription factors and mediator proteins
Activators
Promoter
Gene A
RNA polymerase

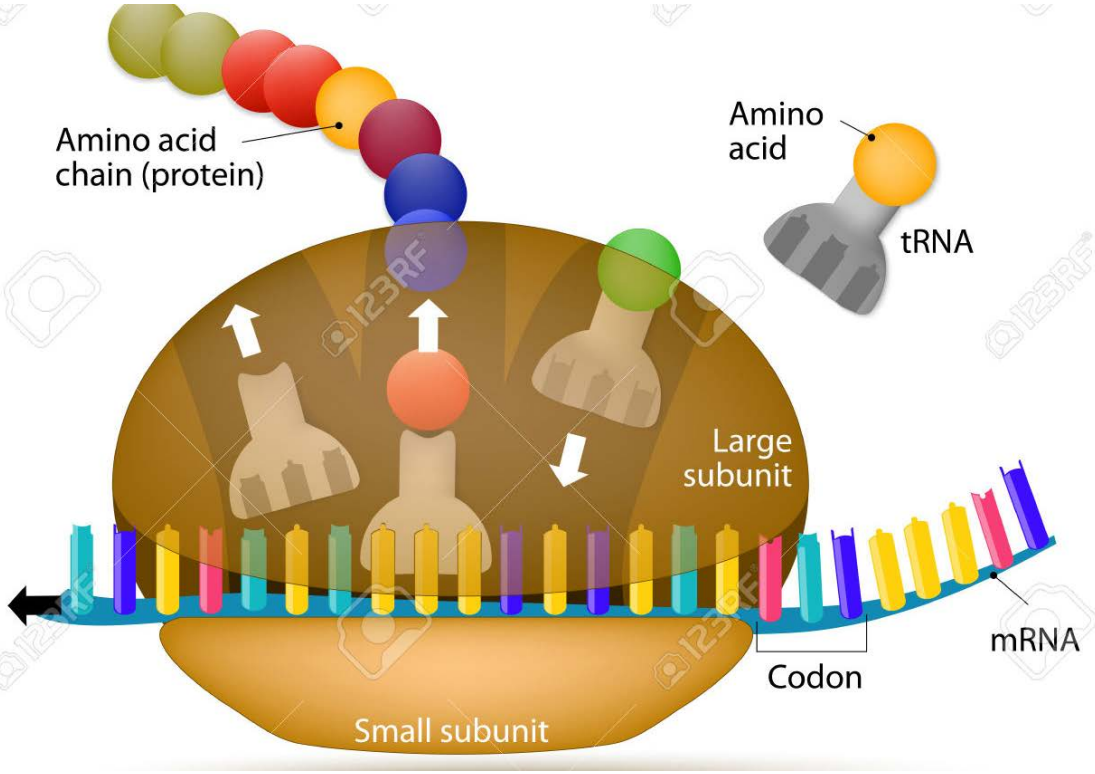
RNA-Dependent Synthesis of DNA
or

REVERSE TRANSCRIPTION

(Manual on Biochemistry)

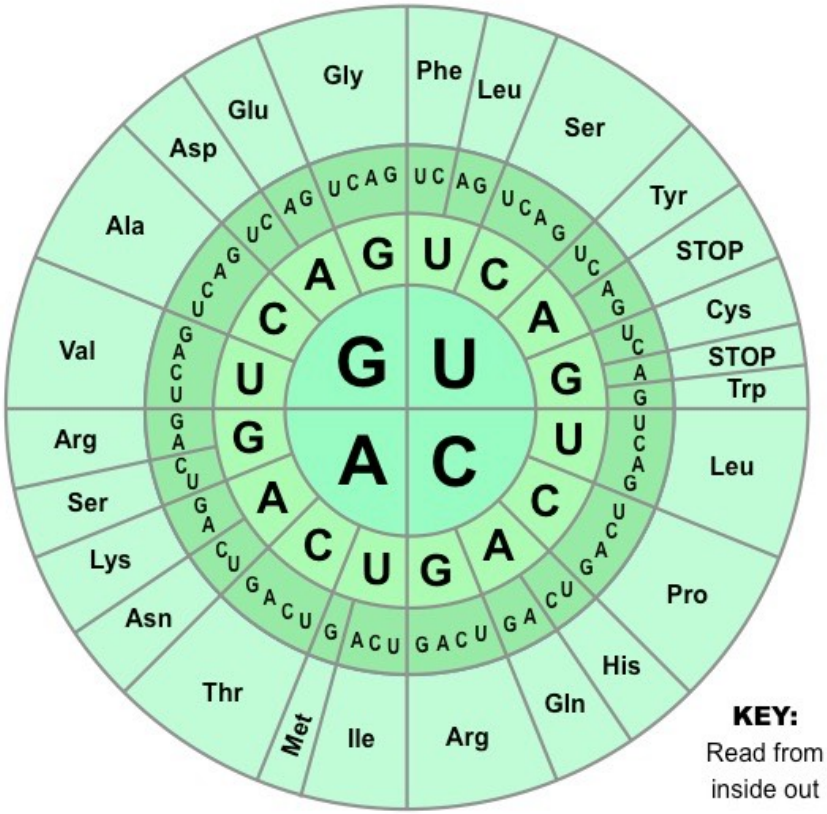

Life Cycle
1. Binding
2. Fusion
CD4 Cell
3. Reverse Transcription
4. Integration
5. Replication
6. Assembly
7. Budding

PROTEIN SYNTHESIS
(TRANSLATION)



The nucleotide sequence of an mRNA contains code words for each amino acid – the genetic code

1st letter	Second Letter: U		Second Letter: C		Second Letter: A		Second Letter: G		3rd letter
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G



Features of the Genetic Code

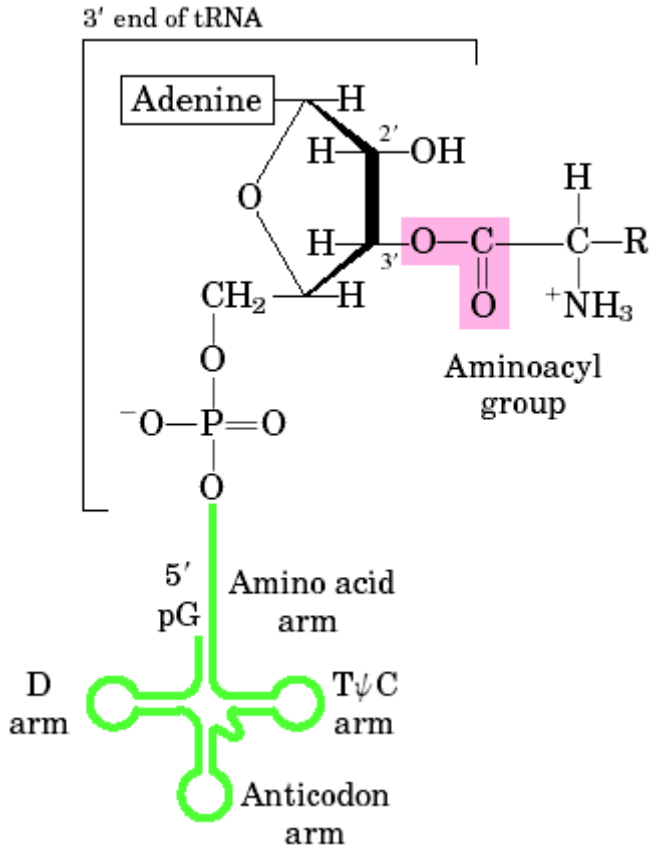
- **Triplet**
- **Degenerate**
- **Unambiguous**
- **Nonoverlapping**
- **Not punctuated**
- **Universal**
- **Sence and nonsence codons**
- **The massage is read in direction 5′→3′**

Stages of translation:

- *Activation of amino acids*
- **Initiation**
- **Elongation**
- **Termination**
- *Posttranslation processing of proteins*

Activation of amino acids

Aa + tRNA + ATP → **Aminoacyl-tRNA + AMP+PP**



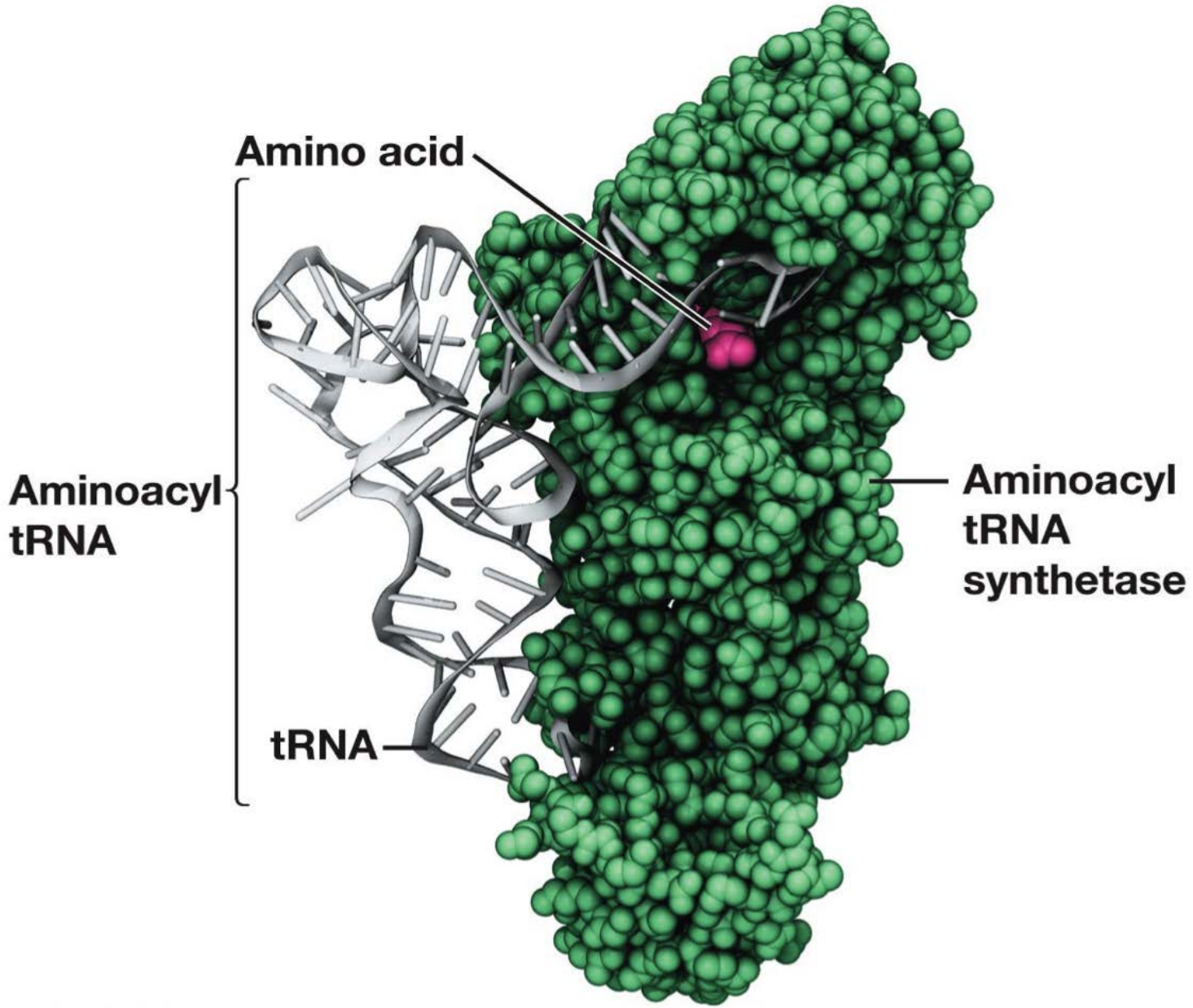
Process is catalised by aminoacyl-tRNA synthetases

The process proceeds in 2 steps:

1. An amino acid reacts with ATP, forming an AMP-amino acid-enzyme complex.

2. This activated amino acid is transferred to the corresponding tRNA

Aminoacyl-tRNA synthetase

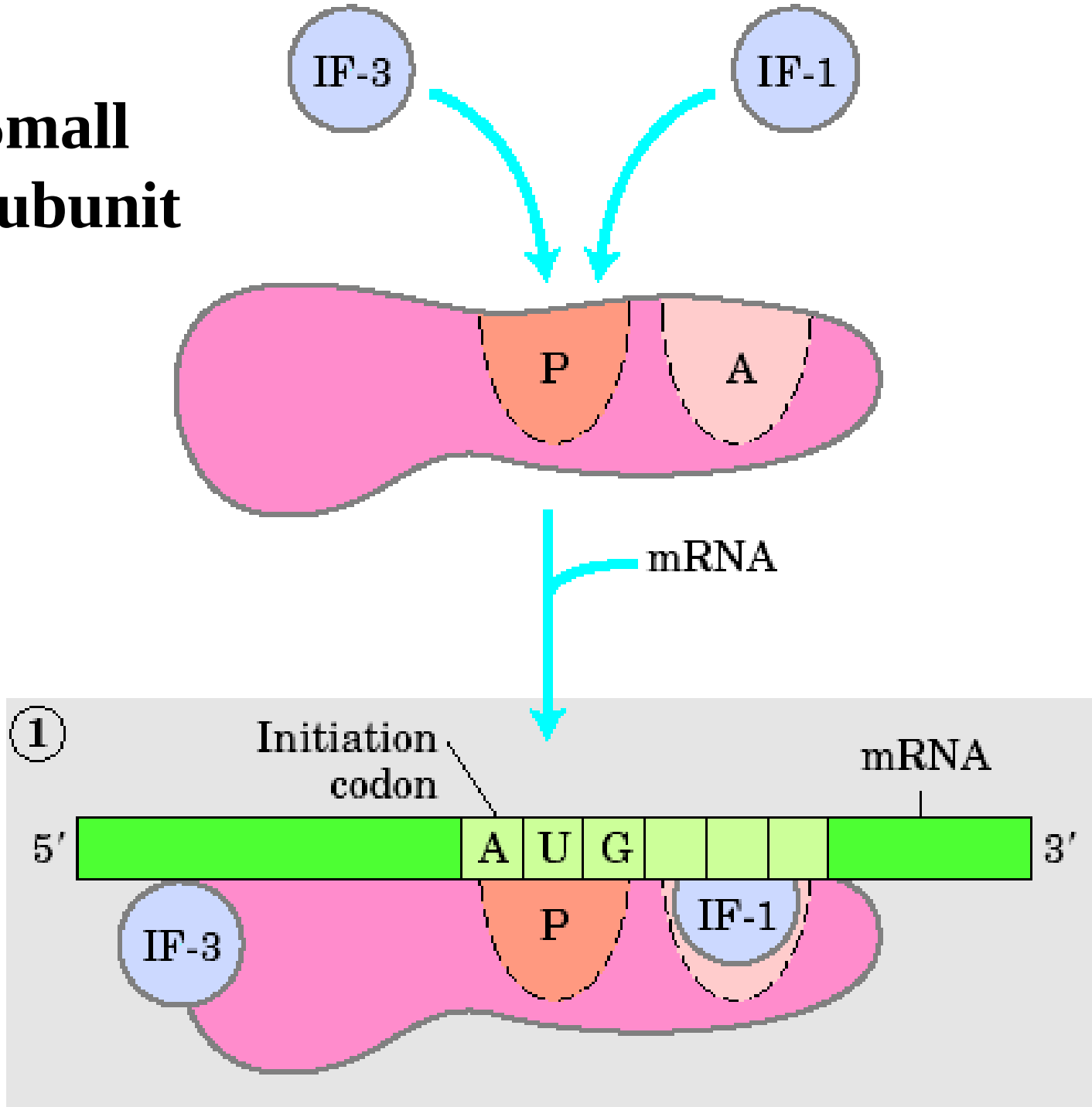


Protein synthesis

Harper's Illustrated Biochemistry

Initiation

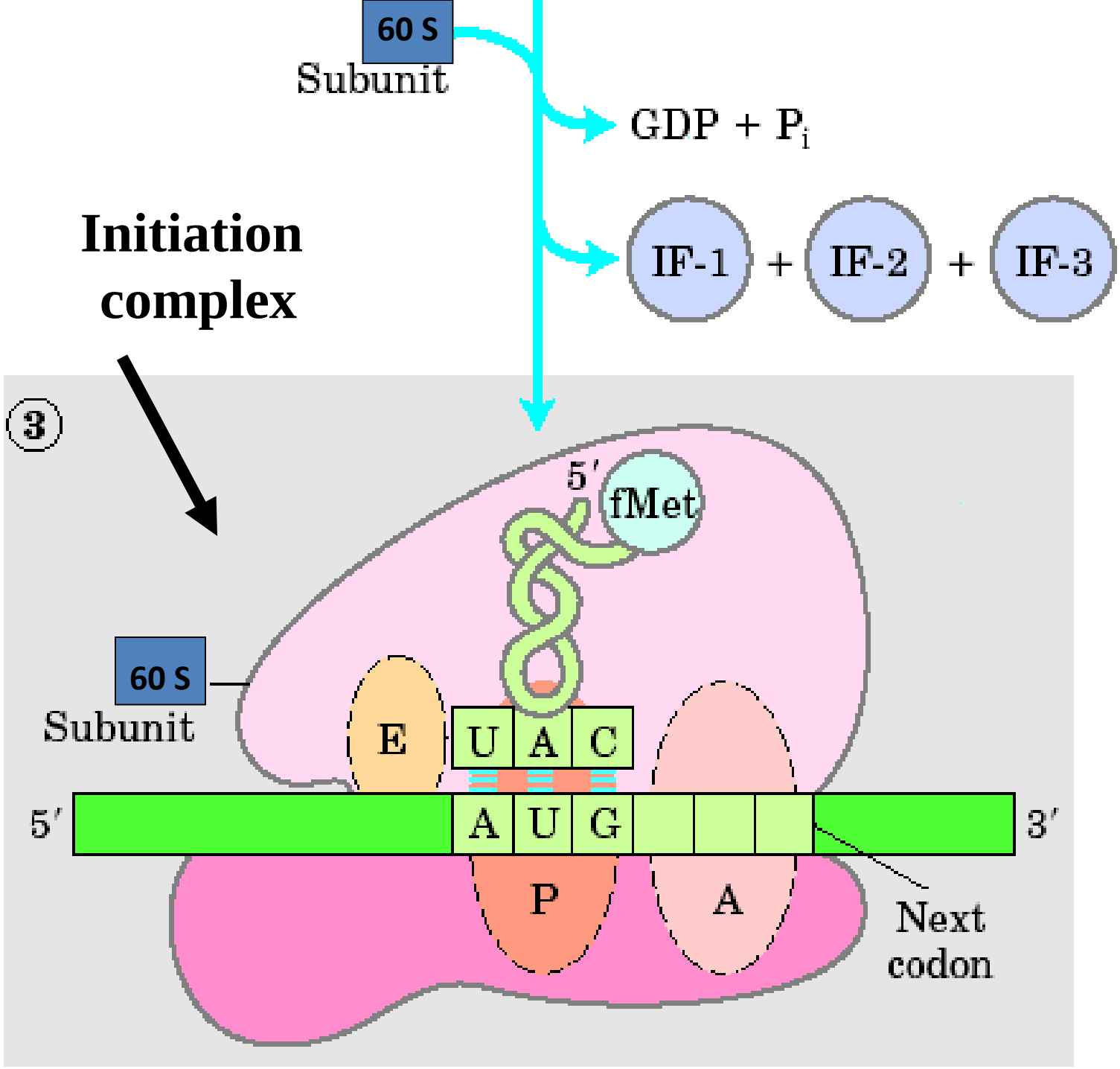
Small subunit



Initiation

2
5′
fMet
IF-2
GTP
U A C
(3′) UAC (5′)
Anticodon
5′
fMet
tRNA
IF-2
GTP
U A C
5′
A U G
3′
IF-3
P
IF-1

Initiation

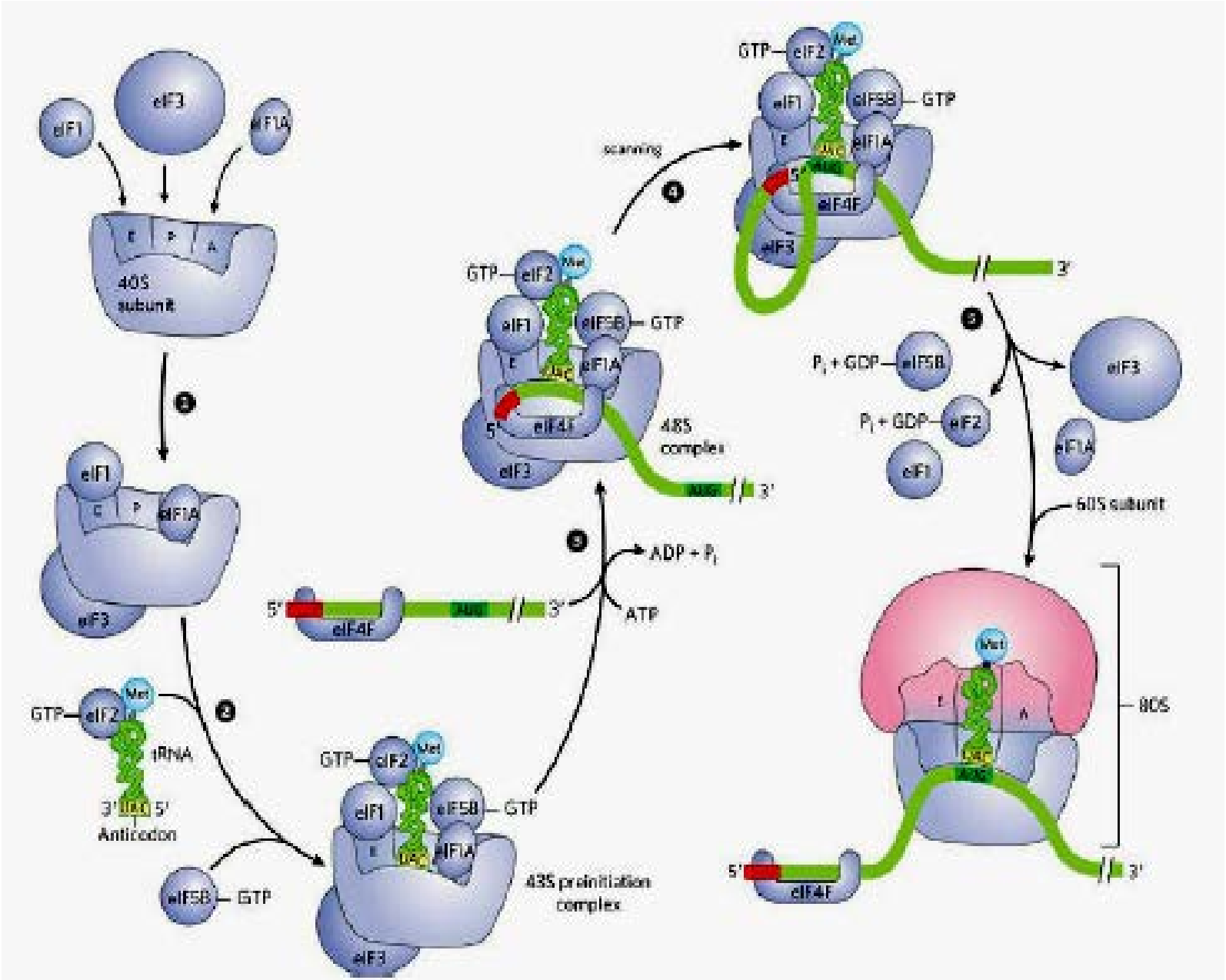

60 S
Subunit
GDP + P_i
IF-1 + IF-2 + IF-3
Initiation complex
3
5′
fMet
60 S
Subunit
E
U A C
5′
A U G
3′
P
A
Next codon

P – peptidyl site

A – aminoacyl site

E – exit site

Initiation in eukaryotes

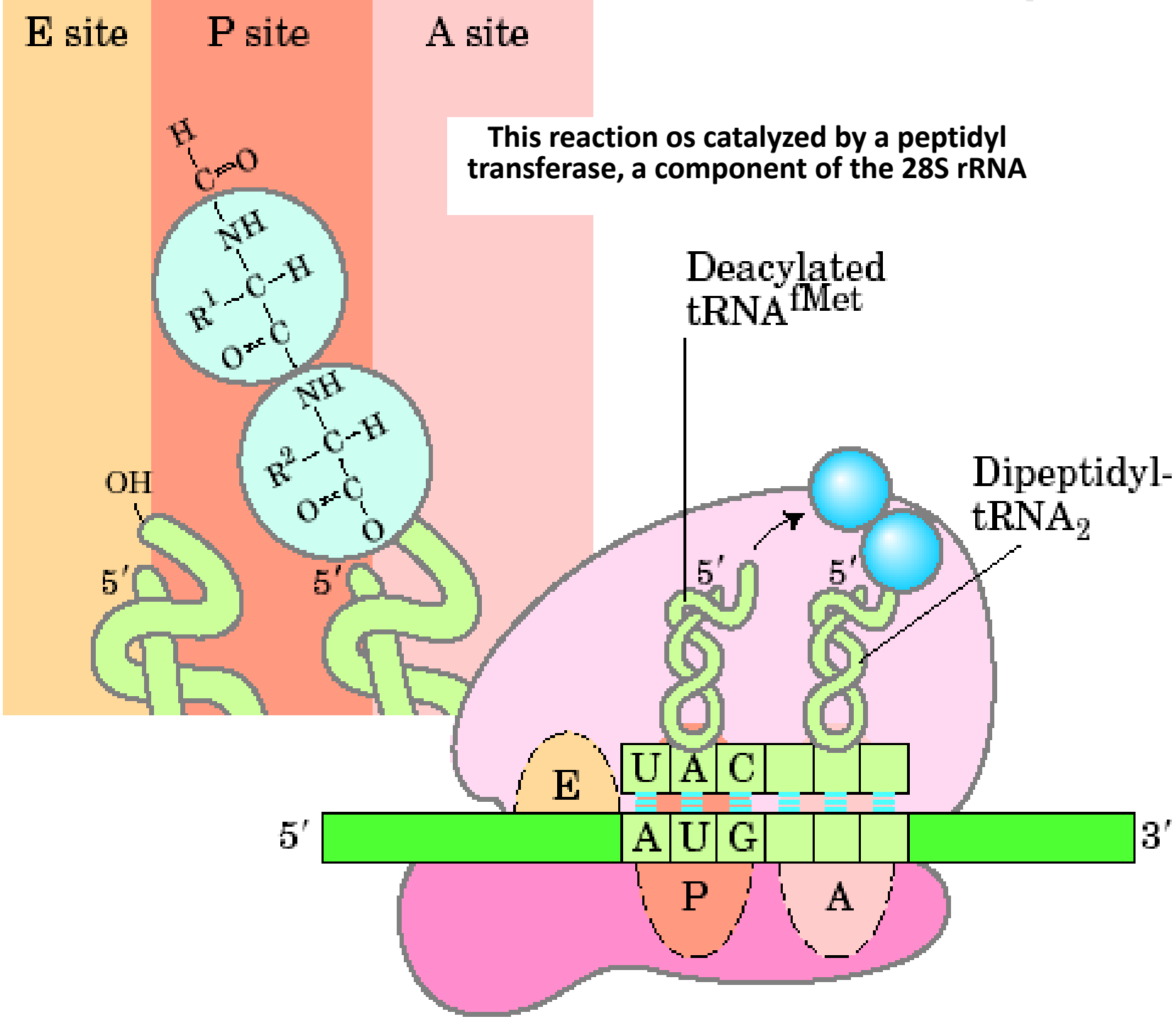


Elongation

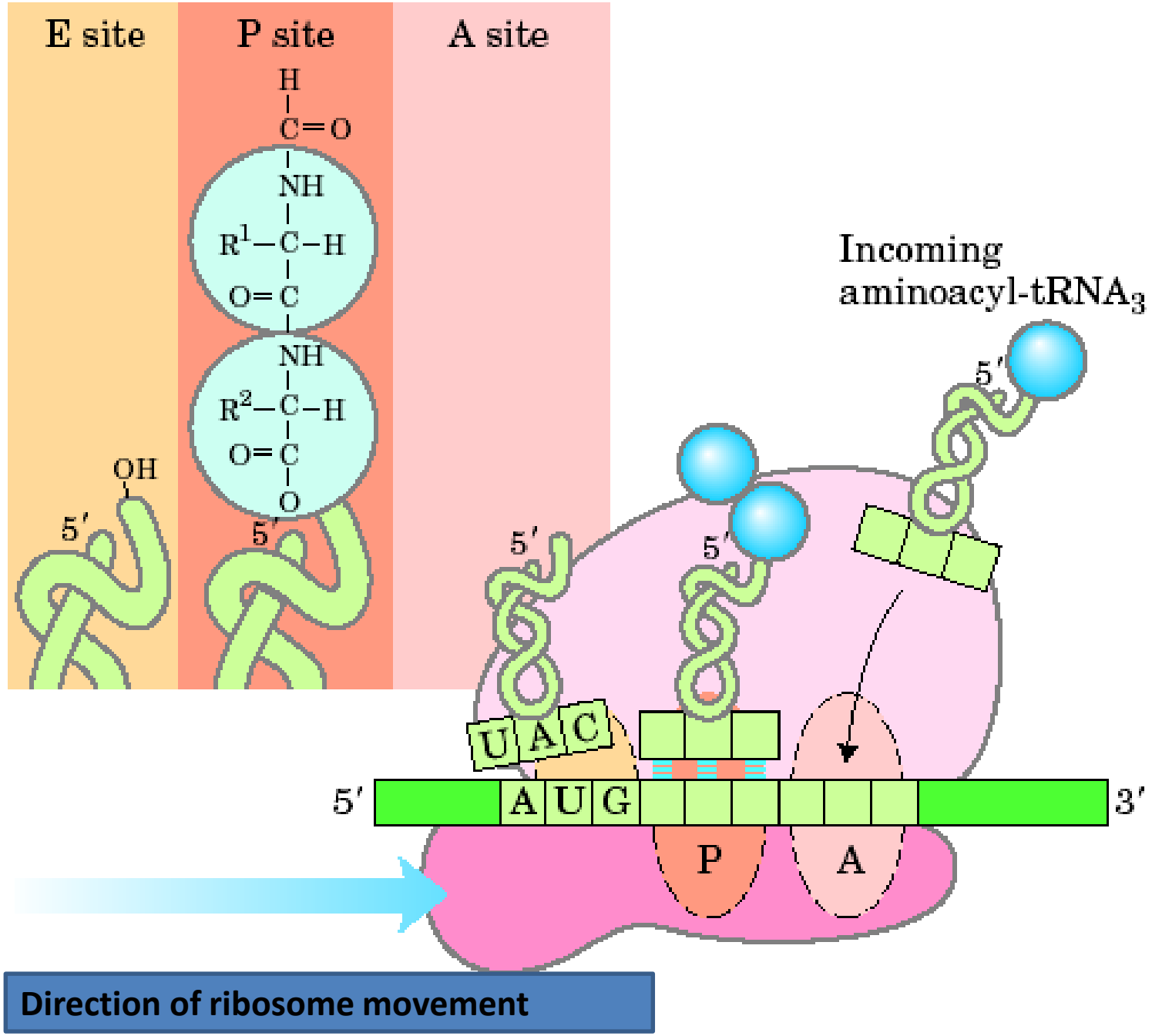
elongation

E site
P site
A site
fMet-tRNA fMet
Aminoacyl-
tRNA2
H
C=O
NH
R1–C–H
O=C
O
5′
NH2
R2–C–H
O=C
O
5′
U A C
A U G
mRNA 5′
3′
E
P
A

Elongation


E site
P site
A site
This reaction os catalyzed by a peptidyl transferase, a component of the 28S rRNA
Deacylated tRNA fMet
Dipeptidyl-tRNA2
OH
5′
5′
5′
5′
E
U A C
A U G
5′
3′
P
A

Elongation

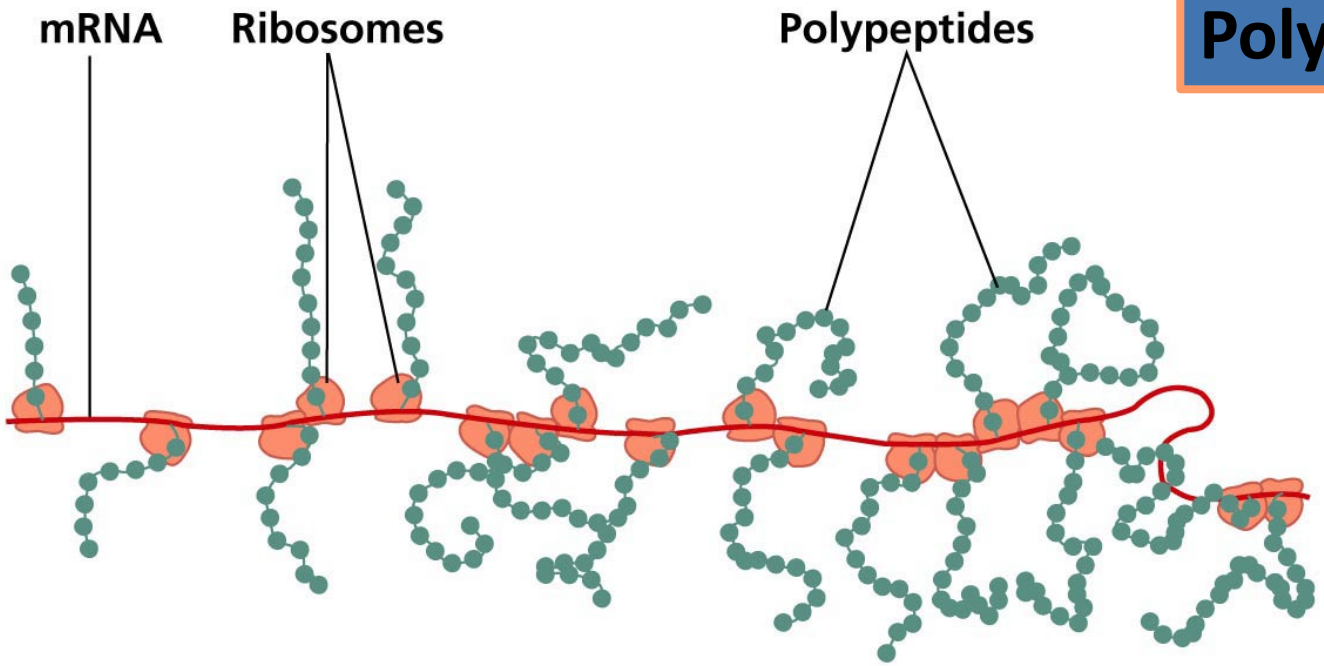

E site
P site
A site
H
C=O
NH
R1—C—H
O=C
NH
R2—C—H
O=C
O
OH
5′
5′
Incoming
aminoacyl-tRNA3
5′
5′
5′
U A C
5′
A U G
3′
P
A
Direction of ribosome movement

Termination

Termination

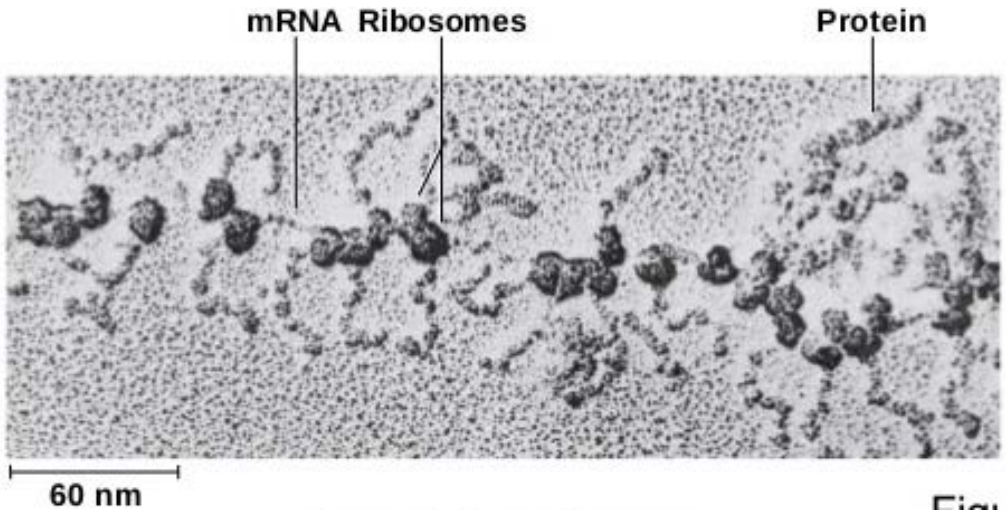
Termination

Polyribosomes



Direction of transcription

(a)



Posttranslation processing of proteins

(Posttranslational Modification)

- **Folding**
- **Removing of methionine from N-terminus**
- **Attachment of prosthetic groups**
- **Chemical modification of amino acids**
- **Limited proteolysis and activation of proteins**
- **The movement of proteins to their points of action**

Regulation of translation

- **Harper's Illustrated Biochemistry**
- **Manual on Biochemistry**

Protein Synthesis Is Inhibited by Many Antibiotics and Toxins

Protein synthesis is a central function in cellular physiology and is the primary target of many naturally occurring antibiotics and toxins.

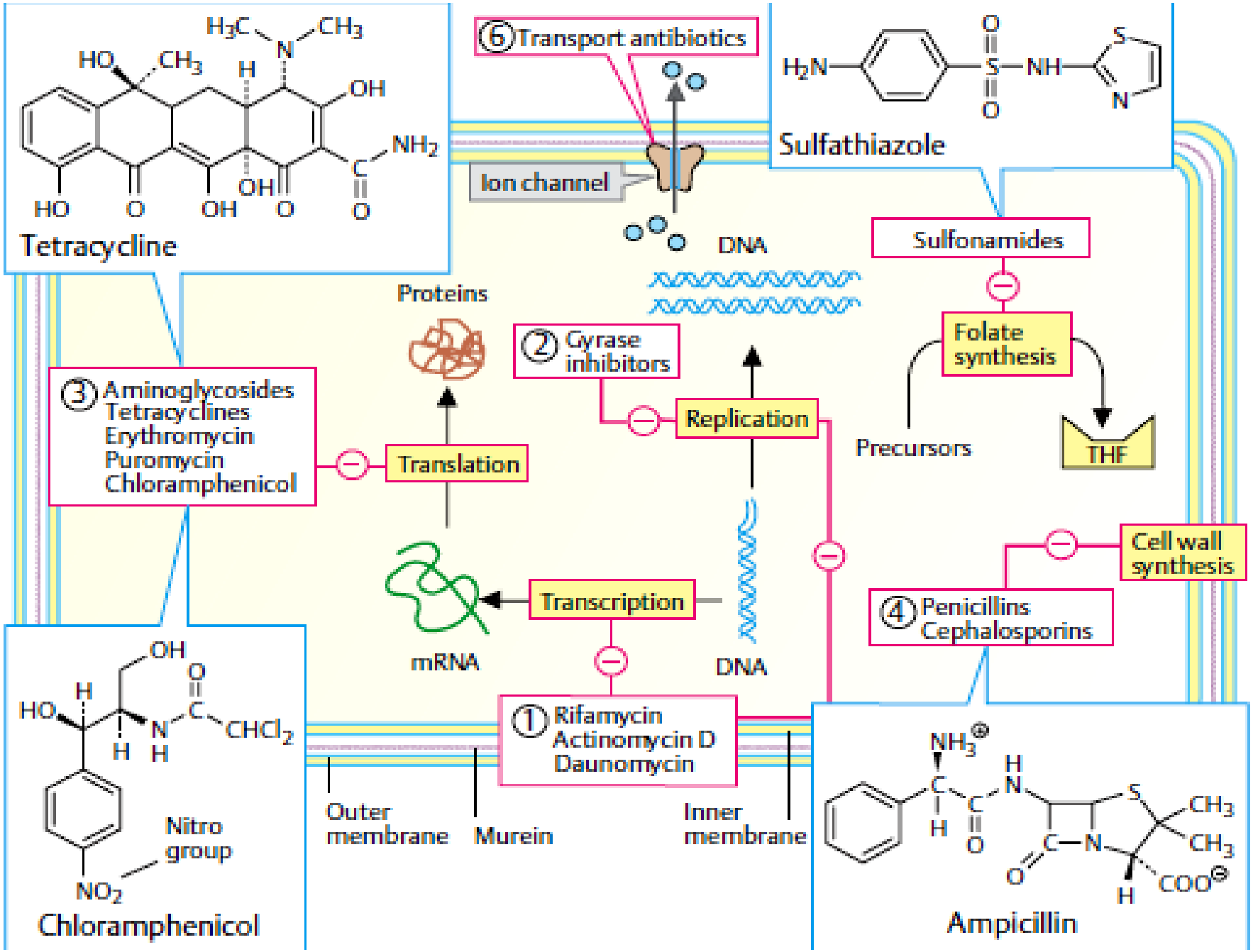
Antibiotics are substances which, even at low concentrations, inhibit the growth and reproduction of bacteria and fungi.

Substances that only restrict the reproduction of bacteria are described as having *bacteriostatic* effects (or *fungistatic* for fungi).

If the target cells are killed, then the term *bactericidal* (or *fungicidal*) is used.

The growth-inhibiting substances are known as *cytostatic* drugs.

Almost all antibiotics are produced by microorganisms and certain fungi. However, there are also synthetic antibacterial substances, such as sulfonamides and gyrase inhibitors.


Tetracycline
⑥ Transport antibiotics
Ion channel
DNA
Sulfathiazole
Sulfonamides
Folate synthesis
Precursors
THF
Proteins
② Gyrase inhibitors
Replication
③ Aminoglycosides
Tetracyclines
Erythromycin
Puromycin
Chloramphenicol
Translation
Transcription
mRNA
DNA
Cell wall synthesis
④ Penicillins
Cephalosporins
① Rifamycin
Actinomycin D
Daunomycin
Outer membrane
Murein
Inner membrane
Nitro group
Chloramphenicol
Ampicillin

Synthetic inhibitors of DNA topoisomerase II, known as *gyrase inhibitors*, restrict replication and thus bacterial reproduction.

A large group of antibiotics attack bacterial ribosomes. These inhibitors of translation include the *tetracyclines*.

Tetracyclines inhibit protein synthesis in bacteria by blocking the A site on the ribosome, preventing the binding of aminoacyl-tRNAs.

The *aminoglycosides*, of which *streptomycin* is the best-known. It binds to the small subunit of the bacterial ribosome irreversibly, interfering with the binding of formyl-methionyl-tRNA to the subunit. This leads to codon misreading, eventual inhibition of protein synthesis and ultimately death of microbial cells.

Erythromycin **impairs the normal functioning of the large ribosomal subunit.**

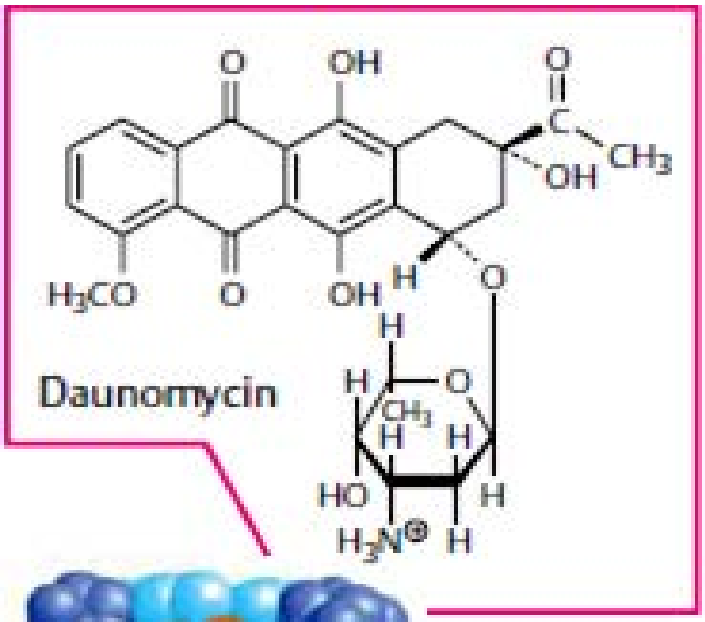
***Chloramphenicol*, one of the few natural nitro compounds, inhibits ribosomal peptidyltransferase in bacteria, it does not affect cytosolic protein synthesis in eukaryotes.**

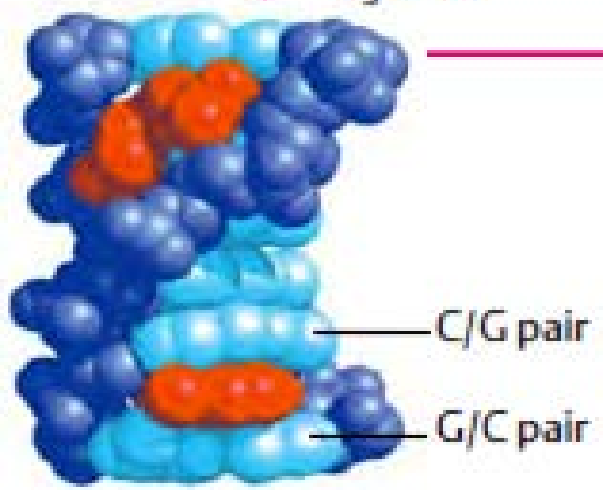
***Puromycin* mimics an aminoacyl tRNA, binds to the ribosomal A site and therefore leads to premature interruption of elongation.**

Substances known as intercalators, such as *rifamycin* and *actinomycin D* are deposited in the DNA double helix and thereby interfere with replication and transcription.

As DNA is the same in all cells, intercalating antibiotics are also toxic for eukaryotes.

They are therefore only used as cytostatic agents.





Cycloheximide

blocks the peptidyl transferase of 80S eukaryotic ribosomes but not that of 70S bacterial ribosomes.

Several other inhibitors of protein synthesis are notable because of their toxicity to humans and other mammals.

Diphtheria toxin

catalyzes the ADP-ribosylation of a diphthamide (a modified histidine) residue of eukaryotic elongation factor eEF2, thereby inactivating it.

Ricin

an extremely toxic protein of the castor bean, inactivates the 60S subunit of eukaryotic ribosomes