

Genetic information

Part 2

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ภาควิชาชีวเคมี

คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

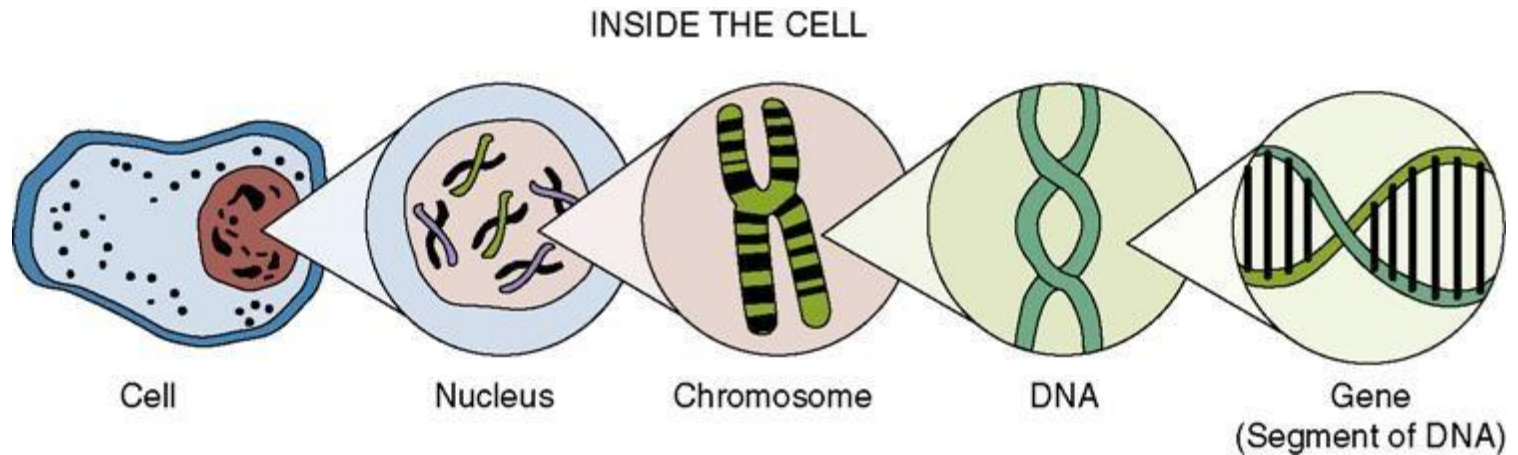
Outline

- DNA replication
- RNA synthesis
 - Initiation of RNA synthesis
 - Termination of RNA synthesis
 - Eukaryote RNA Processing
- Protein synthesis
 - Initiation
 - Elongation
 - Termination
 - Post-translational Modification

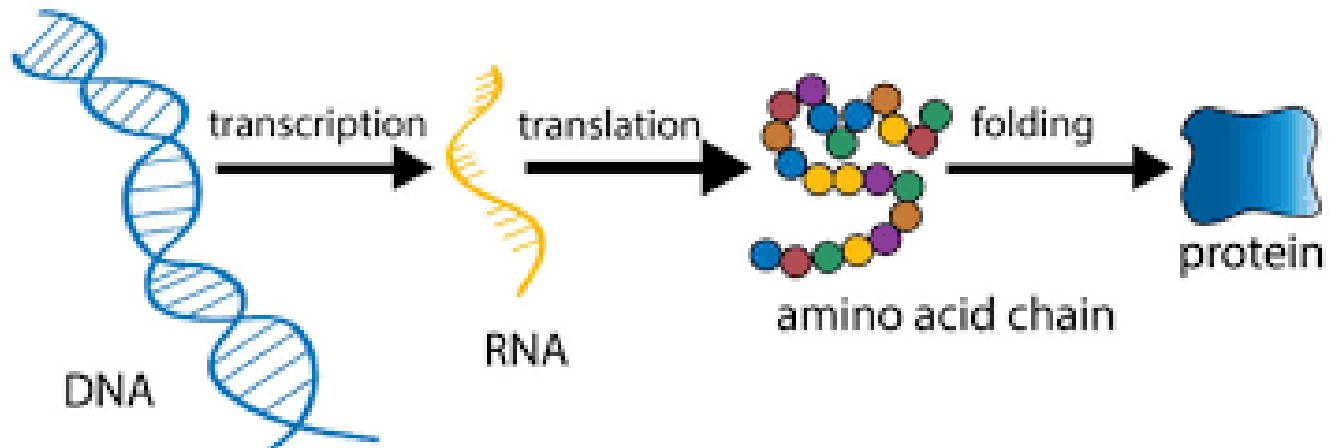
RNA synthesis

(Transcription)

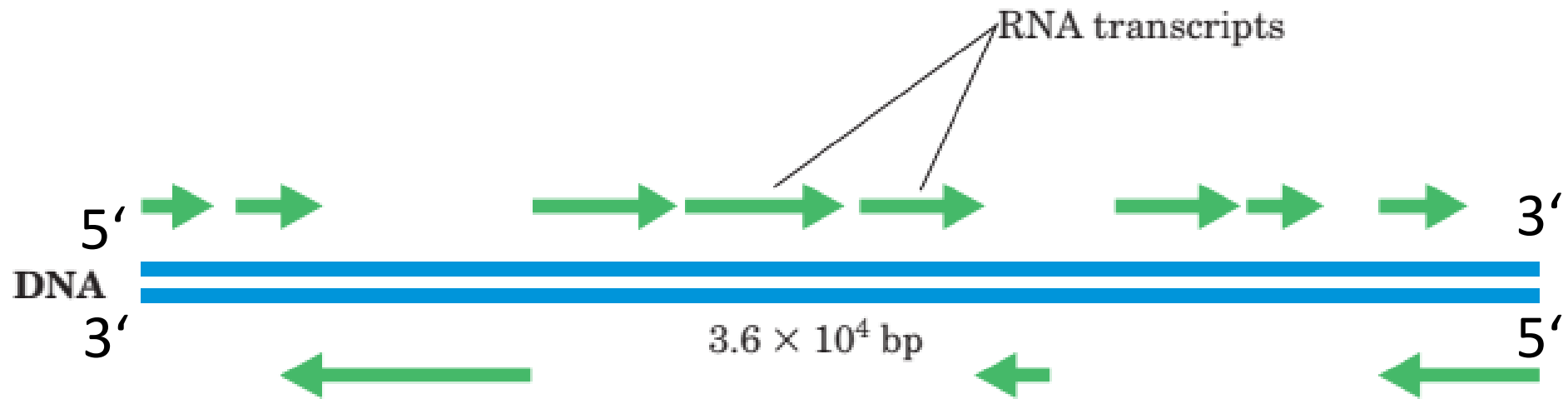
Gene expression



RNA : storage, transfer, catalysis



Organization of coding information



Ingredients of RNA synthesis

- DNA template
- ribonucleoside triphosphate (NTP) (ATP, GTP, CTP, UTP)
- DNA dependent RNA polymerase
- Mg^{2+} , Mn^{2+}
- No primer



Transcription (RNA synthesis)

- Initiated at **promoter** sites on the DNA template
- Consensus / conserved sequence

Transcription is initiated at promoter sites on the DNA template



Consensus
sequence



rrnB P1



trp



lac



recA



araBAD

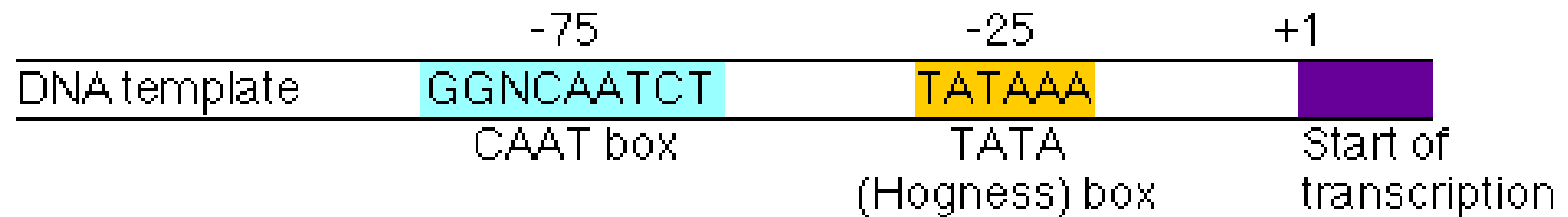


PRIBNOW BOX

Prokaryotic promoter site:

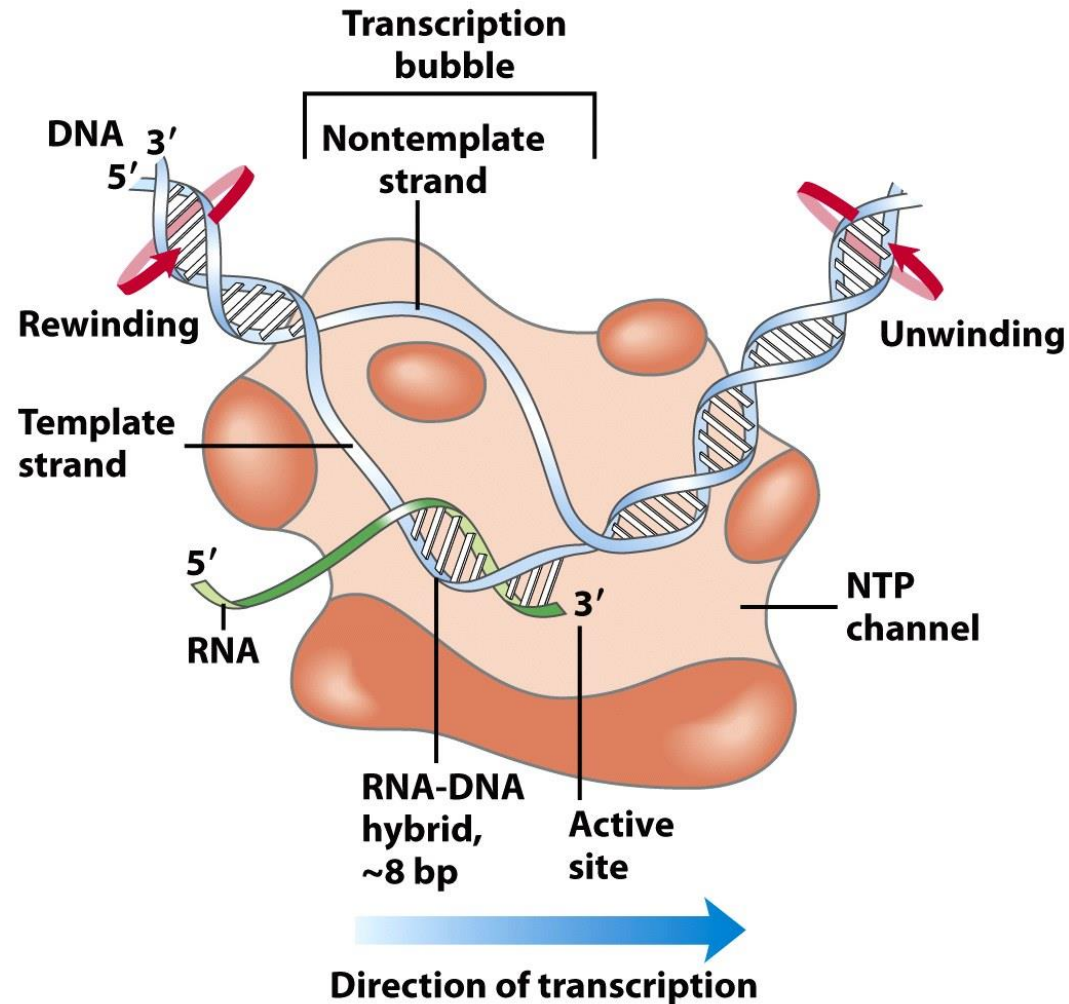


Eukaryotic promoter site:



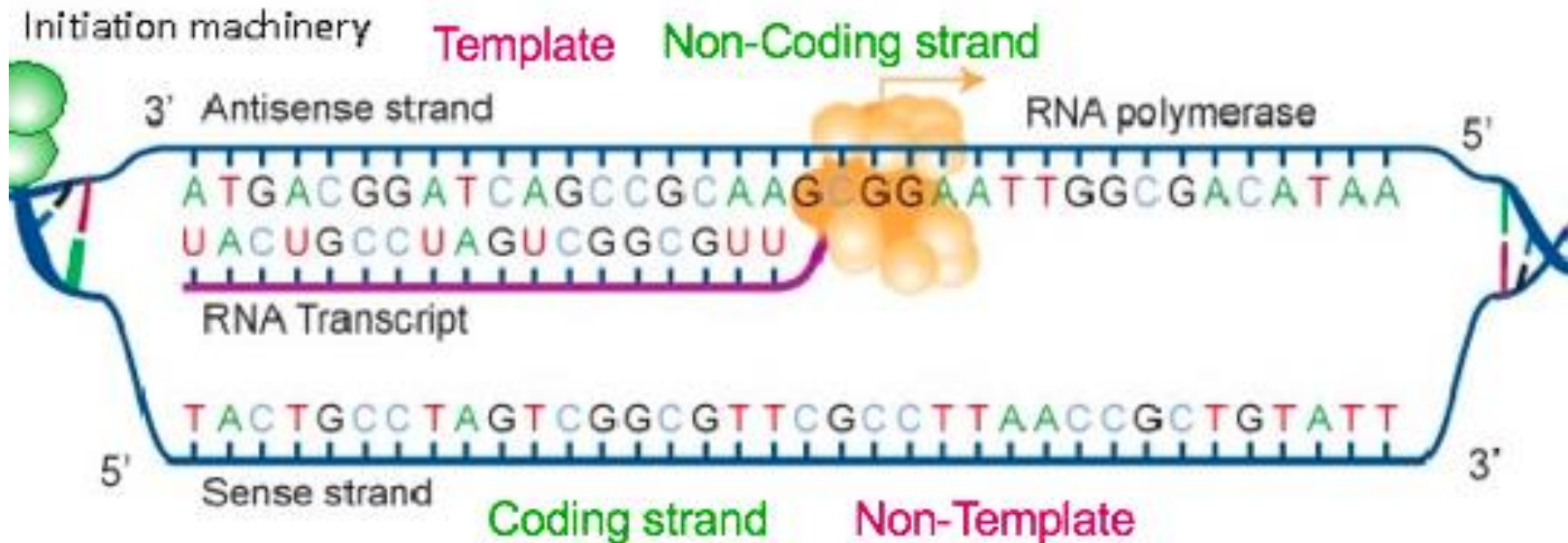
DNA-dependent RNA polymerase (RNA polymerase)

- No proofreading
- Don't need primer
- 5 subunits (α , β , β' , ω , σ)



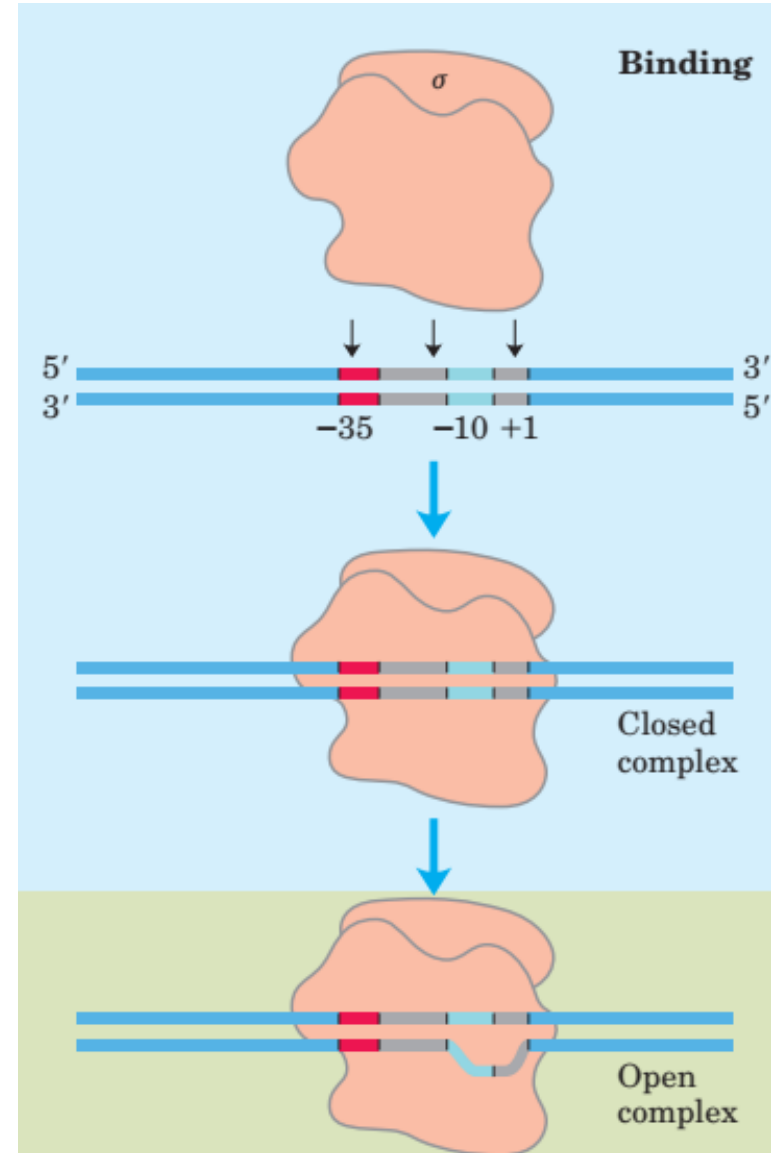
Initiation of RNA synthesis

- The sequence of RNA is complementary with DNA template, anti-sense, non-coding
- The sequence of RNA is similar to non-template, sense or coding strand



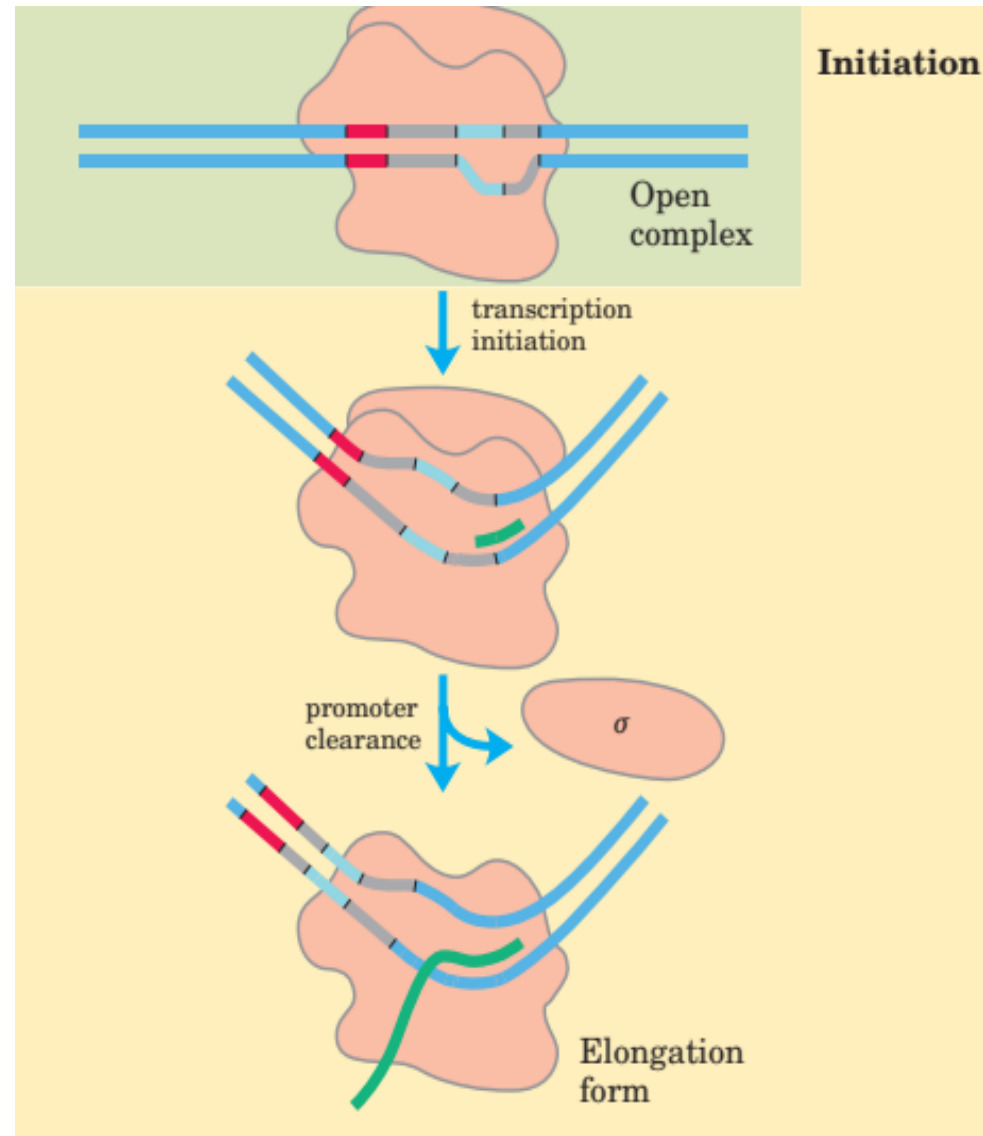
Initiation of RNA synthesis

- Promoter DNA is stably bound
- Require σ factor
- -10 region to position +2 or +3—is then unwound to form an open complex
- Rifampicin inhibit σ factor



Initiation of RNA synthesis

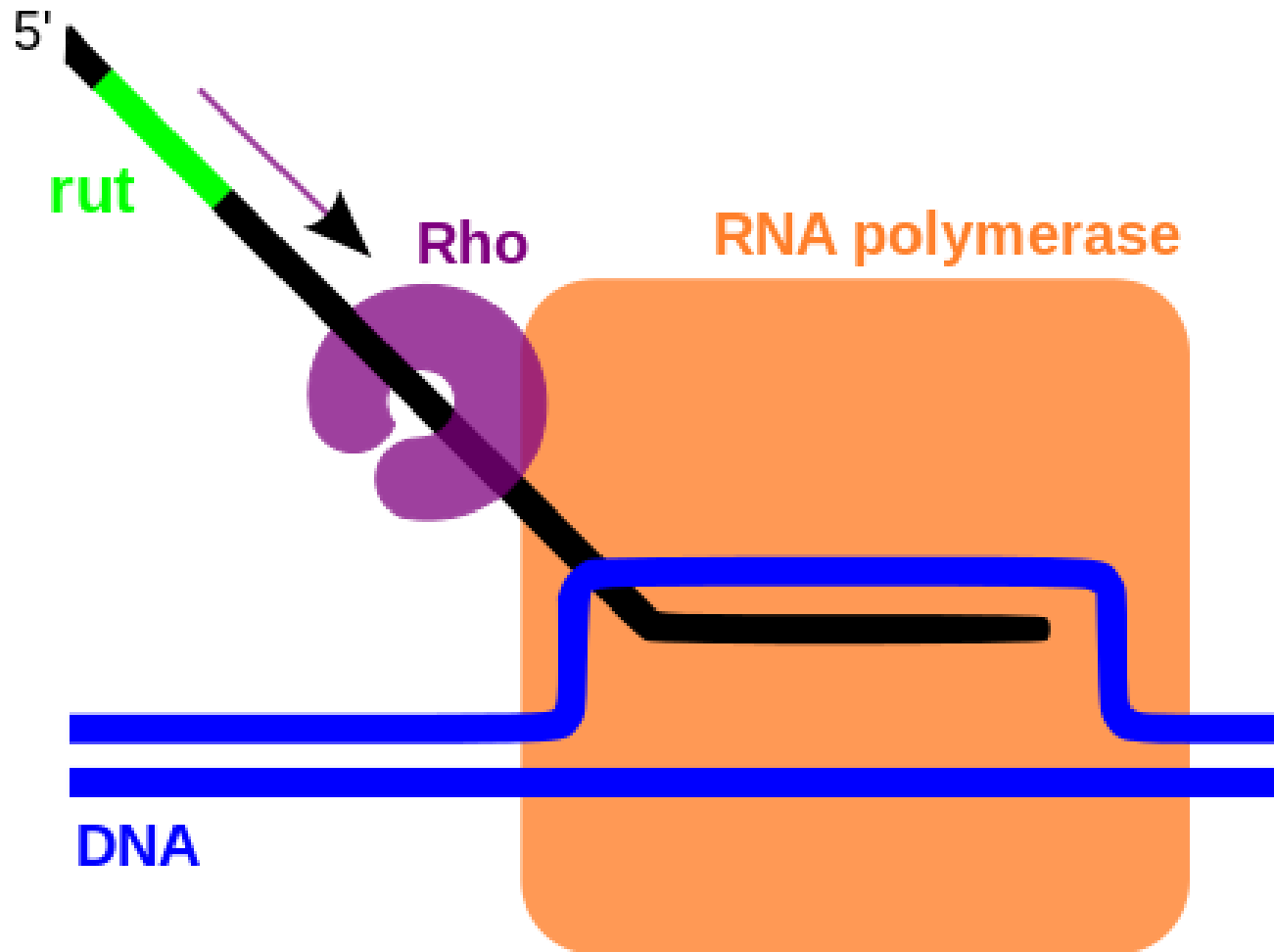
- Sigma subunit (factor) of RNA polymerase binds to promoter located upstream from +1 of structural genes
- Once the first 7 or 10 nucleotides of a new RNA are synthesized, the σ subunit is released
- The polymerase leaves the promoter and becomes committed to elongation of the RNA
- Actinomycin D binds DNA and preventing elongation of RNA chain



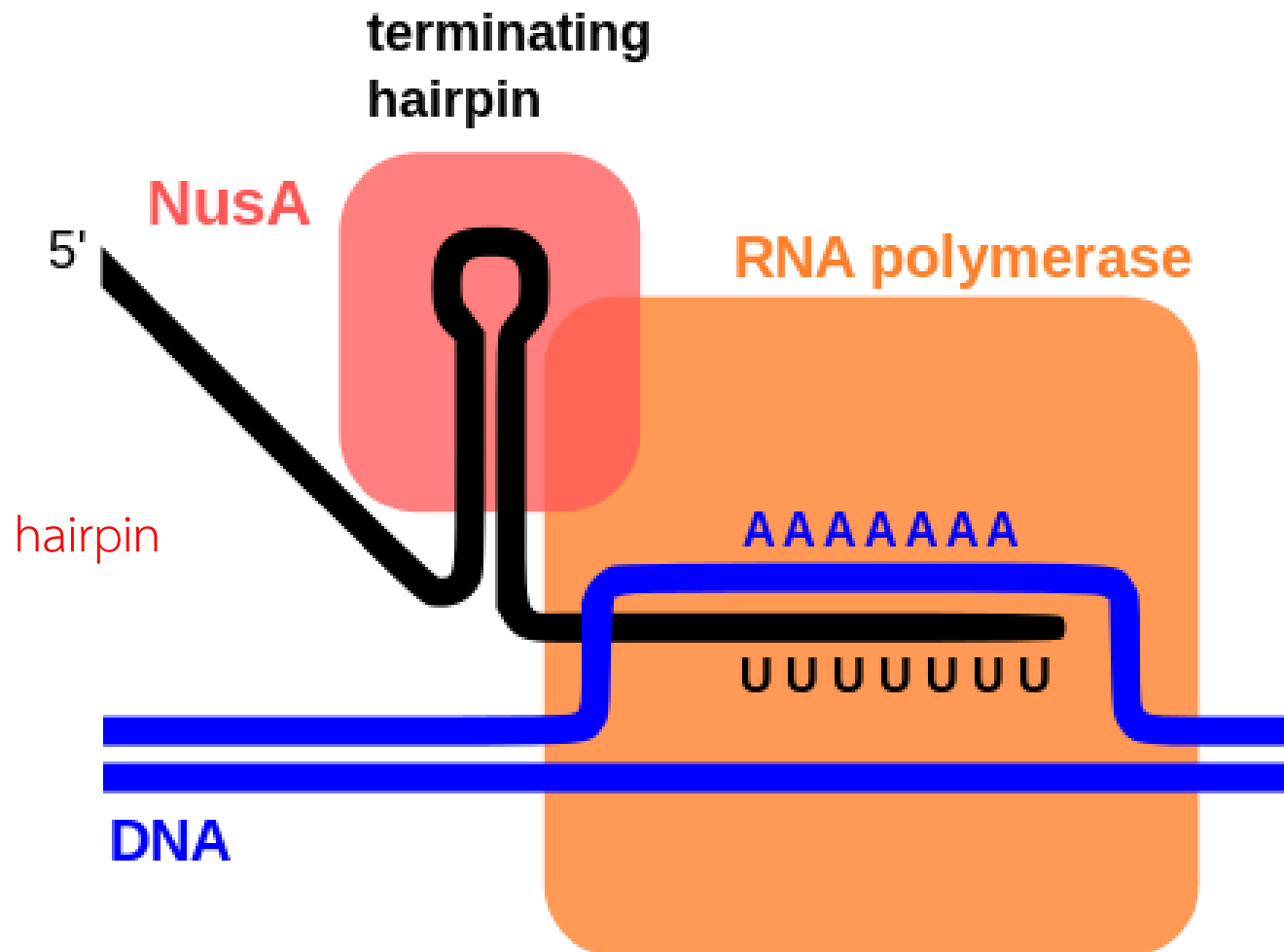
Termination of RNA synthesis

- Termination of RNA synthesis occurred by 2 ways,
- ρ dependent : Rho protein binds act as helicase to push away the RNA polymerase
- rho independent : hairpin loop form at the end and push the enzyme away

ρ (Rho) dependent



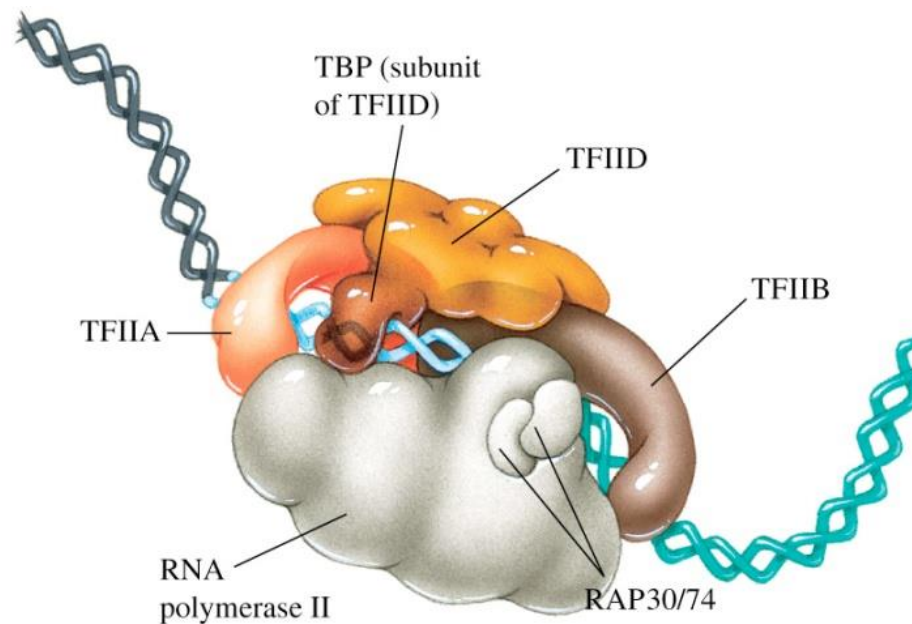
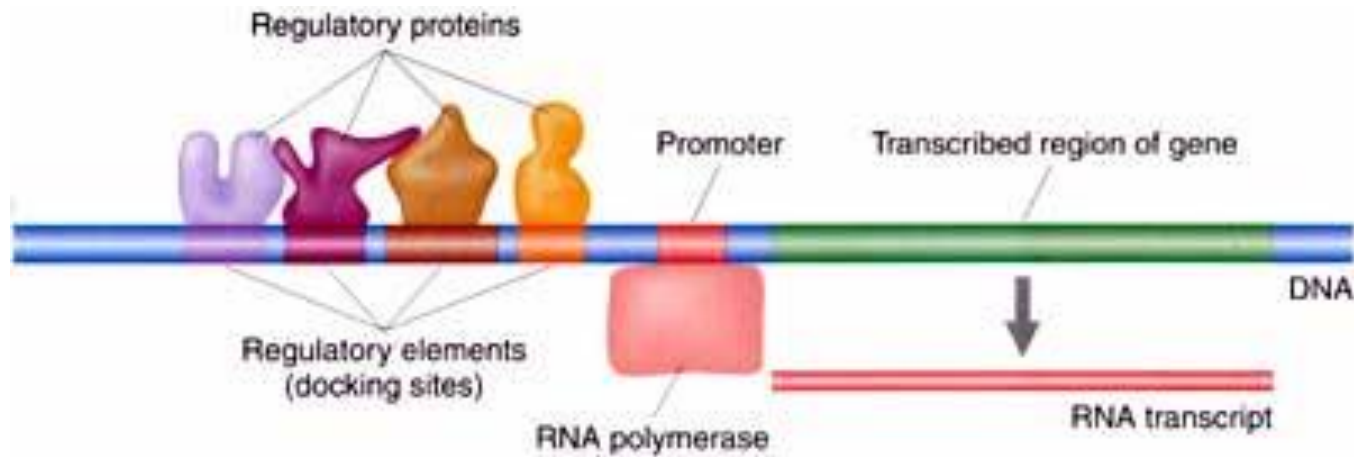
ρ (Rho) independent



Eukaryote RNA Polymerase

- Eukaryote has more than 1 RNA Polymerase and regulation is more complex, requires binding of several regulatory proteins
- Pol I : synthesize pre-ribosomal RNA 18S, 5.8S 28S rRNAs
- Pol II : synthesize mRNAs, 12 sub-unit
- Pol III : synthesize tRNAs, 5S rRNA and small specialized RNA

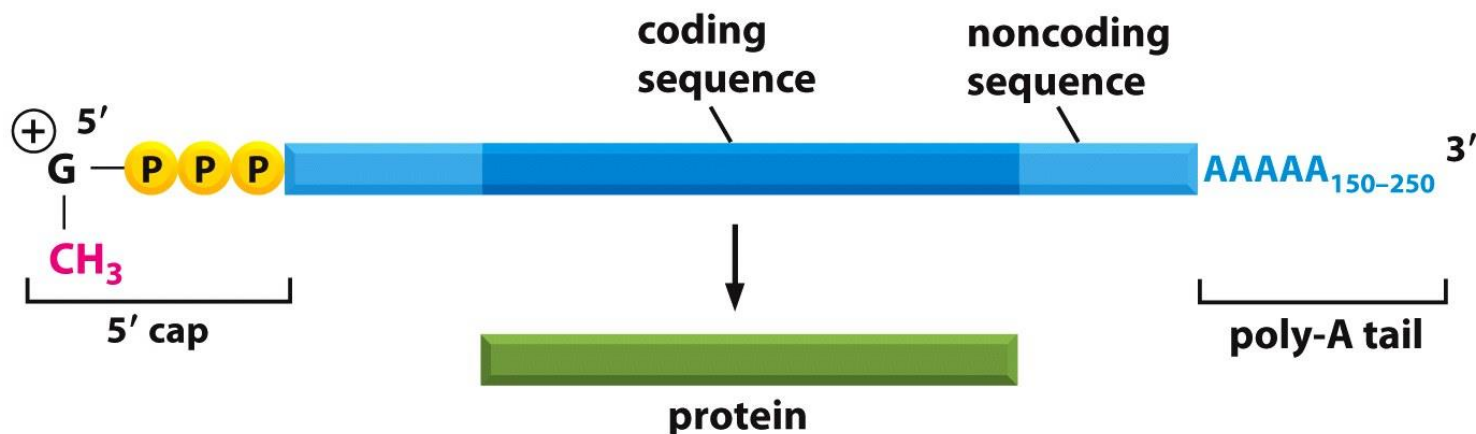
Eukaryote RNA Polymerase



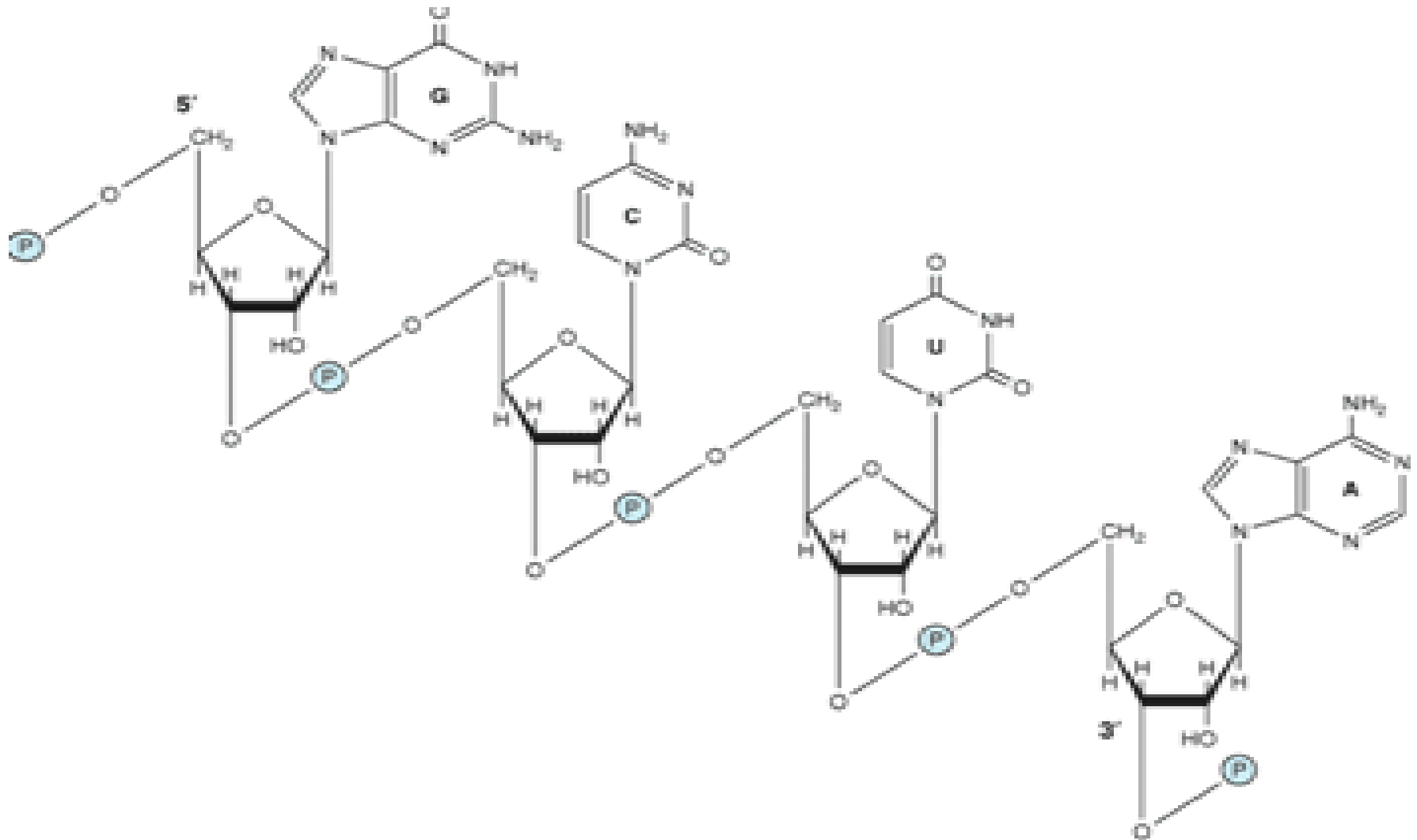
Eukaryote RNA Processing : step1

- Methylation at 5' end and 7-methylguanosine make capped RNA binds to a protein
- Poly A binds to another protein and linked like ring also by another protein to protect mRNA from degradation

RNA capping and polyadenylation

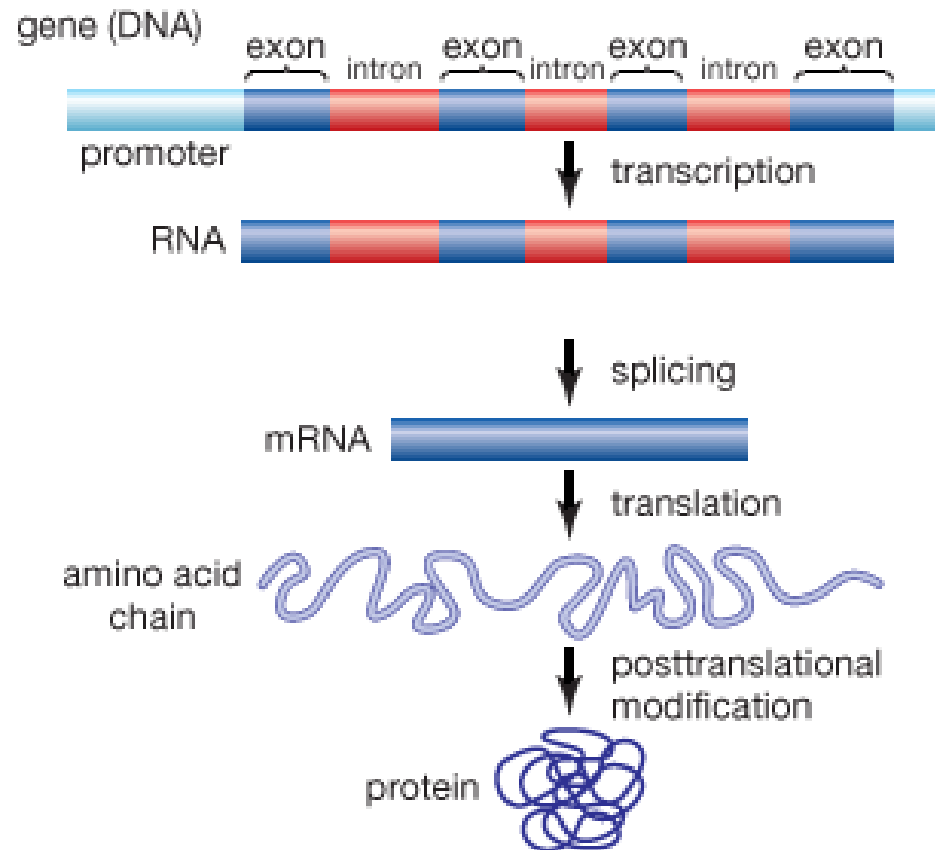


Eukaryote RNA Processing : step1



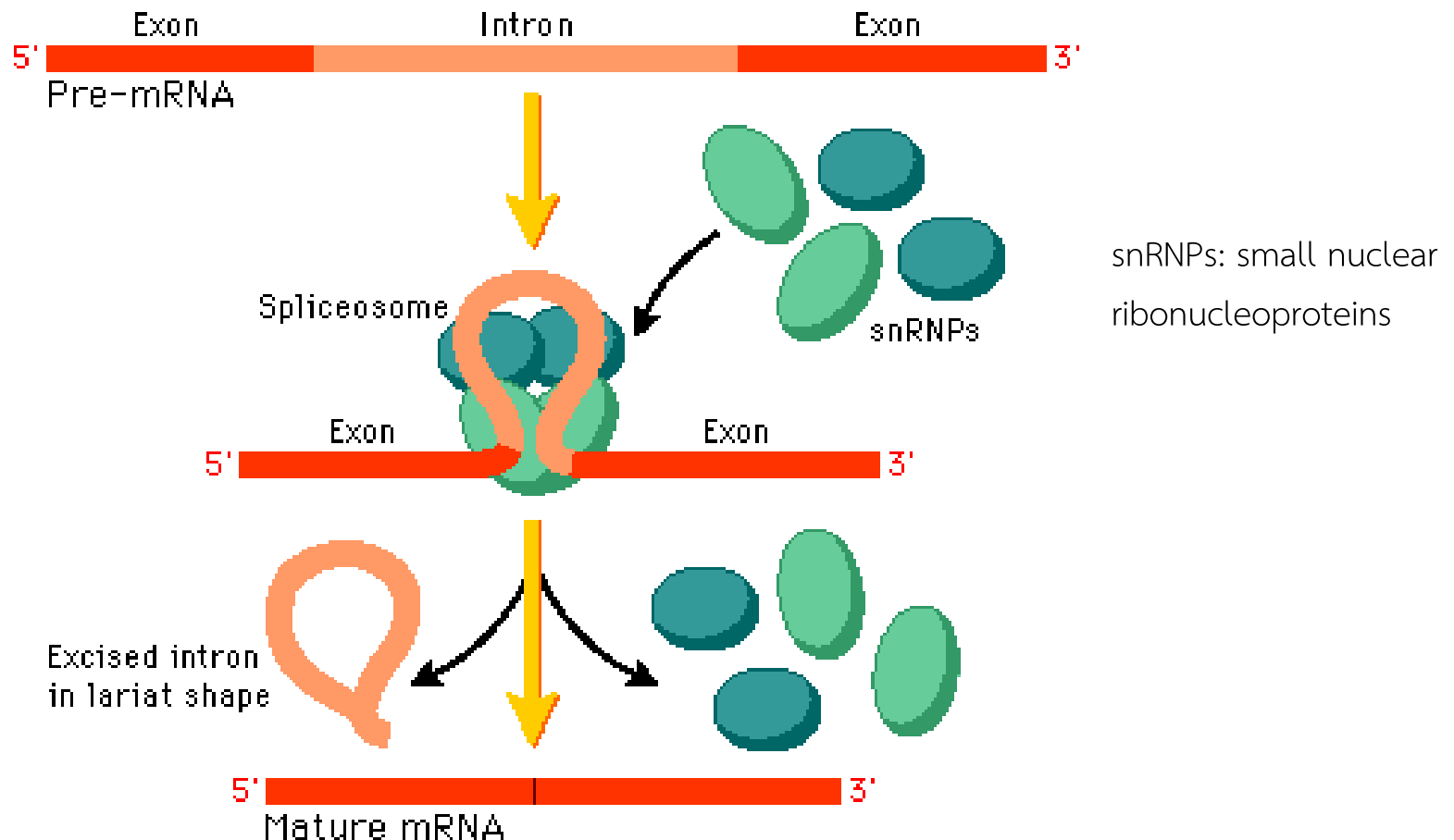
Eukaryote RNA Processing : step2

- Splicing: the non-coding sequences (introns) were removed and left coding sequences (exons) to join as matured mRNA
- This matured-mRNA will get into cytoplasm for translation into a protein



Eukaryote RNA Processing : step2

- Splicing : by enzymes or **non-enzymes**



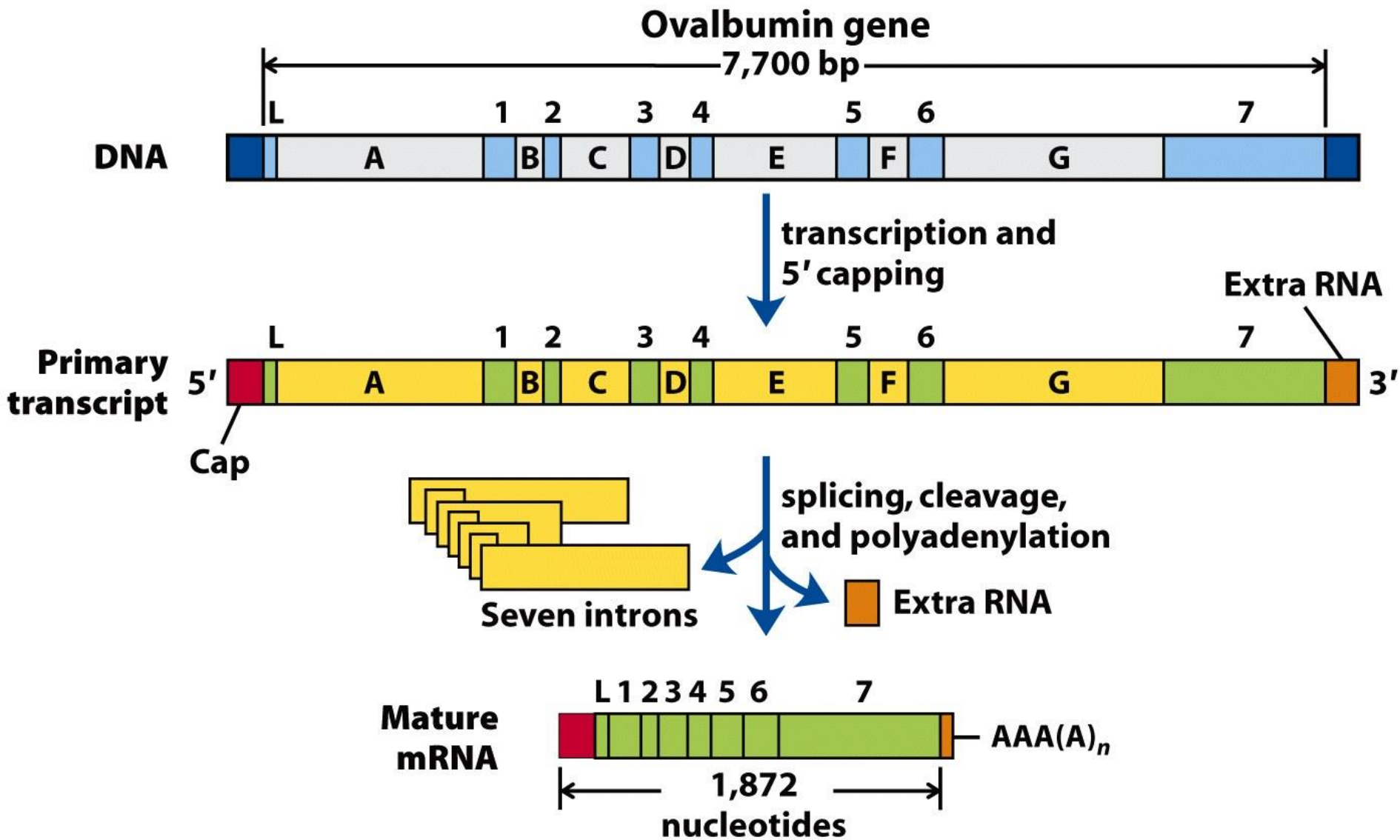
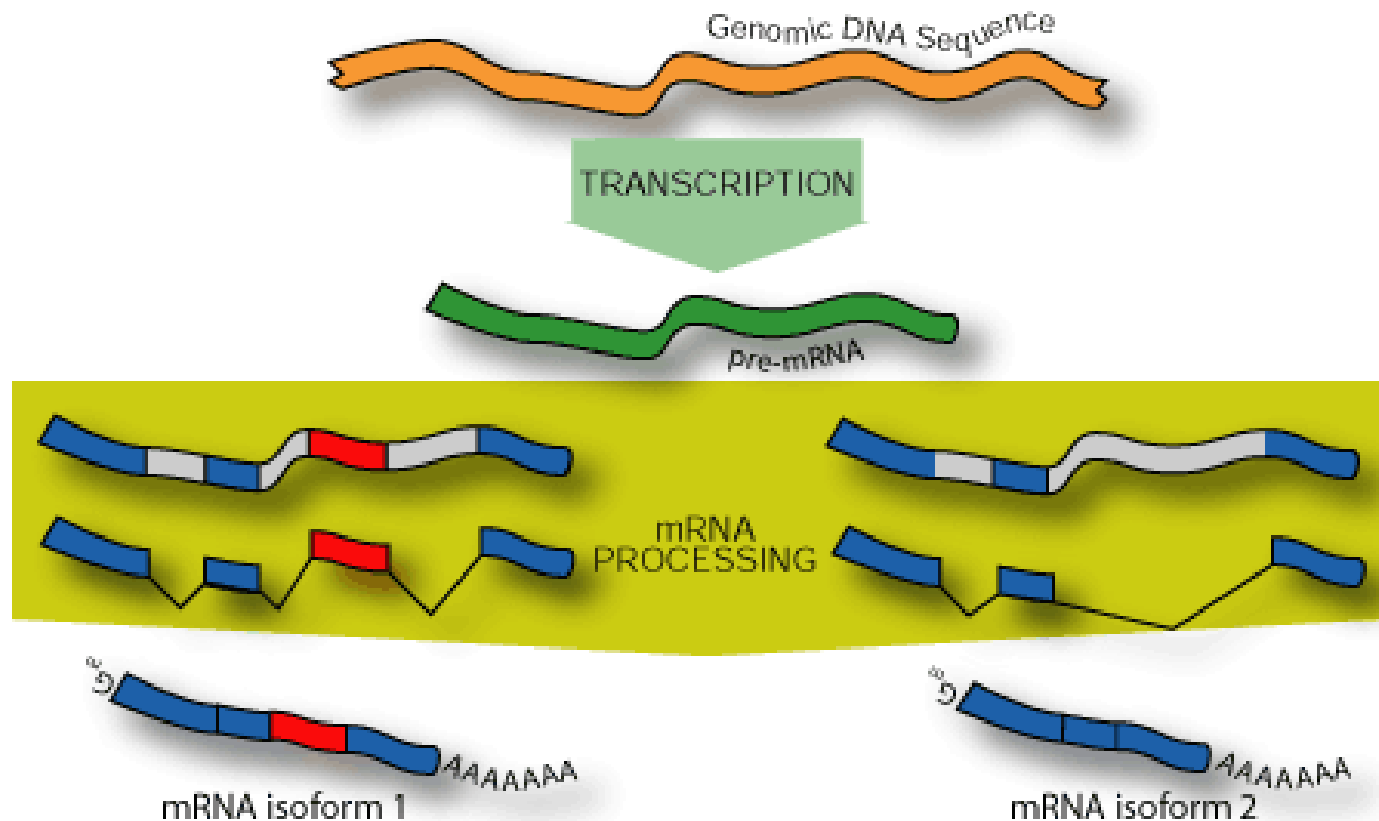


Figure 26-19
Lehninger Principles of Biochemistry, Fifth Edition
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Alternative splicing

- Eukaryote mRNA can be transcribed into more than 1 type of matured-RNA → Isoforms



Template and nontemplate (coding) DNA strands

(5') CGCTATAGCGTTT(3')

DNA nontemplate (coding) strand

(3') GCGATATCGCAAA(5')

DNA template strand

(5') CGCUAUAGCGUUU(3')

RNA transcript

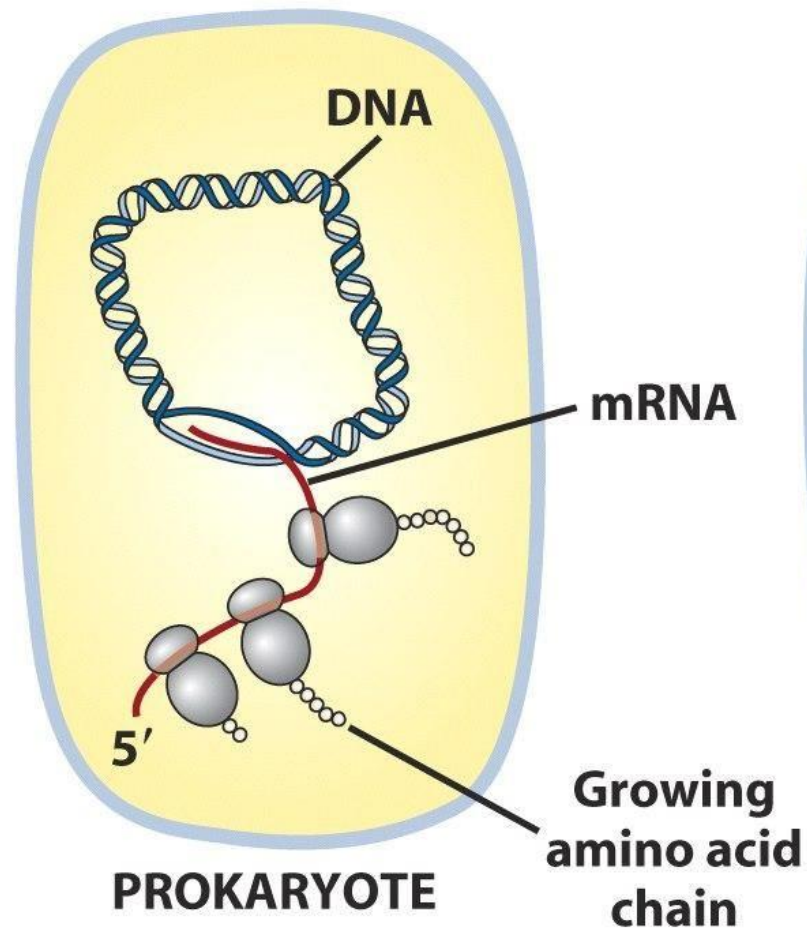
Summary

- A gene composed of control region (promoter) and a structural gene (coding sequence complementary with the DNA template).
- mRNA is synthesized by binding of RNA polymerase to the promoter and produce RNA with sequence complementary to template and similar (except U) to sense strand.
- Processing steps (Capping and tailing + splicing) are required for eukaryote for fully function of RNA (matured mRNA).
- Error in splicing steps is common to cause diseases.

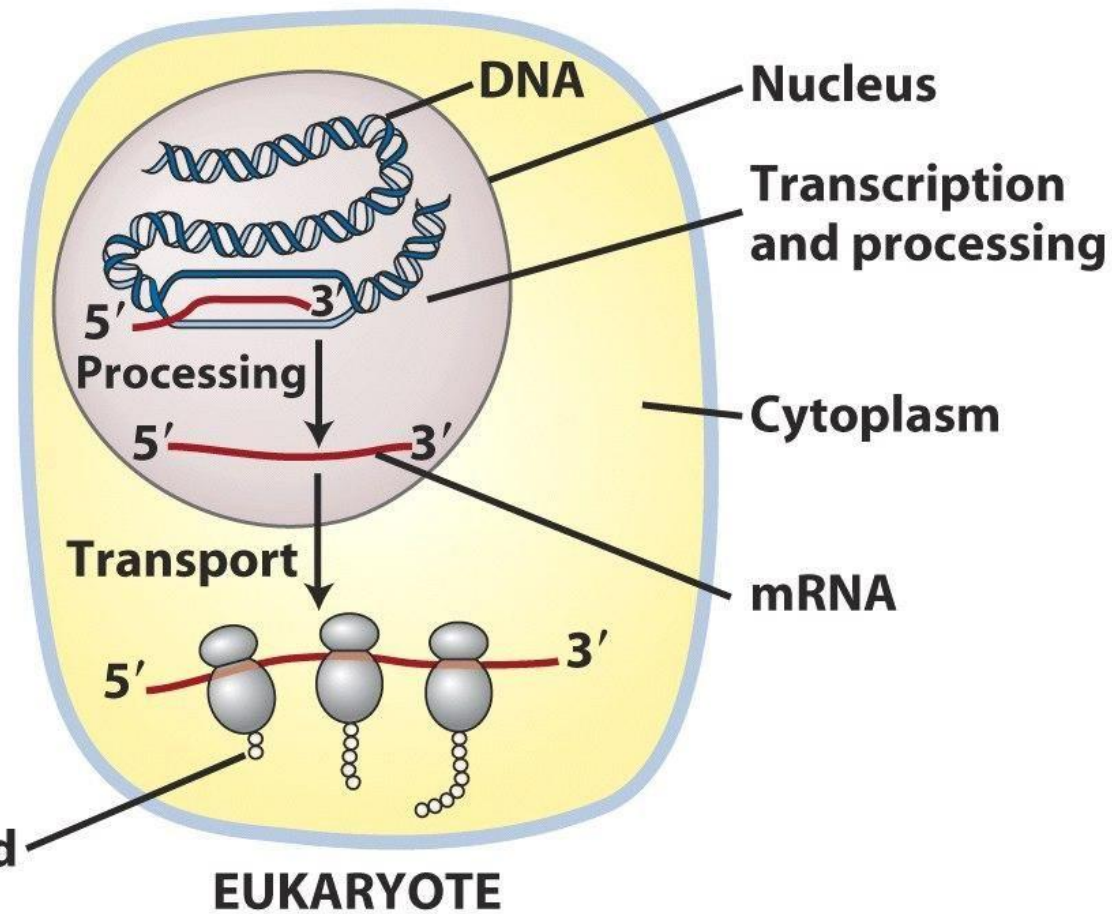
Protein synthesis

Translation and
Posttranslational Modifications

(a)



(b)



Complexity of protein

- Proteins is the end of the information pathway
- 20 amino acids
- NH_2 -terminal \rightarrow COOH -terminal
- Different levels of protein in different cells, organ, cell stages etc. can be different
- Proteins come in different isoforms, are churned through metabolic and degradative pathways, are alternatively spliced, and often link with one another to form complexes made up of multiple proteins

Ingredients of protein synthesis

- L-amino acid
- t-RNA
- Genetic code
- Aminoacyl-tRNA synthetase
- ribosome

The Genetic Code

- How 4 letters genetic alphabet (A, T, C, G) combined to get 20 amino acids
- A two nucleotide code : $4 \times 4 = 16$ possible combination is not enough
- Sets of three nucleotides : would be sufficient ($4 \times 4 \times 4 = 64$ possible combinations)

The Genetic Code

- 1961, Matthaei and Nirenberg combined the synthetic RNA poly-U and see what amino acids will be for it.

U U U U U U U U U U

Phe, Phe, Phe

C A C A C A C A

His, Thr, His

C A A C A A C A A C A A

Gln, Gln, Gln, Gln

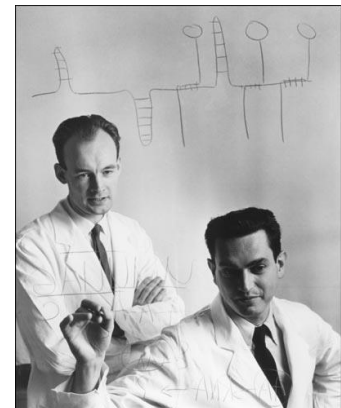
C A A C A A C A A C A A

Asn, Asn, Asn

C A A C A A C A A C A A

Thr, Thr, Thr

Reading frame



The rules of the Genetic Code

- 1. Triplet code: read 3 at a time
- 2. Non-overlapping, commaless

Reading frame #1

5'-AGUCUUACCGCAUUGUGG-3'

AGU	CUU	ACC	GCA	UUG	UGG
-----	-----	-----	-----	-----	-----

Ser--Leu--Thr--Ala--Leu-Ser

Reading frame #2

5'-AGUCUUACCGCAUUGUGG-3'

AGU	CUU	ACC	GCA	UUG	UGG
-----	-----	-----	-----	-----	-----

Val--Leu--Pro--His--Cys

Reading frame #3

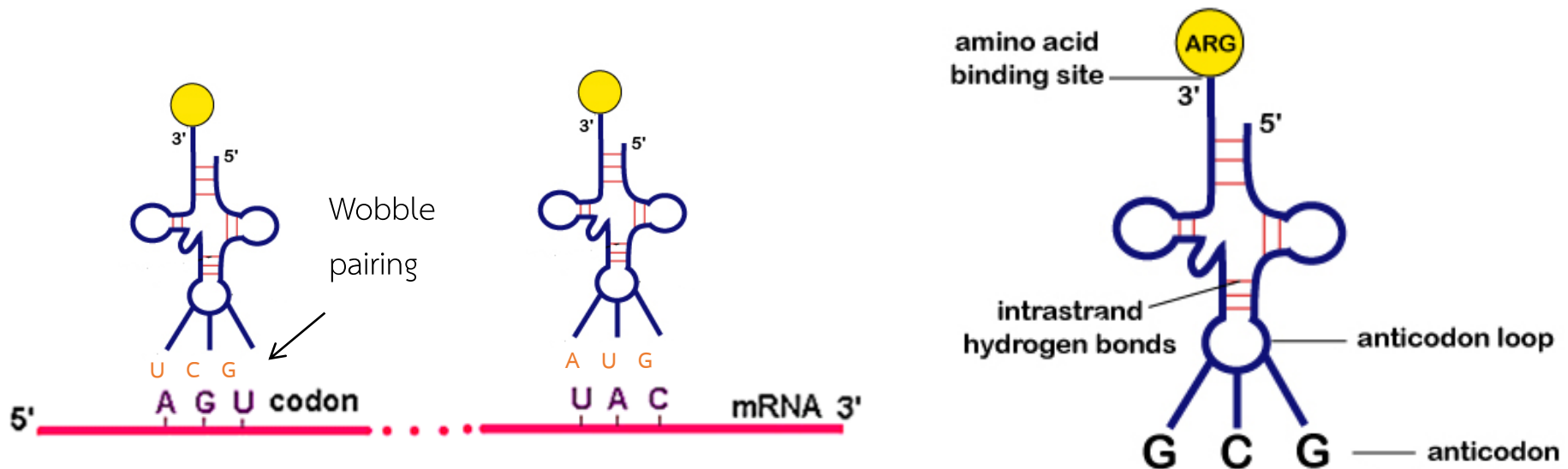
5'-AGUCUUACCGCAUUGUGG-3'

AGU	CUU	ACC	GCA	UUG	UGG
-----	-----	-----	-----	-----	-----

Ser--Tyr--Arg--Ile--Val

Rules of the Genetic Code

- 3. Degenerated code but non ambiguous: 1 amino acid (aa) has more than 1 codon. There are some aa that have just 1
- 4. Universal genetic code: bacteria, plant, human are all using this code

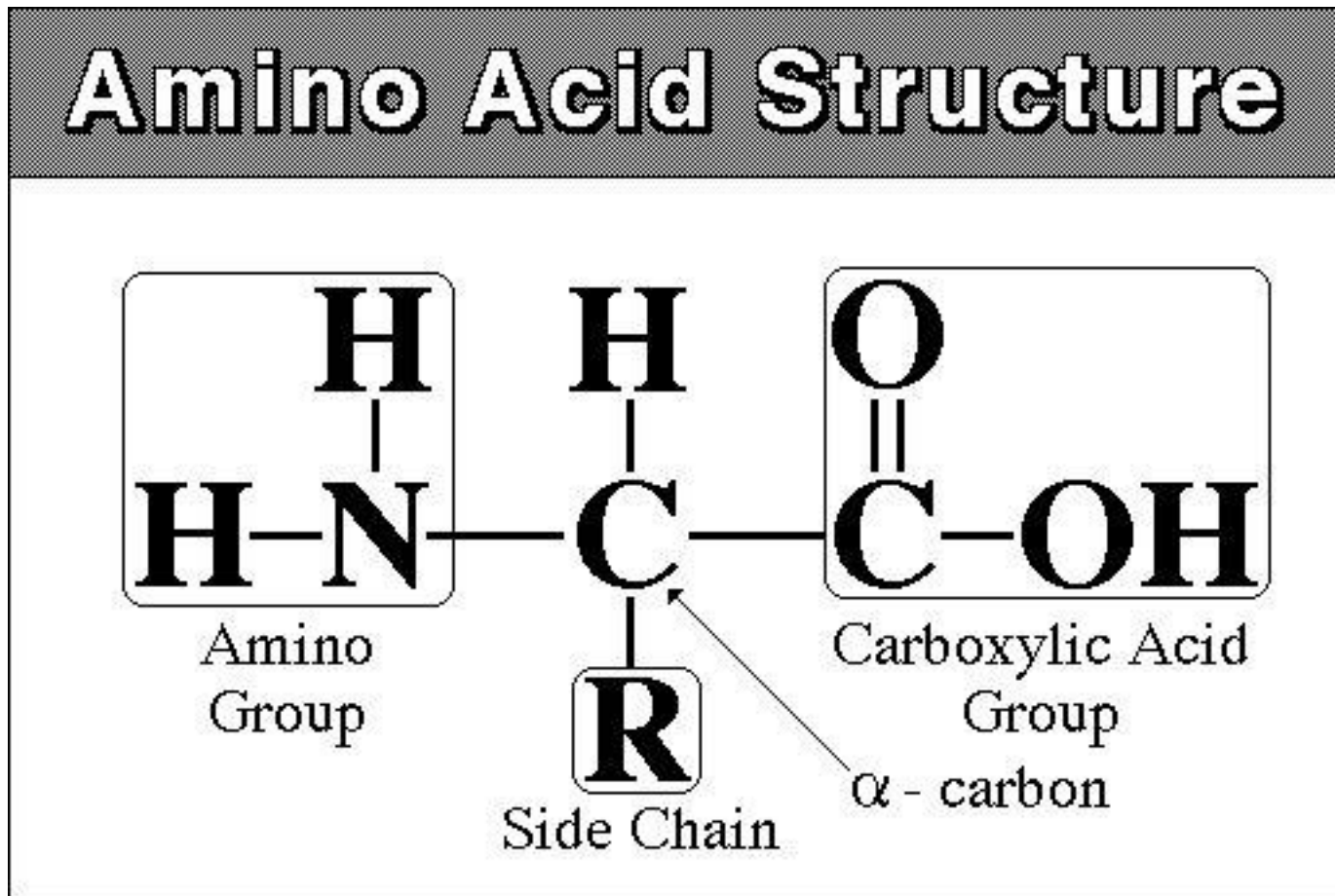


		Second base				
		U	C	A	G	
First base (5' end)	U	UUU] Phe	UCU]	UAU] Tyr	UGU] Cys	U
		UUC]	UCC] Ser	UAC]	UGC]	C
		UUA] Leu	UCA]	UAA Stop	UGA Stop	A
		UUG]	UCG]	UAG Stop	UGG Trp	G
	C	CUU]	CCU]	CAU] His	CGU]	U
		CUC]	CCC] Pro	CAC]	CGC] Arg	C
		CUA]	CCA]	CAA] Gln	CGA]	A
		CUG]	CCG]	CAG]	CGG]	G
	A	AUU]	ACU]	AAU] Asn	AGU] Ser	U
		AUC] Ile	ACC]	AAC]	AGC]	C
		AUA]	ACA]	AAA] Lys	AGA] Arg	A
		AUG Met or start	ACG]	AAG]	AGG]	G
	G	GUU]	GCU]	GAU] Asp	GGU]	U
		GUC]	GCC]	GAC]	GGC]	C
		GUA] Val	GCA]	GAA] Glu	GGA]	A
		GUG]	GCG]	GAG]	GGG]	G
						Third base (3' end)

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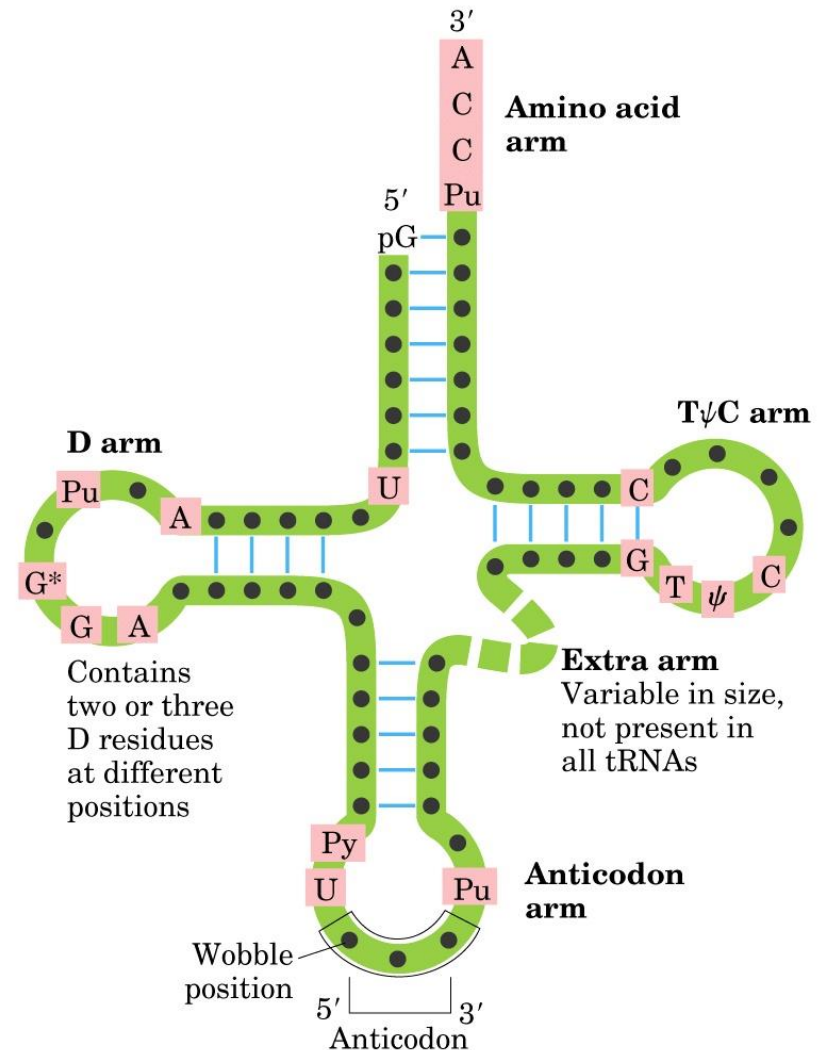
มีกรดอะมิโน 2 ชนิด (methionine และ tryptophan) ที่มีรหัสพันธุกรรมรหัสเดียว

Amino acid



Transfer RNA (tRNA)

- Clover leaf shape
- tRNA : 3 bases complementary with codon (on mRNA) : called **anticodon**
- Wobble position



amino acid
binding site

ARG

3'

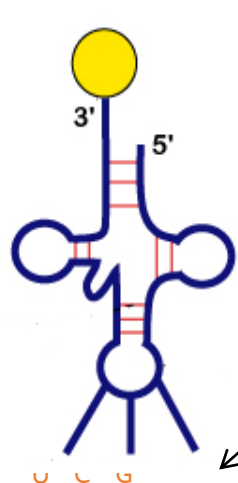
5'

intrastrand
hydrogen bonds

anticodon loop

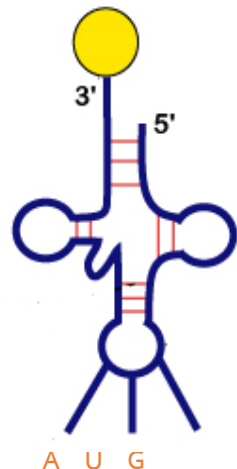
G C G

anticodon



Wobble
pairing

A G U codon



U A C

mRNA 3'

Wobble position

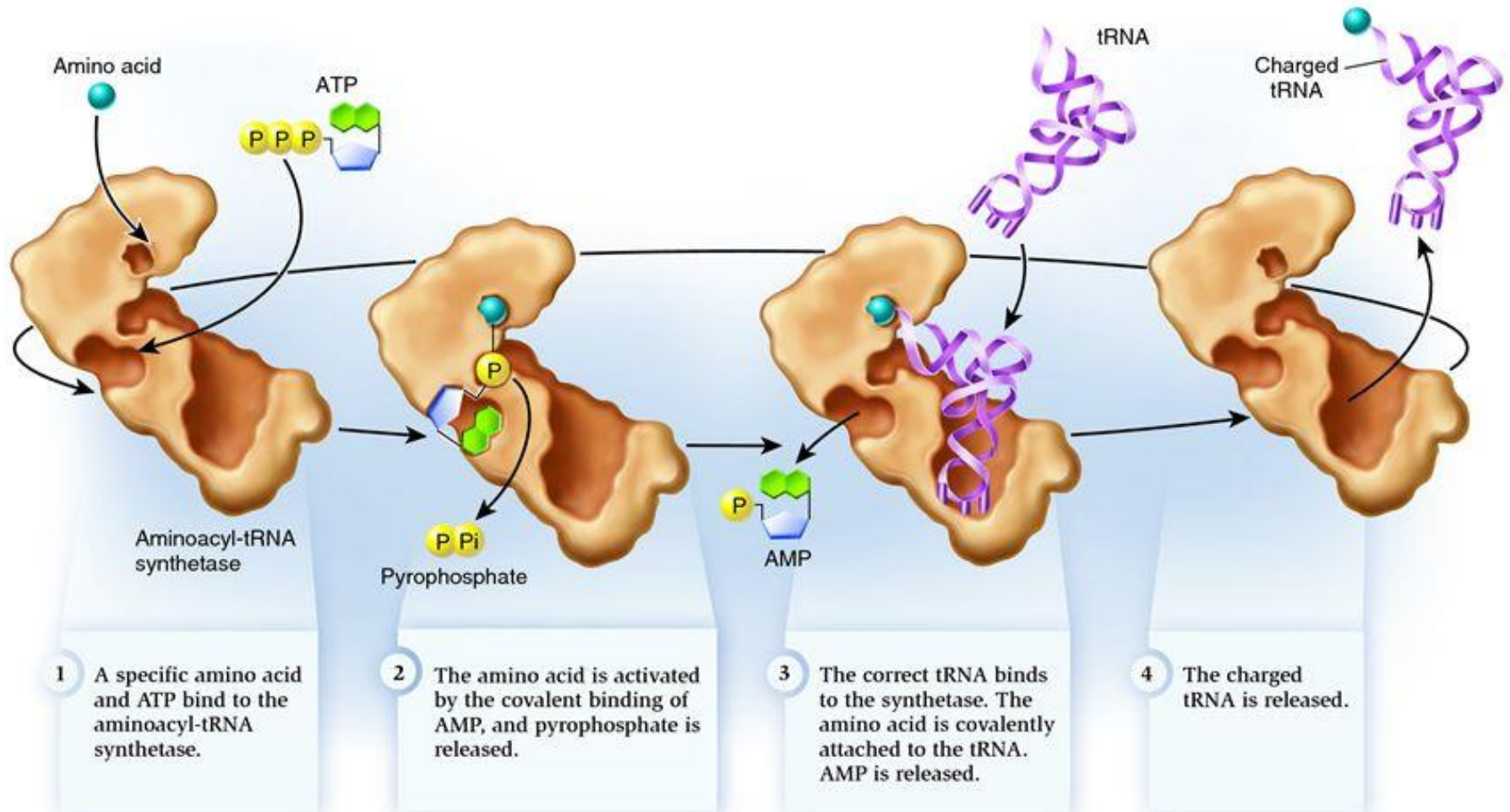
- Wobble position : bases at 3' of codon and bases at 5' of anticodon
- wobble base → tRNA 1 ชนิด จับกับ codon ของ mRNA ได้มากกว่า 1 codon

5' base of anticodon	3' base of codon
C	G
A	U
U	A or G
G	C or U
I	U or C or A

Aminoacyl-tRNA synthetase

- Enzyme that attaches the appropriate amino acid onto its tRNA (esterification of a specific cognate amino acid to one of all its compatible cognate tRNAs)
- Sometimes called "charging" or "loading" the tRNA with the amino acid
- ขบวนการนี้อาศัย Mg^{2+} เป็น cofactor และต้องการ ATP (2 ATP)
- $\text{Amino Acid} + \text{tRNA} + \text{ATP} \rightarrow \text{Aminoacyl-tRNA} + \text{AMP} + \text{PPi}$
- 20 different aminoacyl-tRNA synthetases
- α -COOH group ของ กรดอะมิโน กับ 3'-OH group ของ tRNA

Charged tRNA



The Genetic Code

- Arg, Leu, Ser : have 6 codons
- Met, Trp have only 1 codon
- One tRNA, carry the same aa, can bind to more than 1 codon by Wobble pairing hypothesis
- This way, 20 tRNA with 20 aa can pair with 61 codons.

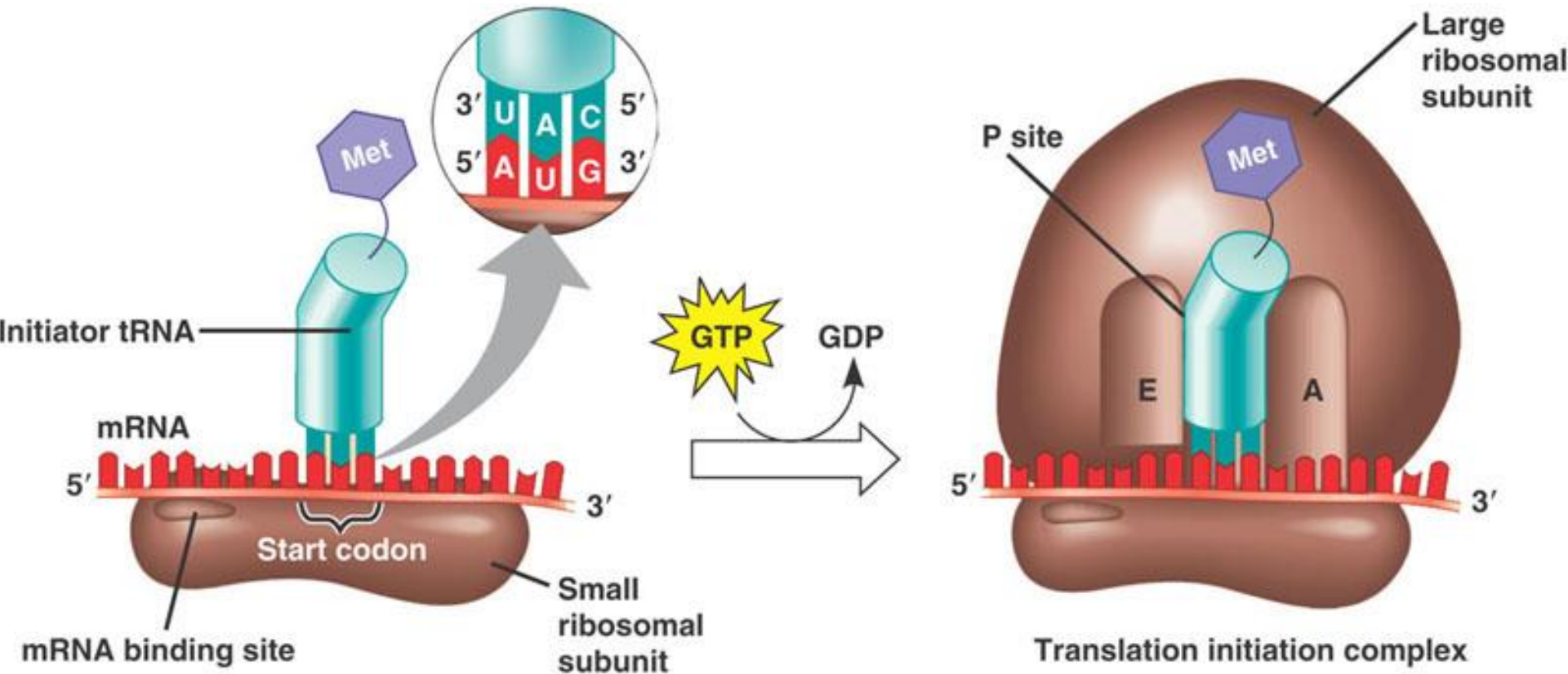
Ribosome

- Site of biological protein synthesis (translation)
- 2 subunits (small & large subunit)
- Small sub unit: binds to a larger subunit and the mRNA pattern
- Large subunit : binds to the tRNA, the amino acids, and the smaller subunit
 - P site : เป็นบริเวณที่เกิดสาย polypeptide
 - A site : เป็นบริเวณที่ aa-tRNA มาเกาะ
- Prokaryotic ribosome : 70S (50S & 30S)
- Eukaryotic ribosome : 80S (60S & 40S)

Steps in protein synthesis at the Initiation step

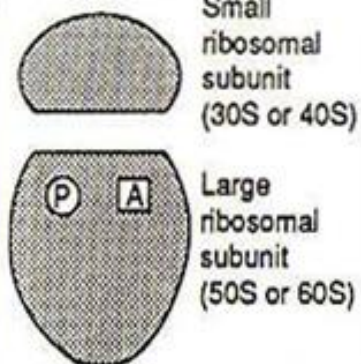
- 1. 30S ribosome uses its 16SRNA to bind to Shine-Dalgarno Sequence (SD) on 5' end of mRNA.
 - Binding of SD-ribosome Fixed the reading frame of protein synthesis
- 2. Next to the SD is the first codon, AUG,that code for Methionine. fMet-tRNA binds to AUG and the large ribosome subunit come

Steps in protein synthesis at the Initiation step

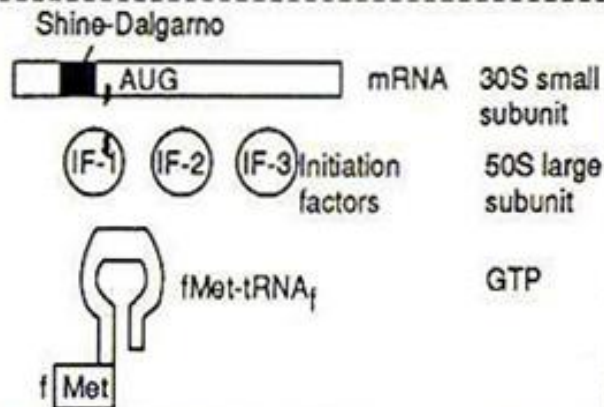


Steps in protein synthesis at the Initiation step

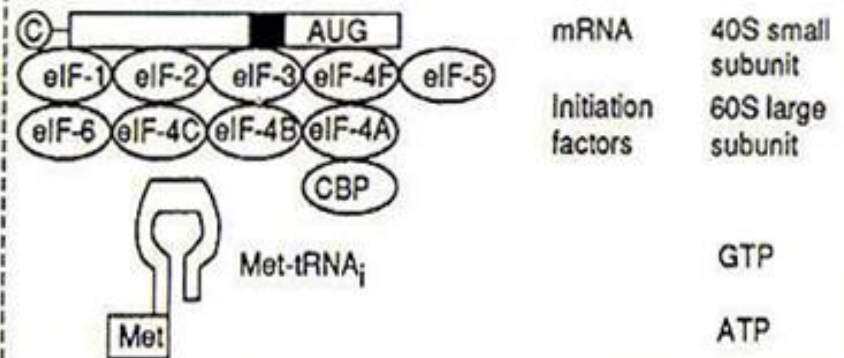
Initiation components



Prokaryotic

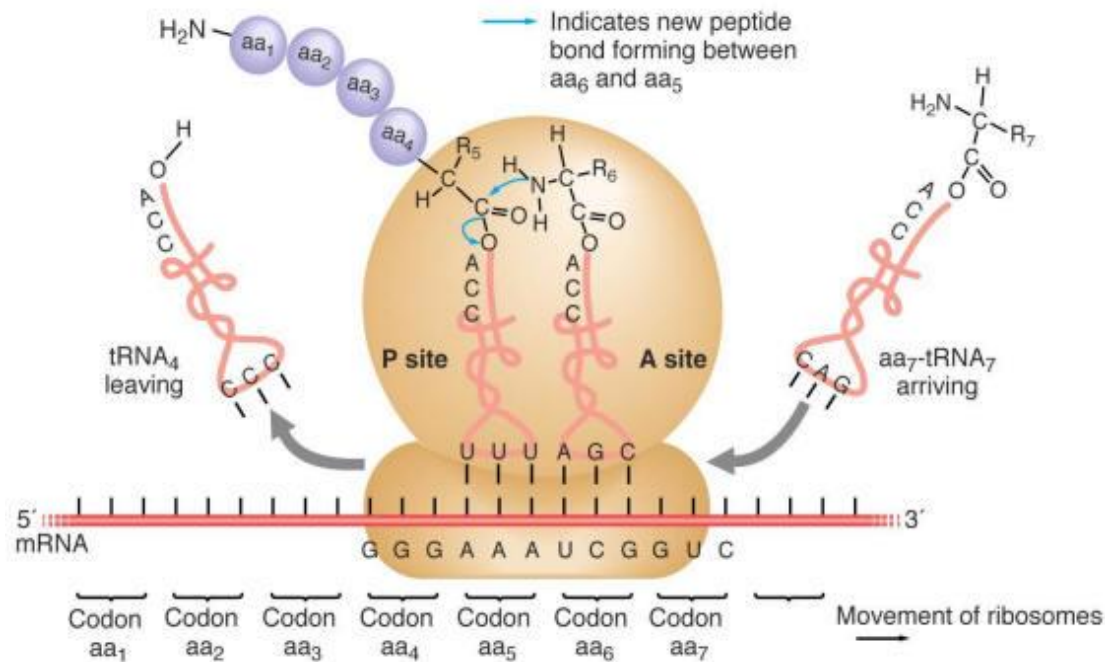


Eukaryotic



Elongation step

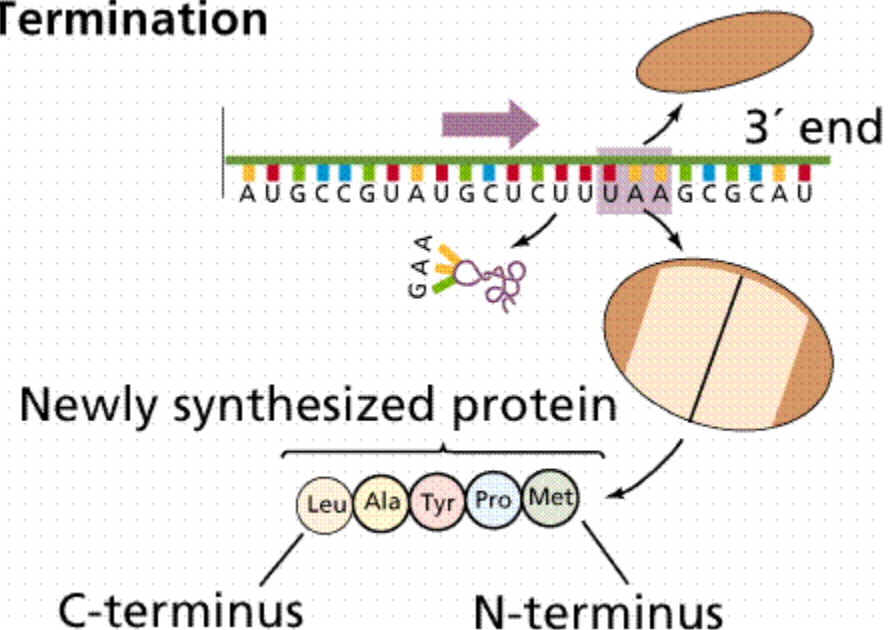
- Ribosome moves along the mRNA
- tRNA with anti-codon complementary with the next codon comes with aa. Peptide bond is formed between fMet and the next aa and
- tRNA without aa is leaving



Termination

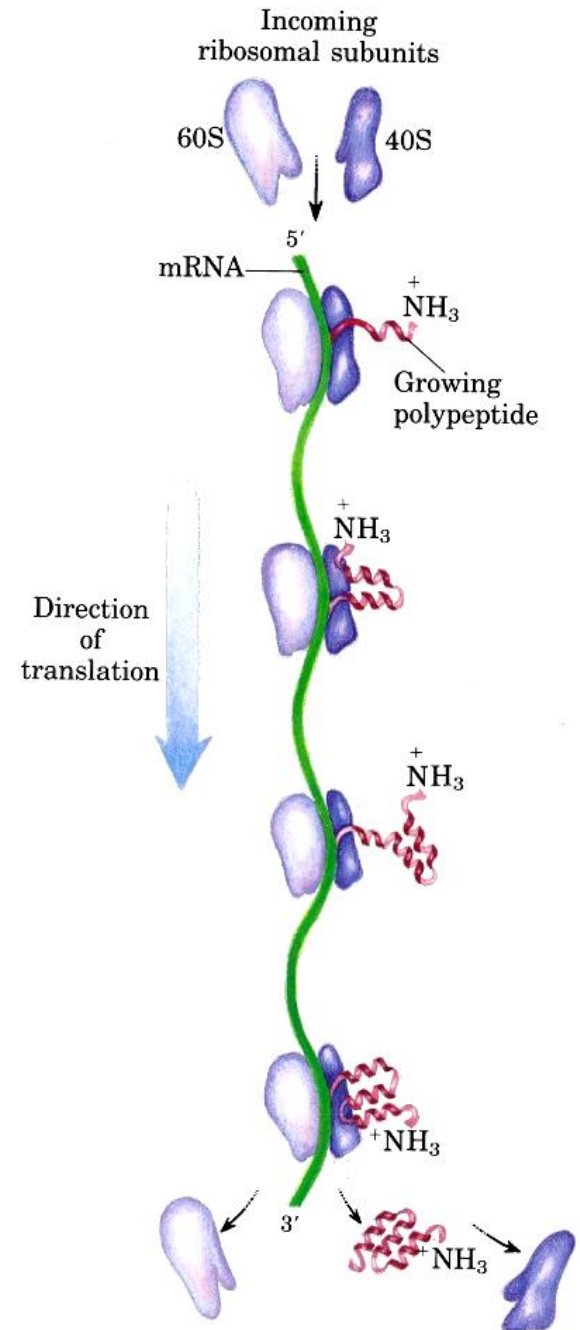
- Ribosome encounters termination codons (UAG, UAA or UGA) → Translation of a protein ends
- Releasing factors: come to bind with the stop codon and all ribosome separated.
- RF-1 : UAA, UAG
- RF-2 : UAA, UGA
- RF-3 : GTP

Termination



Polyribosome (polysome)

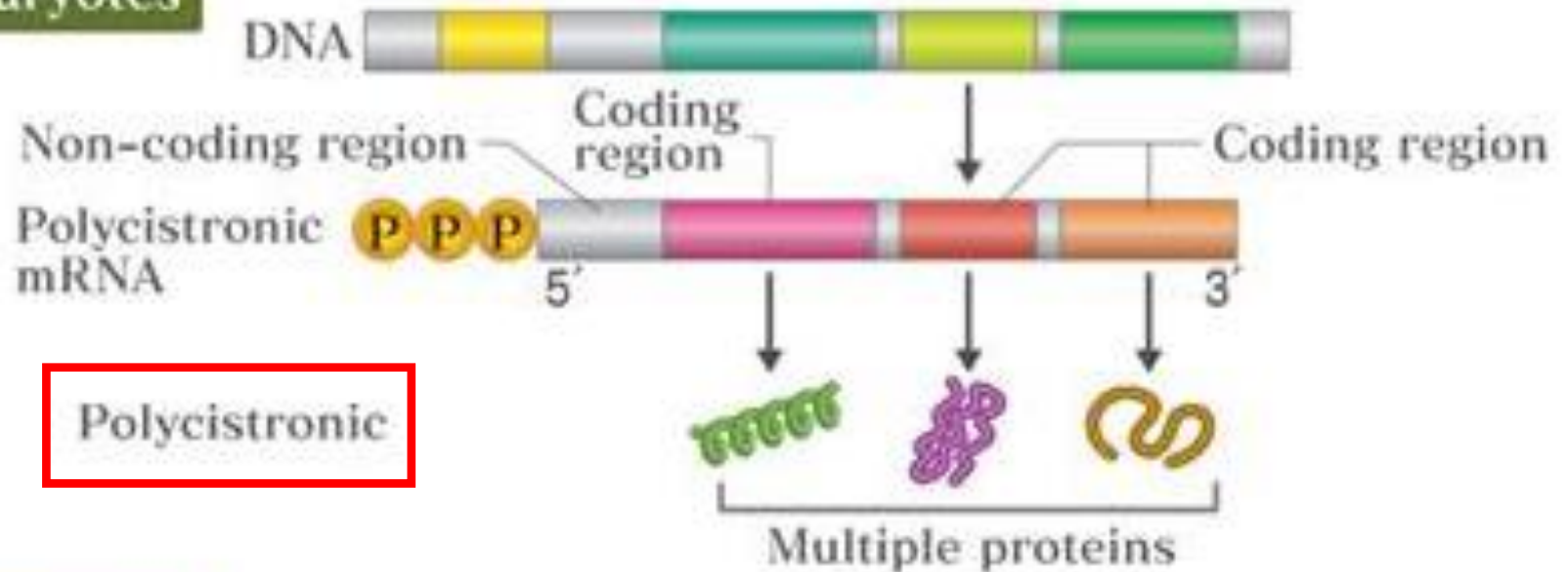
- After protein synthesis initiation : new ribosome comes and binds the same mRNA for starting new protein synthesis initiation
- Polyribosome → cell can speed up the rate of protein synthesis



Polycystronic / monocystronic mRNA

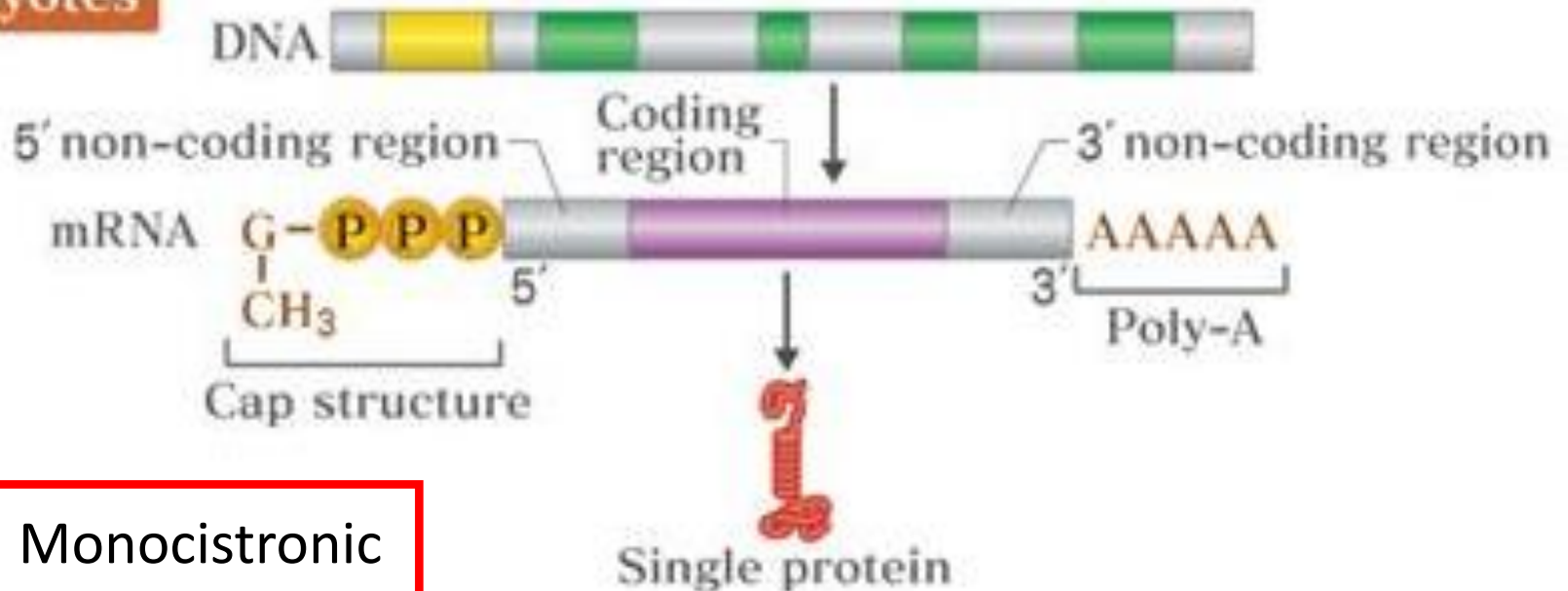
- Prokaryote organized their gene that function together to be under the same controller, called operon. One mRNA translates to more than one Protein (polycystronic mRNA)
- Eukaryote produce one mRNA that will Translate into one protein (monocystronic)

Prokaryotes



Polycistronic

Eukaryotes

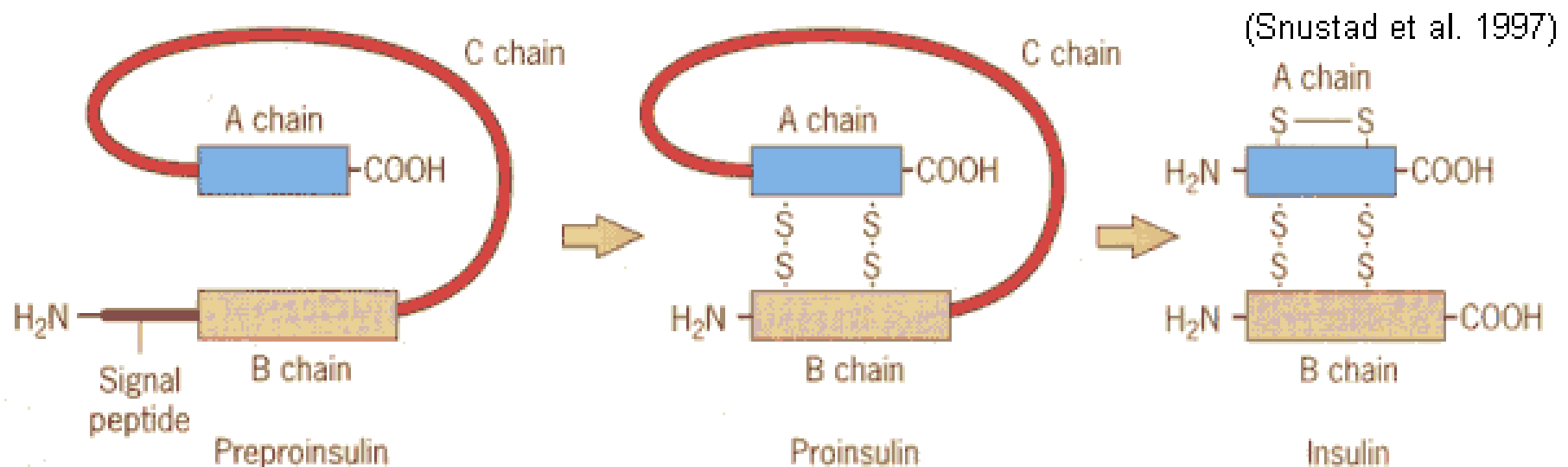


Monocistronic

Post-translational Modification

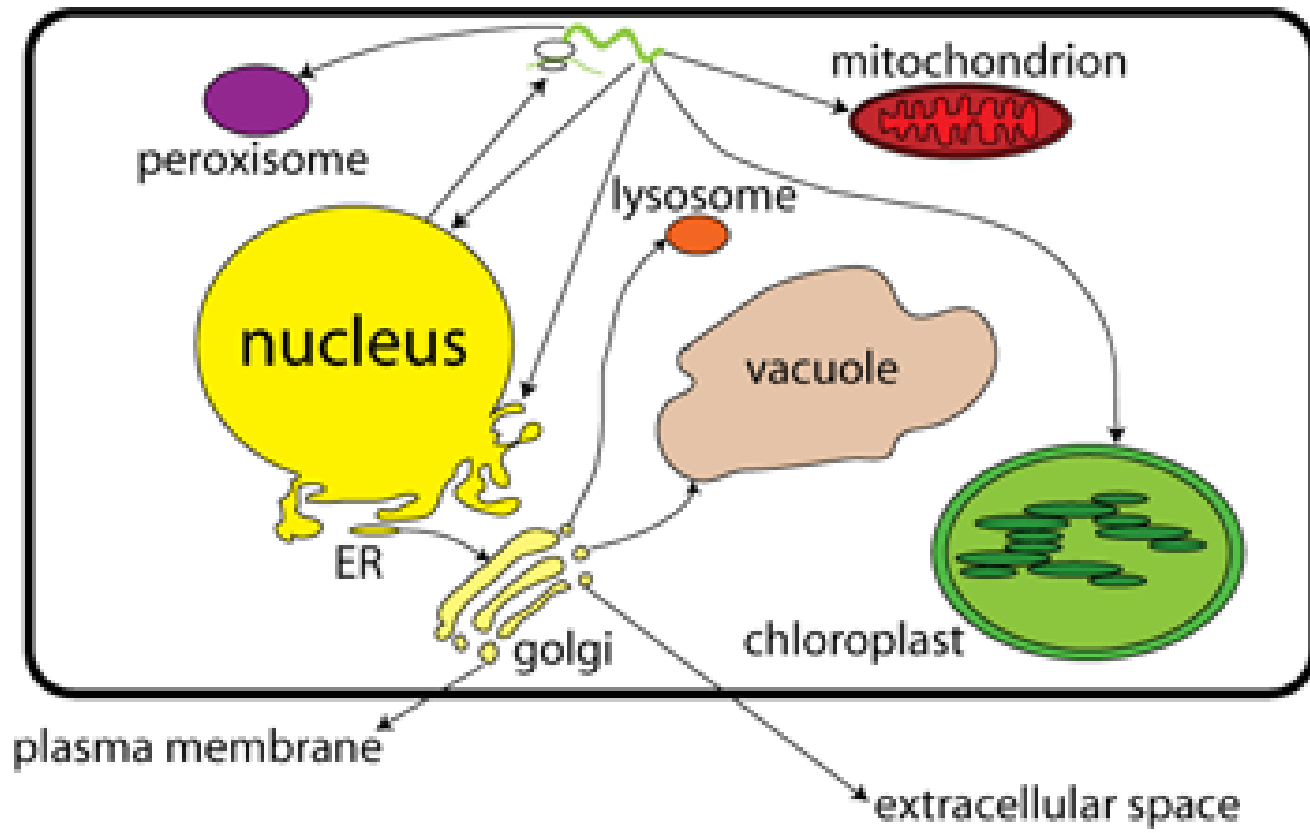
- Generally enzymatic modification of proteins during or after protein biosynthesis called post-translational modification.
- Correct folding / chemical modification for biological active function
- Phosphorylation (add phosphate)
- Endoplasmic reticulum (ER) add oligosaccharides to protein (glycoprotein)
- Disulfide linkage, substitute groups exp. acetylation, methylation, ubiquitylation, hydroxylation etc.

Insulin : Post-translational modification

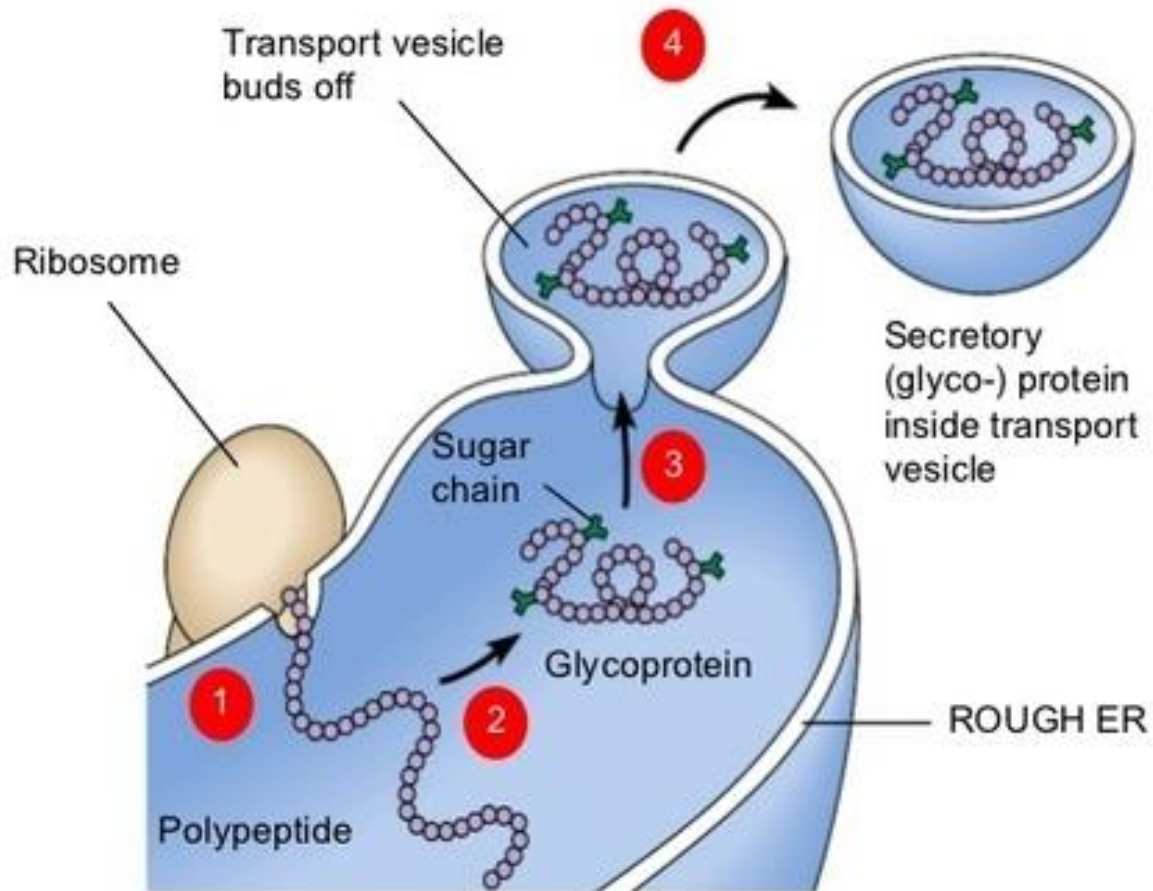


Protein translocation (protein targeting)

1. Post-translational translocation
2. Cotranslational translocation

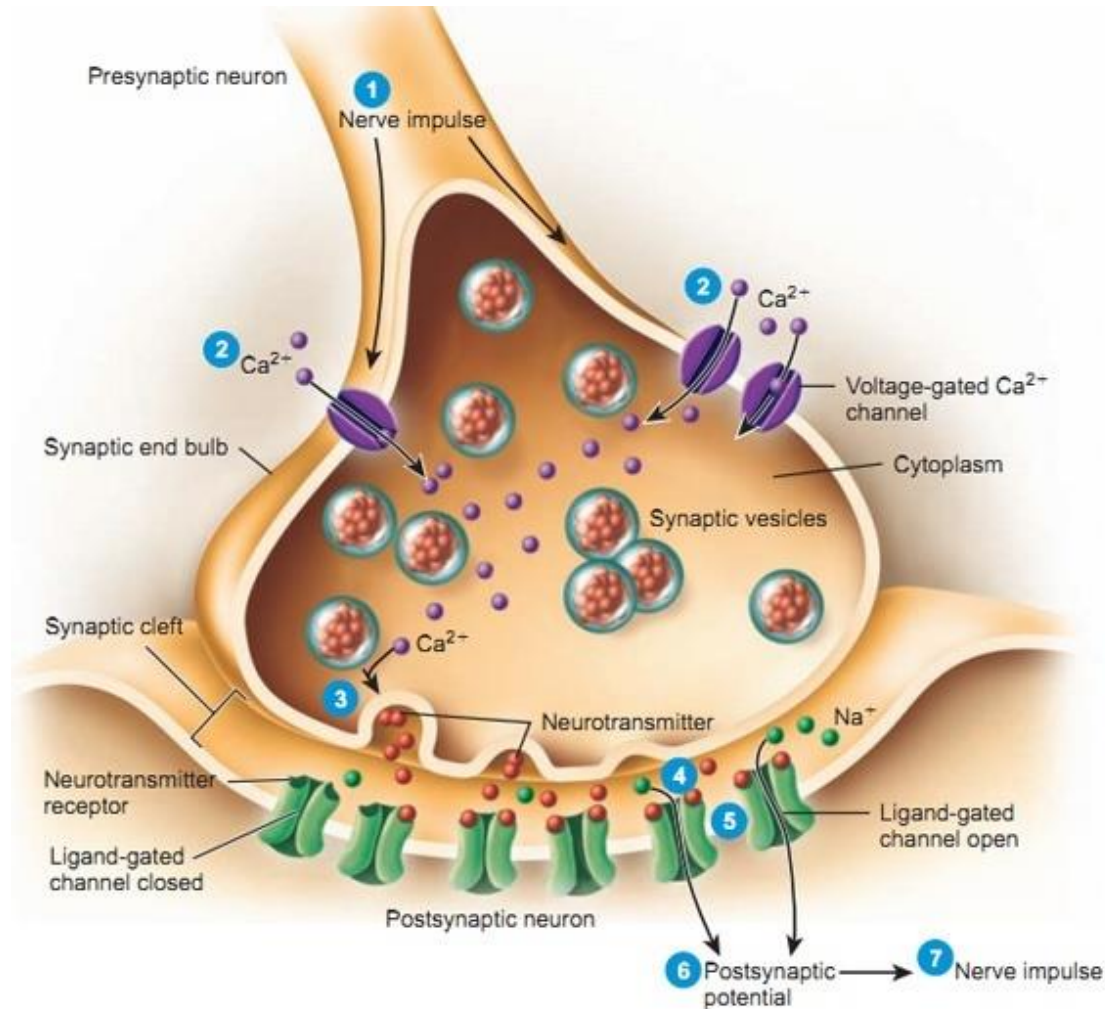


Glycoprotein and secreted protein



Endoplasmic reticulum

Proteins such as neurotransmitter will be secreted out of the cell



Inhibitors of protein synthesis

- Affect on prokaryotes : streptomycin, tetracycline, chloramphenicol & erythromycin
- Affect on eukaryotes : cycloheximide & diphtheria toxin
- Affect both prokaryotes & eukaryotes : puromycin

Inhibitors of protein synthesis

Inhibitors	Mechanism of action
streptomycin	binds to 30 s ribosomal subunit, preventing formation of the initiation complex
tetracycline	binds to 30 s ribosomal subunit, inhibits binding of aa-t RNA to A site
chloramphenicol	inhibits peptidyl transferase activity of 50s ribosomal subunit
erythromycin	binds to 50 s ribosomal subunit, prevents translocation
cycloheximide	inhibits peptidyl transferase in eukaryotes
diphtheria toxin	inhibit translocation of peptidyl-tRNA from A to P site
puromycin	prematurely terminates synthesis

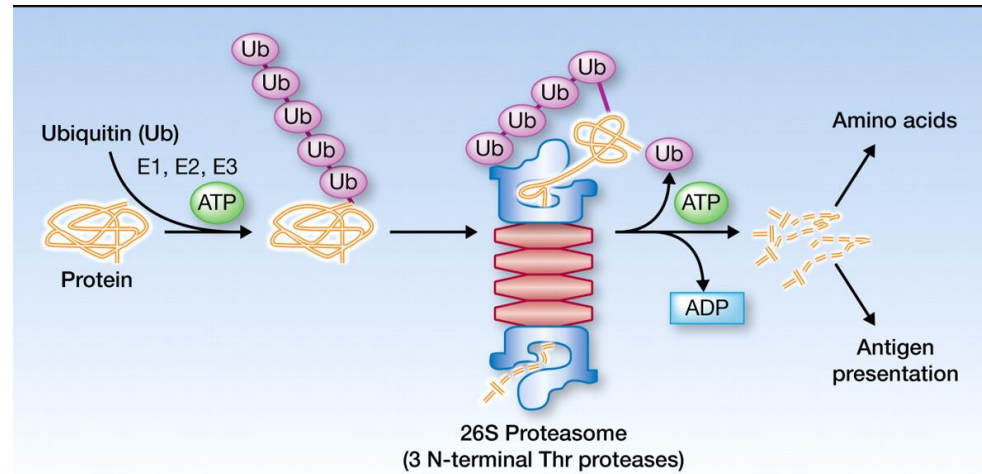
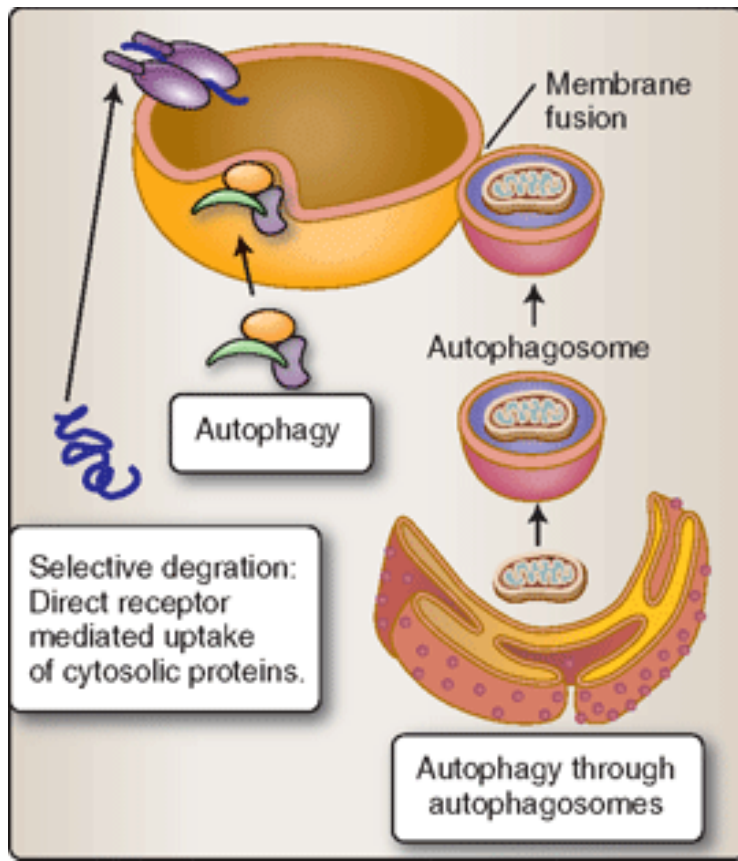
Protein degradation

- Protein has half-life, exp. N-Met 20 min, N-Pro 3 min degraded after synthesize.
- Protein containing more PEST sequence (proline-glutamate-serine-threonine) → shorter half-life
- Protein is degraded with several purpose
 - Food, pathogen that engulf into the cell is degraded to get energy or prevent cell from destruction by pathogen
 - Get rid of unwanted or folding defect proteins
 - To slow/stop biochemical reaction
 - Control metabolism exp. Autophagy (stress, starvation) destroy its own organelle

Protein degradation

Two ways:

Lysosomal proteolysis : Engulf pathogen, Food fused with Lysosome and destroyed by Enzymes (endocytosis, autophagy)



Miss-folding of protein is Destroyed in cytosolic by **proteasome pathway/Ubiquitin-ATP dependent pathway**

Summary

- Proteins are complex: variety, various in their amount
- Has many functions in our body
- Post-translational modification, alternative splicing occurred in eukaryote: makes them function properly
- Protein targeting bring proteins to their work place

Suggested reading

