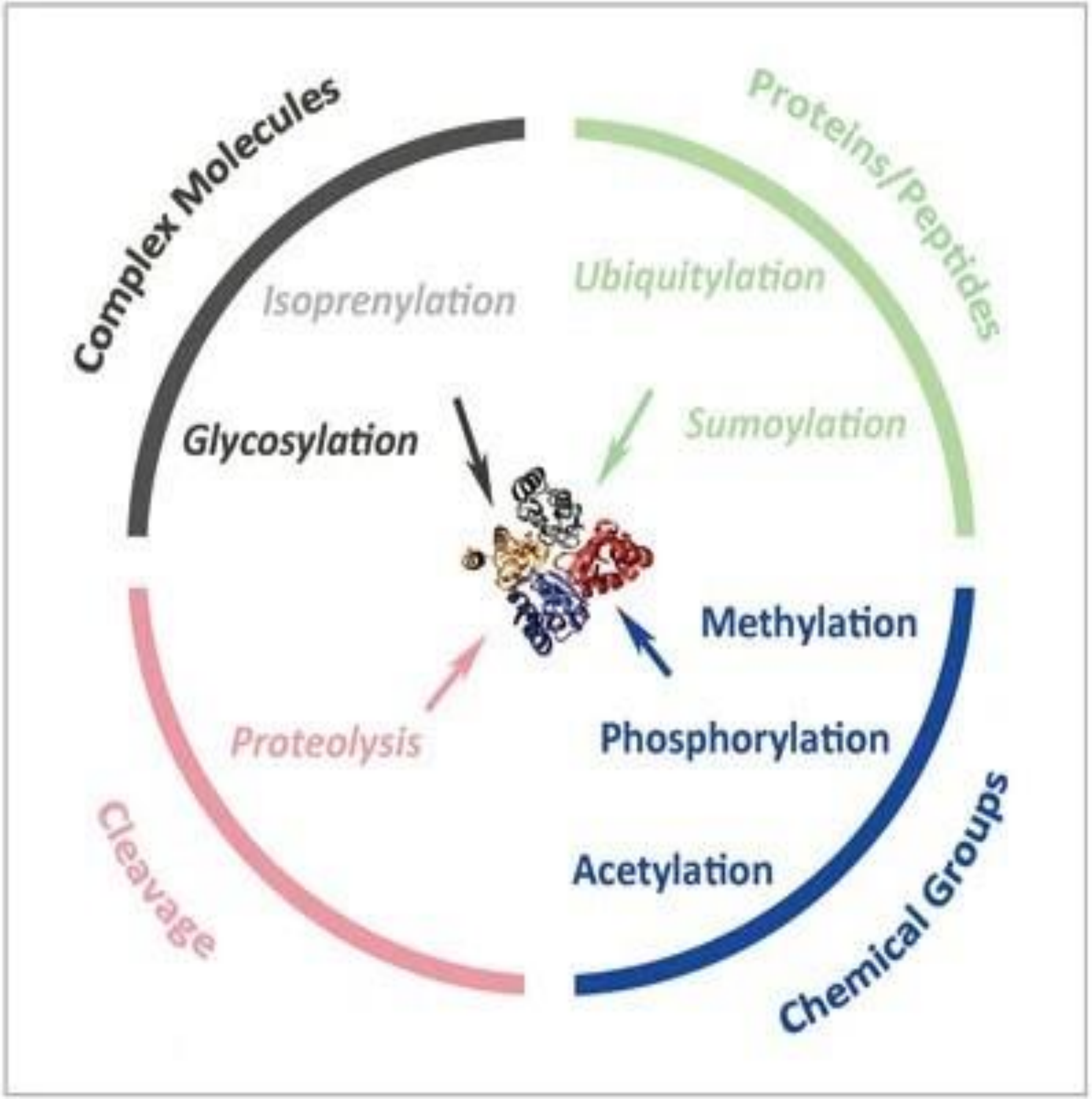
Post-translational modifications & Inhibitors of protein synthesis



By:
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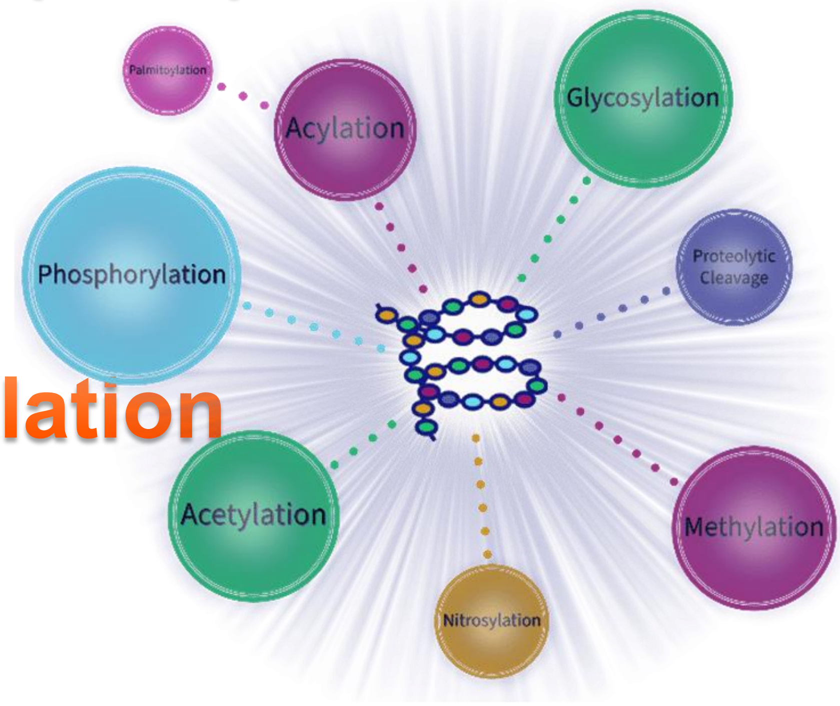
Learning Outcomes

At the end of this lecture, student should be able to:

- Define **Post-translational modification (PTM).**
- Identify **Importance of PTM.**
- Differentiate between **types of PTM.**
- List different **inhibitors of protein translation both Pro**karyotic or **Eu**karyotic.

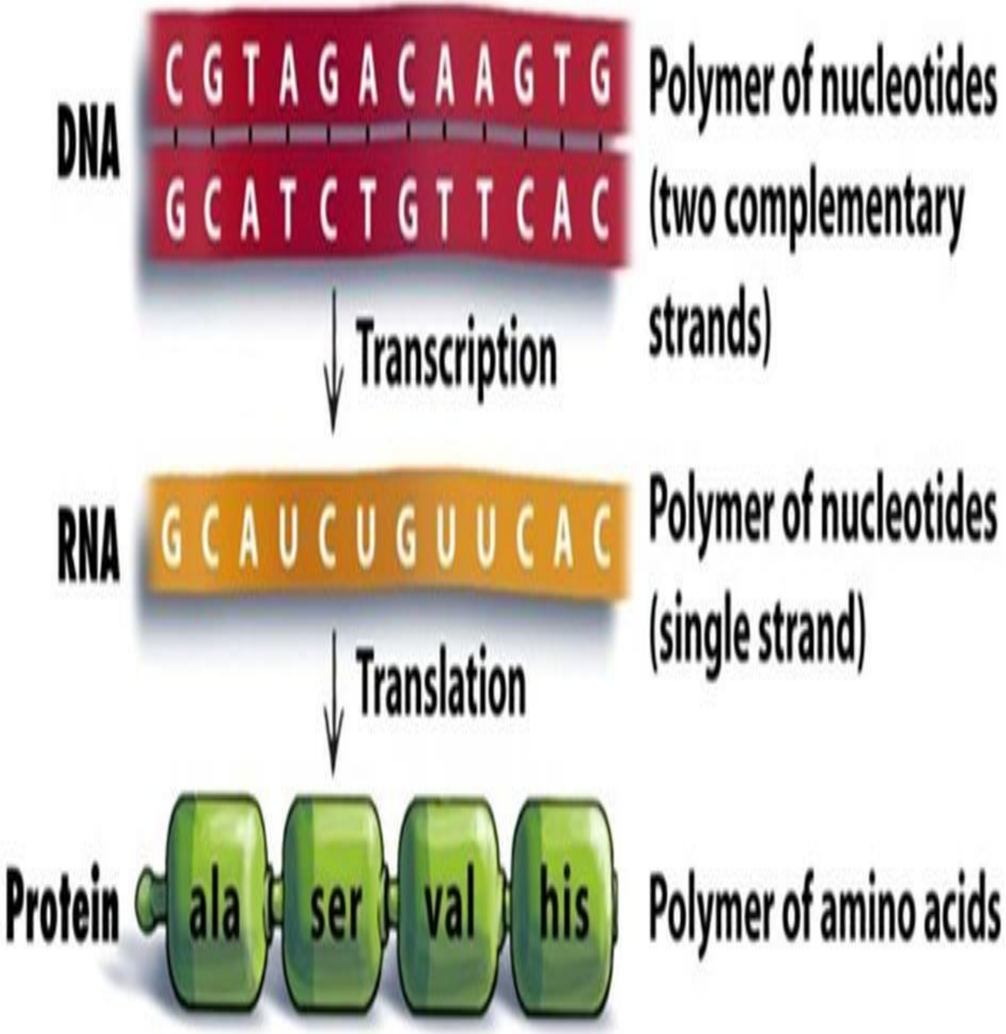


Case Scenario

- A 22-year-old dental student presents to the clinic with **pain, swelling, and pus discharge** around a partially erupted lower third molar (wisdom tooth). He is diagnosed with **pericoronitis**, and a bacterial culture reveals infection with *Streptococcus* species. The patient reports a history of **penicillin allergy**, so the dentist prescribes **erythromycin**. **How does erythromycin help treat this dental infection?**

- Translation is often referred to as the "last step" of the "central dogma" of biology, whereby DNA is converted to RNA and then to protein.

- However, there are additional steps involved after protein synthesis that are necessary for a cell, tissue and organism to achieve its functional biology and diversity.

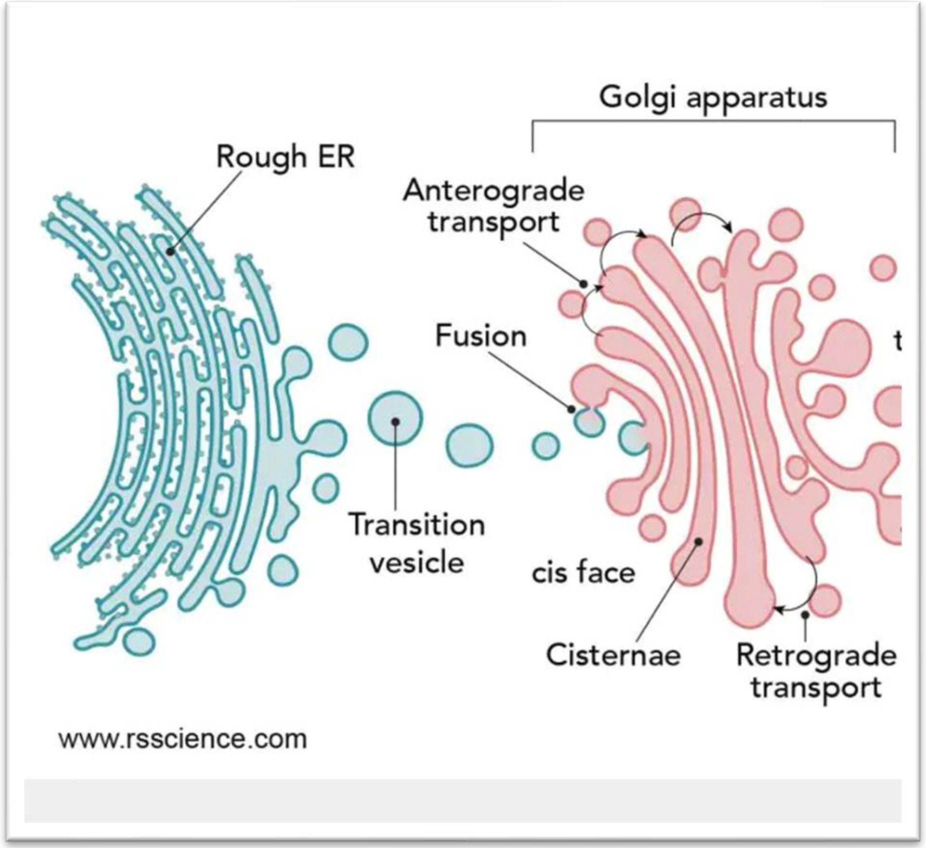


Protein still needs modifications to be able to perform normal function

What is Post-translational modification (PTM)?

- **Definition:** It is the chemical modification of the polypeptide or certain amino acid residues of a protein after its synthesis.

- **Site:** occur mostly in ER and Golgi apparatus.



Why PTM is important ?

- In order for individual cells to perform their functions, and respond to environmental stimuli, a variety of different proteins must be expressed at different timepoints across cells, tissues and organs.

- The diversity of the proteome is achieved by several different mechanisms; **post-translational modifications** are one example.

Types of post translational modifications (PTMs)

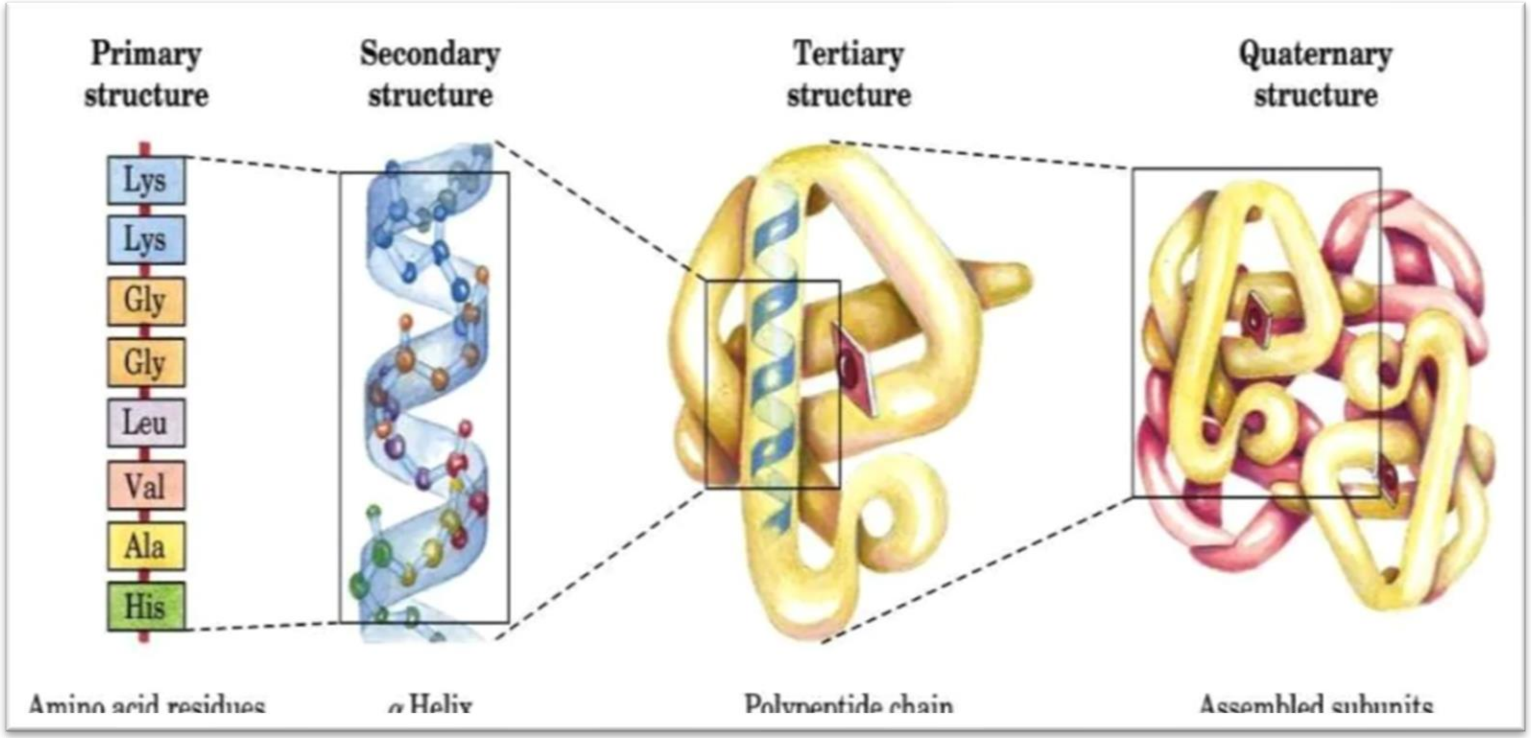
1- Protein folding

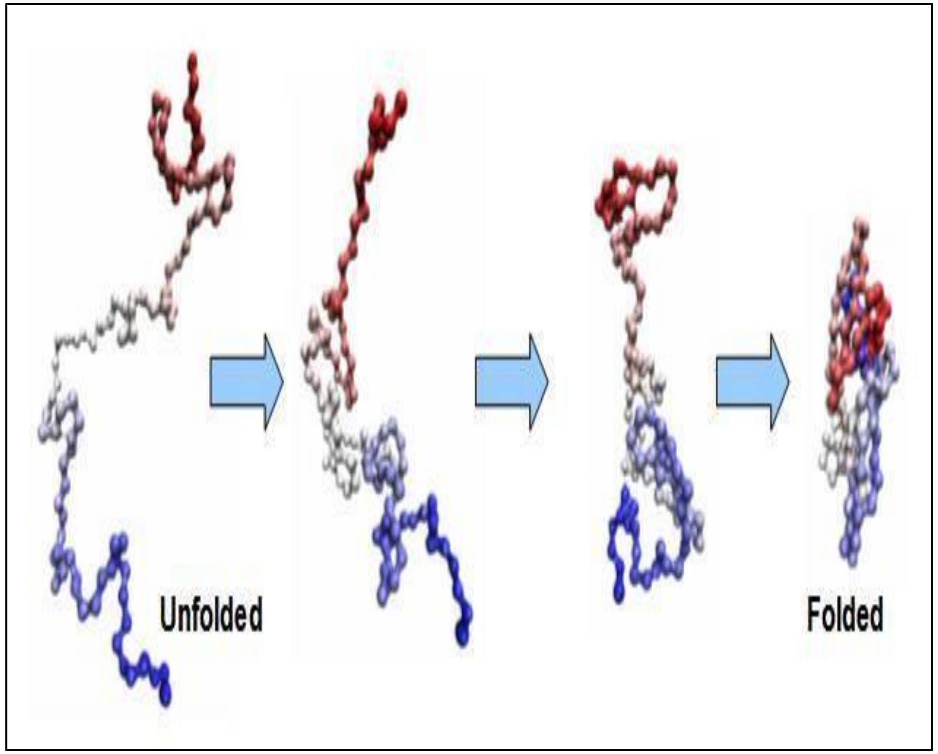
2- Trimming

3- Covalent modifications

1- Protein Folding

- Folding of polypeptide primary structure to get secondary, tertiary +/- quaternary structure is needed to become functioning.

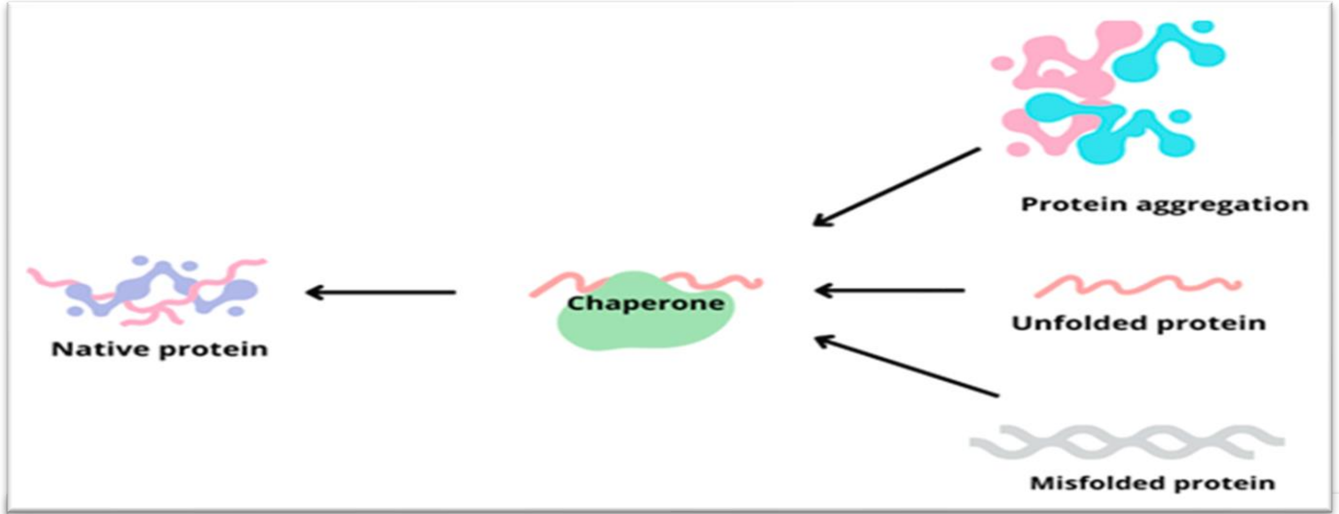


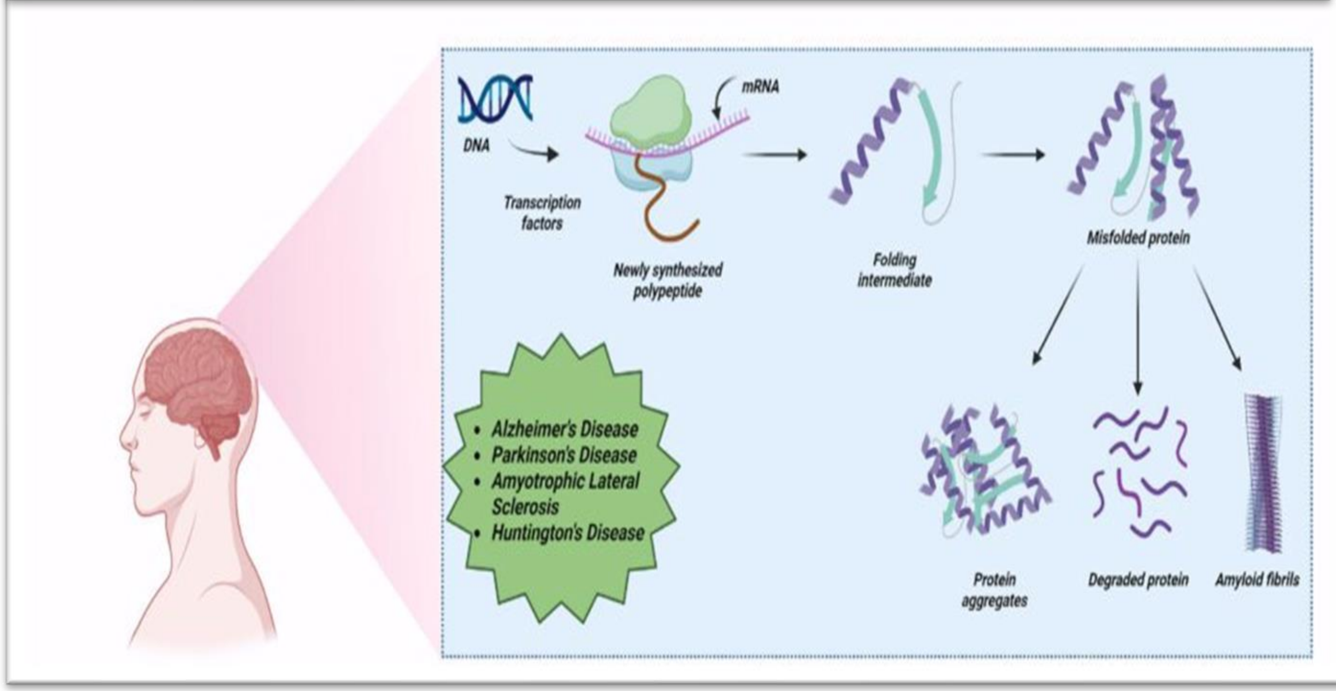


1- Protein Folding

- Not all proteins fold spontaneously; some proteins need chaperones to fold correctly.

- Abnormal or misfolding of proteins cause disease as Alzheimer's disease





2- Trimming (proteolysis)

- **Definition:** removal of part of the peptide chain and conversion of <u>inactive</u> protein to <u>active</u> one.

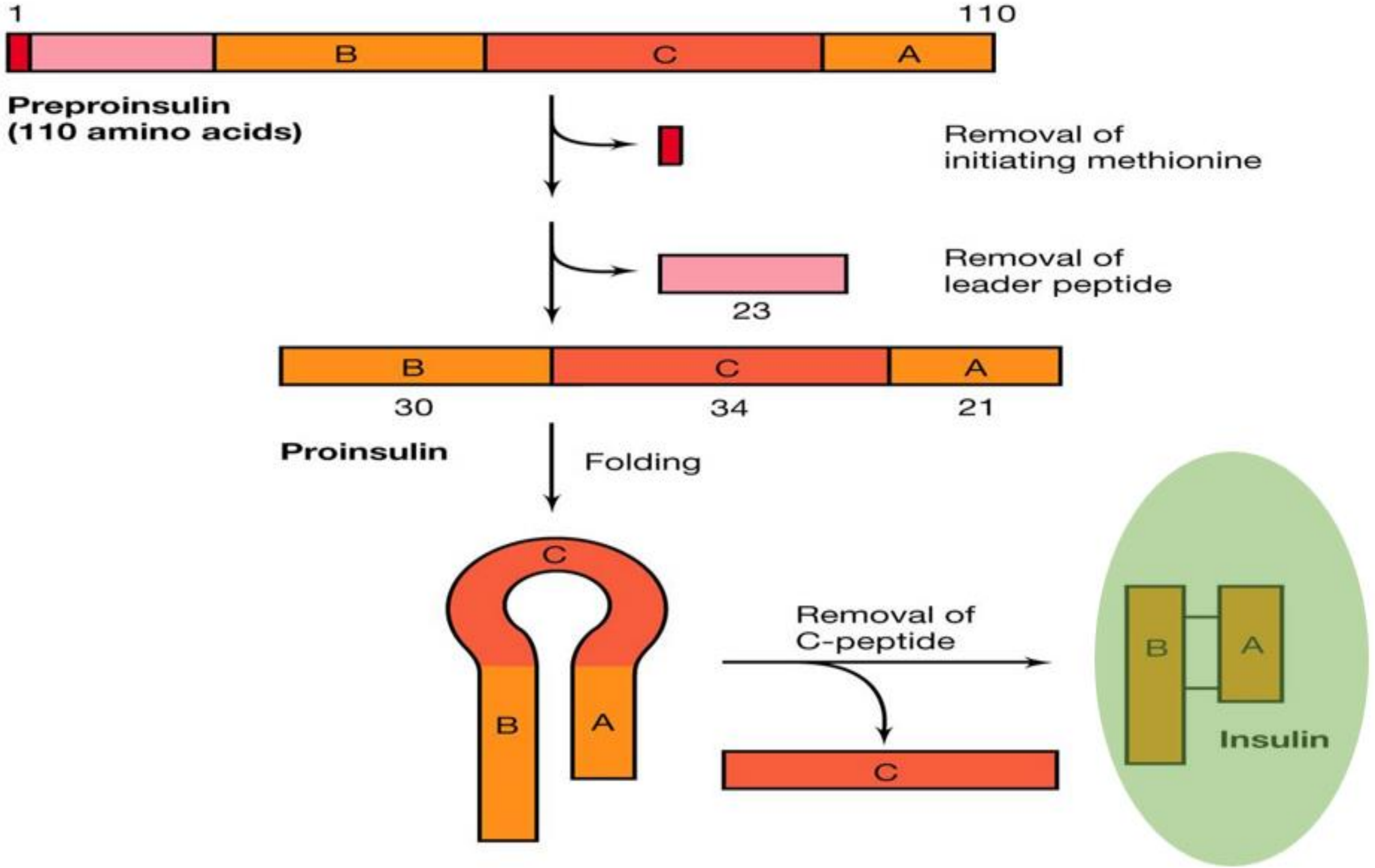
- Many proteins are formed as a large precursor molecules that are functionally inactive and part of the chain must be removed to become active molecules.

2- Trimming (proteolysis)

❑ Examples of trimming

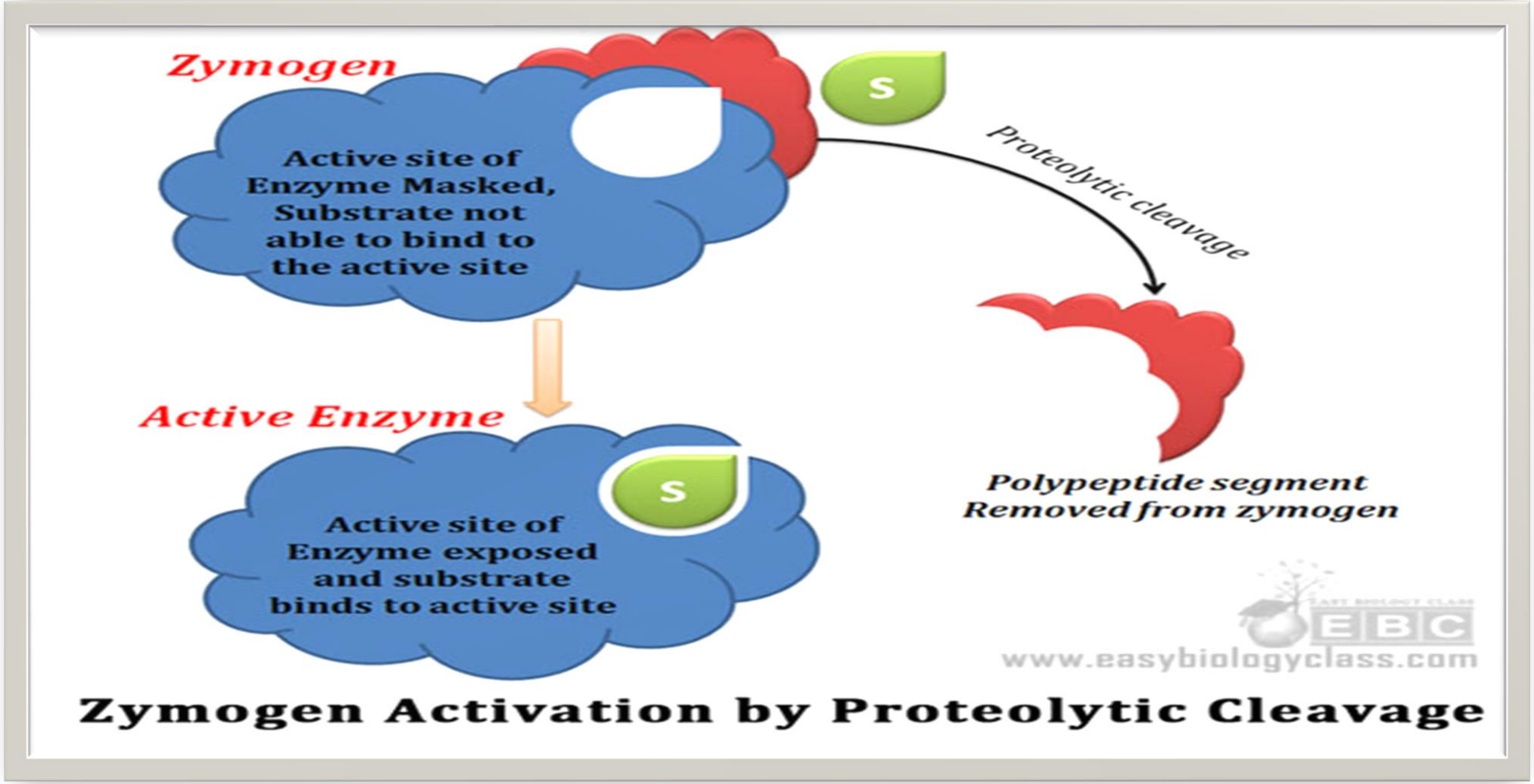
1-Conversion of Pre-proinsulin to Insulin

Pre-Proinsulin
↓
Proinsulin
↓
Insulin
cleaved C peptide



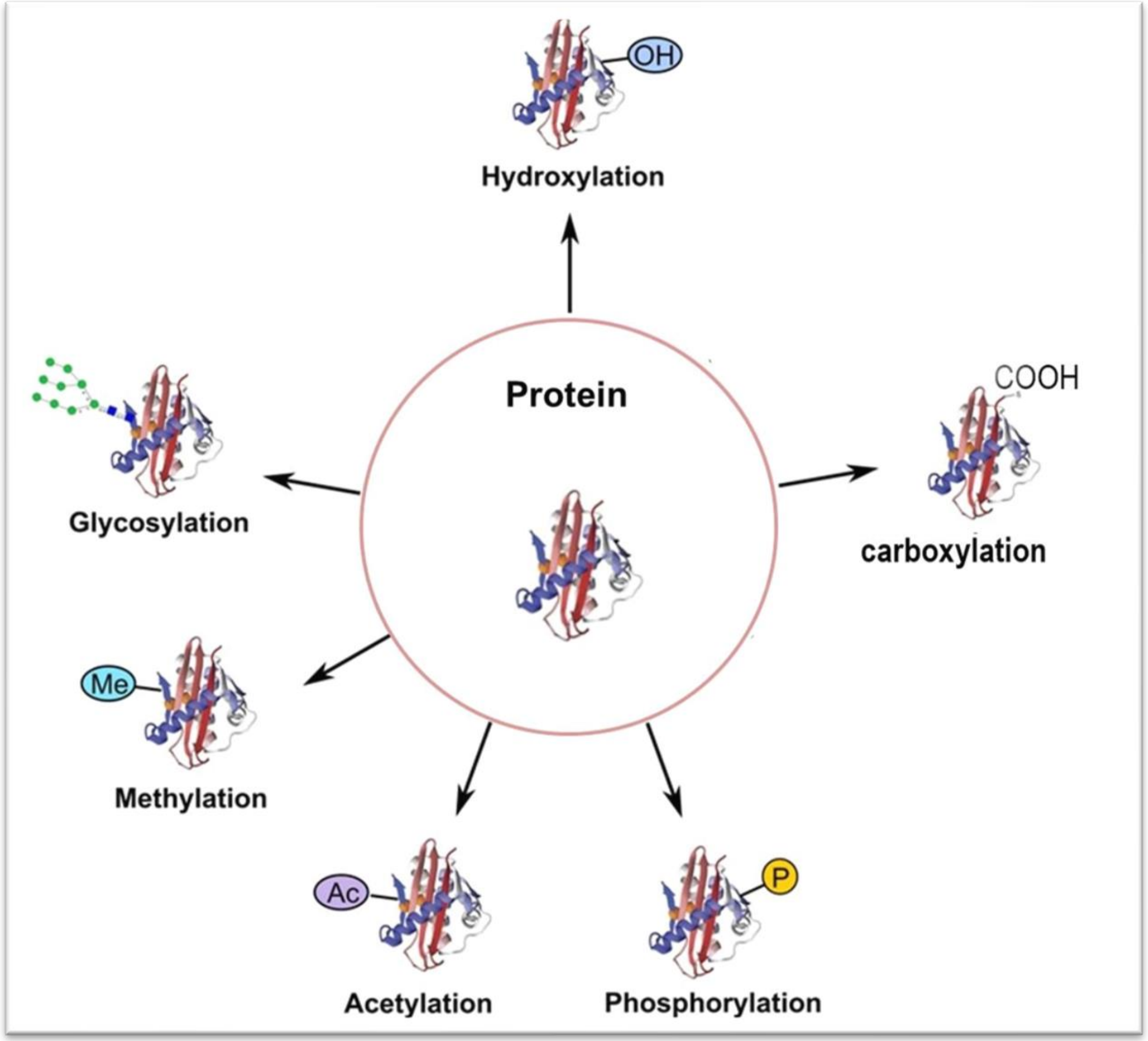
2- Conversion of Proenzymes of GIT to active enzymes

For example: inactive pancreatic trypsinogen become active trypsin



3- Covalent Modifications

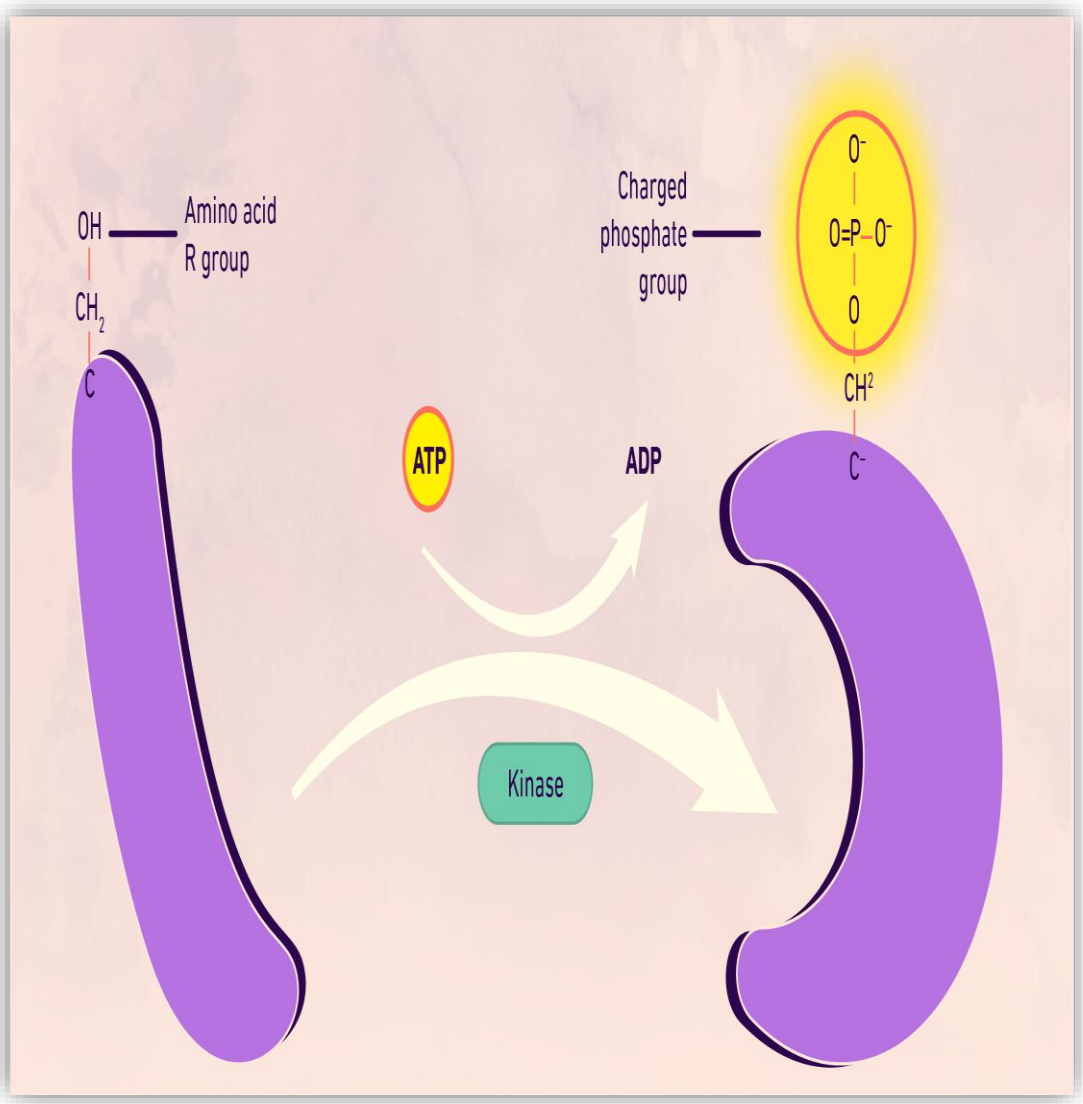
Definition: **Activation** or **inactivation** of Proteins by covalent attachment of a variety of chemical groups to their composing amino acids.



Examples of covalent modifications:

A) Protein phosphorylation

- Protein phosphorylation is one of the most common PTM.
- By addition of a phosphate to hydroxyl groups of serine, threonine or tyrosine residues.
- Proteins phosphorylation occurs by protein kinases and reversed by phosphatases.
- It is important for cell regulation and the activation and deactivation of proteins (e.g. enzymes and receptors).



Proteins may be activated or inactivated by phosphorylation

- **Enzymes (proteins) activated by phosphorylation**

❑Glycogen phosphorylase.

❑Hormone sensitive lipase.

❑Citrate lyase.

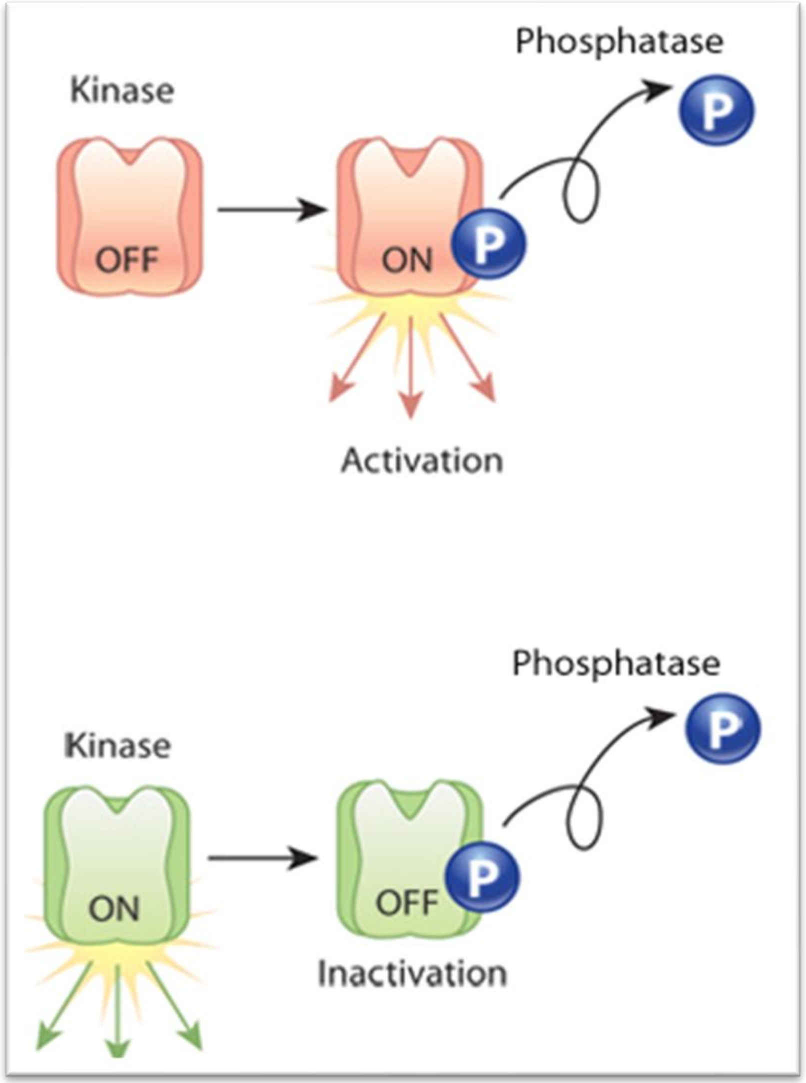
- **Enzymes (proteins) inactivated by phosphorylation**

❑Glycogen synthetase.

❑Pyruvate kinase.

❑Pyruvate dehydrogenase.

❑Acetyl CoA carboxylase.

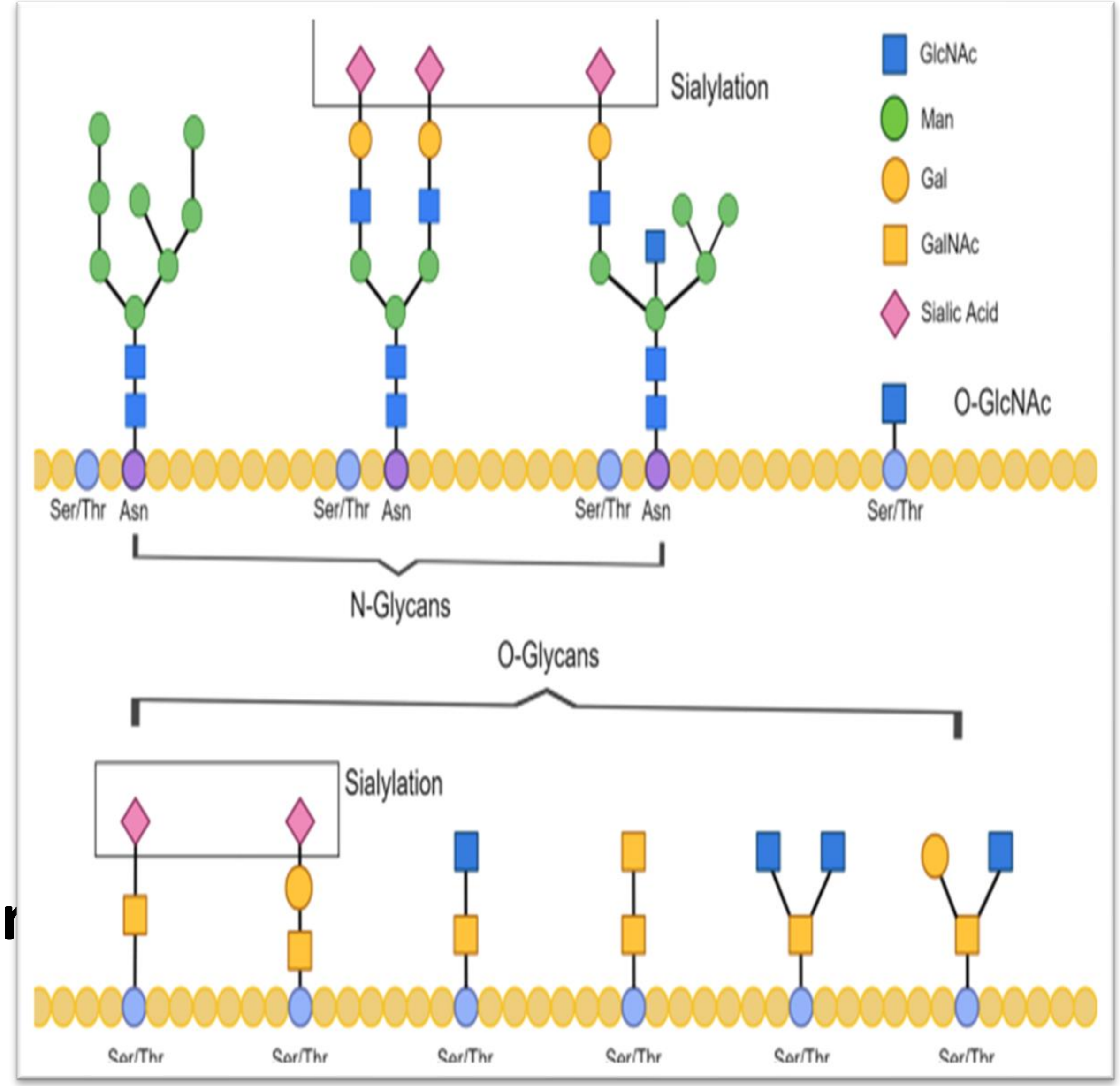


Examples of covalent modifications:

B) Protein Glycosylation

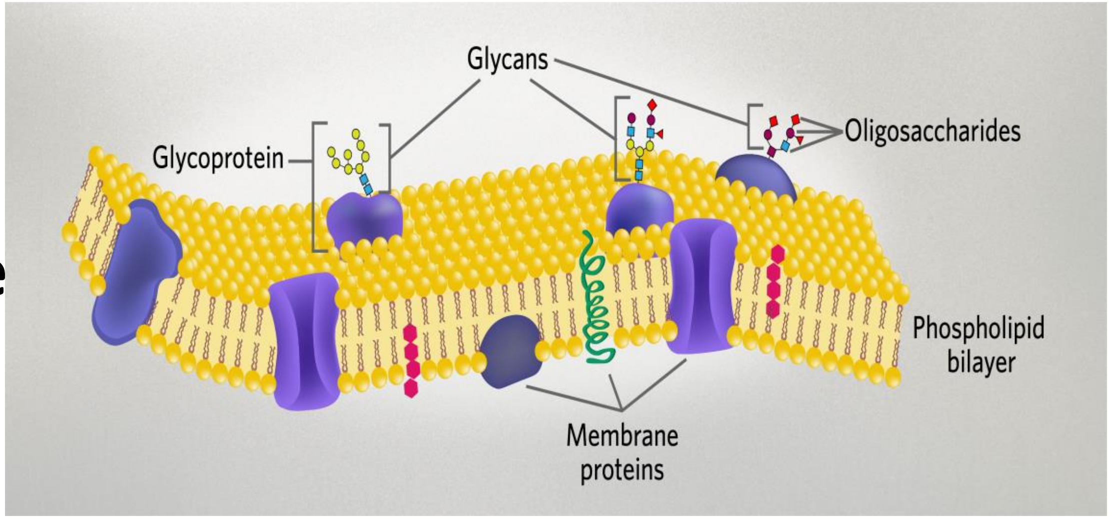
- **It involves** the covalent addition of a CHO part (glycans) to an amino acid, forming a glycoprotein.

- **Two major types:**
  - **N-linked,** whereby a sugar molecule is attached to the amide nitrogen of **asparagine**
  - **O-linked**, where a sugar molecule is attached to the oxygen atom of **serine or threonine.**

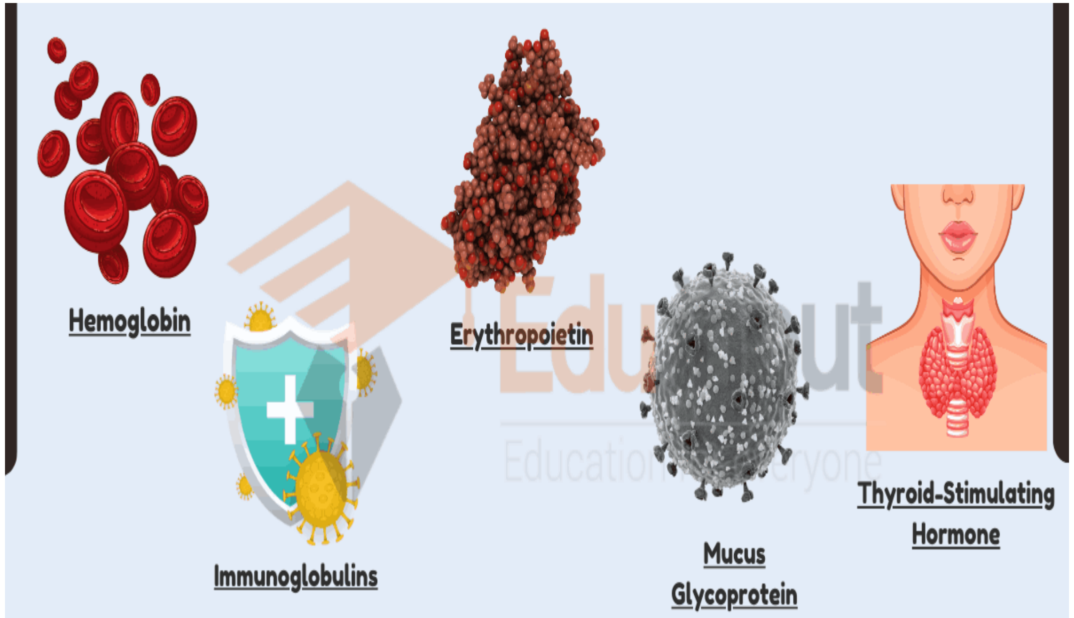


Examples of covalent modifications:

❑Importance of Glycosylation:



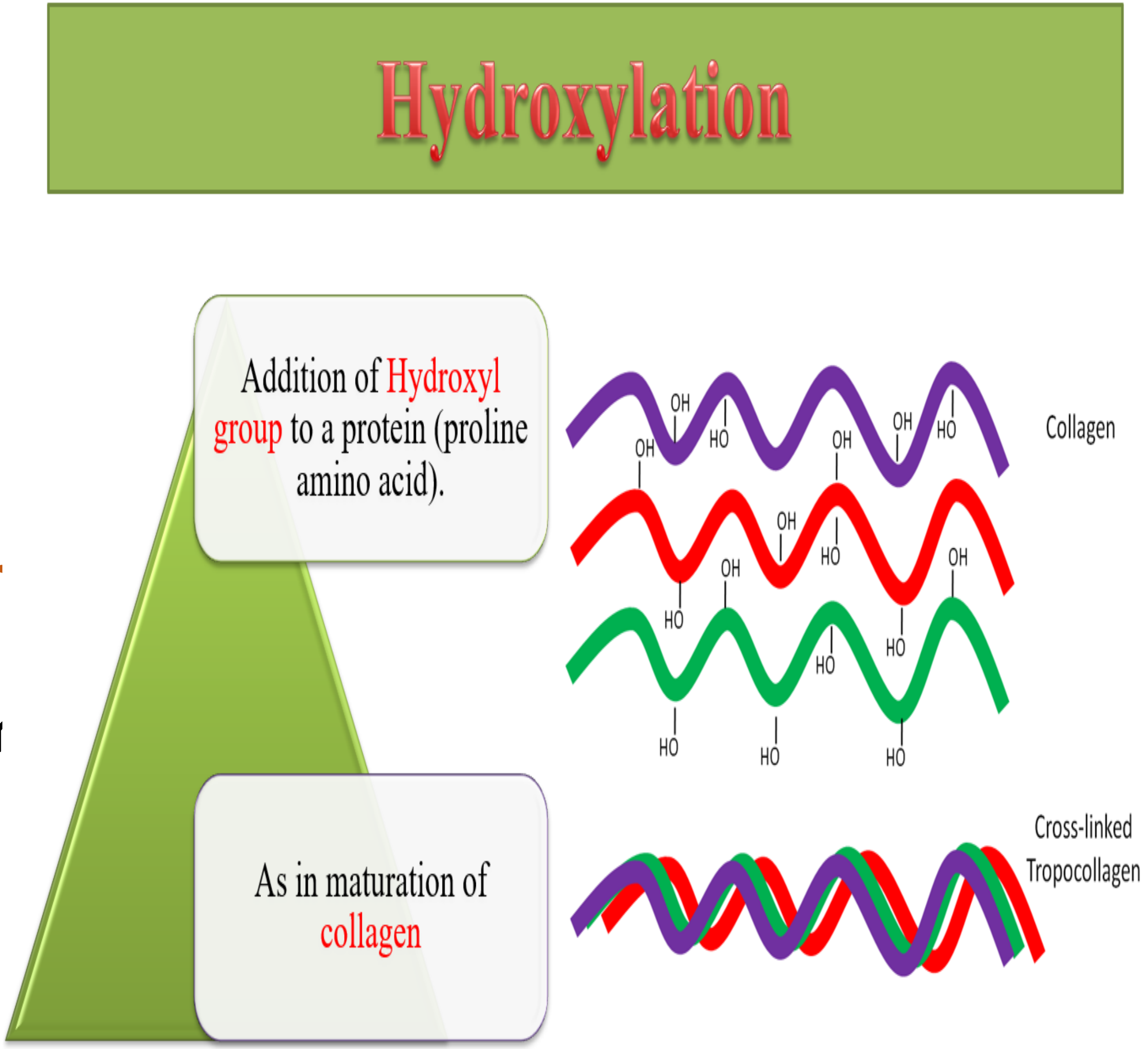
- Structural functions: e.g. in cell membrane
- Hemoglobin
- immunoglobulins : immunity
- human blood group types.
- Some hormones & their receptors



Examples of covalent modifications:

C) Hydroxylation:

- During collagen maturation, proline and lysine of procollagen are hydroxylated to give hydroxyproline and hydroxylysine present in collagen
- This occurs in endoplasmic reticulum and requires vit. C

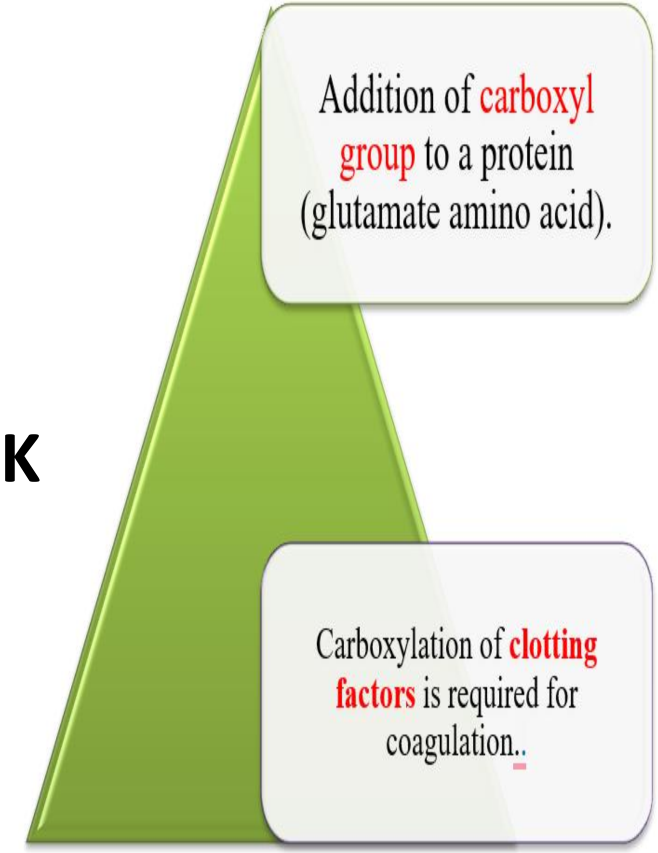


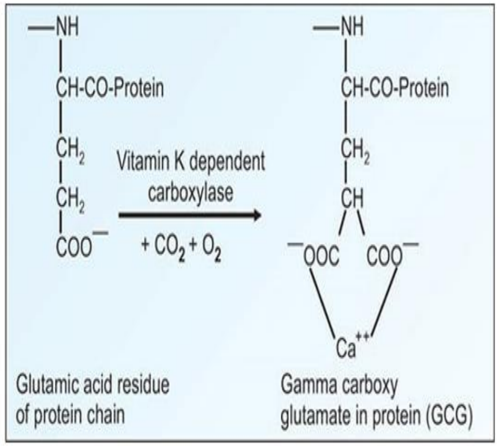
Examples of covalent modifications:

D) Carboxylation

- γ-carboxylation of glutamic acid residues in prothrombin to form γ-carboxyglutamate.
- This occurs in liver and requires vit. K

Carboxylation

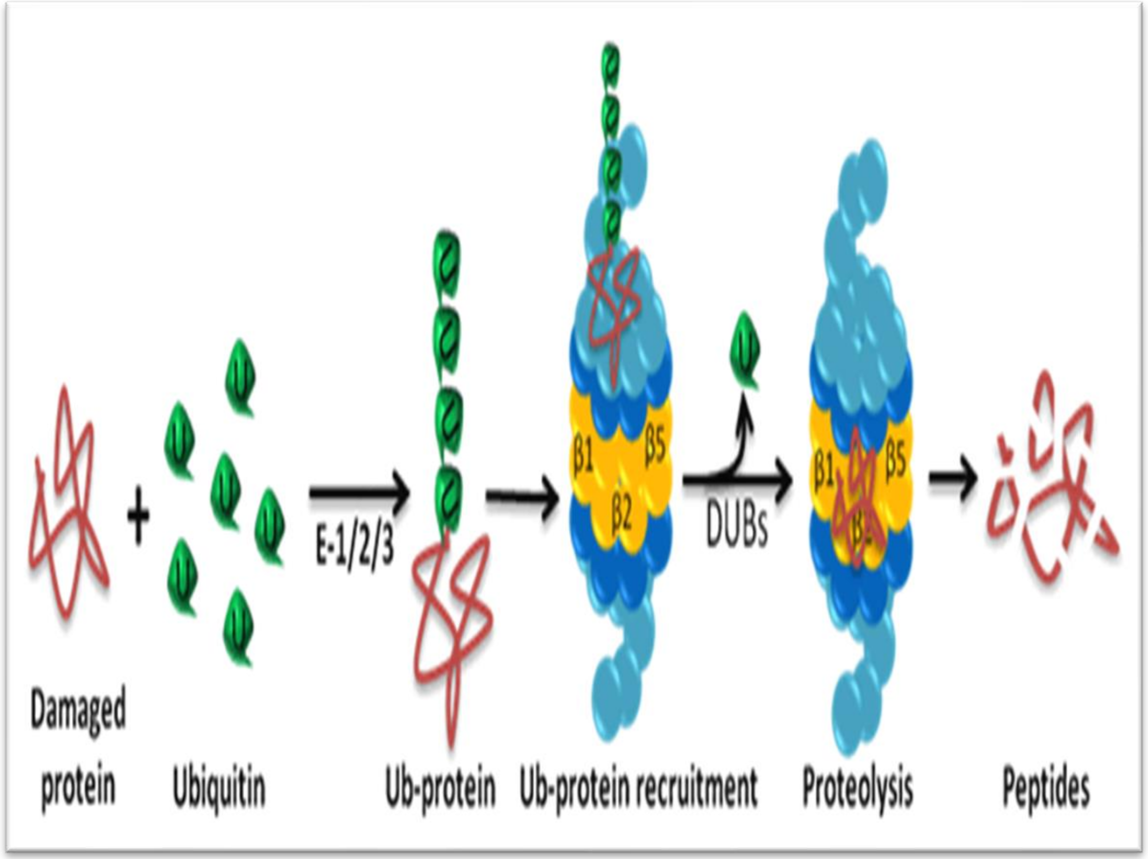


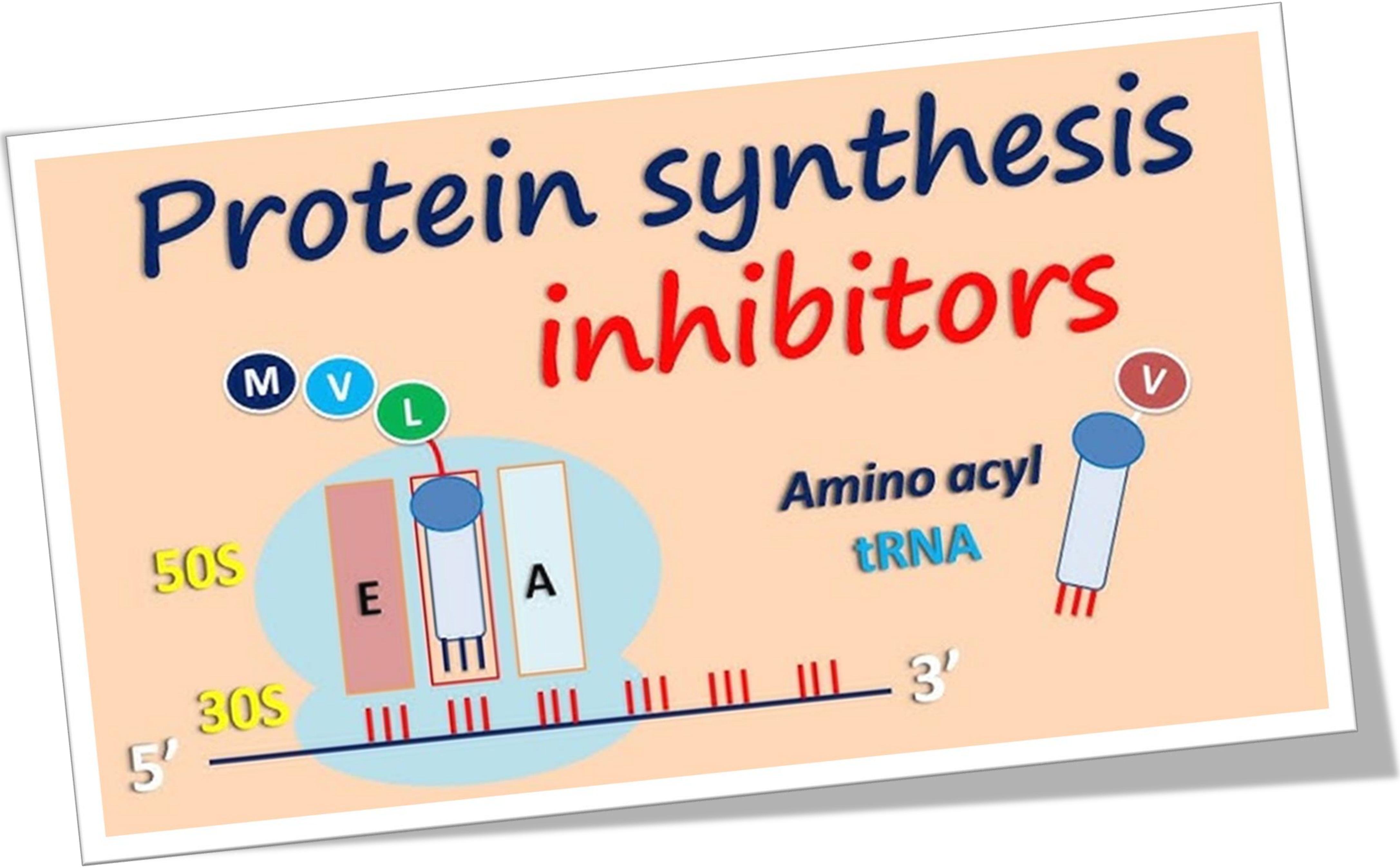


Examples of covalent modifications:

E) Protein ubiquitination

- Binding of Ubiquitin, a small protein molecule to lysine residues of a specific protein
- Ubiquitin addition acts as cellular "eat-me" signals, to mark protein for degradation by proteasomes.
- Dysregulation of this system has been implicated in diseases such as cancer and neurodegeneration.

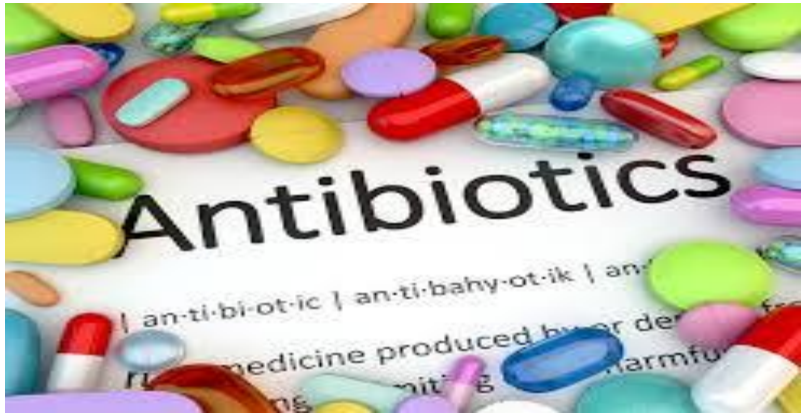



Protein synthesis
inhibitors
M
V
L
50S
E
A
30S
5′
3′
Amino acyl
tRNA
V

Inhibitors of translation

1. Inhibitors of Prokaryotic Protein Synthesis

- Protein synthesis in bacteria is a primary target for antibiotics



- Bacteria need to produce proteins to:
  - Grow
  - Reproduce
  - Maintain their structure

- By blocking protein synthesis, antibiotics essentially **“starves” the bacteria of essential proteins**, stopping infection spread.

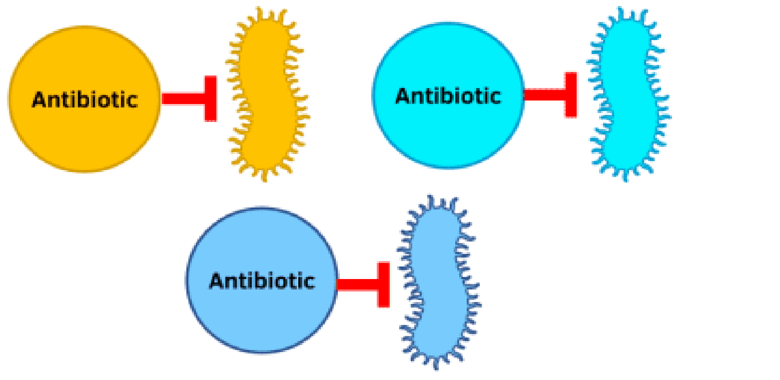


Fig 1. Antibiotics are specific. Each bacteria requires a specific antibiotic.

Inhibitors of translation

1. Inhibitors of Prokaryotic Protein Synthesis

a) Reversible Inhibitors **(bacteriostatic effect):**

- Tetracycline
- Chloramphenicol
- Macrolides (Erythromycin)

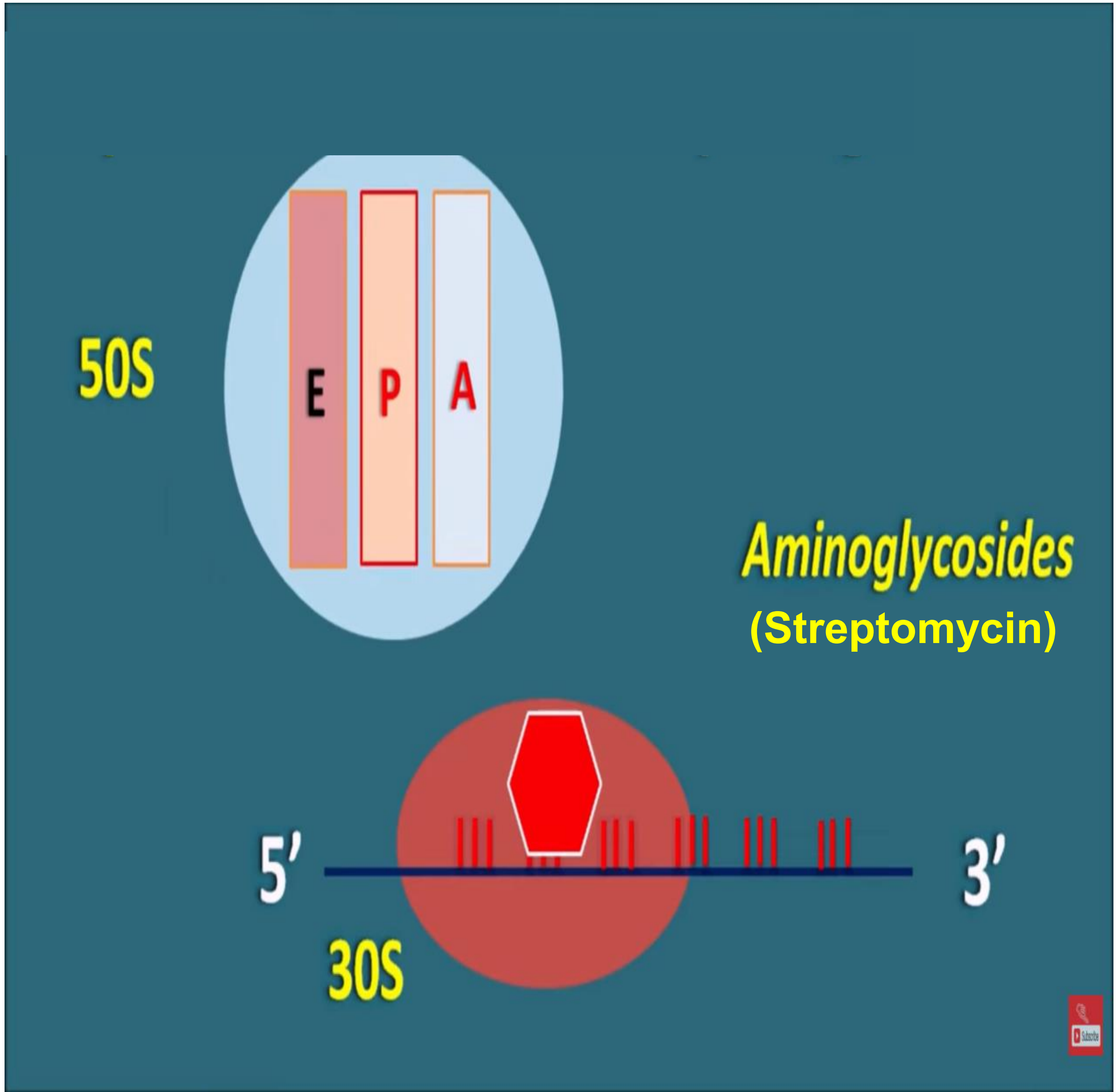
b) Irreversible inhibitors **(bactericidal effect):**

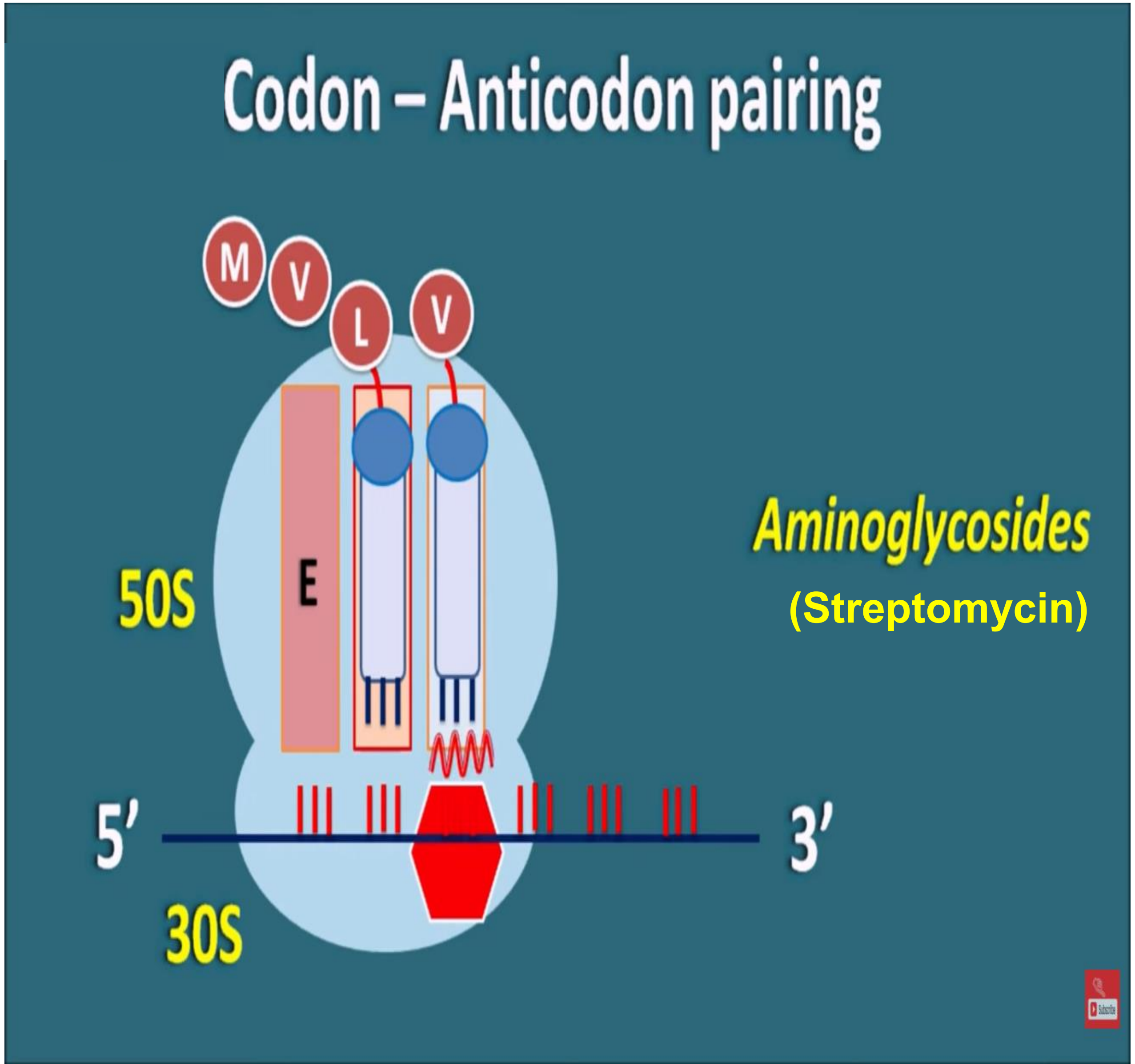
Aminoglycosides eg: streptomycin, neomycins and gentamycins

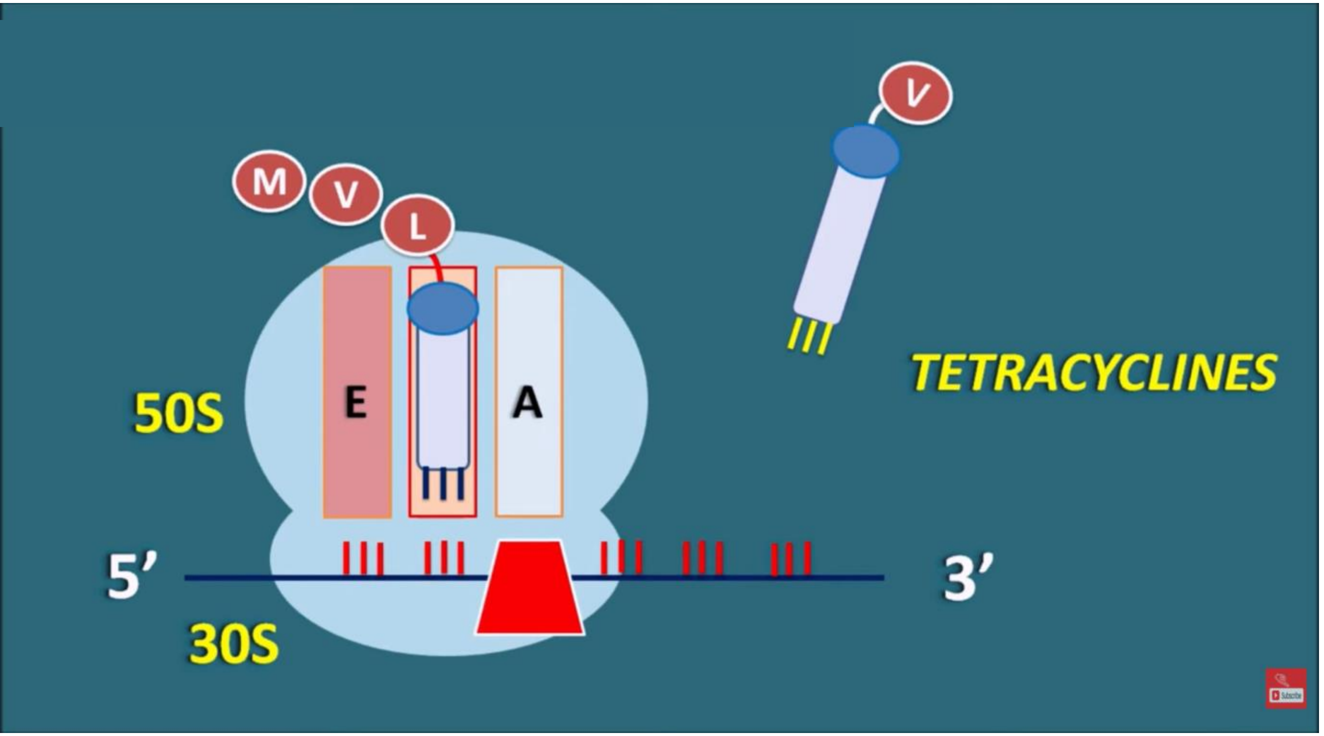
Antibiotics & inhibition of translation

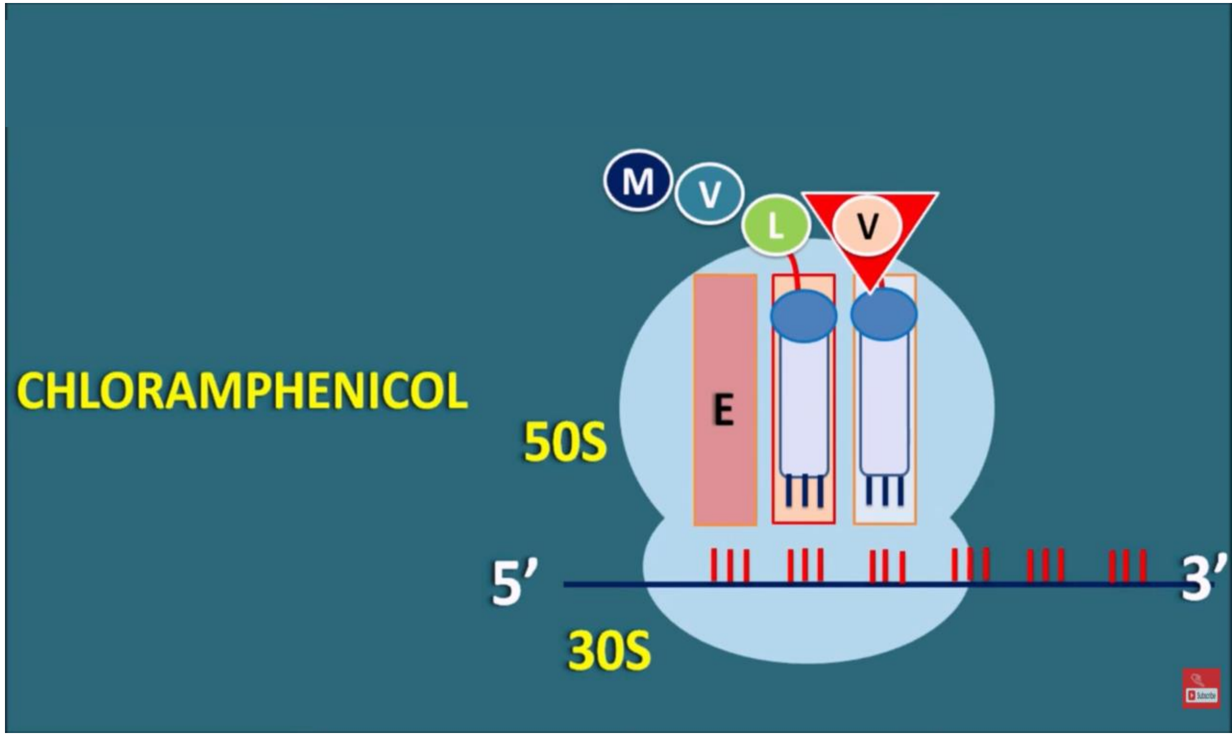
Name	Target (binds to)	Function
❑ Streptomycin (Aminoglycosides)	30S	Interfering with the initiation step, or misread mRNA
❑ Tetracycline	30S	Block the A site to prevent binding of aminoacyl-tRNA
❑ Chloramphenicol	50S	Inhibits peptidyl transferase (prevent peptide bond formation).
❑ Erythromycin (Macrolides)	50S	Block elongation by interfering with translocation step
❑ Puromycin	ribosome of Pro and Eukaryotes	Premature termination

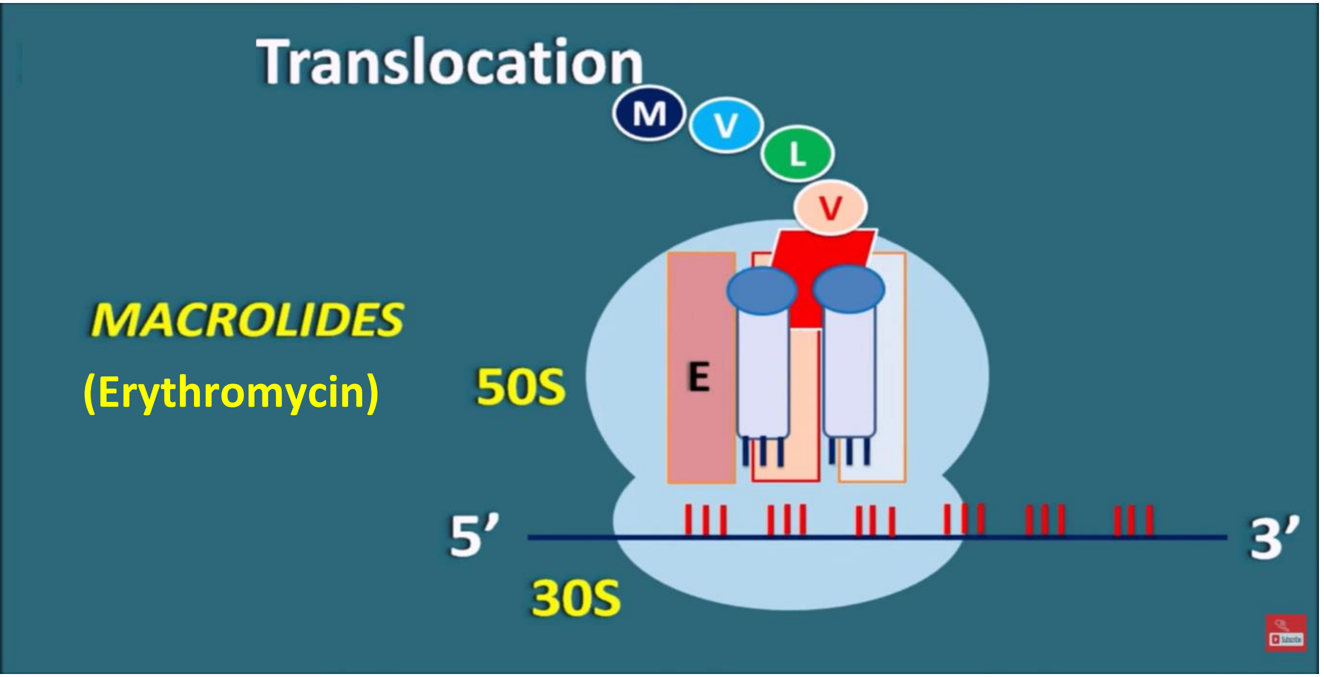
Antibiotics & inhibition of translation

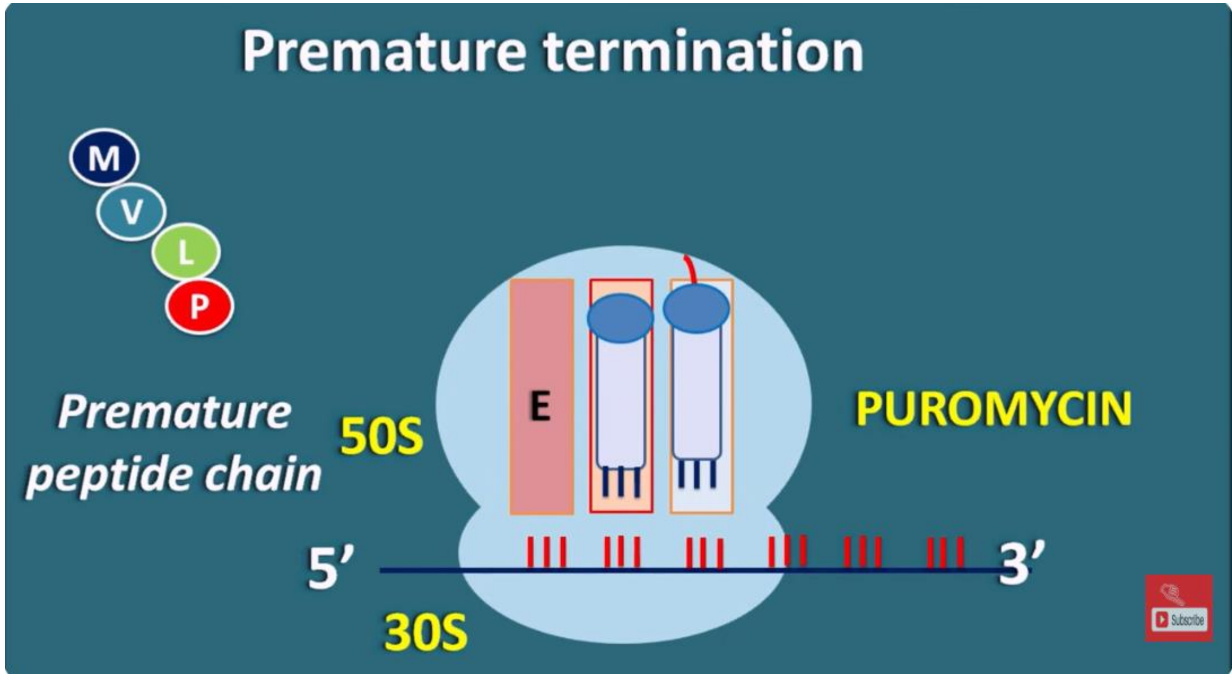





V
M
V
L
E
A
50S
TETRACYCLINES
5′
3′
30S

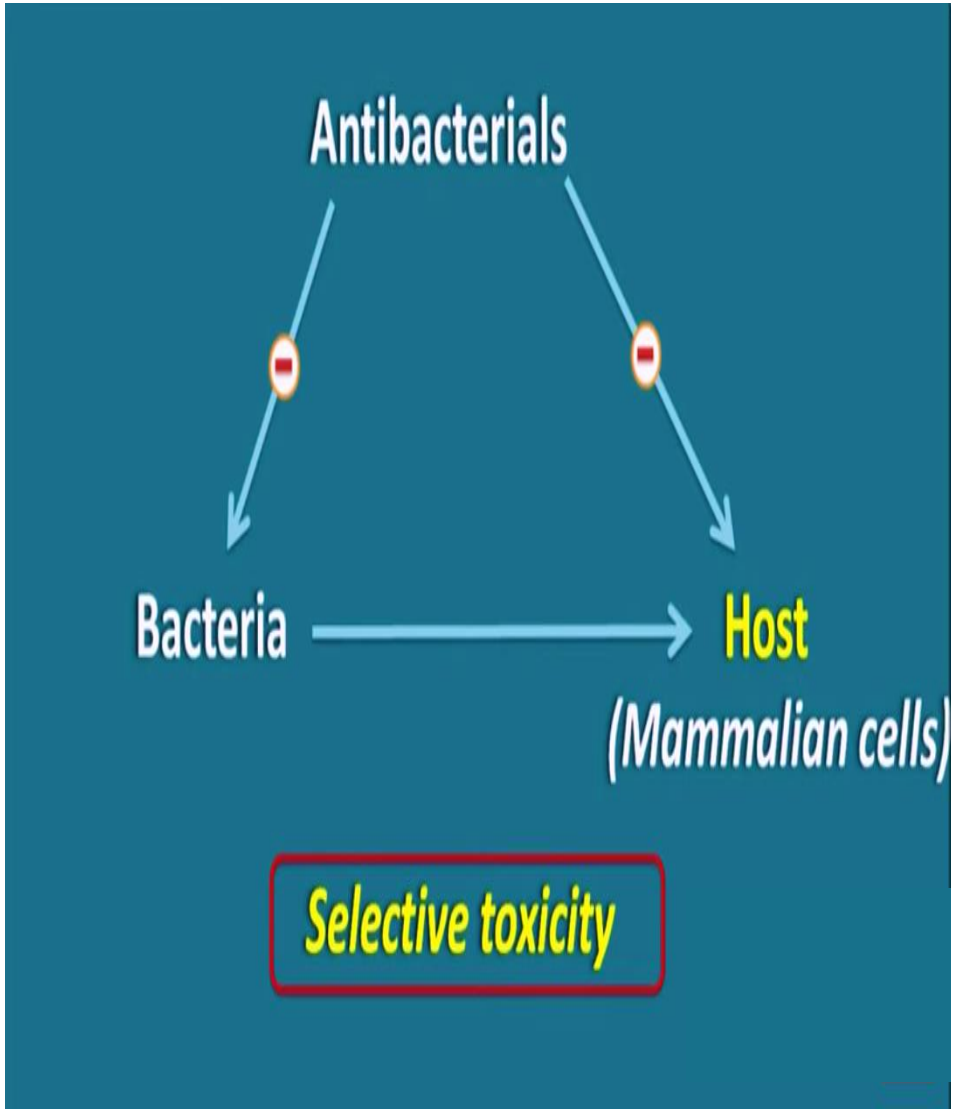

M
V
L
V
CHLORAMPHENICOL
E
50S
5′
3′
30S


Translocation
M
V
L
V
MACROLIDES
(Erythromycin)
50S
E
5′
3′
30S


Premature termination
M
V
L
P
Premature
peptide chain
50S
E
PUROMYCIN
5′
3′
30S

Antibiotics & inhibition of translation

- You use antibiotics like **erythromycin** safely because they **don't affect human ribosomes**
- But if a drug inhibits **eukaryotic protein synthesis**, it can damage:
  - Oral mucosa
  - Bone marrow
  - Healing tissues
- That's why selectivity is critical in pharmacology



Inhibitors of translation

2. Inhibitors of Eukaryotic Protein Synthesis

- Unlike antibiotics (which selectively target **prokaryotic ribosomes 70S**), inhibitors of **eukaryotic protein synthesis (80S ribosomes)** are generally **Toxic to human cells** , Therefore **NOT used as antibiotics.**

- **So where are they used?**

Inhibitors of translation

2. Inhibitors of Eukaryotic Protein Synthesis

1 Cycloheximide (Highly toxic and non-selective)

- Inhibits peptidyl-transferase in 60S subunit → block protein translation.
- commonly used in research to study protein degradation and induce apoptosis

2 Toxins: e.g. Diphtheria toxin

- Produced by *Corynebacterium diphtheriae.*
- Inhibits **elongation factor (EF-2)** → Stops protein synthesis → cell death

Case Report and Clinical Correlates

- Student's infection was treated with erythromycin.
- Erythromycin binds to the 50S ribosomal subunit of bacteria.
- It prevents the translocation step.


Thank You