ENZYMOLOGY - V

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Regulation of enzyme activity

Several ways to regulate enzyme activity:

1. Modulation of enzyme activity ➔ Changing the enzyme activity from being inactive to active or vice versa by editing enzyme structure.

A- Covalent modification ➔ Adding/removing parts to the enzyme usually phosphate group to transfer the enzyme from inactive to active or vice versa.

B- Allosteric modulation ➔ Adding inhibitors/activators at the allosteric site.

2. Proteolytic cleavage of proenzymes ➔ Removing some parts to the enzyme to transfer it from inactive to active.

Regulation of enzyme activity Cont:

3. Compartmentation ➔ Reactions take place at different sites of the same cell.

4. Enzyme production ➔ The most tough in regulating enzymatic activity because it is acting on the amount of enzyme produced // genetic level regulation.

5. Feedback inhibition ➔ There are two types feedback inhibition and feedback regulation.

IMPORTANT NOTES

- Note1: Phosphorylation / dephosphorylation mechanism is one of different mechanisms to regulate enzymatic activity that are considered as Hormonal regulatory mechanism.

- Note2: there are two types of hormones:

1. **Hydrophobic hormones**: able to penetrate (cross) the cell membrane // their receptors are located inside the cell.
2. **Hydrophilic hormones**: unable to penetrate (cross) the cell membrane // their receptors are extracellular or Trans membrane.

PHOSPHORYLATION / DEPHOSPHORYLATION MECHANISM

1. The hormone binds to its receptor, Once the hormone is binding to its receptor, they form Hormone-Receptor Complex.
2. This binding activates an integral membrane protein called G-protein:

- G-protein consist of 3 subunits (α, β, γ) as long as the subunits are linked together ➔G-protein is inactive. (Have quaternary structure).
- To turn G-protein to its active form a dissociation of G-protein to α-subunit and β, γ-subunits must occur. As if β, γ-subunits are blocking the activity of the α-subunit.
- α-subunit in the inactive form is linking a molecule of guanosine diphosphate.
- when the α-subunit dissociate β, γ-subunits from a substitution of the guanosine diphosphate on the α-subunit with guanosine triphosphate occur thus α-subunit is activated.

PHOSPHORYLATION / DEPHOSPHORYLATION MECHANISM

3. Active α-subunit activate an enzyme inside the cell called **adenylate (adenylyl) cyclase enzyme** which form a Signal transduction: formation of secondary messengers such as cAMP.

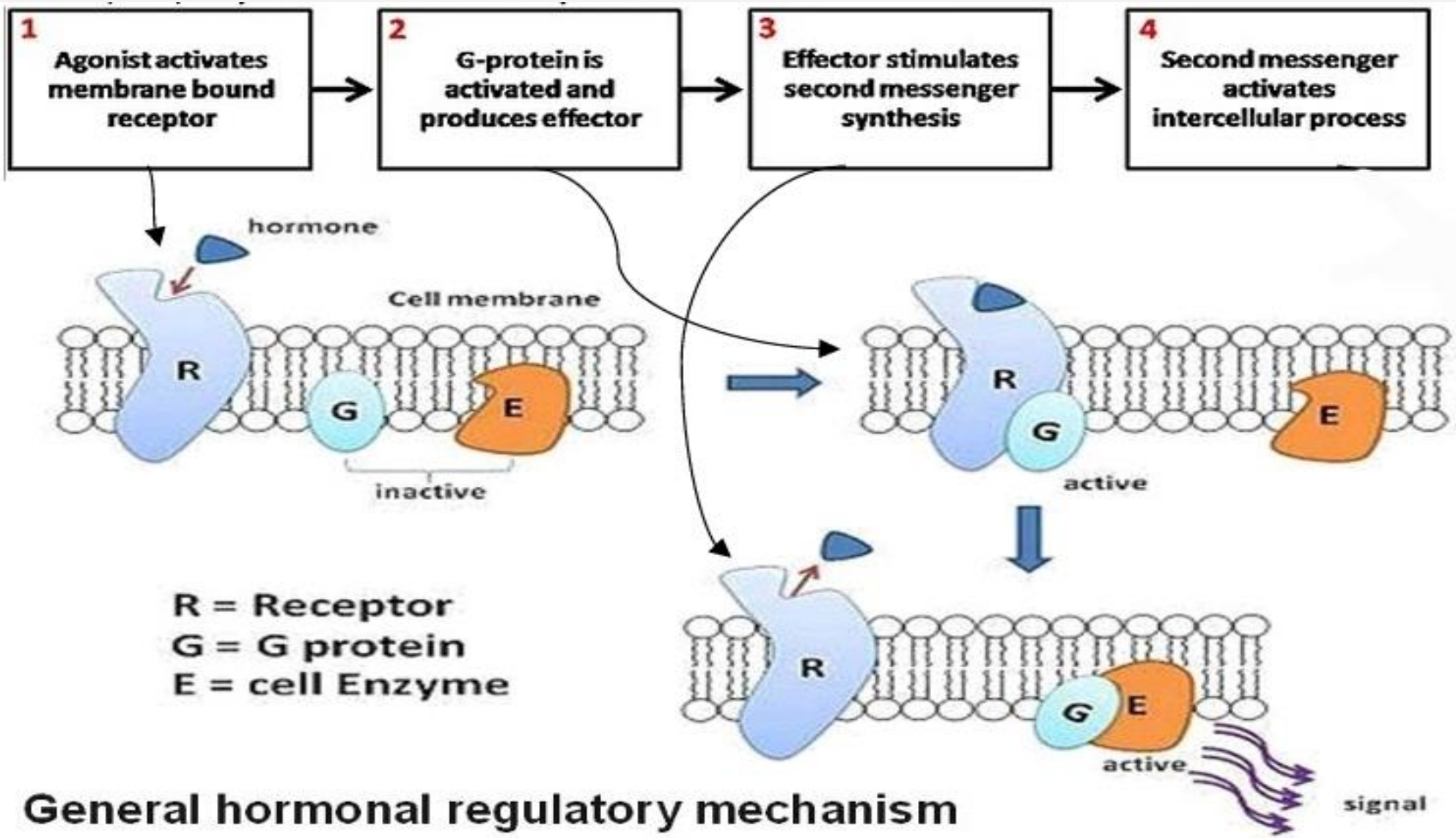
- In phosphorylation / dephosphorylation mechanism **adenylate cyclase enzyme** transfer part of ATP molecules contained in the cell into **cAMP** (cyclic adenosine monophosphate).

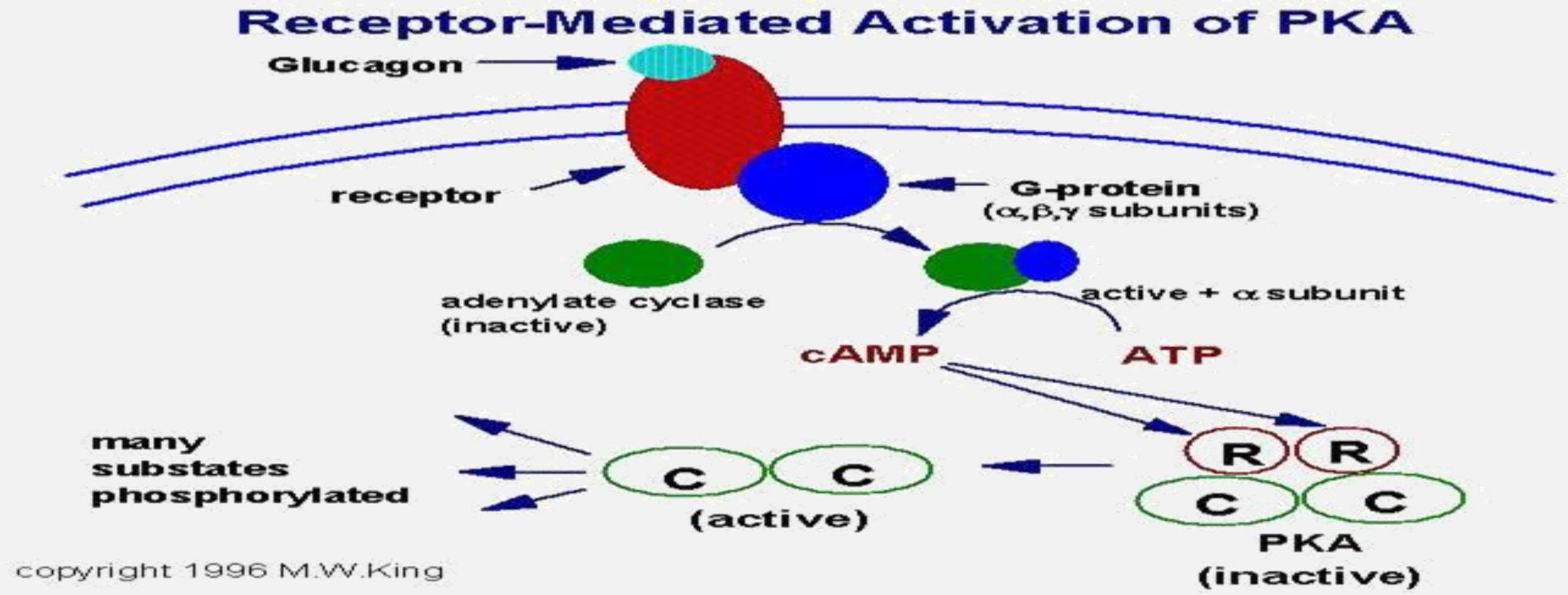
4. cAMP activate **protein kinase enzyme:**

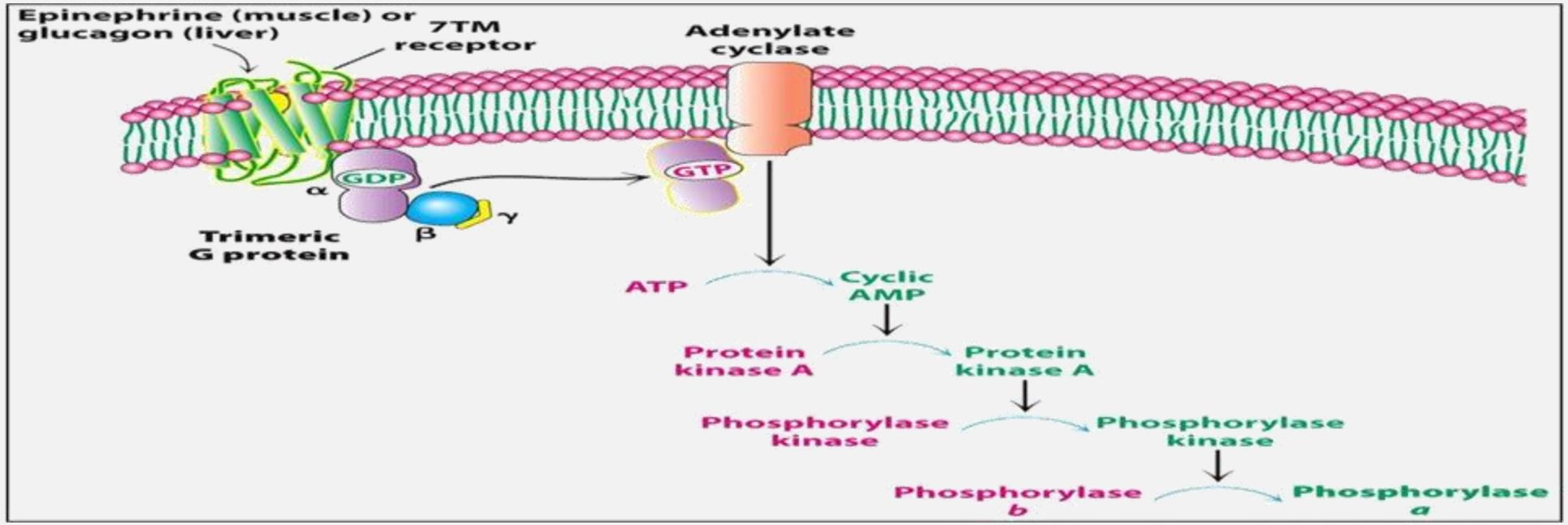
- **Protein kinase enzyme** consist of 4 protein subunits, 2R and 2C as long these dour subunits are linked together this enzyme is in active. As if R-subunits are controlling the activity of enzyme.
- The function of cAMP is to separate the 2R-subunits from the 2C-subunits thus the enzyme is activated.

5. Activate protein kinase enzyme start to phosphorylate a group of enzymes inside the cell.

Signal transduction: a cascade of reactions that start by forming secondary messenger like cAMP and by causing a change in the cell such as phosphorylation some of the enzymes.




Receptor-Mediated Activation of PKA
Glucagon
receptor
G-protein
(α,β,γ subunits)
adenylate cyclase
(inactive)
active + α subunit
cAMP
ATP
many
substates
phosphorylated
C
C
(active)
R
R
C
C
PKA
(inactive)
copyright 1996 M.W.King


Epinephrine (muscle) or
glucagon (liver)
7TM
receptor
Adenylate
cyclase
GDP
GTP
α
β
γ
Trimeric
G protein
ATP
Cyclic
AMP
Protein
kinase A
Protein
kinase A
Phosphorylase
kinase
Phosphorylase
kinase
Phosphorylase
b
Phosphorylase
a

B- <u>Allosteric regulation</u>:

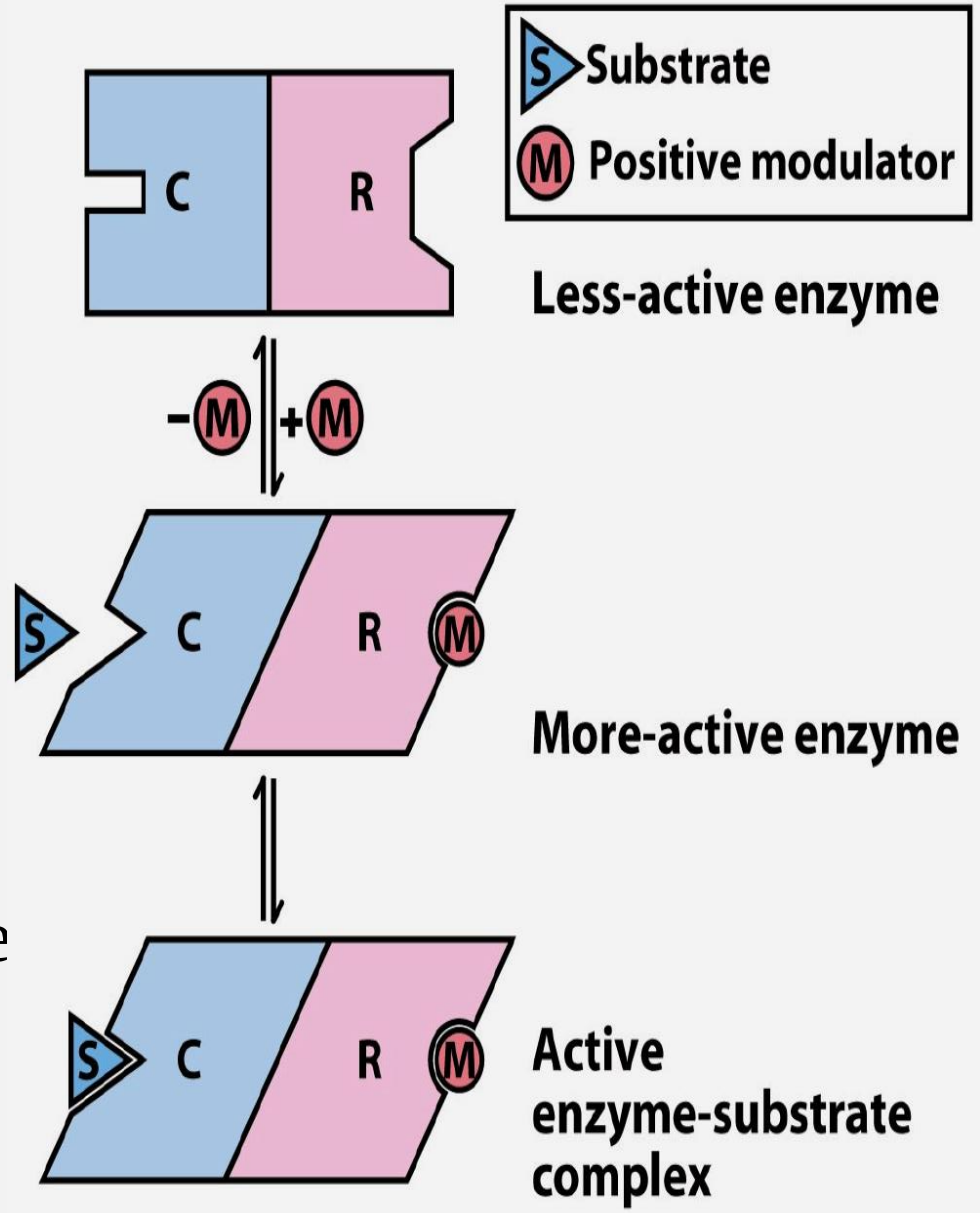
- Allosteric regulation is the term used to describe case where an enzyme is functioning at one site, then affected by binding of a regulatory molecule at another site.
- Allosteric regulation may either inhibit or stimulate an enzyme activity by changing the enzyme either to its active or inactive forms.

-The binding of an allosteric activator stabilizes its active form, while binding the allosteric inhibitor stabilizes the inactive form of the enzyme.

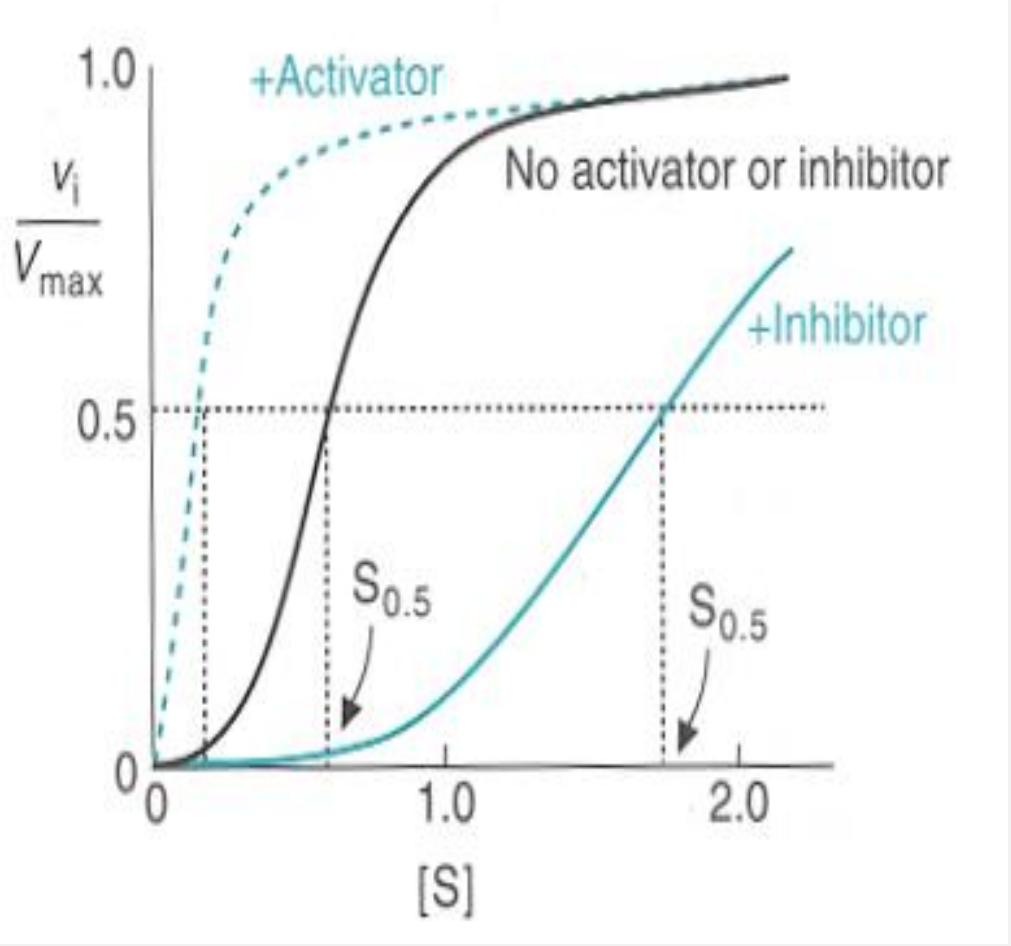
- End products are often inhibitors.
- Often allosteric modulators do not resemble the substrate or the product of the enzyme catalyzing the reaction.

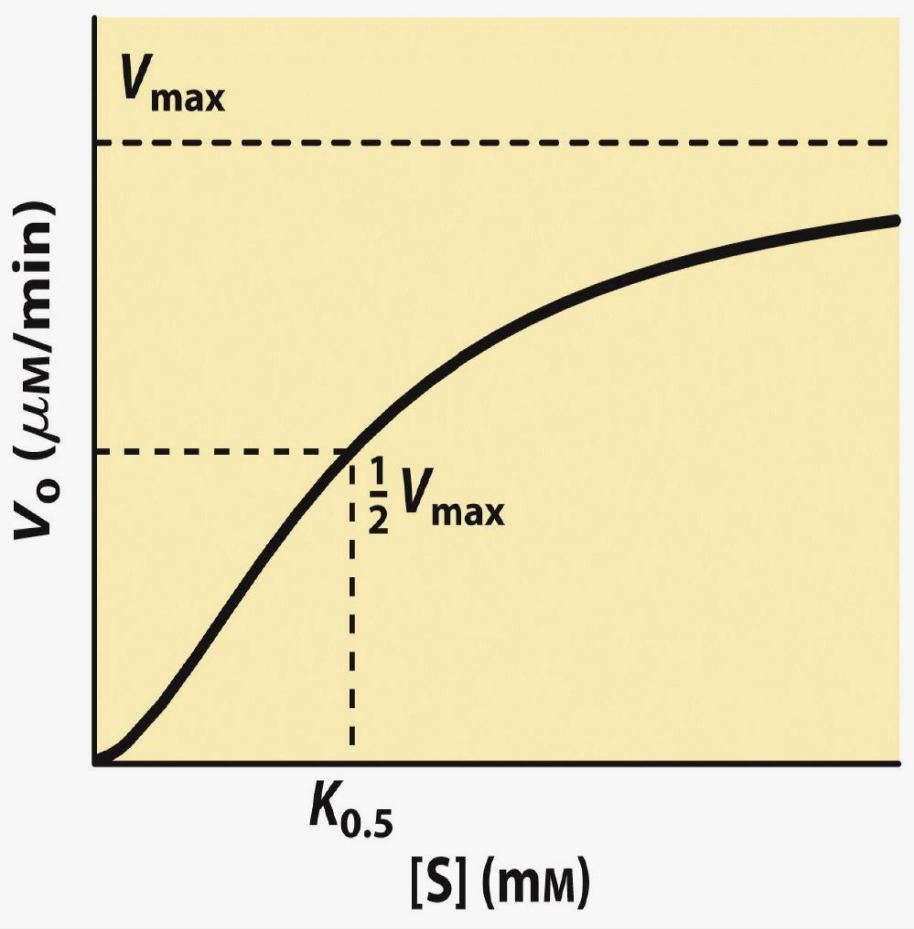
- Allosteric modulators bind non-covalently to the enzyme at a site rather than the substrate binding site.

- Allosteric enzymes usually have quaternary structure
- Allosteric enzymes do not exhibit typical Michaelis- Menton kinetics.
- Instead, the curve is sigmoidal, which indicates that the binding of substrate to the enzyme changes (e.g. increases) the affinity of the enzyme for substrate.
- Some allosteric modulators alters the Km, the Vmax remains constant.
-The modulators are not altered by the enzyme.



- **C = Catalytic (active site).**
- **R = Regulatory (allosteric binding).**
- Positive modifier: improves the active site for the substrate
- Negative Modifier: disrupt the active site





Allosteric regulation gives sigmoidal curve
Effects of a positive (+) and a negative (-) modulator that alter the Km without altering the maximum velocity Vmax

Activator = same Vmax – decrease Km (Faster)

Inhibitor = same Vmax – increase Km (Slower)

2- PROTEOLYTIC CLEAVAGE OF PROENZYME:

- Zymogens activation: certain proteins are synthesized and secreted as inactive precursor proteins known as **proproteins**. (thus, there is also proteins that are not enzymes that have immature form such as collagen ➔ procollagen)

- - The proproteins of enzymes are termed **proenzymes** or **zymogens**.

- - Selective proteolysis converts a proprotein by one or more successive proteolytic "**clips**" to a form that exhibits the characteristic activity of the mature protein, such as , its enzymatic activity. such as, its enzymatic activity.

- - The digestive enzymes pepsin, trypsin, and chymotrypsin (proproteins = pepsinogen, trypsinogen, and chymotrypsinogen, respectively), several factors of the blood clotting and blood clot dissolution cascades, are examples of Zymogen activation.

EXAMPLES OF ZYMOGEN ACTIVATION:

1. The **digestive enzymes** pepsin, trypsin, and chymotrypsin (proproteins = pepsinogen, trypsinogen, and chymotrypsinogen, respectively).

2. Several **factors of the blood clotting** and **blood clot dissolution cascades.**

When synthesized (through RER)	While its stored (in Golgi apparatus)	When secreted
preproinsulin	proinsulin	insulin
preprocollagen	procollagen	collagen

- Enzymes which work on proteins (digestive enzymes) such as (insulin, pepsin, etc.) have zymogens, why?

❖ Imagine that these types of enzymes where secreted or stored in there active form inside the secretory vesicles in the Golgi apparatus thus they will start digesting the proteins of the secretory vesicles which cause a defect.

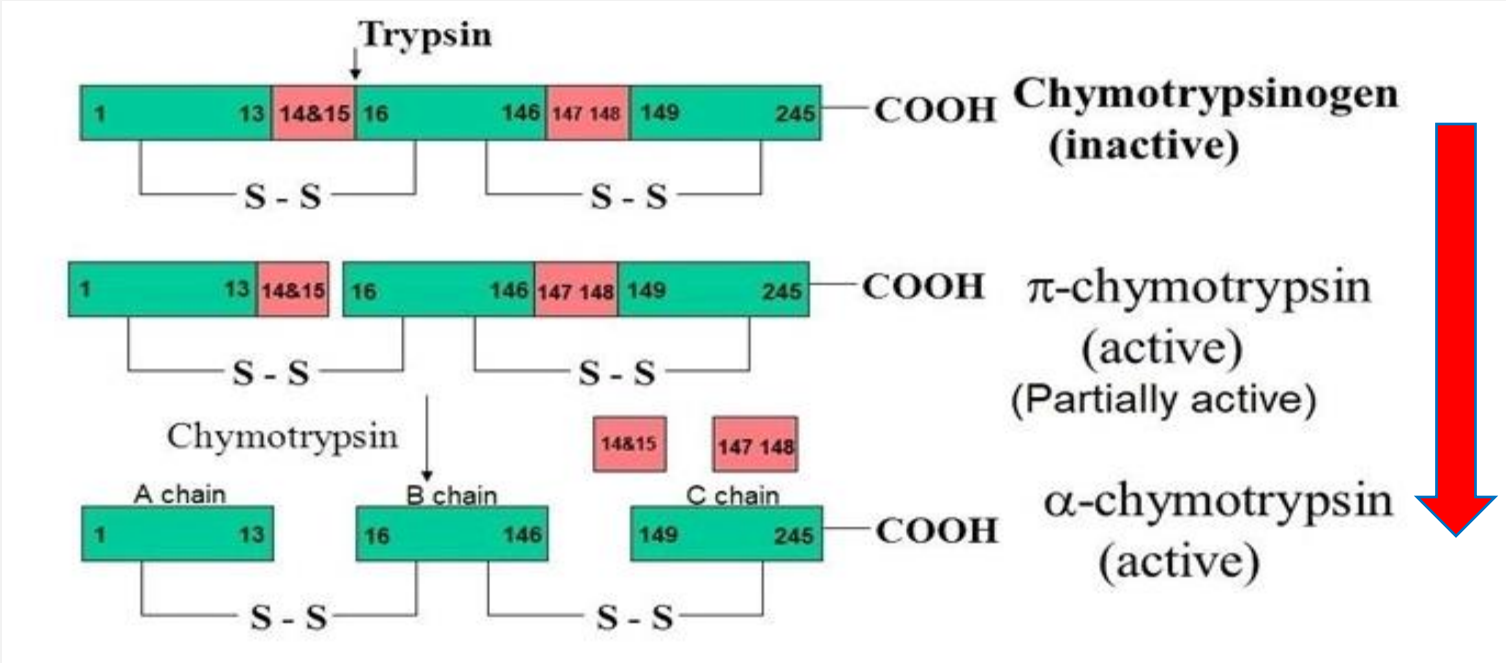
- **Chymotrypsin** activation by proteolytic cleavage:

❖ Activation requires removal of a blocking peptide by proteolytic cleavage.

❖ α-chymotrypsin chains are linked together due to bonds such as disulfide bond.

❖ This method is also known as clips.

❖ This method can be acted on other proteins as well, such as: coagulation of blood



Enzyme/substrate Compartmentation:

- Compartmentation **ensures metabolic efficiency** & **simplifies regulation (Importance).**
- Segregation of metabolic processes into distinct subcellular locations like the cytosol or specialized organelles (nucleus, endoplasmic reticulum, Golgi apparatus, lysosomes, mitochondria, etc.) is another form of regulation.

- Biological membrane: is the barrier which separate reactions from each other.

Examples of reactions and where does it take place

Plasma membrane	Amino acid transport systems, Na^+-K^+ ATPase
Cytosol	Glycolysis, glycogenesis and glycogenolysis, hexose monophosphate pathway, fatty acid synthesis, purine and pyrimidine catabolism, aminoacyl-tRNA synthetases
Mitochondria	Tricarboxylic acid cycle, electron transport and oxidative phosphorylation, fatty acid oxidation, urea synthesis
Nucleus	DNA and RNA synthesis
Endoplasmic reticulum (rough and smooth)	Protein synthesis, steroid synthesis, glycosylation, detoxification
Lysosomes	Hydrolases
Golgi apparatus	Glycosyl transferases, glucose-5-phosphatase, formation of plasma membrane and secretory vesicles
Peroxisomes	Catalase, D-amino acid oxidase, urate oxidase

- Question: Why there is a Segregation of metabolic processes into distinct subcellular locations is a form of regulation?

- Answer:

Because every enzyme inside the cell favor special conditions which at work at its maximum activity thus changing the enzyme location from one site to another will affect the enzyme so its activity would decrease or even the enzyme stop working.

Example:

In cytosol glycolysis take place then the products get transported through mitochondria membranes via transporting proteins in order for the electron transport chain (ETC) to take place.

But if the cell doesn't need to continue the (ETC) the transporting proteins won't transport the substrates from the cytosol to the mitochondria.

4- <u>Enzyme production (hormonal regulation)</u>:

- Enzyme synthesis (transcription and translation of enzymes genes) can be induced or decreased by hormonal activity that controls the genes.

-This mechanism of enzyme regulation is slower than other mechanisms (**long-term regulation**), i.e. covalent and allosteric modulation of enzyme activity.

- Causes changes in the concentration of certain "inducible enzymes" (are adaptive, i.e. synthesized as needed by the cell). (Constitutive enzymes synthesis is at a constant rate).
- Induction occurs usually by the action of hormones, (e.g. steroid and thyroxine) and is exerted by changes in the expression of gene encoding the enzymes.

- More or less enzyme can be synthesized by hormonal activation or inhibition of the genes.

ENZYME PRODUCTION (HORMONAL REGULATION/ GENETIC LEVEL REGULATION):

- **Genetic Level Regulation**: we have 2 types of genes:

1. **Constitutive genes**: gene is active all the time and is giving its products at constant rate (need no inducer or inhibitor).
2. **Induced genes**: need inducer and inhibitor // carried out under effect of hormones.

- Enzyme synthesis genes are considered Induced genes thus ➔
- Enzyme synthesis (transcription and translation of enzymes genes) can be induced or decreased by hormonal activity that controls the genes.
- But also, we have some constitutive enzymes that are synthesized at a constant rate.
- This mechanism of enzyme regulation ➔ causes changes in the concentration of certain "**inducible enzymes**" (are adaptive, i.e. synthesized as needed by the cell), whereas "**Constitutive enzymes**" are synthesized is at a constant rate.

ENZYME PRODUCTION (HORMONAL REGULATION/ GENETIC LEVEL REGULATION) CONT:

- **Induction** occurs usually by the action of hormones, (e.g. steroid and thyroxin) and is exerted (applied) by changes in the expression of gene encoding the enzymes.
- More or less enzyme can be synthesized by hormonal activation or inhibition of the genes
- **Example**:
- Hyperglycemia stimulate the secretion of Insulin in order to decrease the amount of glucose in the blood through all possible mechanisms ➔ reach normal level.
- Hypoglycemia stimulate the secretion of Glucagon to increase the amount of glucose.
- In **hyperglycemia**:

1. Insulin induces increased synthesis of enzymes: glucokinase, glycogen synthase and PFK-1
2. Insulin decreases the synthesis of several key gluconeogenic enzymes (amino acid ➔ glucose).

- In **hypoglycemia:**

1. glucagon approximately do the opposite function of insulin

FEEDBACK INHIBITION VS. FEEDBACK REGULATION:

1. Feedback Inhibition:

- End product act as inhibitor which attack the rate limiting step enzyme ➔ attach at a specific site ➔ goes some conformational changes ➔inhibit the binding of the substrate ➔switching off the production of mediates.
- If the mediates are not metabolized in any other metabolic pathway their synthesis (after the inhibition of the pathway) will lead to accumulation thus will cause defects.

2. Feedback Regulation:

- Very similar to the hormonal regulation but instead of being under the effect of hormones it will be under the effect of substrates.
- Feedback regulation may affect any step during protein (enzyme) synthesis:

A) At DNA level: changing gene structure

B) At transcription level: controlling the rate of transcription

C) At translation level: modulating the translation

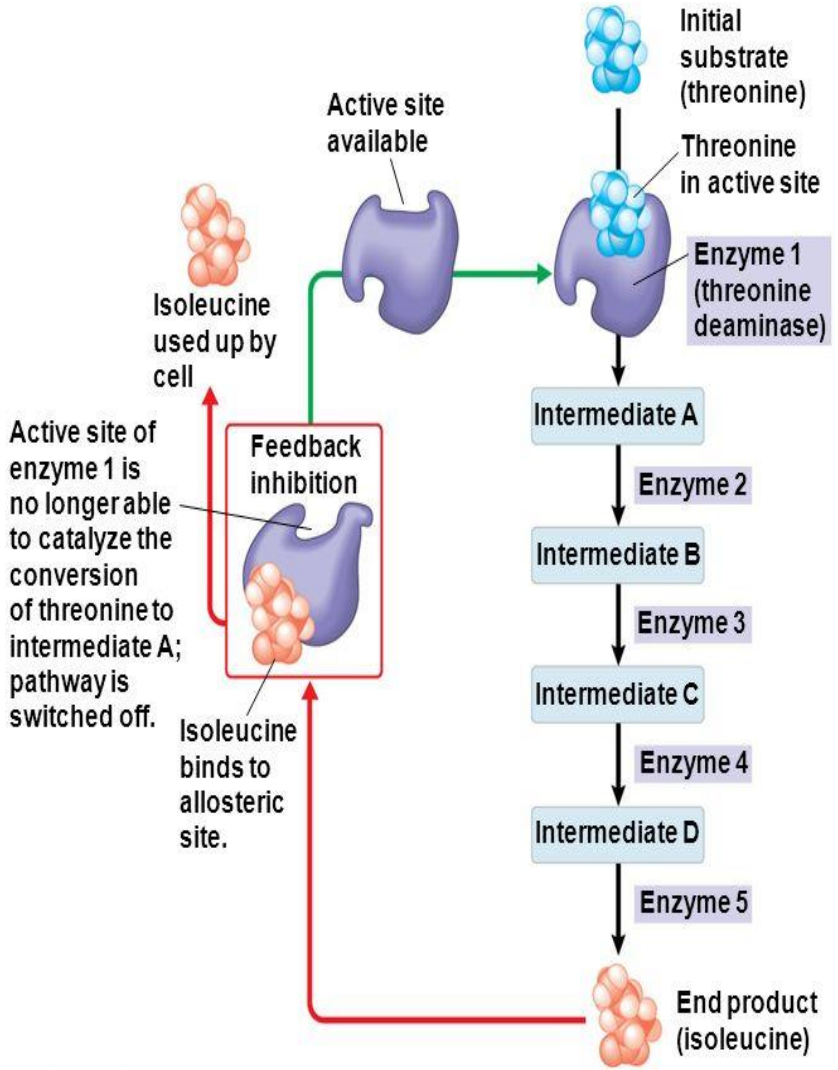
- Or even after protein synthesis: instead of being small and effective it can be grouped with other molecules of the proteins making them unable to function.

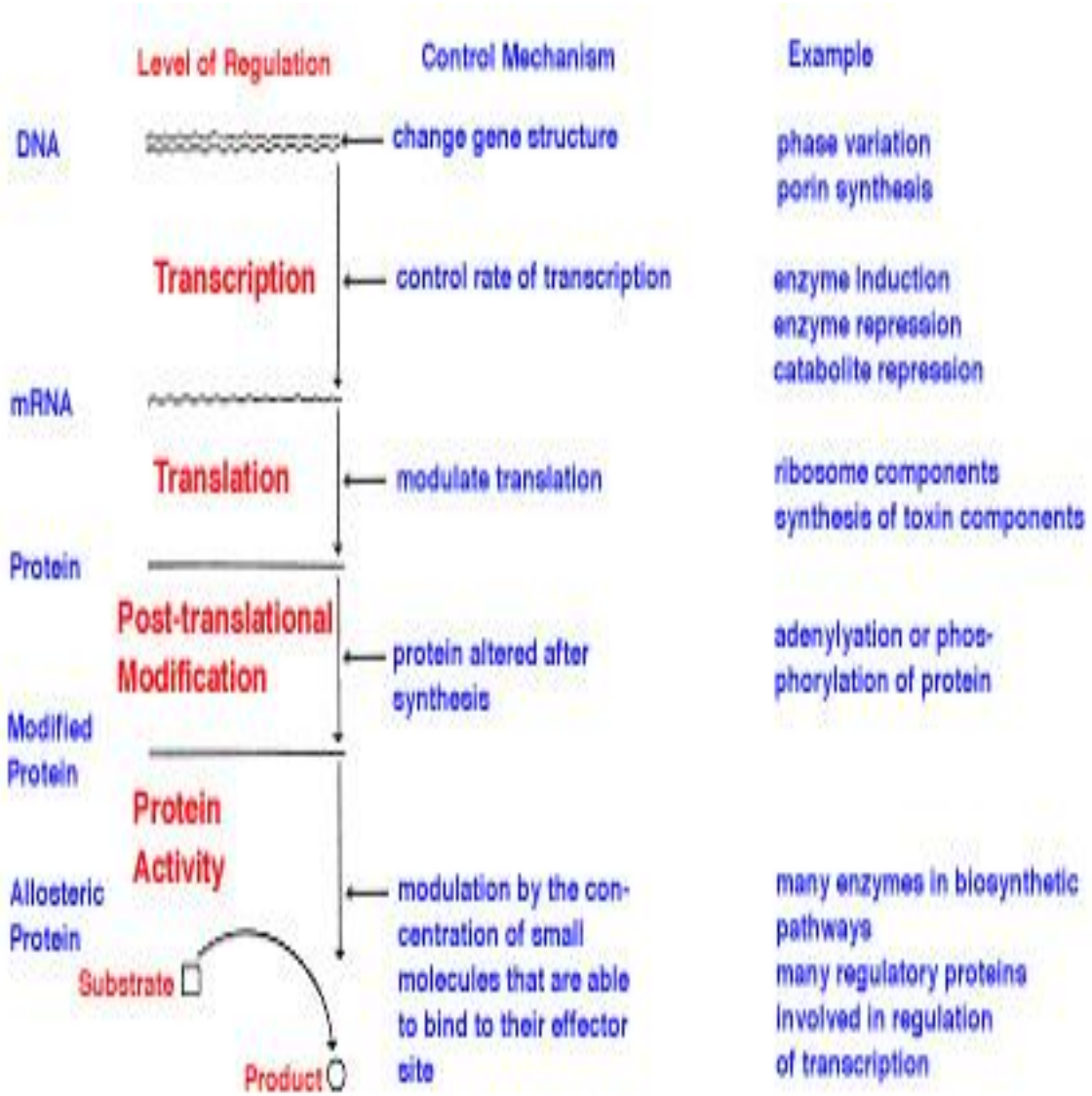
FEEDBACK INHIBITION VS. FEEDBACK REGULATION CONT:

- An example of Feedback inhibition: **Cholesterol**
- We need around 1 g of cholesterol every day regardless dietary or synthesized
- Dietary cholesterol decreases hepatic synthesis of cholesterol, (feedback regulation not feedback inhibition).
- HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis, is affected, but cholesterol does not feedback-inhibit its activity.
- Regulation in response to dietary cholesterol involves the effect of cholesterol or a cholesterol metabolite on the expression of the gene that encodes HMG-CoA reductase (enzyme repression).
- Dietary cholesterol ➔ Feedback regulation at translation level

LEVELS OF REGULATION

Figure 8.21





THANK YOU