Enzymology- An overview-4

Regulation of enzyme activity

This indicates that enzymes are not active all the time

Several ways to regulate enzyme activity:

1. Modulation of enzyme activity:

   A- Covalent modification.

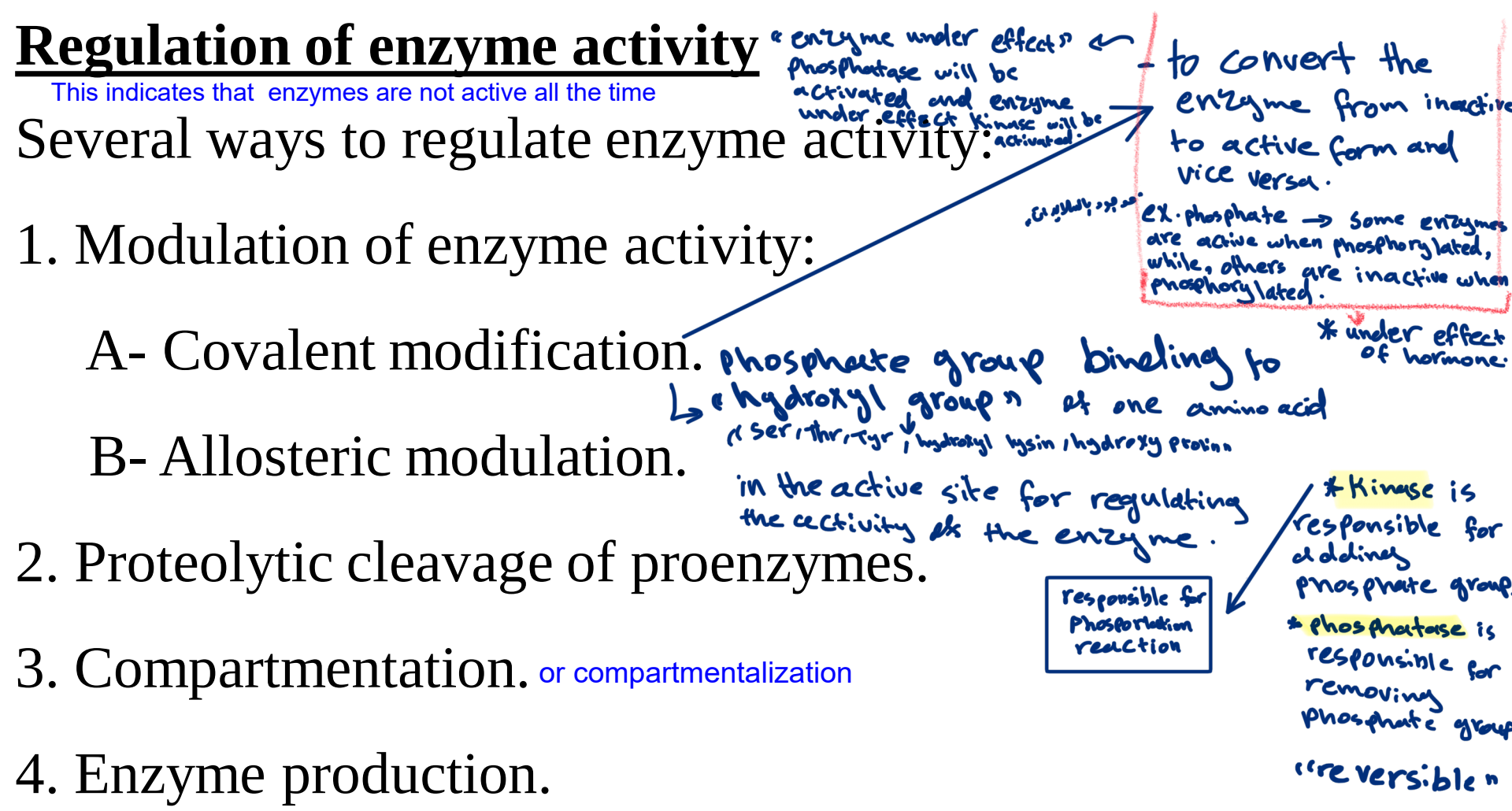
   B- Allosteric modulation.

2. Proteolytic cleavage of proenzymes.

3. Compartmentation. or compartmentalization

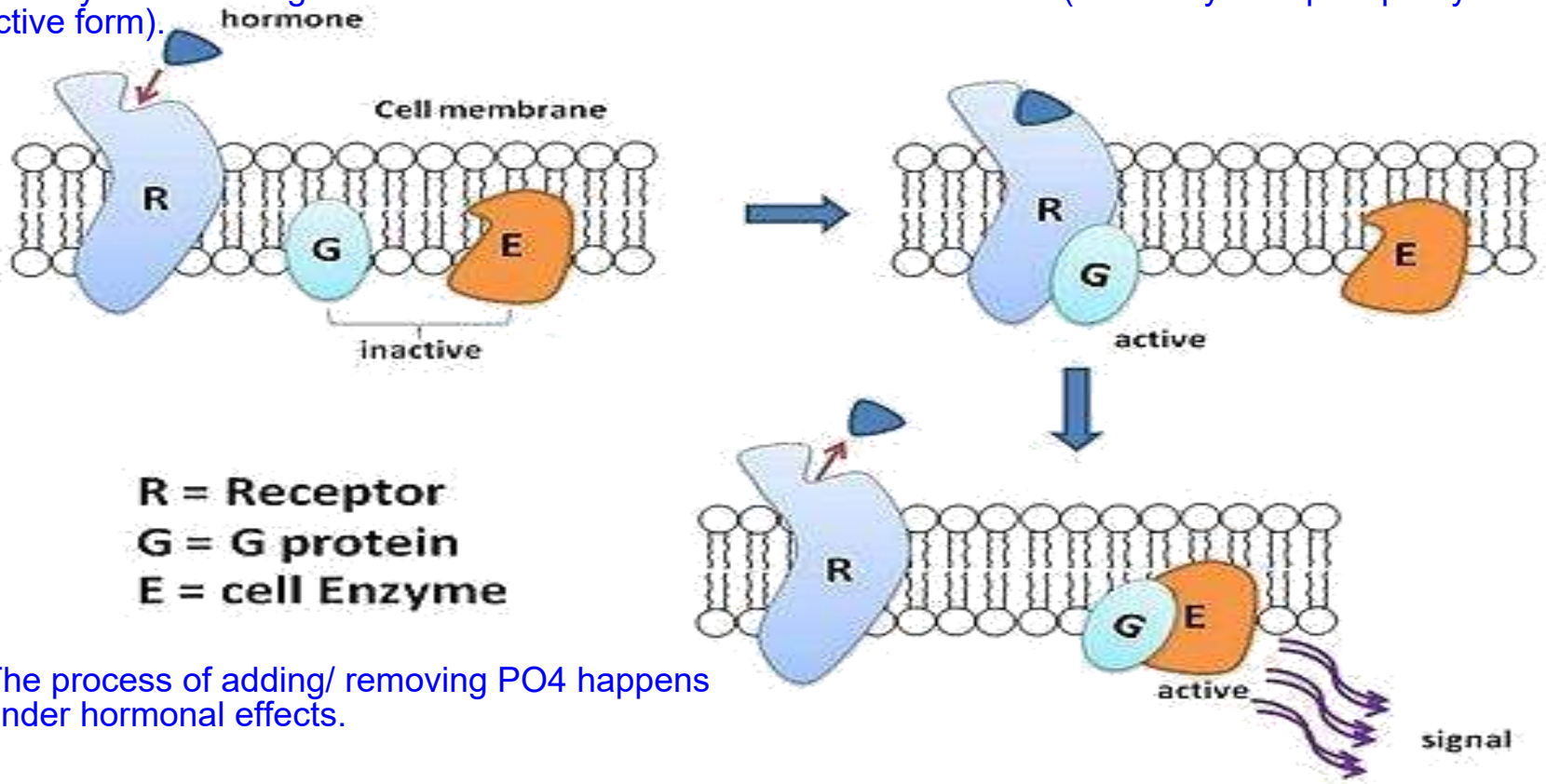
4. Enzyme production.

5. Feedback inhibition



- Usually by the addition of or lysis of phosphate (PO4) groups to and from enzymes.
- Some enzymes are active when phosphorylated, while, others are inactive when phosphorylated.

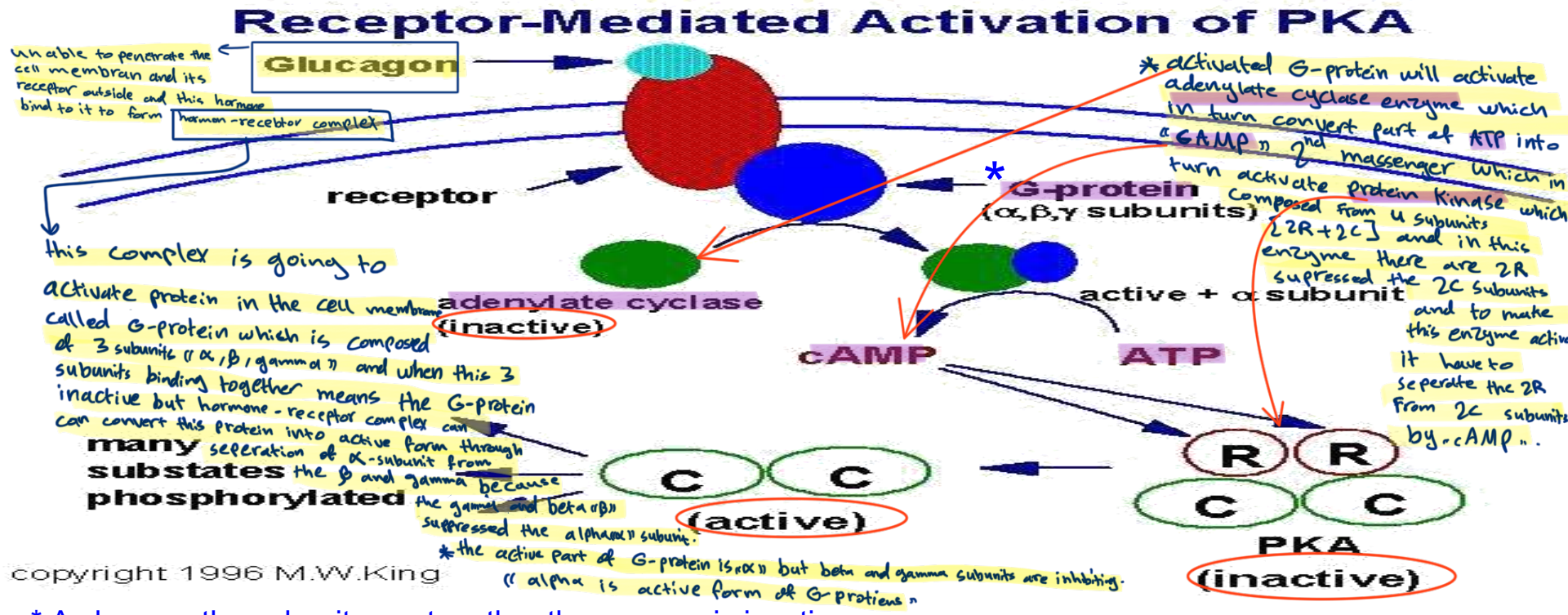
- Adding "by kinase enzyme" - or removing "by phosphatase enzyme"- phosphate group (PO4) to amino acids of an enzyme will change its status from active to inactive and vice versa (not always the phosphorylated form is the active form).



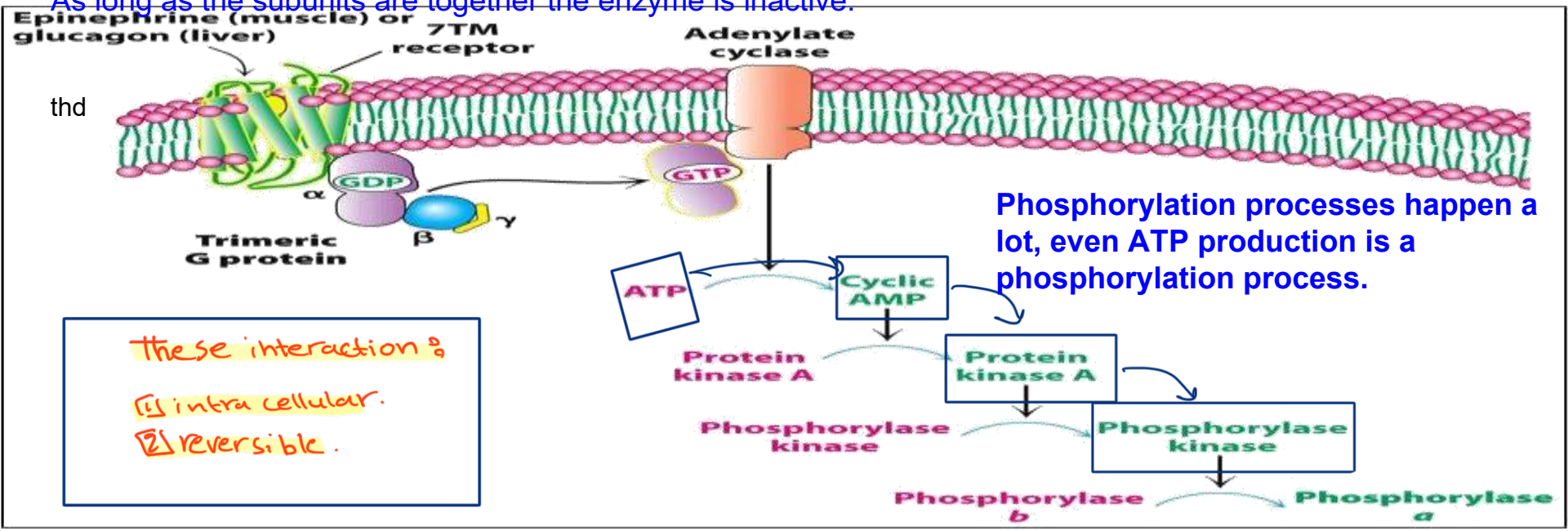
R = Receptor
G = G protein
E = cell Enzyme

The process of adding/ removing PO4 happens under hormonal effects.

Receptor-Mediated Activation of PKA



* As long as the subunits are together the enzyme is inactive.



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Phosphorylation processes happen a lot, even ATP production is a phosphorylation process.

these interaction is
1) intra cellular.
2) reversible.

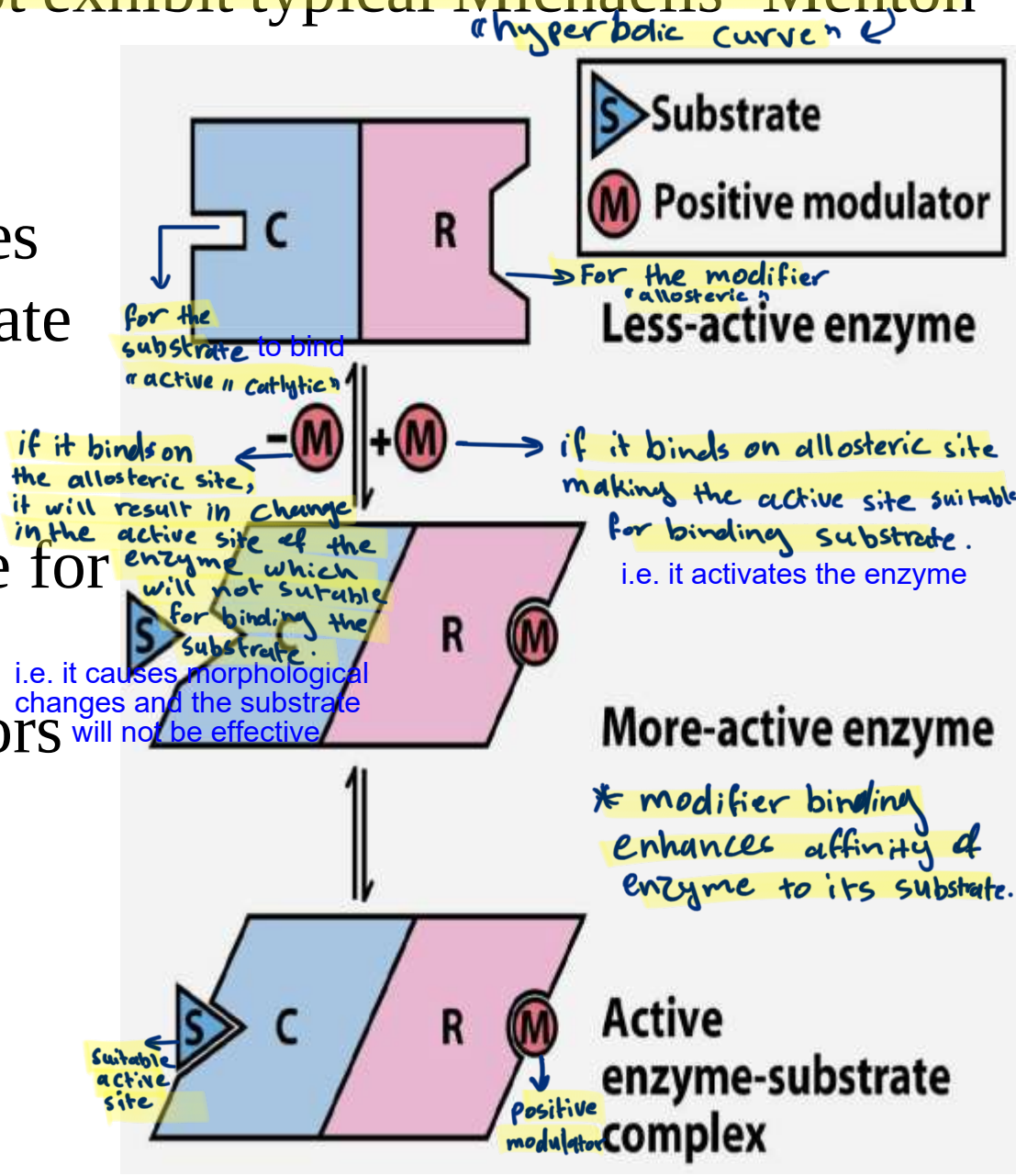
B- Allosteric regulation:

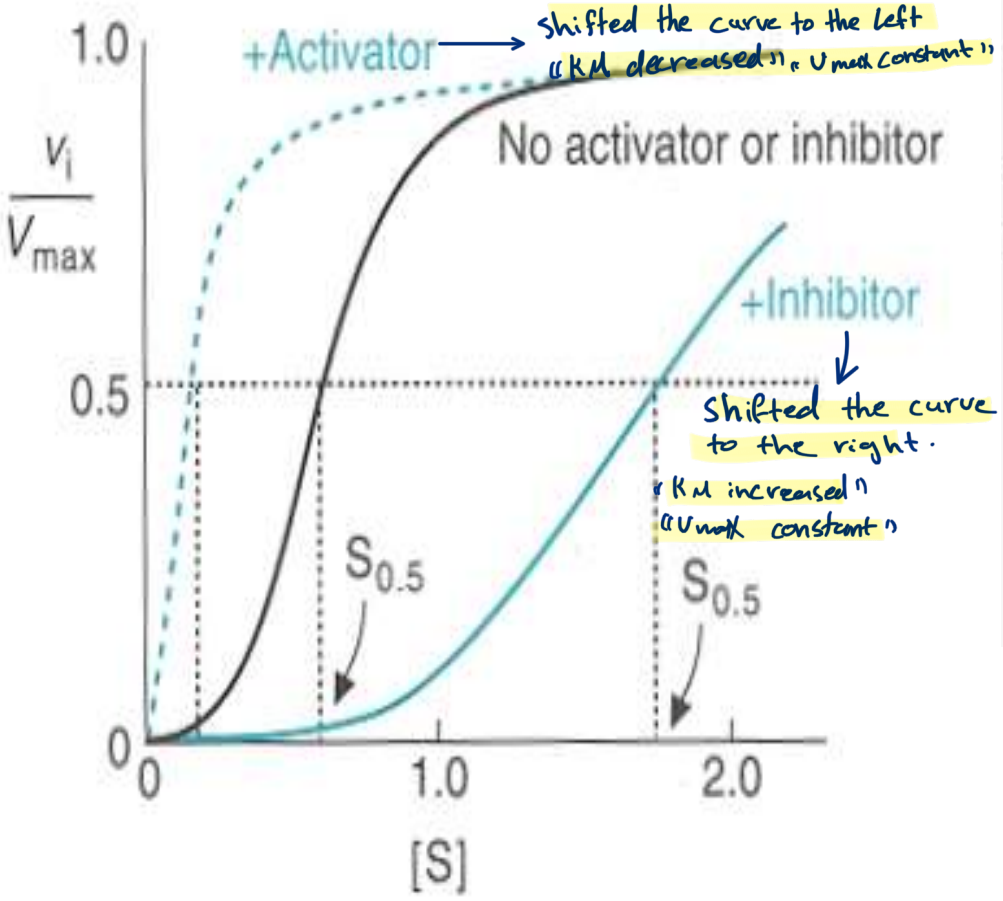
- Allosteric regulation is the term used to describe cases where an enzyme is functioning at one site, then, affected by binding of a regulatory molecule at another site.
- Allosteric regulation may either inhibit or stimulate an enzyme activity by changing the enzyme either to its active or inactive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

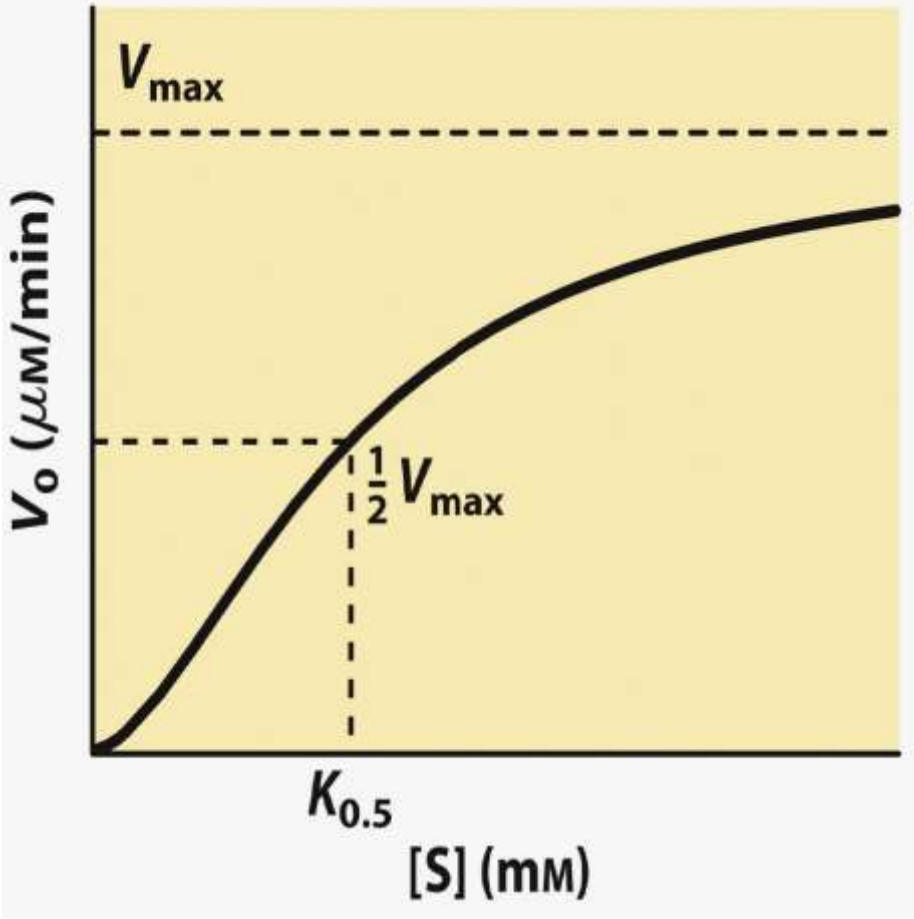
« modifier »

« active 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.







Allosteric regulation gives sigmoidal curve

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

2- **Proteolytic cleavage of proenzyme**:

- Zymogens activation: certain proteins are synthesized and secreted as inactive precursor proteins known as **proproteins**.

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

- 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.

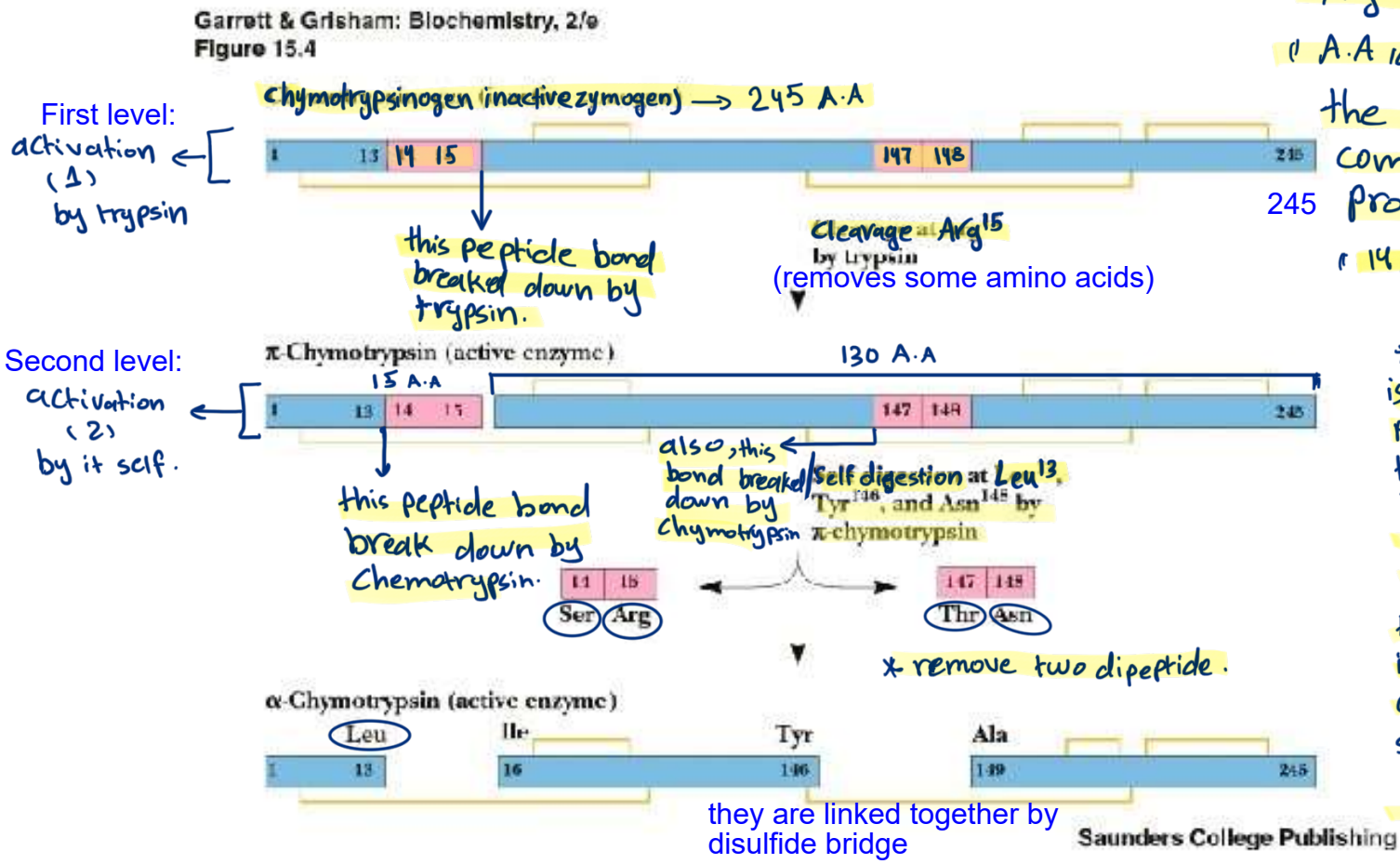
- The digestive enzymes pepsin, trypsin, and chymotrypsin (proproteins = pepsinogen, trypsinogen, and chymotrypsinogen, respectively), several factors of the blood clotting and blood clot dissolution cascades, are examples of Zymogen activation. (those are called digestive proteins; from the pancreas; i.e. coagulation factors are only activated when we are injured)

* any enzyme's name ends with «gen» or starts with «pro» means this enzymes are inactive « contain extra parts «amino acid» should be removed to be active.

* The digestive enzymes should be inactive at some points so they do not destroy stomach lining (walls).

Proteolytic cleavage of proenzyme(zymogen)

Activation happens in two levels:



*trypsin enzyme which is secerated by pancreas is reponsible for cutting between "Arg 15" and "A.A 16", then the chymotripsin complete the process by removing "14, 15, 147, 148" A.A

*chymotrypsinogen is secreted from pancreas then transported into intestines and this enzyme contain 4 A.A which are the reason of inactivation of this enzymes [14,15,147,148] should be removed for the activation of this enzyme by protelytic enzyme.

Enzyme/substrate Compartmentation:

- Compartmentation **ensures metabolic efficiency** & **simplifies regulation**
- 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

(not in the same location)

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

Ex. if a reaction started in the cytoplasm and finishes in the mitochondria it has to cross a double membrane, this is called "Pathway Regulation"

4- Enzyme production (hormonal regulation):

- 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.

Example:

activation to synthesis of all enzyme that can utilize glucose.

decrease sugar blood level by increase the reaction which need glucose.

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

* glucagon ⟶ increase sugar blood level by increase the reactions which give glucose such as gluconeogenic → formation of glucose.

5- Feed back inhibition v/s feed back regulation:

enzyme isn't synthesized → caused by entrance of molecule into the nucleus then bind to DNA to prevent producing of enzyme.

↳ the enzyme is existing but the final product inhibits its activity.

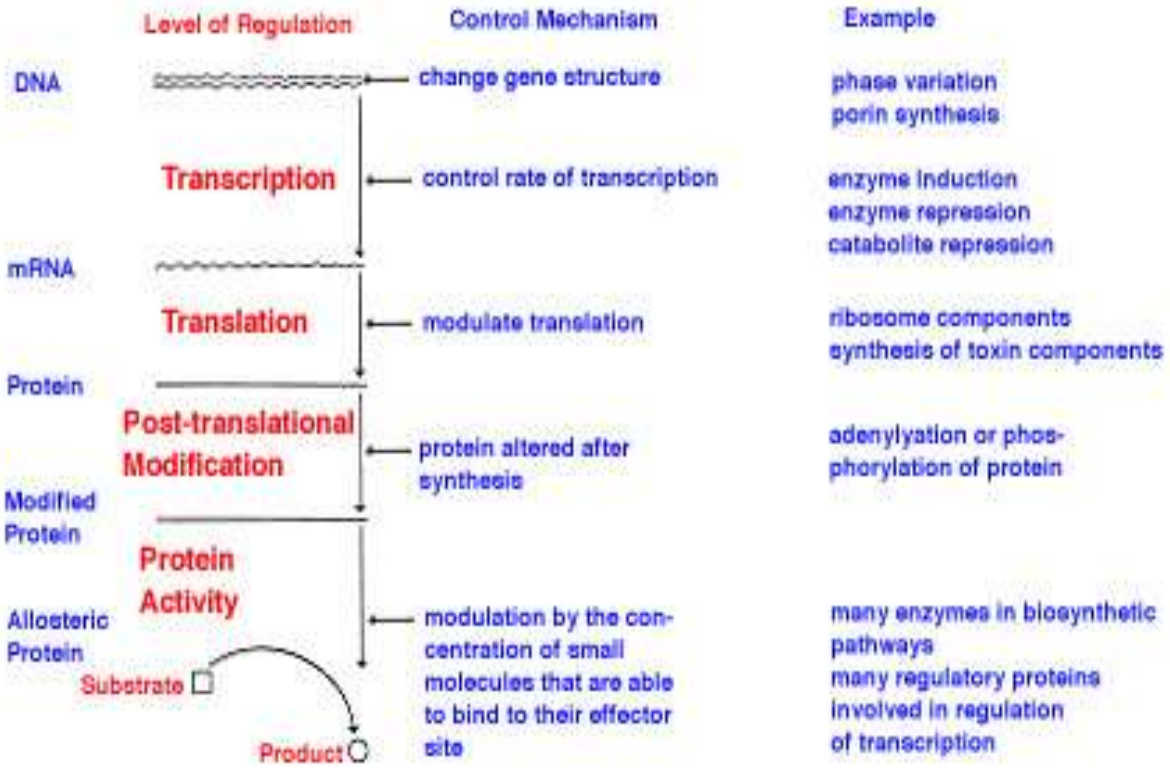
- It is the regulation of a metabolic pathway by using end product as an inhibitor within the pathway to keep cells from synthesizing more product than necessary.
- Dietary cholesterol decreases hepatic synthesis of cholesterol, (feedback regulation not feedback inhibition).
- HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis, is affected, but cholesterol does not feedback-inhibit its activity.

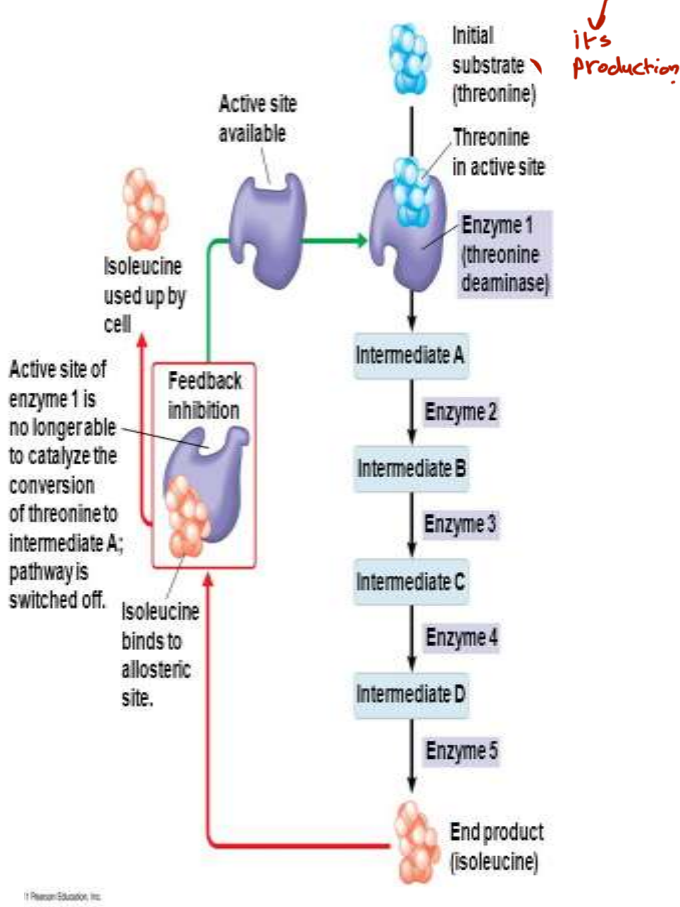
« enzyme isn't synthesized » « genetic level regulation »

ex. sterol regulatory element binding protein.

is inhibited by feedback regulation by molecule called sterol regulatory element binding protein. when the cell contain insufficient amount of cholestrol, this molecule is going enter the nucleus to bind to particular part to the DNA called « hormone respons element » to stop the transcription of the gen encoding for « MG-CoA » and stop

- 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).





Sterol Regulatory Element Binding Proteins (SREBP) penetrates the nuclear membrane and extends to the nucleus where it binds a sequence of DNA called Hormone Response Element (HRE) which stops the transcription of genes itself.