# Enzymology 4: Regulation of enzyme activity

There are 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 active to inactive)
3. Compartmentation. ➔ (reactions take place at different sites of the same cell)
4. Enzyme production. ➔ (the most tuff in regulating enzymatic activity because it is acting on the amount of enzyme produced // genetic level regulation)
5. Feedback regulation ➔ (there are two types feedback inhibition and feedback regulation)

**Notes:**

1) Enzymes inhibitors (as a substances) can be studied inside the body or at test tubes, whereas enzymes regulation only take place inside the cells.
2) The prefix (PRO-) and the suffix (-GEN) indicates that this form of enzyme is inactive or immature.

| *Type of regulation* | *speed* | *Period lasting* |
|---|---|---|
| *1.Allosteric modulation* | Fast | Doesn't last long |
| *2.Covalent modification* | Medium | Medium |
| *3.Genetic level regulation (hormonal regulation/ enzyme regulation)* | Slow | Last Long (long-term regulation) |

## 1. Regulation by modulation of enzyme activity:

### A- Covalent modification:

- Characteristics:
  - ❖ Reversible but not with the same enzyme ➔ require one enzyme for activation and one enzyme for inactivation.
  - ❖ Covalent modification is under hormonal regulation.
  - ❖ The most common covalent modification is **Phosphorylation / Dephosphorylation**.

- Phosphorylation / dephosphorylation:
  - ➢ Usually by the addition of or lysis of phosphate (PO4) groups to and from enzymes.
  - ➢ Amino acids with –OH groups are targets for phosphorylation.
  - ➢ In phosphorylation the phosphate group attach to the enzyme through ester bonds with hydroxy containing amino acids.
  - ➢ Involves protein kinases (add phosphate group) / phosphatases (remove phosphate group).
  - ➢ Some enzymes are active when phosphorylated, whereas, others are inactive when phosphorylated.

- Protein kinase Vs. Phosphatases:

- **Protein kinase:**
  - Function: phosphorylation (transfer a phosphate group from donor to acceptor).
  - Enzyme class: Transferase (We need a donor).
    - Phosphate group donor: ATP
    - Phosphate group acceptor: dephosphorylated enzyme
  - Irreversible with the same enzyme (kinase) ➔ because the bond between the third phosphate group (the one to be removed) and the second one is a high energy bond ➔
  - Accompanied by loss of energy (use energy).
  - Phosphorylation is under hormonal regulation (hormone activate enzymes according to the cell need).
- **Phosphatases:**
  - Function: dephosphorylation (remove phosphate group).
  - Enzyme class: Hydrolyses.

- Phosphorylation / dephosphorylation mechanism:

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

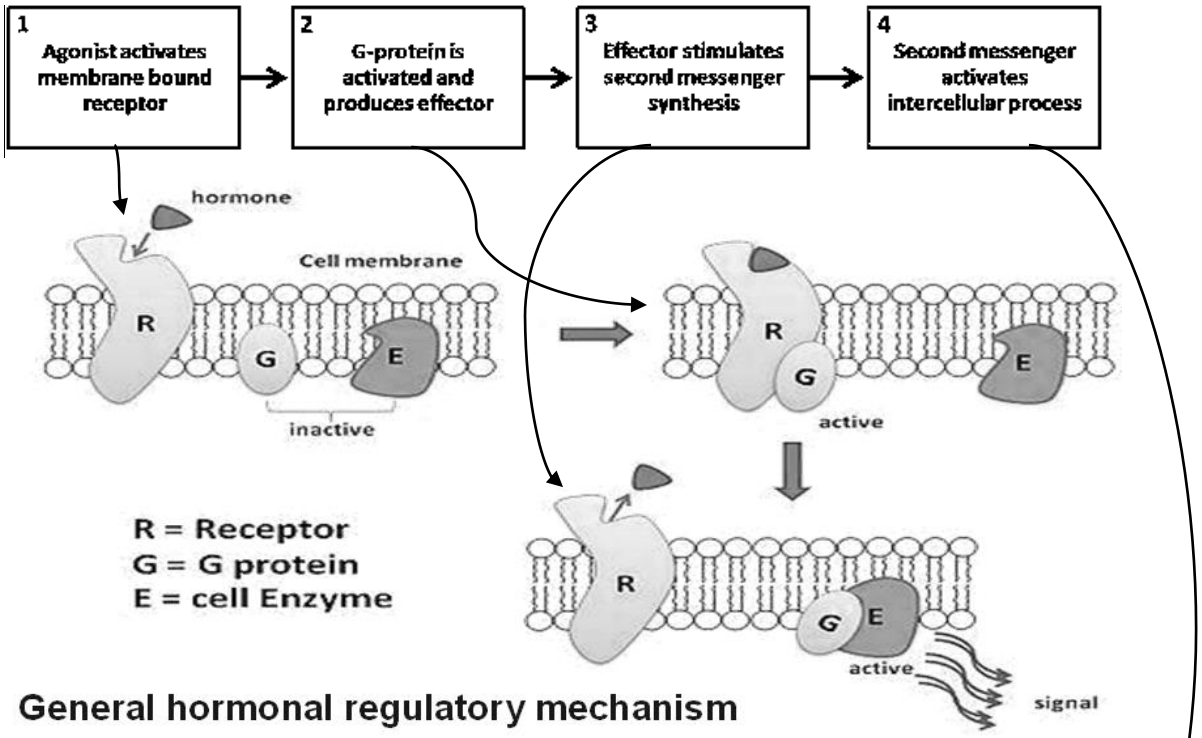
**Note2:** there are two types of **hormones**:

- Hydrophobic hormones: able to penetrate (cross) the cell membrane // there receptors are located inside the cell
- Hydrophilic hormones: unable to penetrate (cross) the cell membrane // there receptors are extracellular or Trans membrane

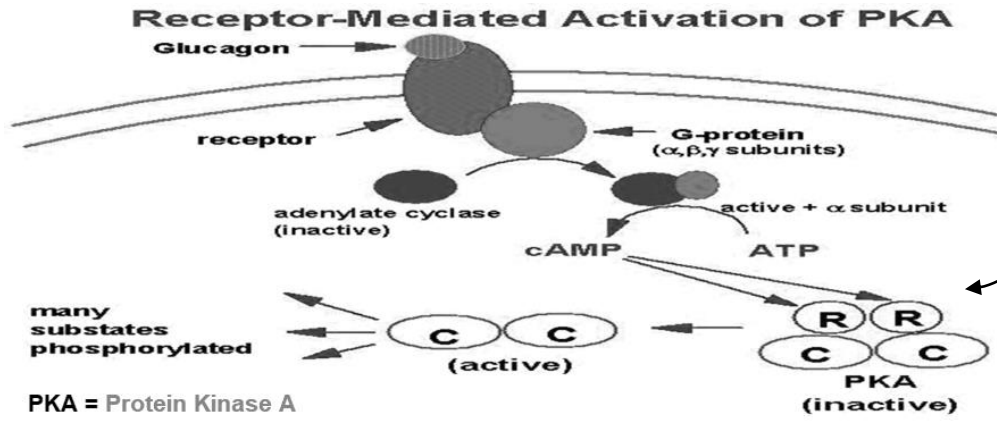
Phosphorylation / dephosphorylation mechanism steps:

1) The hormone bind to its receptor Once the hormone is binding to its receptor they form **Hormone-Receptor Complex**
2) This binding activate 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
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 subunit, **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.

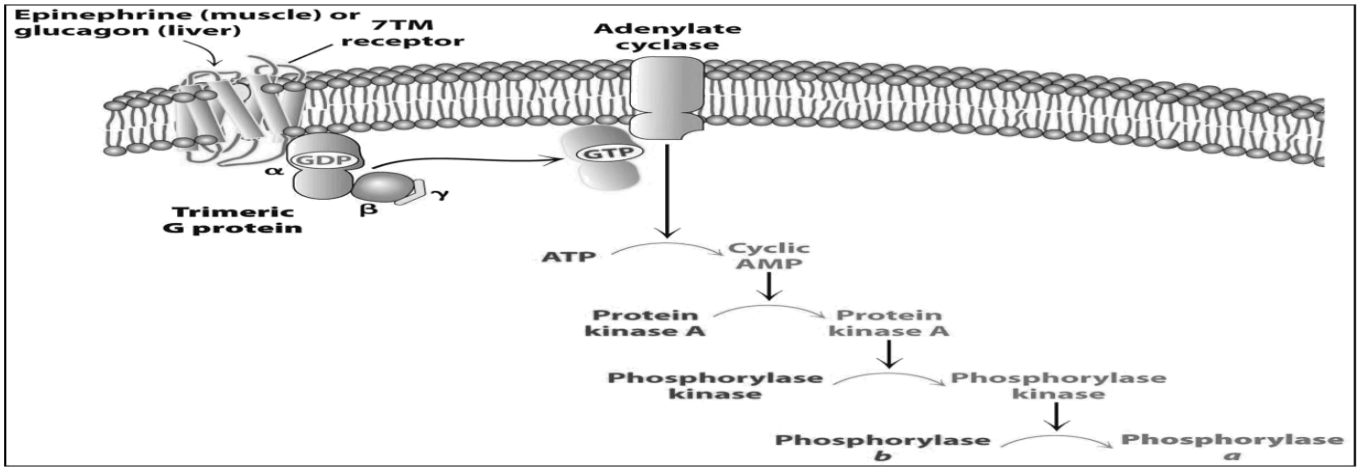
***Signal transduction:*** a cascade of reactions that start by forming secondary messenger like cAMP and by causing a change in the cell such as phosphorylation some of the enzymes




General hormonal regulatory mechanism



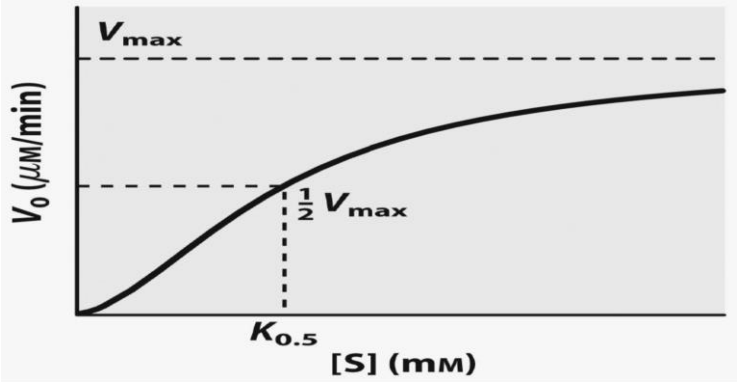

PKA = Protein Kinase A

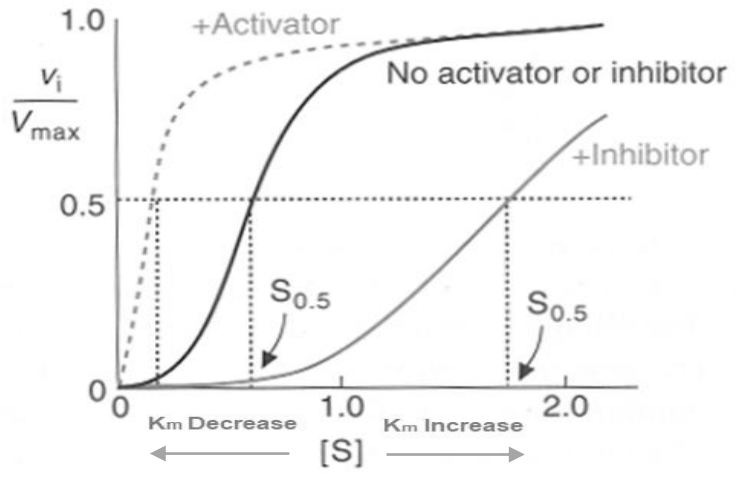


1. Regulation by modulation of enzyme activity:

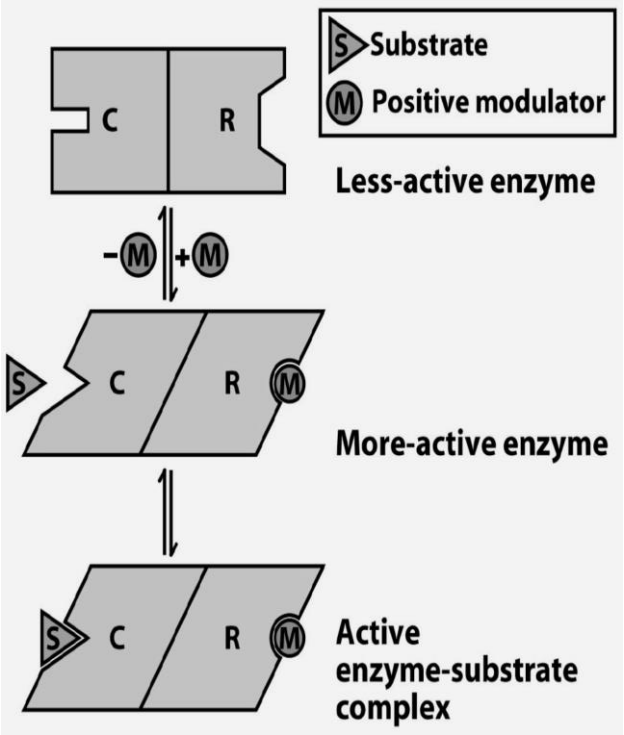
## B- Allosteric regulation:

- **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 enzyme:** Just to remember!!
  (1) *Allosteric enzymes* usually have quaternary structure
  (2) *Allosteric enzymes* do not exhibit typical Michaelis- Menton kinetics.
  (3) 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**
- **Allosteric modulators:**
  (1) Often *allosteric modulators* do not resemble the substrate or the product of the enzyme catalyzing the reaction.
  (2) *Allosteric modulators* bind **non-covalently** to the enzyme at a site rather than the substrate binding site. (because if it binds covalently it would be considered covalent modification)
  *(3)* Some *allosteric modulators* alters the Km, *the Vmax remains constant.*
  (4) The *modulators* are not altered by the enzyme.
  (5) End products are often inhibitors.
- **Allosteric regulation characteristics:**
  (1) Allosteric regulation may either inhibit or stimulate an enzyme activity by changing the enzyme either to its active or inactive forms.
  (2) The binding of an allosteric activator stabilizes its active form, whereas binding the allosteric inhibitor stabilizes the inactive form of the enzyme.
- **Example of Allosteric regulation: Hemoglobin**
  - Hemoglobin has two forms: Tight form (inactive form) and Relax form (active form).
    - **Tight form** ➔ 2α and 2β linked together ➔ every α and β subunits form a dimer with strong bonds (covalent, disulfide, etc.) ➔ 2 dimers are linked together by the same bonds linking between the subunits of the dimer.
    - To transfer the tight form to the relax form ➔ a gradually breaking of bonds between the two dimmers and within the dimmer itself – by adding oxygen molecules ($O_2$) gradually to hemoglobin some bonds partially broke – thus the tight form begin to transform to the relax form gradually.




**Activator =** same $V_{max}$ – decrease $K_m$
**Inhibitor =** same $V_{max}$ – increase $K_m$




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

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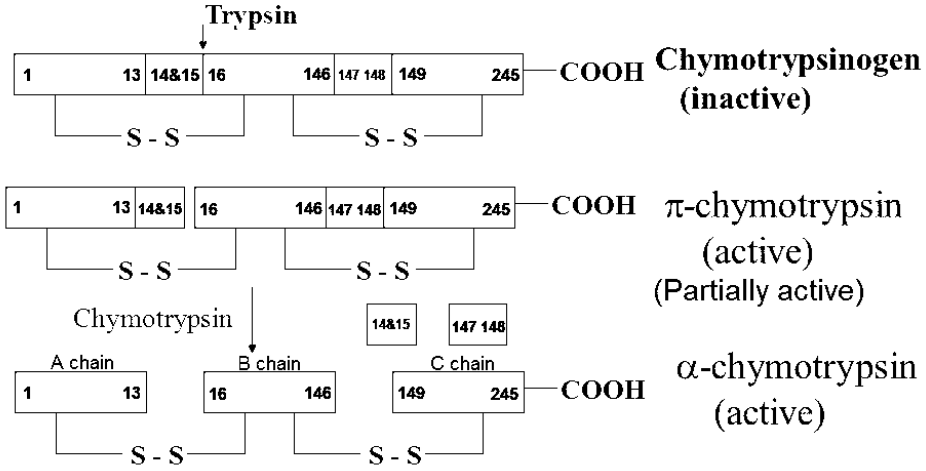
## 2. Proteolytic cleavage of proenzyme (Zymogens activation):

- 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 ➔ precollagen)
- 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
- **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

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## 3. Enzyme/substrate Compartmentation:

- **Compartmentation:** means there is **segregation** of metabolic processes into distinct subcellular locations like the cytosol or specialized organelles (nucleus, endoplasmic reticulum, Golgi apparatus, lysosomes, mitochondria, etc.).
- **Biological membrane** is the barrier which separate reactions from each other
- **Importance:** ensures 1.metabolic efficiency & 2.simplifies regulation

**Q:** Segregation of metabolic processes into distinct subcellular locations is a form of regulation why?

**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

**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 |

## 4. 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.
- **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:**
  (a) Insulin induces increased synthesis of enzymes: glucokinase, glycogen synthase and PFK-1
  (b) Insulin decreases the synthesis of several key gluconeogenic enzymes (amino acid ➔ glucose).
- **In hypoglycemia** glucagon approximately do the opposite function of insulin

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## 5. Feedback Inhibition Vs. Feedback Regulation:

### A- Feedback Inhibition:

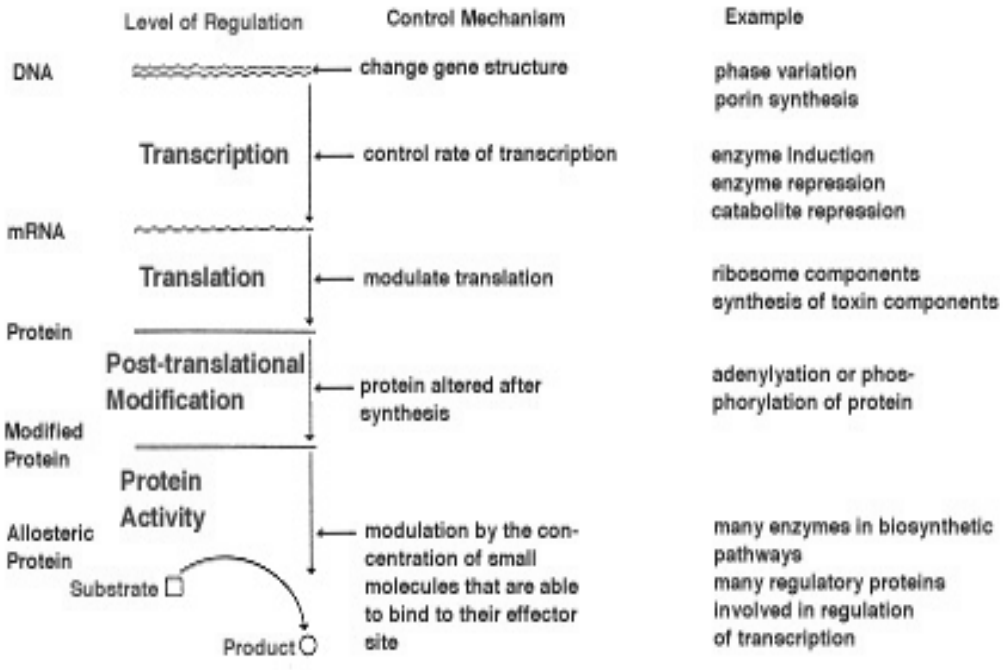
- End product act as inhibitor which attack the rate limiting step enzyme ➔ attach at a specific site ➔ goes some conformational changes ➔inhibit the binding of the substrate ➔switching off the production of *mediates*
- If the mediates are not metabolized in any other metabolic pathway their synthesis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

### B- 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:**
  (1) **At DNA level:** changing gene structure
  (2) **At transcription level:** controlling the rate of transcription
  (3) **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
- **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

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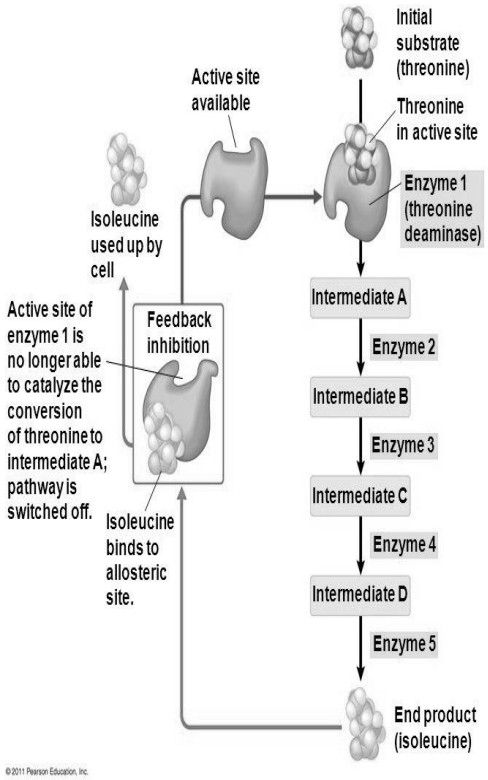


Qualities you need to get through medical school and residency:

- Discipline.
- Patience.
- Perseverance.
- A willingness to forgo sleep.
- Ability to weather crises of faith and self-confidence
- Accept exhaustion as a fact of life
- Addiction to caffeine a definite plus
- Unfailing optimism that the end is in sight