بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

BioChemistry

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

Final | Lecture 16

Enzymes Pt.6 (Regulation Pt.3)

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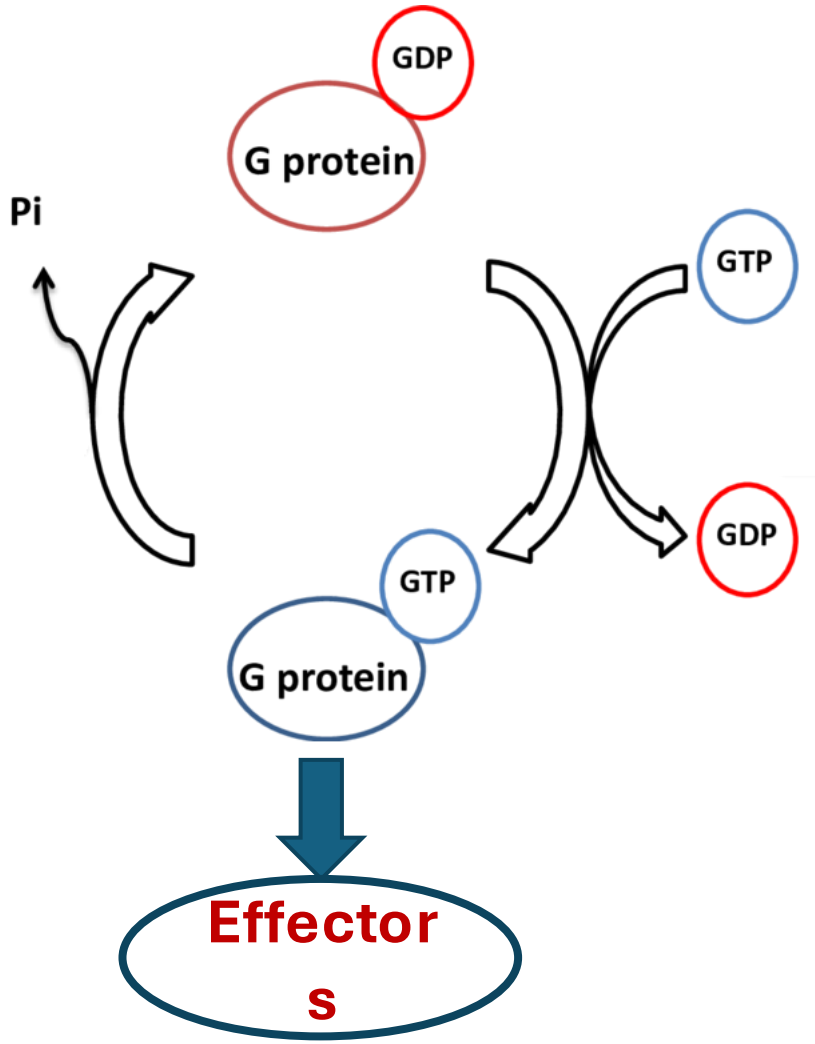
Color coding used in the modified:

- *Black: the original slides*
- *Blue: the doctor's explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Large and small regulatory modulators

Small monomeric G proteins

- When GTP (small) is bound, the conformation of the small monomeric G protein (large) allows it to bind target proteins, which are then activated or inhibited.
- The small monomeric G protein hydrolyzes a phosphate from GTP to form GDP, which changes the G protein conformation and causes it to dissociate from the target protein.
- GDP is exchanged for GTP, which reactivates the small monomeric G protein.



Irreversible covalent modification (Proteolytic activation)

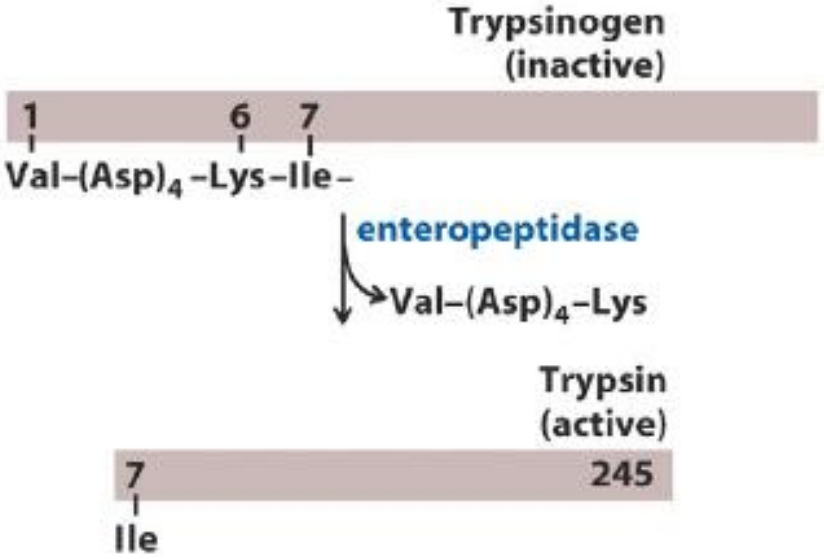
Proteolytic activation is a very specific modification that cannot be turned over; it cannot be reversed.

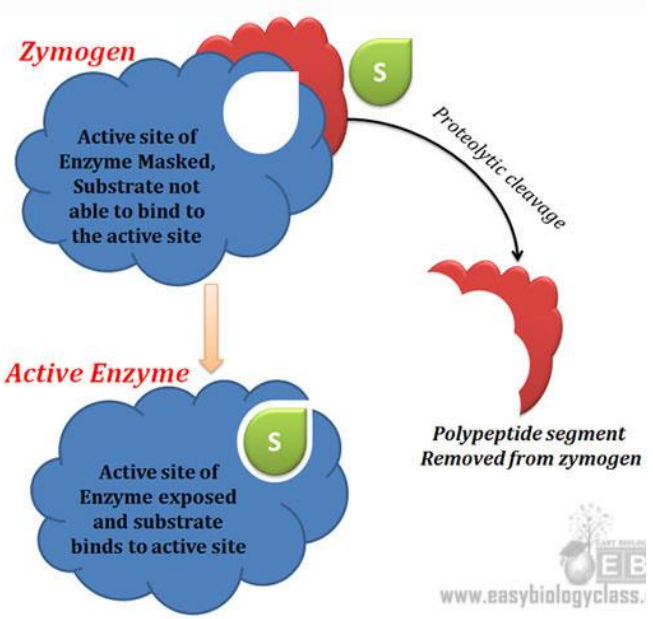
Zymogens

A zymogen is an enzyme that requires proteolytic activation. This means that we need a protease which is a type of hydrolase to cleave the protein. The protease removes a portion of the protein and this makes the protein active. Therefore, this process is irreversible. The portion that is removed from the protein cannot be added back again. This is different from phosphorylation regulation which can be reversed.

- Zymogens or proenzymes are **inactive** precursors of enzymes.
- Activation is done by **irreversibly** removing part of the enzyme (usually known as the pro region present at the N-terminus).
- Examples: **digestive enzymes** such as chymotrypsin, trypsin, and pepsin that get activated when food is ingested.
  - Trypsinogen (zymogen) is activated via removal of the first six amino acids at the N-terminus.

The digestive proteases have a specific portion at the N-terminus. This portion is removed, allowing the protease to become active. In other words, the N-terminal portion blocks the active enzyme. It prevents access to the active site. Once this portion is removed, the process is irreversible, and the active site becomes accessible. The enzyme then becomes active.





- Trypsin is present in the pancreas in an inactive form called trypsinogen. As soon as we put a bite of food into our mouth, a signal is sent to the pancreas, telling it to release trypsinogen, along with the other digestive enzymes, such as chymotrypsinogen, proelastase, prolipase, and pro-saccharidases.

- We previously discussed the meaning of the prefix pro- . Some proteins are synthesized as pro-proteins. These proteins have a portion at the N-terminus that keeps the protein immature and inactive. When this portion is removed, the protein becomes mature and active.

- The digestive enzymes are produced in the pancreas in their inactive forms. This allows the digestive system to be prepared for the arrival of food. As soon as food enters the digestive tract, these enzymes are released, activated in the intestines, and begin digestion. If we had to wait for the pancreas to produce the digestive proteases and then release them after the food had already reached the digestive tract, it would take hours. We put food into our mouth, and we cannot wait for hours for it to reach the intestine before digestion begins. Instead, as soon as we eat, the digestive enzymes are released and they become activated in the intestines, so they are ready when the food reaches them, and they can immediately begin to function.

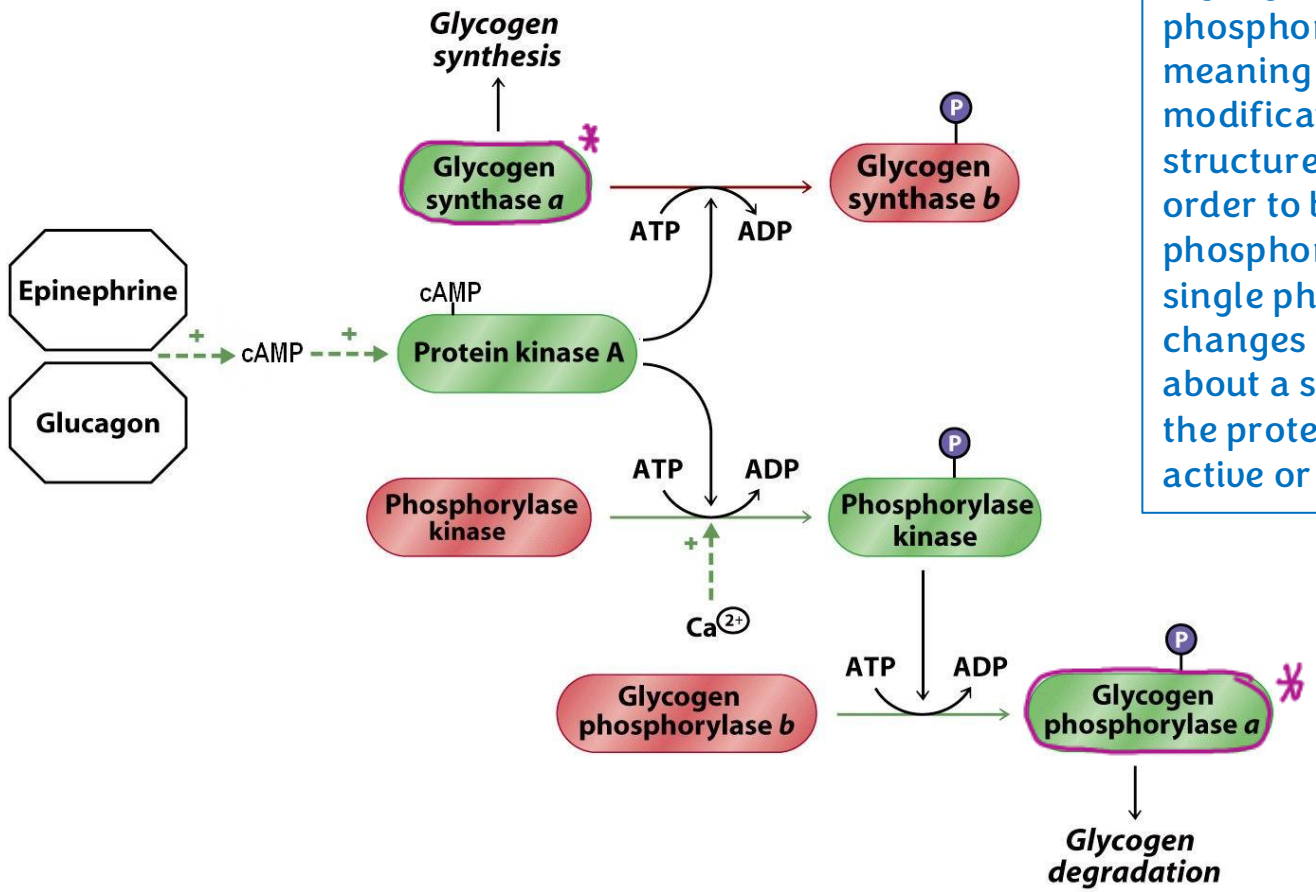
- If they become activated inside the pancreas, before food is present, they would begin acting on proteins within the cells and tissues. They would be released and start breaking down the tissues, which would damage the pancreas. Therefore, as a protective mechanism for our tissues, these enzymes are released in their inactive forms and are activated in the appropriate location after food has entered the digestive system.

Allosteric regulation

Allosteric proteins, including enzymes, require a small modification in their structure to become active, or to become more active or less active.
For example, T-state hemoglobin can bind to oxygen, but its affinity for oxygen is very low. Therefore, it represents the less active form of hemoglobin. It can be converted into a more active form, the R state, in which its affinity for oxygen increases.
The same concept applies to enzymes that are allosteric enzymes. These enzymes only require a **small modification** of their structure to become more active. Therefore, they can transition from a less active state, the T state, to a more active state, the R state, when a small modification occurs in their structure.

Phosphorylation cascade

Remember this cascade (means a series of proteins acting sequentially) from the previous lecture.



Principles of Biochemistry, 4/e
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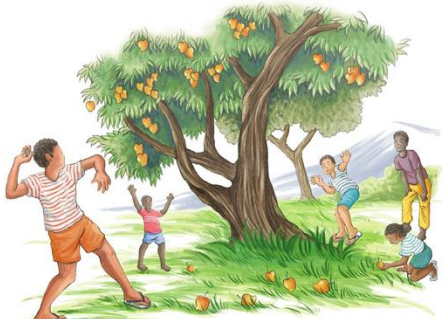
Glycogen synthase and Glycogen phosphorylase are allosteric enzymes, meaning that they require a small modification or change in their structure, similar to hemoglobin, in order to become active or inactive by phosphorylation. The addition of a single phosphate group to the protein changes its structure. We are talking about a small structural shift causing the protein to shift toward either the active or inactive form.

Glycogen synthase becomes inactive by phosphorylation, whereas phosphorylase kinase becomes active by phosphorylation.

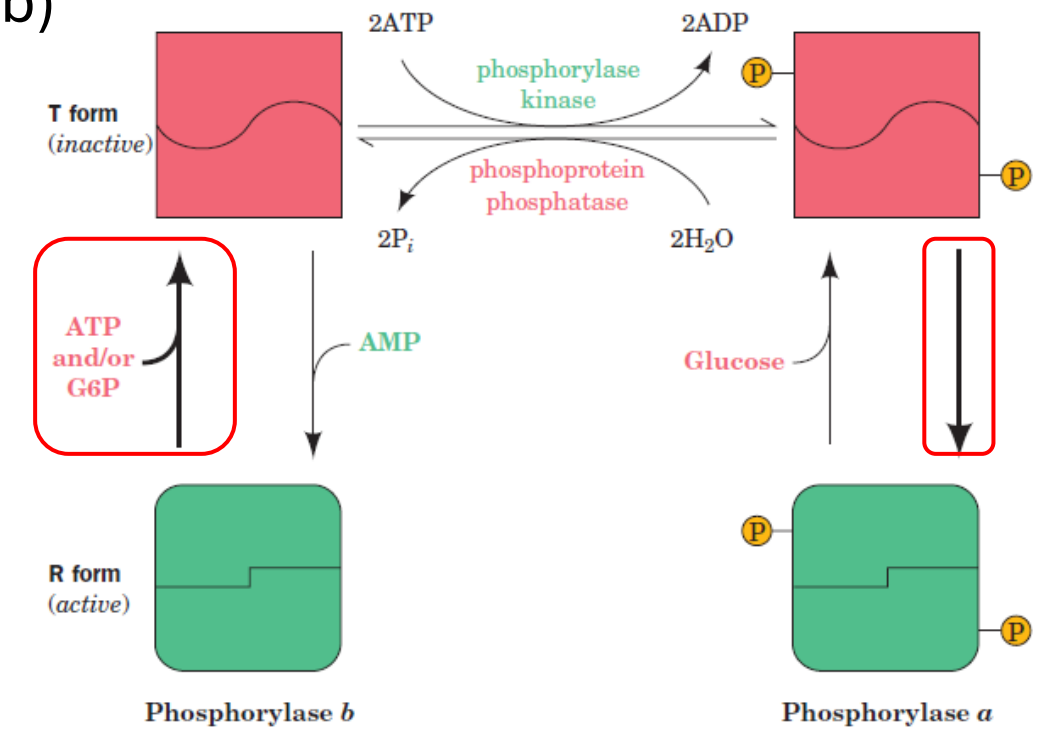
$$\text{glycogen}_{(n\ \text{residues})} + P_i \longrightarrow \text{glycogen}_{(n-1\ \text{residues})} + \text{glucose 1-P}$$

Example: Glycogen phosphorylase

- GP catalyzes removal of glucose molecules from glycogen.
- The enzyme exists in four forms:
  - T (inactive) and R (active) states
  - Phosphorylated (a) and dephosphorylated (b)



- *When phosphorylated, it is known as phosphorylase a.*
- *When dephosphorylated, it is known as phosphorylase b.*



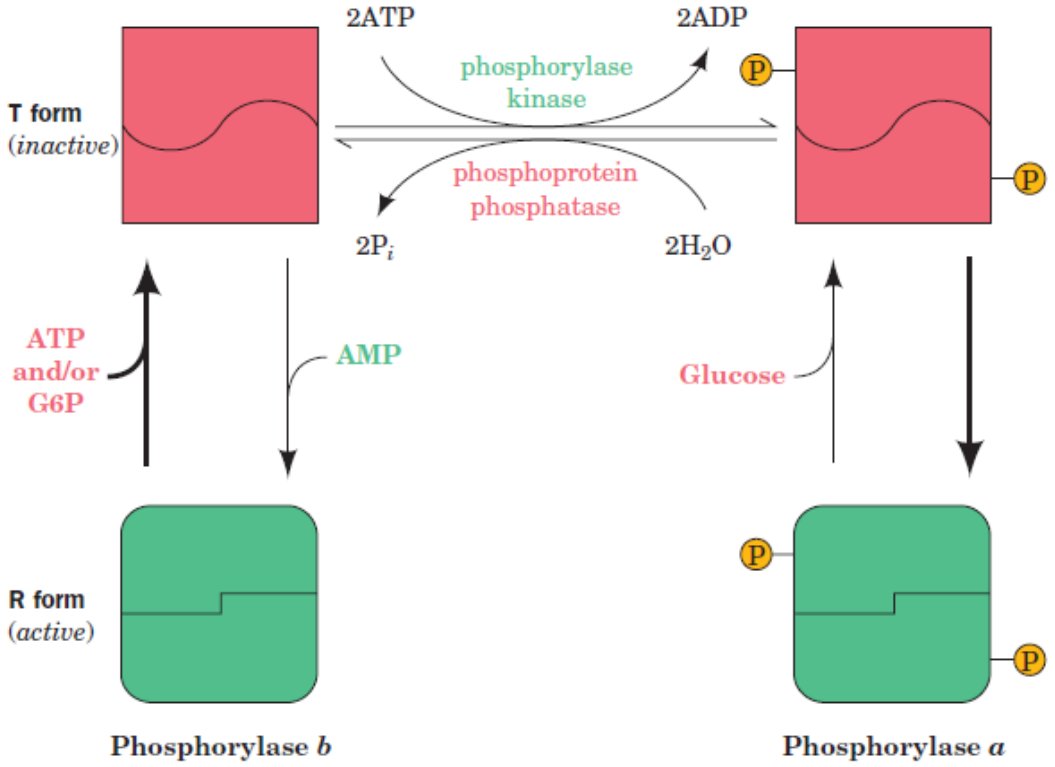
when phosphorylation occurs on this enzyme, a small change in its structure takes place, causing glycogen phosphorylase to become active.

When the enzyme is dephosphorylated, it exists predominantly in the T form and When the enzyme is phosphorylated, it exists predominantly in the R form. So,phosphorylation shifts the protein toward the R form, making the enzyme more active. Therefore, there is an equilibrium between the T and R forms. When the enzyme is dephosphorylated, the equilibrium shifts toward the T form. When the enzyme is phosphorylated, the equilibrium shifts toward the R form.

And note that this does not mean when the protein is dephosphorylated, cannot be in the R form no actually it exists in a very small fraction compared to the T stat.

The two forms of each form

- Both phosphorylase b and phosphorylase a exist in equilibrium between an active R state and a less-active T state.
- Phosphorylase b is usually inactive because the equilibrium favors the T state.
- Phosphorylase a is usually active because the equilibrium favors the R state.



The transition of phosphorylase b between the T and the R state is controlled by the energy charge (ATP and AMP) of the muscle cell and availability of glucose-6-phosphate.

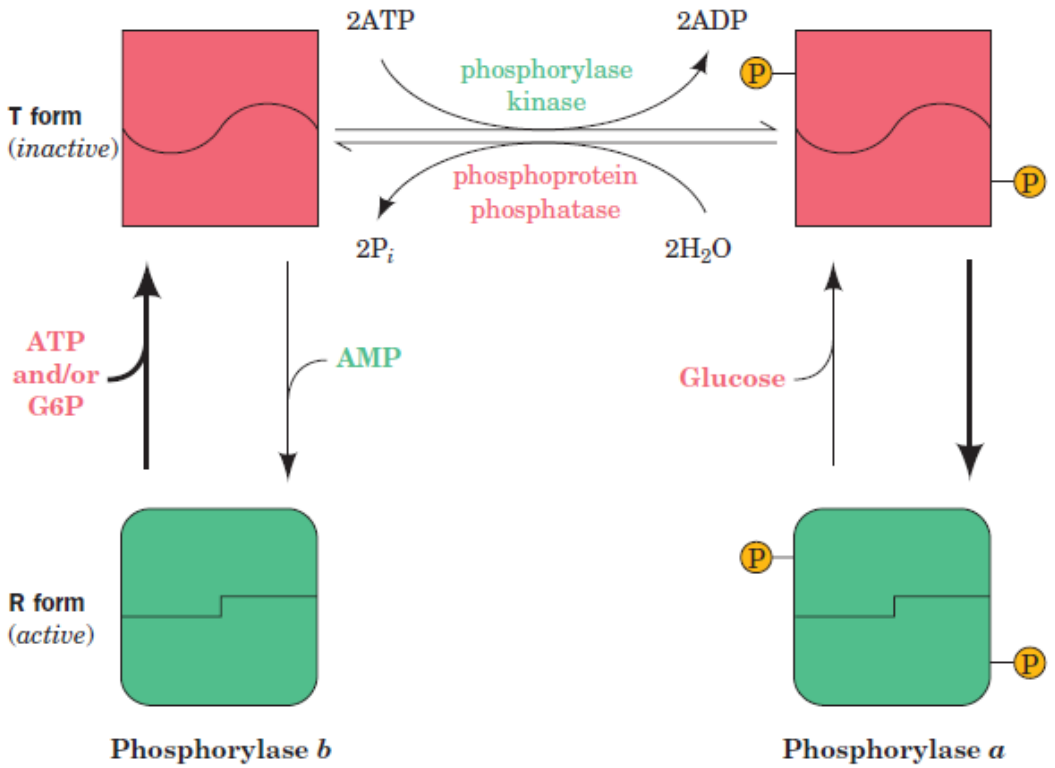
These are the regulators, similar to the allosteric effectors that we discussed previously for hemoglobin

- In the presence of ATP or glucose-6-phosphate, the phosphorylase enzyme is shifted from the **R state to the T state**, particularly when the enzyme is dephosphorylated, making it less active. So when ATP levels or glucose-6-phosphate levels are high, It **indicates** that the cell has **enoughe energy**, so there is no need to break down glycogen.

- Also, when glucose levels are high, it also shifts the enzyme from the R state to the T state, when the protein is phosphorylated.

- When AMP levels are high, AMP shifts the enzyme from the **T state to the R state** when the enzyme is dephosphorylated. Because high AMP levels are an indication to the cell that there is **not enough ATP**. The cell needs more ATP. Therefore, a high AMP level signals to the cell: break down glycogen to produce more energy.

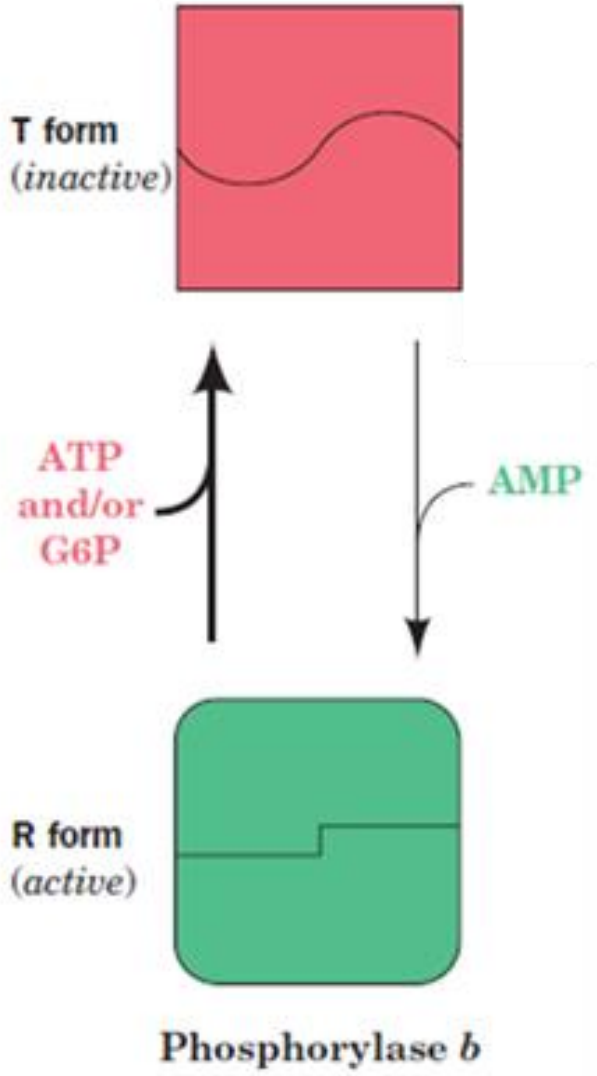
- These are **allosteric regulators,** and they can have either negative or positive effects on the enzyme.



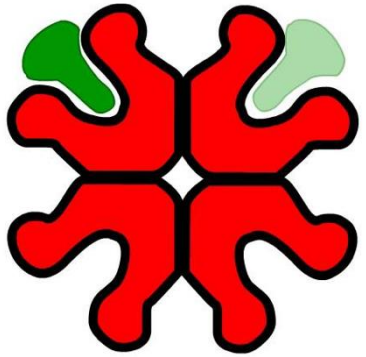
What do ATP and AMP do?

- Muscle phosphorylase b is active only in the presence of high concentrations of AMP, which binds to a nucleotide-binding site and stabilizes the conformation of phosphorylase b in the R state.
- ATP acts as a negative allosteric effector by competing with AMP and so favors the T state.

Glucose 6-phosphate also favors the T state of phosphorylase *b*, an example of feedback inhibition.

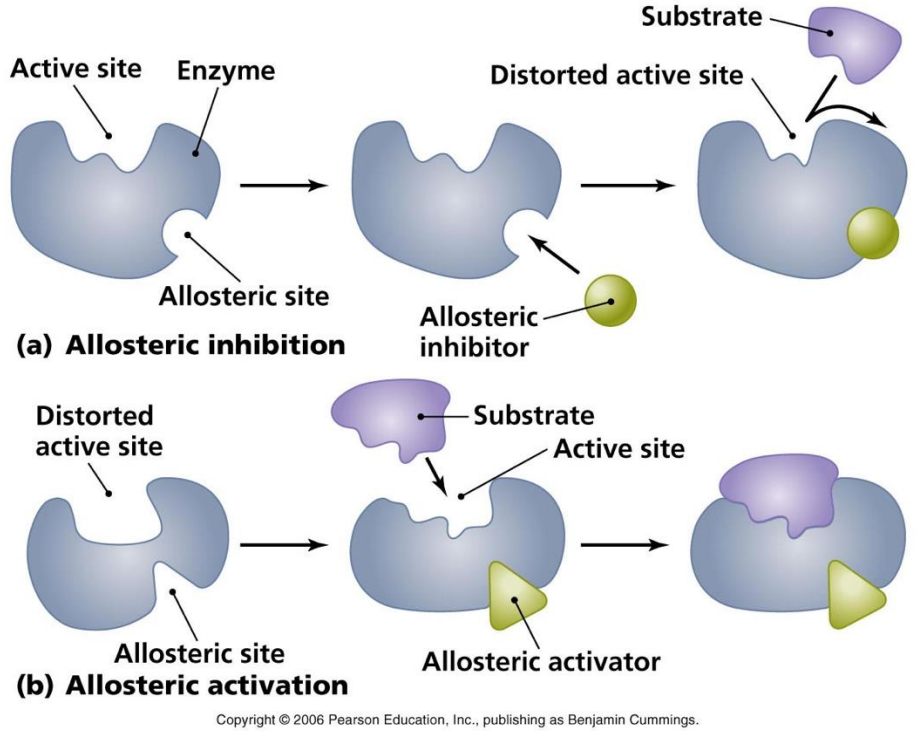


Allosteric enzymes and their modifiers



- Allosteric enzymes are multi-subunit proteins;
  - **One subunit contains the active site (catalytic subunit) and another containing the regulatory site (regulatory subunit).** The regulatory sites can change the shape of the enzyme, making it more active or less active.
  - Multiple active sites can exist on multiple subunits.
- The binding of regulatory molecules triggers conformational changes in the active site via modifying non-covalent interactions.
- Allosteric enzymes bind **modifiers** at the allosteric site, a site that is physically separate from the catalytic site. cause a slight change in the structure of the enzyme.
  - A **negative** allosteric modifier (inhibitor) causes the enzyme to have **less activity**.
  - A **positive** allosteric modifier (activator) causes the enzyme to be **more active**.

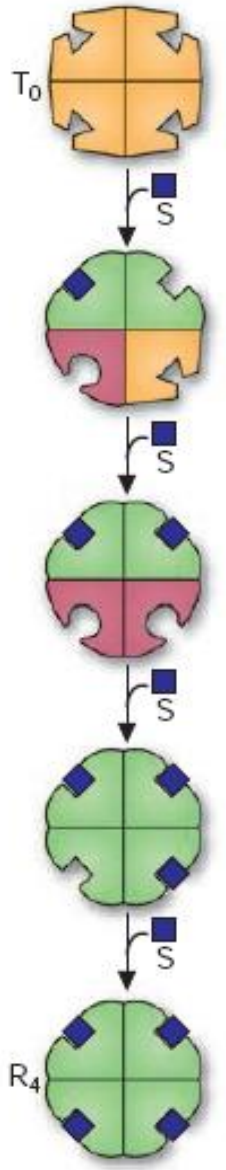
One of the features or characteristics of allosteric proteins is that they have a quaternary structure, meaning that they are made up of multiple subunits



More on modifiers

- When the modifier is a molecule **other** than the substrate, then it is known as **heterotropic**.
- If the modifier is **same** as the substrate, then it called **homotropic**.
  - The binding of the substrate causes the enzyme to become more active and binds to a second substrate at a different active site with more ease.
  - This is called "**positive cooperativity**".
  - T to R conformation
  - There is also **negative cooperativity**.

The enzyme becomes progressively more active as more modifier molecules bind.



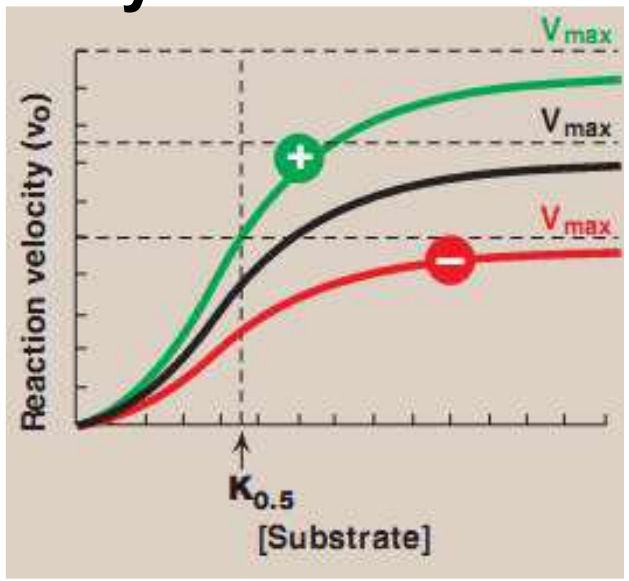
Types of allosteric enzymes

- The Michaelis-Menten model cannot explain the kinetic properties of allosteric enzymes.
- $K_{0.5}$ is used instead of K_M.

$K_{0.5}$ is the measurement of affinity for allosteric enzymes, K_m is used for enzymes that follow Michaelis–Menten kinetics, which have a hyperbolic curve.

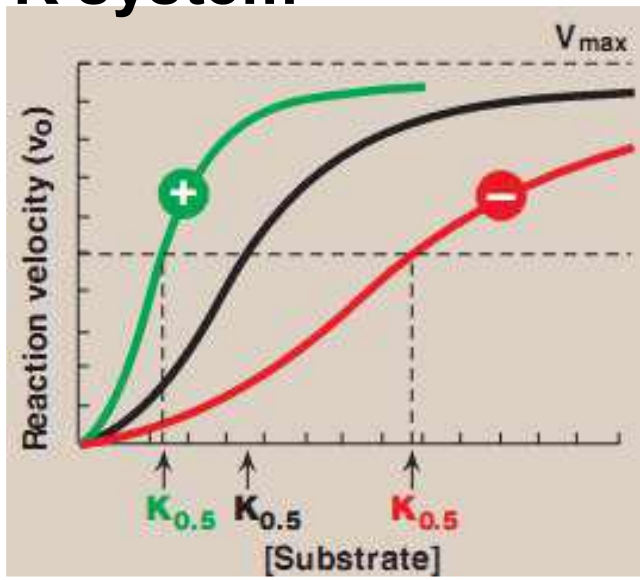
Note that we will not talk about the V system

V system



Same K0.5, Different Vmax.

K system



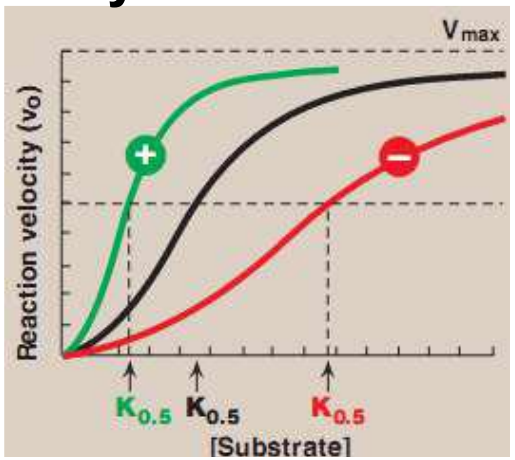
Different K0.5, same Vmax.

Note near-hyperbolic plot with activators

- We will focus only on the K-system enzymes.
- Basically, the idea is that we have the enzyme without any modifier. Notice that on the X-axis we have the substrate concentration, and on the Y-axis we have the reaction velocity, just as in the Michaelis-Menten plot.

K system

- When we add a positive modifier, the curve shifts to the **left**, the $K_{0.5}$ becomes **lower** and the affinity becomes **higher**, making it **easier** for the enzyme to reach the Vmax .



Different K0.5, same Vmax.

- When we add a negative modifier, the curve shifts to the **right**, the $K_{0.5}$ becomes **higher** and the affinity becomes **lower**.

- The curve is **sigmoidal**, not hyperbolic like the Michaelis-Menten curve. This is a characteristic of allosteric proteins that are composed of more than one subunit.

- The sigmoidal shape has an **advantage**, similar to what we see with the hemoglobin molecule. We have a point at which the enzyme transitions between the R state and the T state, or from the T state to the R state. Around this point, there can be a very large change in the activity of the enzyme. Therefore, any change in the substrate concentration, whether the curve shifts to the right or to the left, can cause a very large change in the activity of the enzyme. This is one of the characteristics of allosteric enzymes.

- If the substrate is present at a low concentration, the enzyme can be active, but it is not very active. It has some activity. However, as soon as the substrate concentration increases slightly, there is a shift toward the left, and the activity becomes greatly amplified because these are allosteric enzymes.

Allosteric enzymes and metabolism

- Allosteric inhibitors usually have a much **stronger effect** on enzyme velocity than competitive and noncompetitive inhibitors.

Notice that the modifiers of allosteric enzymes, whether negative or positive, are not called competitive or non-competitive inhibitors. We call them allosteric modifiers.

- Allosteric enzymes are **not limited** to regulation through inhibition whereby allosteric effectors may function as **activators**.
- The allosteric effector needs **not** bear any resemblance to substrate or product of the enzyme.

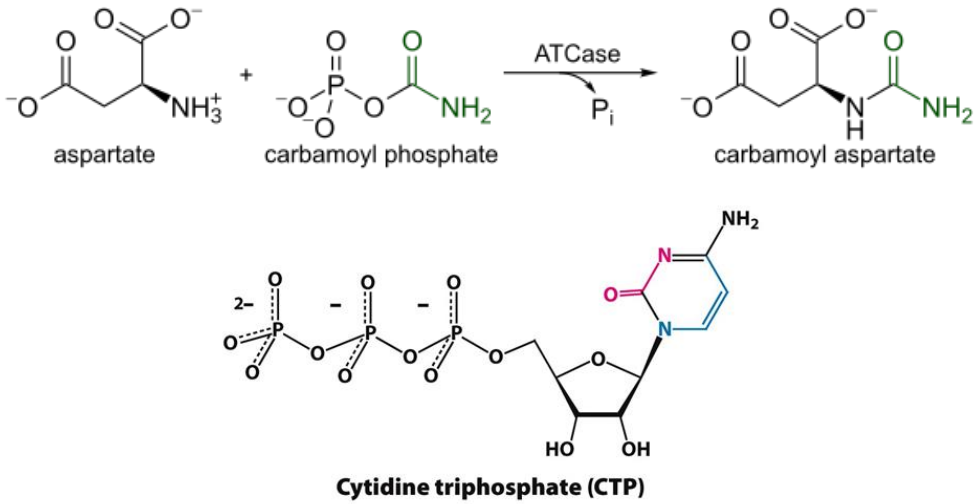
For example, with glycogen phosphorylase, we have glucose-6-phosphate, which is different from glycogen and does not resemble glycogen. The same applies to AMP and ATP; they are different molecules with structures that are different from the substrate or product.

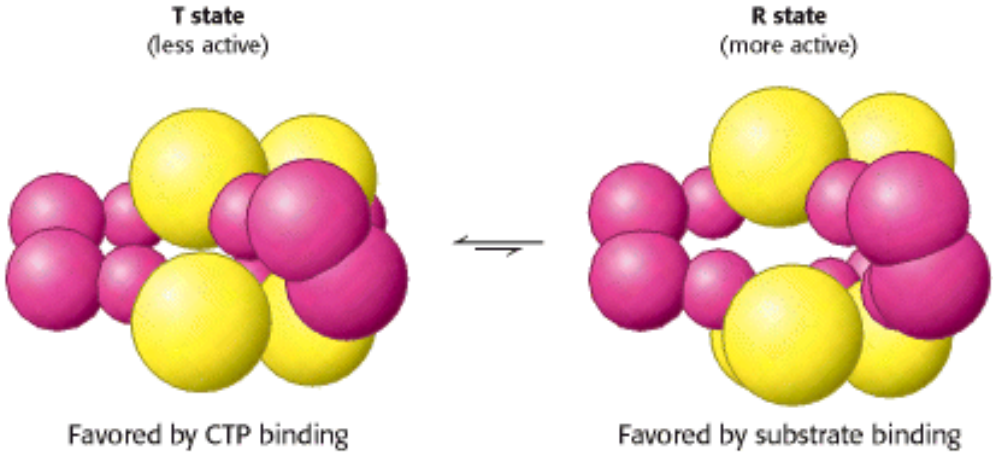
- The effect of an allosteric effector is **rapid** occurring as soon as its concentration changes in the cell.
  - Feedback regulation of metabolic pathways by end products or by signal molecules that coordinate **multiple** pathways.

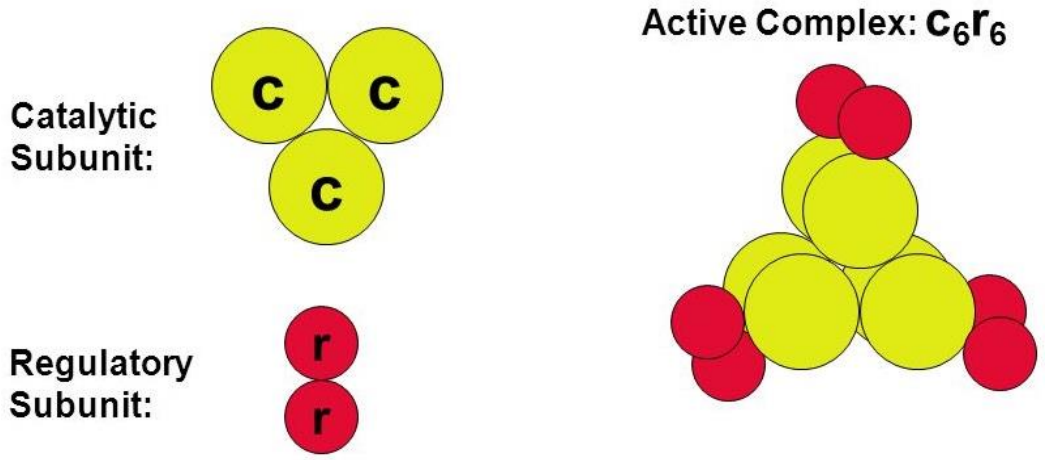
With Michaelis-Menten enzymes, inhibitors generally act on a particular enzyme and rarely affect a different enzyme. In contrast, molecules such as ATP, AMP, or glucose-6-phosphate can act on a group of enzymes. Therefore, they can regulate different enzymes involved in different metabolic pathways.

Aspartate transcarbamoylase

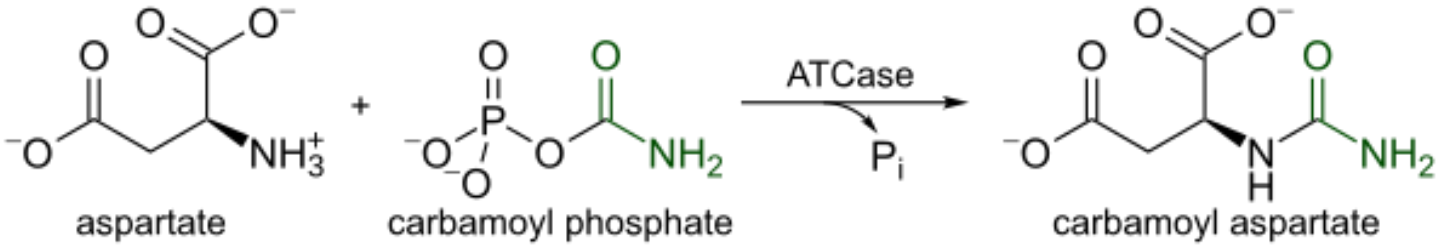
- Aspartate transcarbamoylase (ATCase) catalyzes the first step in the synthesis of pyrimidine nucleotides.
- ATCase consists of **12 polypeptide** chains: **six catalytic subunits (two trimers)** and **six regulatory subunits (three dimers)**.
- It exists in two forms: T state (less active) and R state (more active).



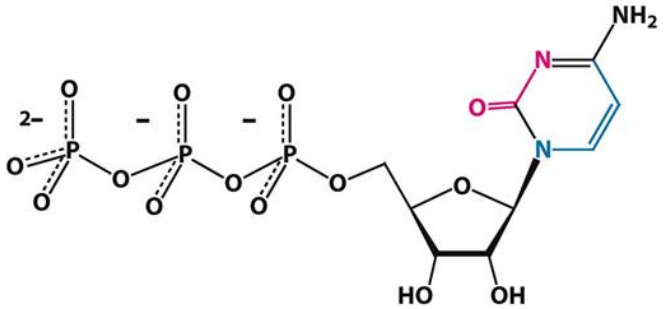




- The first reaction is part of a metabolic pathway. When we talk about a metabolic pathway, we mean a series of reactions in which one molecule is converted into another: A is converted into B, B into C, C into D, D into E, and so on. In this pathway, the first reaction involves **aspartate and carbamoyl phosphate**. These two molecules are converted into **carbamoyl aspartate**.



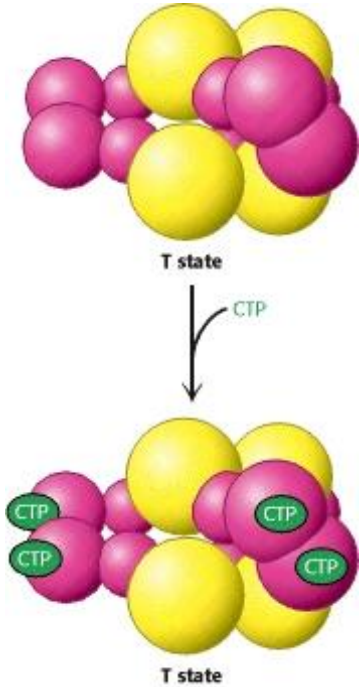
- Then, **carbamoyl aspartate** undergoes further reactions, leading through several steps until, the **final product** is **cytidine triphosphate (CTP). (pyrimidine nucleotides)**



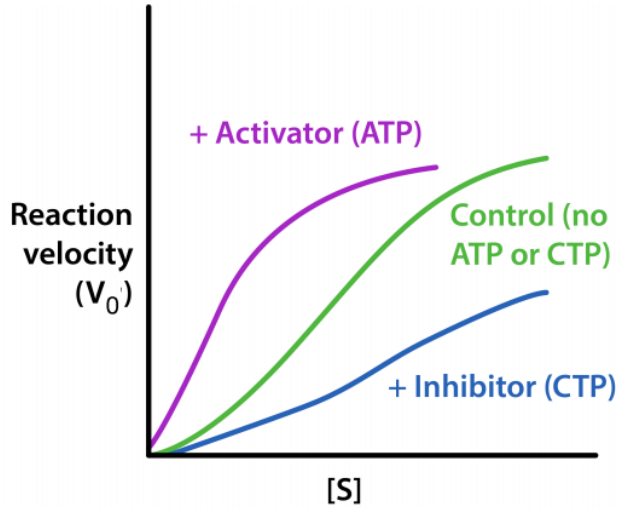
Aspartate transcarbamoylase-regulation

- ATCase is inhibited by CTP, the end-product
  - inducing a major rearrangement of subunit positions
  - stabilizing the T state of the enzyme.
  - decreasing binding affinity for Asp (substrate) at active sites on catalytic subunits
  - increasing K0.5 (K system)
    - Note: a non-competitive inhibitor changes K0.5
    - On the other hand, ATP, a purine, heterotypically activates the enzyme in order to balance the rate of synthesis of purines and pyrimidines in cells.

How regulation for this enzyme happens??
ATP: activator
CTP: inhibitor
Notice that the inhibitor is the same as the final product and exclusively we have ATP as activator: why? Because it's a purine nucleotide



So the cell here says we want to synthesize DNA So we have to synthesize ATP and CTP and TTP and GTP.
We have two purine nucleotides and two pyrimidines .
There Should be balance between pyrimidines and purines.
So this enzyme (CTP synthase)
ATP ↑ Aspartate transcarbamoylase is more active (More CTP, more pyrimidine)=> balance between purines and pyrimidines.
CTP ↑ Aspartate transcarbamoylase activity is decreased to obtain balance between nucleotides
CTP: switch The enzyme to T state (decrease the affinity)
ATP: switch the enzyme to R state By changing the K system (Affinity for substrate) => heterotropic effector
Eventually they will reach Vmax because substrate concentration is very high



So now we are done with regulation for individual enzymes

Modes of metabolic regulation

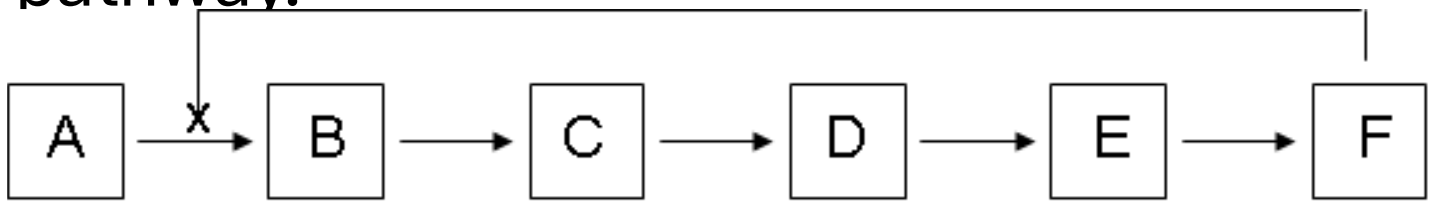
Metabolism is a pathway مسار
A->B->C->D etc..

So now we do not discuss regulation for a single enzyme but rather a thorough pathway; including glycolysis for example which is the break down of Glucose to the form of pyruvate by 10 reactions.
Some reactions are reversible, doesn't need regulation. However, there are other reactions that are like bottleneck; their regulation is very strict

Feedback inhibition

The last substrate does inhibition for the enzyme that catalyzes the first reaction.

- Feedback inhibition or negative feedback regulation: an enzyme present early in a biochemical pathway is inhibited by a late product of pathway.



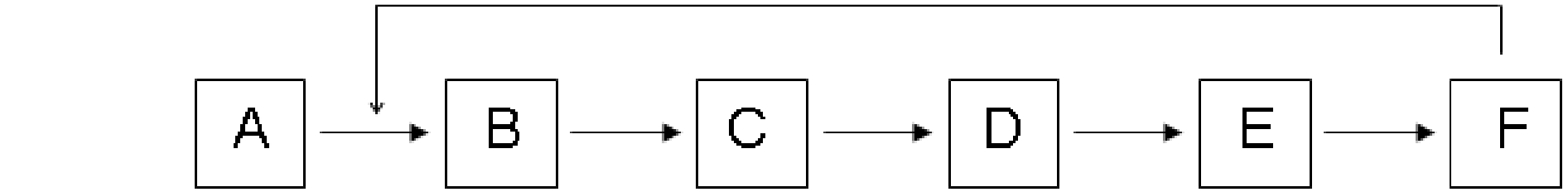
AT REST
(glycolysis inhibited)

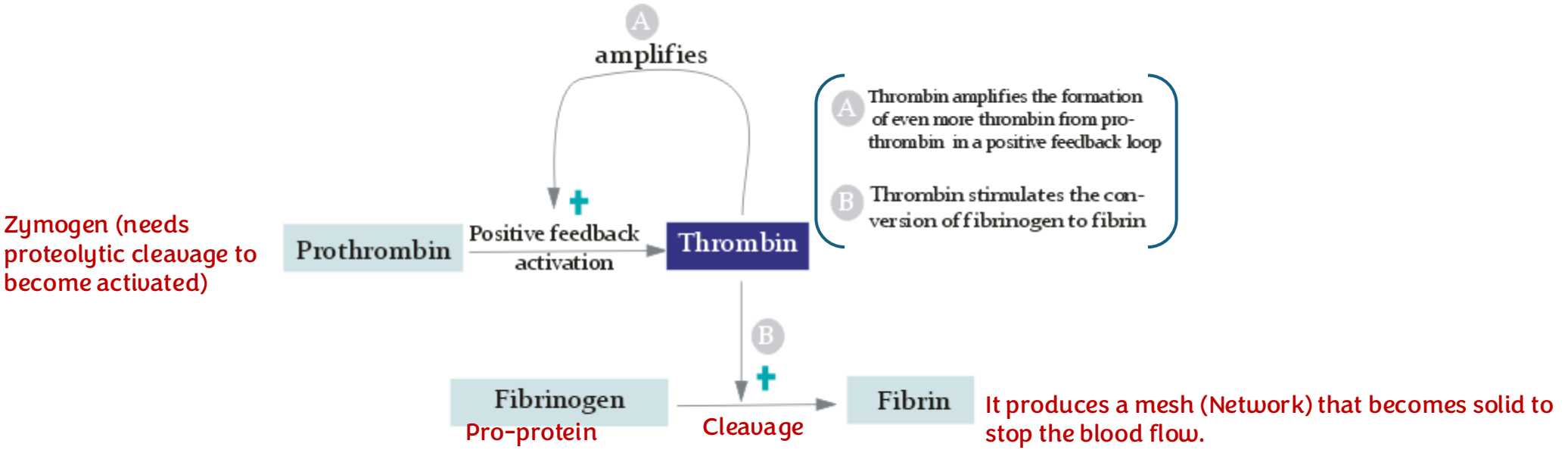
For example, aspartate transcarbamoylase (ATCase), the final product which is CTP affects the first enzyme that does the first reaction-> "Feedback inhibition".
So the last substrate inhibits the first enzyme, sometimes it's the product of the reaction itself(of the first enzyme) not the last substrate But it is called "Product inhibition" So the product regulates the enzyme that catalyzed its reaction (sarcastically ناكر للجميل).

Feedback activation

Blood coagulation تخثر الدمenzymes are a good example. Blood coagulation is simply solidifying blood due to some injury. During these reactions, thrombin (enzyme) is activated

- Positive feedback regulation: a product stimulates the activity of an enzyme.





Zymogen (needs proteolytic cleavage to become activated)

It produces a mesh (Network) that becomes solid to stop the blood flow.

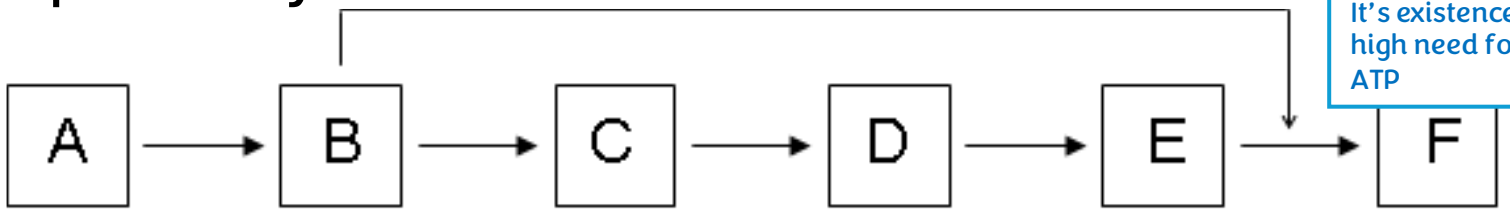
Positive feedback activation of more prothrombin into thrombin

Substrate activates a later enzyme.

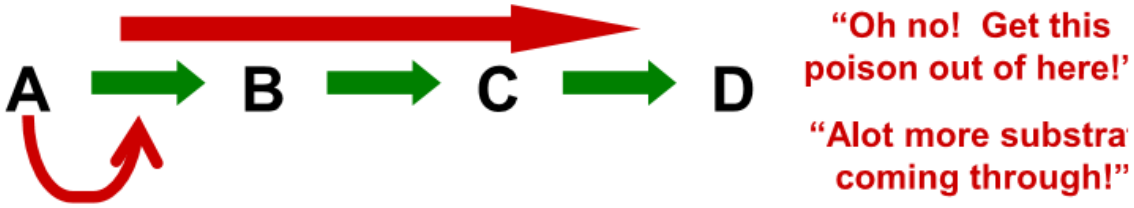
Feed-forward activation

Highly regulated

- Feed-forward regulation: a substrate produced early in a pathway activates an enzyme downstream of the same pathway.

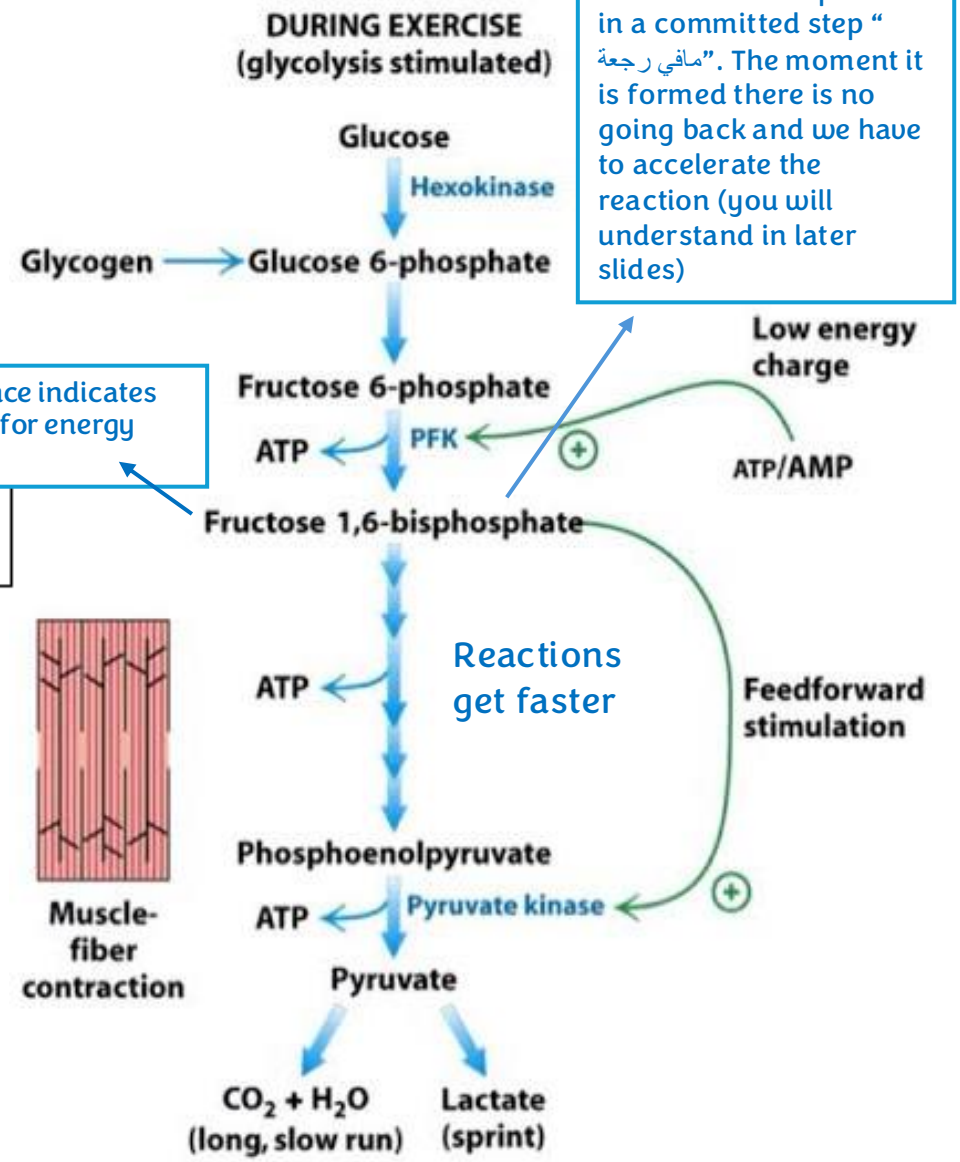


- Glycolysis
- Poisoning



So here Fructose 1,6-bisphosphate does the forward activation because it is the product in a committed step "مافي رجعة". The moment it is formed there is no going back and we have to accelerate the reaction (you will understand in later slides)

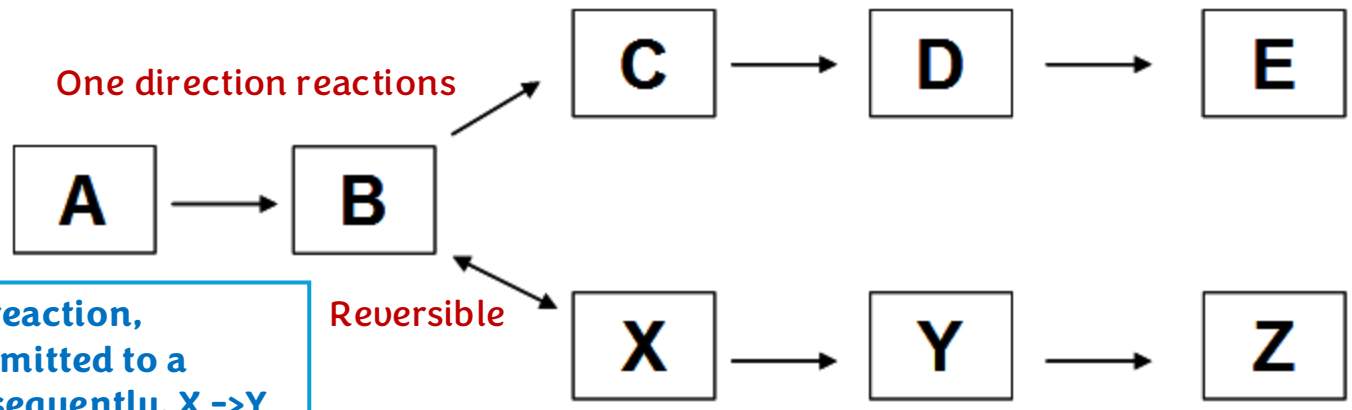
It's existence indicates high need for energy ATP



ملتزم No going back

A committed step

- A committed step in a metabolic pathway is the first **irreversible** reaction that is unique to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return.
- Committed steps are exergonic reaction.
- For example, the committed step for making product E is (B → C), not (A → B).



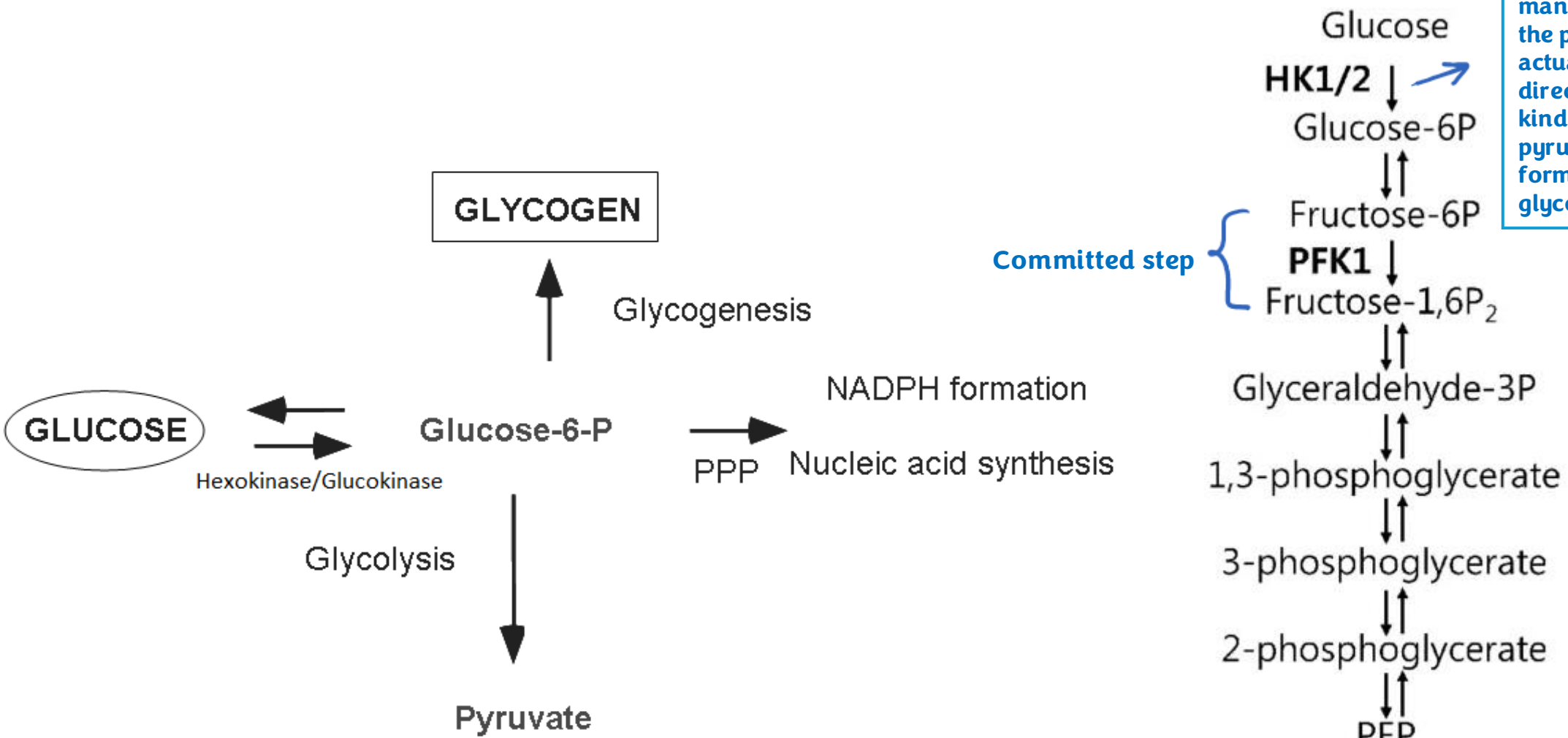
B <->X is a reversible reaction, meaning it is not committed to a single direction. Consequently, X ->Y is the committed step for this pathway

What is the committed step for reactions from A->E?
B->C

A-> B is not a committed step because B is not obligated to one fate it can produce either C or X, while B->C is . Why the subsequent reactions are not a committed steps? Because reactions that are after committed step are called downstream steps.

PFK, not HK/GK, is the committed step

Even though it is a non reversible reaction it is not a committed step because glucose 6-phosphate is used in many other reactions so the product is not actually fated to one direction in the cell it's kind of produce pyruvate or ribose to form DNA or put it in glycogen.

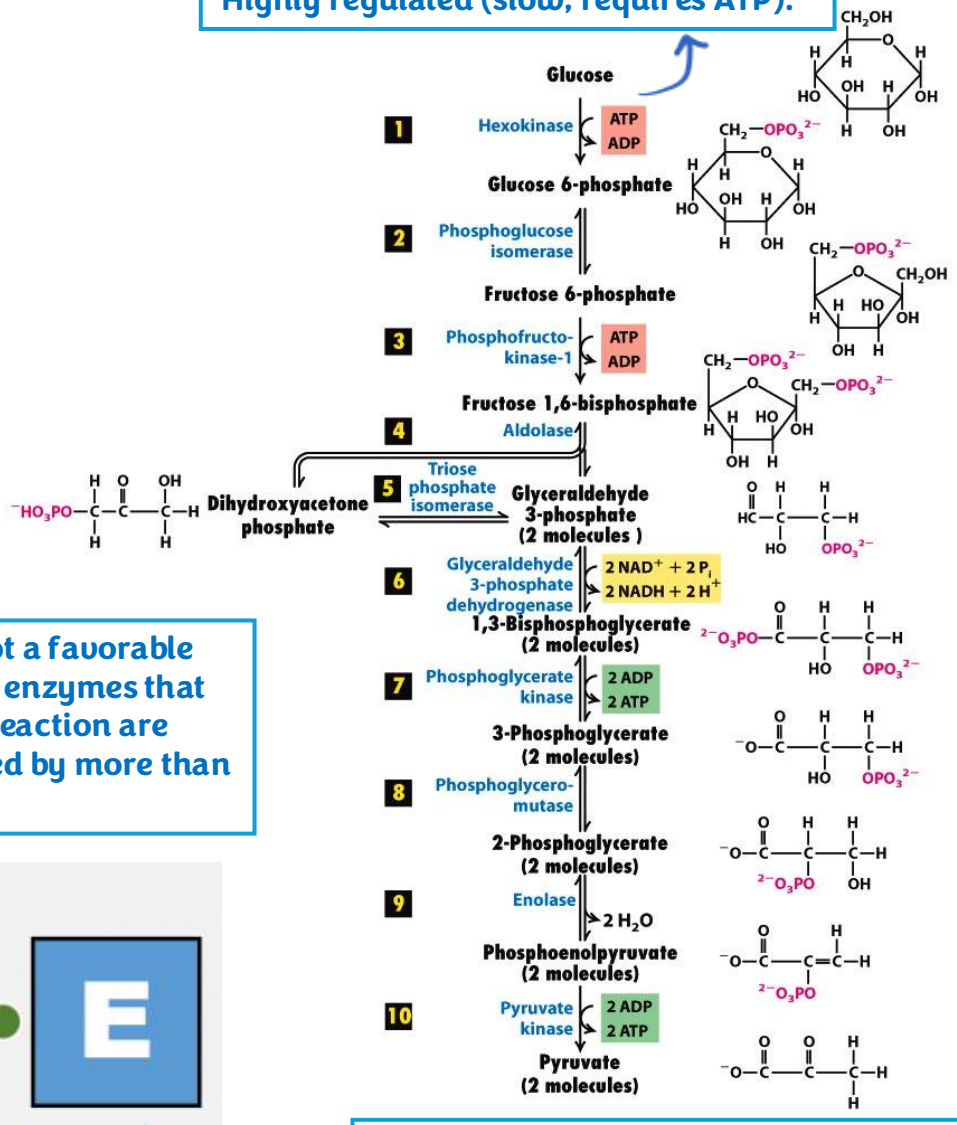


Limits the speed/rate of metabolic pathways

Rate-limiting reactions

- Rate-limiting reactions slow down rate of reactions because:
  - requirement for high amount of energy
  - strict regulation of enzymes
  - high Km values of enzyme towards its substrate
  - And usually they are allosteric enzymes
- These reactions are also usually, but not necessarily, committed steps.

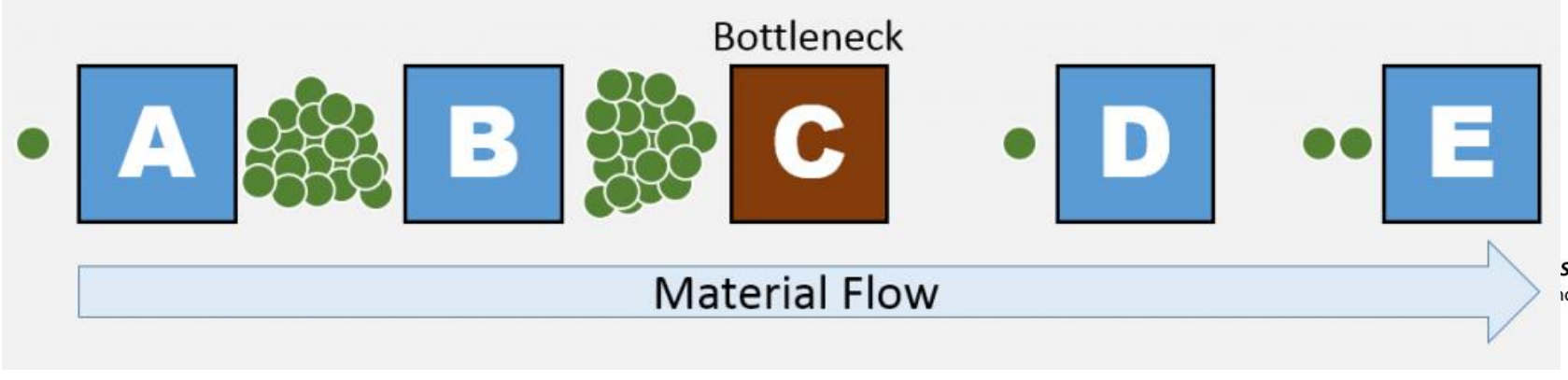
Highly regulated (slow, requires ATP).



Slow reactions but not necessarily a committed step, in the pathway we can have one committed step but more than one rate-limiting reaction

Notice we have a lot of rate limiting reactions but one committed step(fructose 6 phosphate-> Fructose 1,6 bisphosphate)

Using ATP is not a favorable process, so the enzymes that catalyze such reaction are highly regulated by more than on factor



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Basically any reaction that requires energy and spending ATP is highly regulated

Enzymes in disease diagnosis

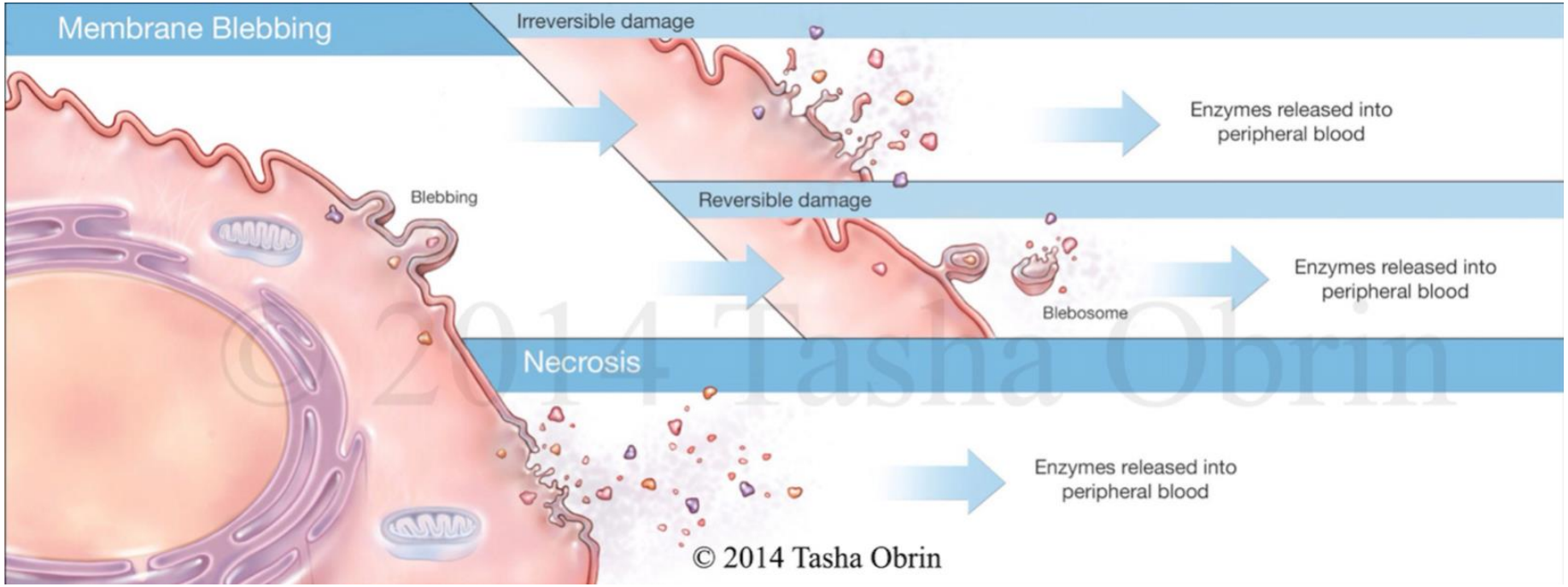
Enzymes can be used in disease diagnosis

Concept

Usually enzymes are inside the cell, cells undergo renewal. When tissue damage happens (either in liver, kidney or heart) there is more dead cells and release for these enzymes.

So when we do blood test for liver enzymes function, there is a percentage of liver enzymes in blood which is normal, it becomes pathological (abnormal) when the percentage is high-> indication of infection in liver (Bacterial infection, tissue damage).
Alcoholic people have liver damage.

- The presence of enzymes in serum indicates that tissue or cellular damage.
- The measurement enzyme amount in serum is of diagnostic significance.



Phosphorylate creatine and used to generate ATP.

Example: Creatine phosphokinase (CPK)

Recall : isozymes: enzymes that catalyze same reaction BUT come from different genes, regulated differently, different kinetics and are tissue specific

Enzyme exists in many tissues and has more than one isozyme

- CPK is found primarily in heart and skeletal muscle as well as the brain.
- Three tissue-specific isozymes of CPK:
  - CPK3 (CPK-MM)
  - CPK2 (CPK-MB)
  - CPK1 (CPK-BB)

They look for the level in the serum when someone actually runs or play sports, we will have a damage for the skeletal muscles and a release for the MM

Serum	Skeletal Muscle	Cardiac Muscle	Brain
			Very specific for brain
0 trace BB	0 trace BB	0% BB	97% BB
<6% MB	1% MB	**20% MB**	3% MB
>94% MM	99% MM	**80% MM**	0%MM

High-level of MB is an indicator for heart attack because it is the predominant in the cardiac tissue and of course, we will have a raise in the MM but the MB will be predominant.

Lactate dehydrogenase LDH1:LDH2 (LDH2 should be higher) If ratio is switched-> heart tissue damage but this is not used anymore because troponin is the gold standard (Specific protein for the heart)

Test yourself

Additional Resources:

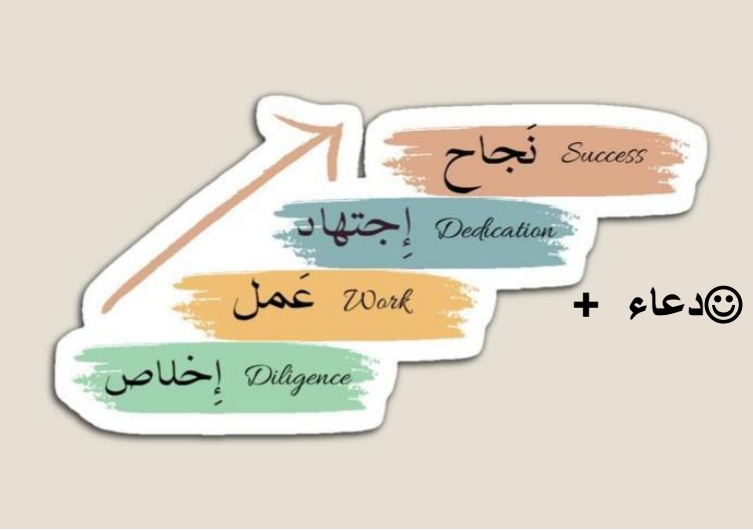
رسالة من الفريق العلمي:

Reference Used:

Prof. Mamoun Ahram recorded lecture

Extra References for the Reader to Use:

Marks' Basic Medical Biochemistry (6th edition)



"وَقُل رَّبِّ زِدْنِي عِلْمًا"

طريق الدراسة وطلب العلم قد يخلله شيء من التعب أو الشعور بالضغط، لكن تذكر دائماً أن كل خطوة تخطوها في هذا الطريق تُقربك من تحقيق أهدافك وبناء مستقبل مشرق.

نصيحة لك :نظم وقتك، واجعل لكل ساعة دراسة وقتاً للراحة، فالاستمرارية بذكاء أفضل بكثير من الجهد المفرط بلا توجيه.

دعاء :اللهم لا سهل إلا ما جعلته سهلاً، وأنت تجعل الحزن إذا شئت سهلاً، يسر لك أمورك وشرح صدرك لكل علم نافع.

لا تنسونا ووالدينا والأمة الإسلامية من صالح دعائكم، ولا تنسوا أهلنا في غزة والمسلمين في كل بقاع الأرض -نسأل الله أن يفرّج عنهم وينصرهم ويثبّت أقدامهم -

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			