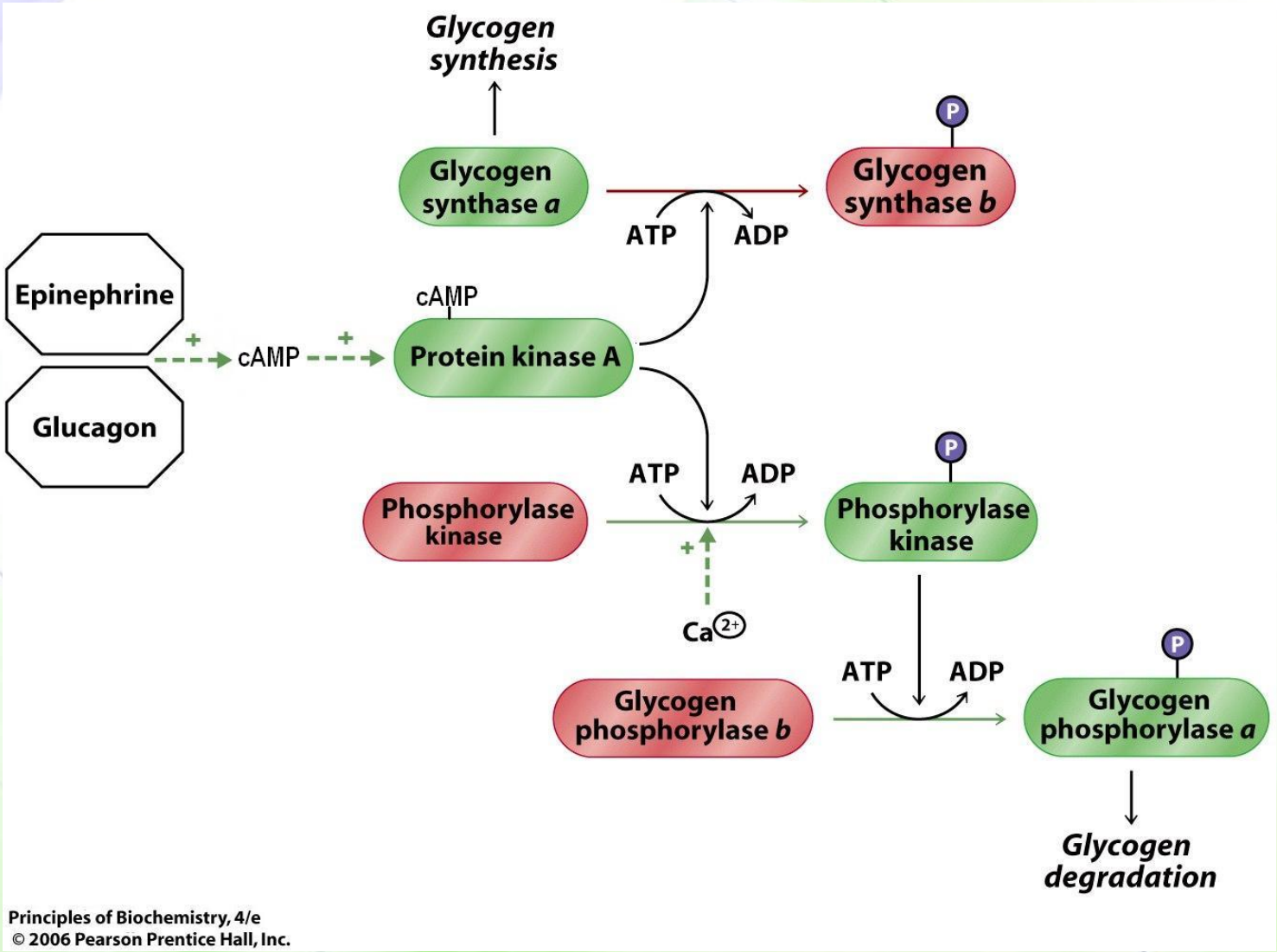
Enzymes
Part III: regulation II

Prof. Mamoun Ahram
Dr. Diala Abu Hassan
Summer 2019

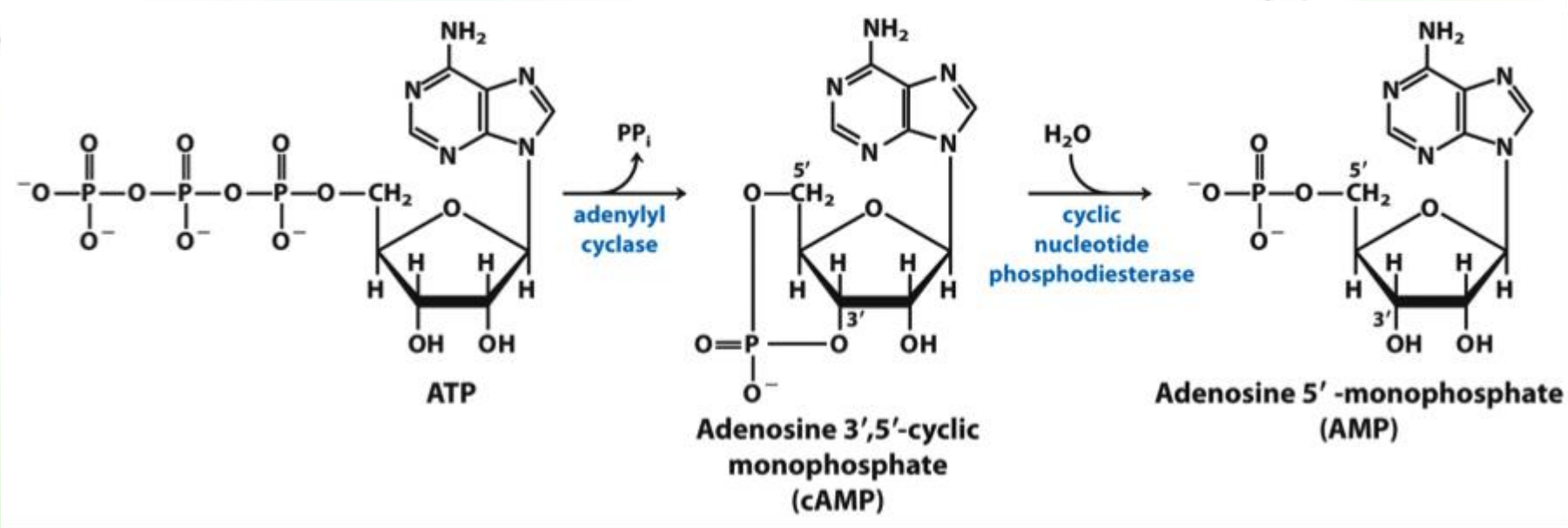
Regulation via modulators

Phosphorylation cascade



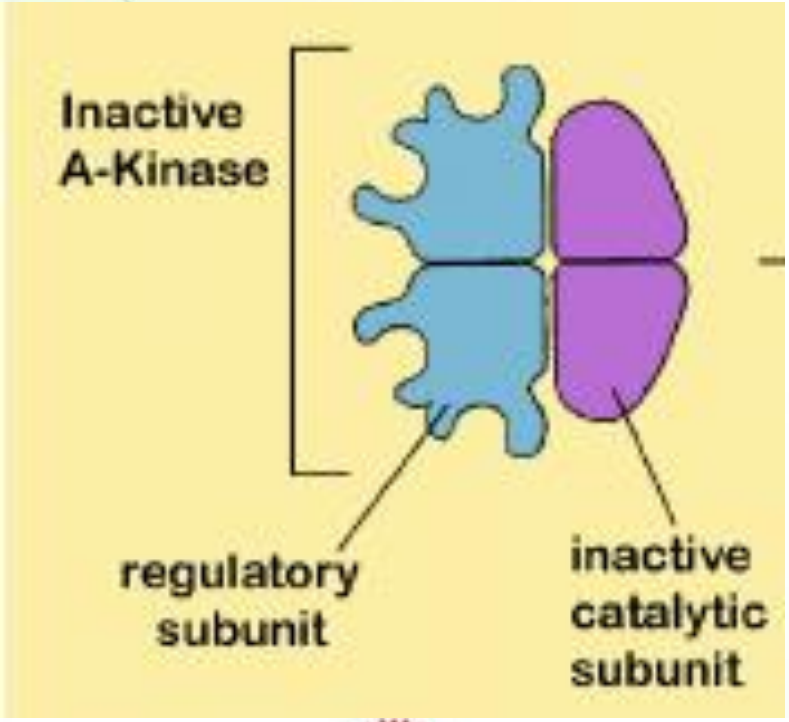
cAMP and protein kinase A (PKA)

- Small-molecule modulators can have dramatic effects on enzymes.
- For example, cAMP, which is structurally modified AMP, can activate protein kinase A (PKA).



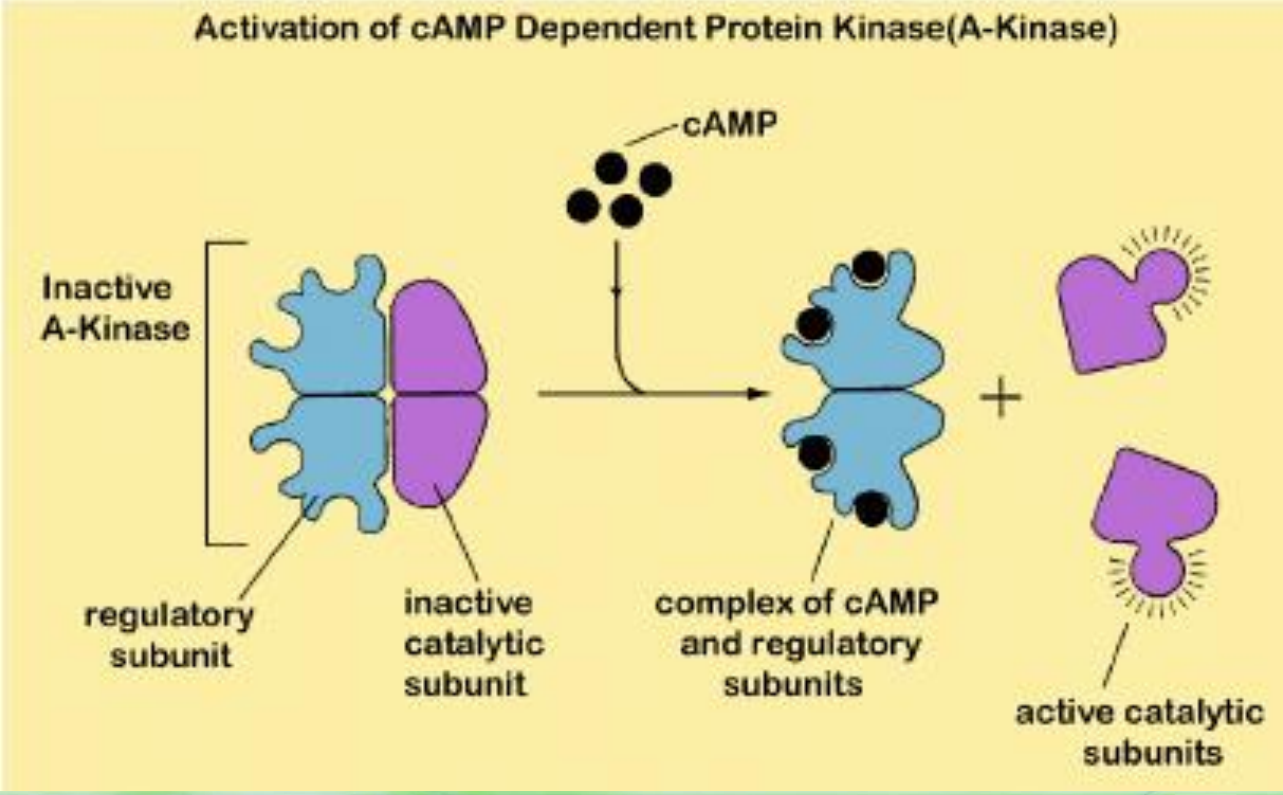
PKA-structure and regulation

- Protein kinase A (PKA), a serine/threonine protein kinase, phosphorylates several enzymes that regulate different metabolic pathways.
  - Example: glycogen phosphorylase kinase
- When inactive, PKA consists of four subunits
  - Two regulatory (R) subunits with high affinity for cAMP,
  - Two catalytic (C) subunits



When cAMP binds

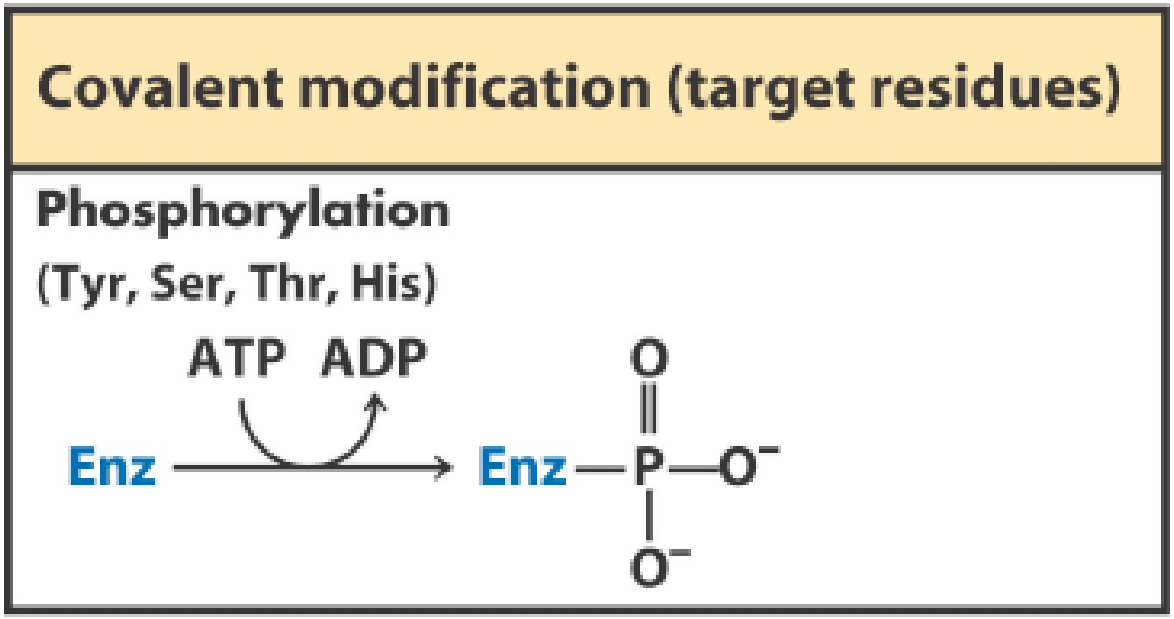
- The binding of two molecules of cAMP to the regulatory subunits leads to the dissociation of R2C2 into an R2 subunit and two active C subunits.



Reversible covalent modification

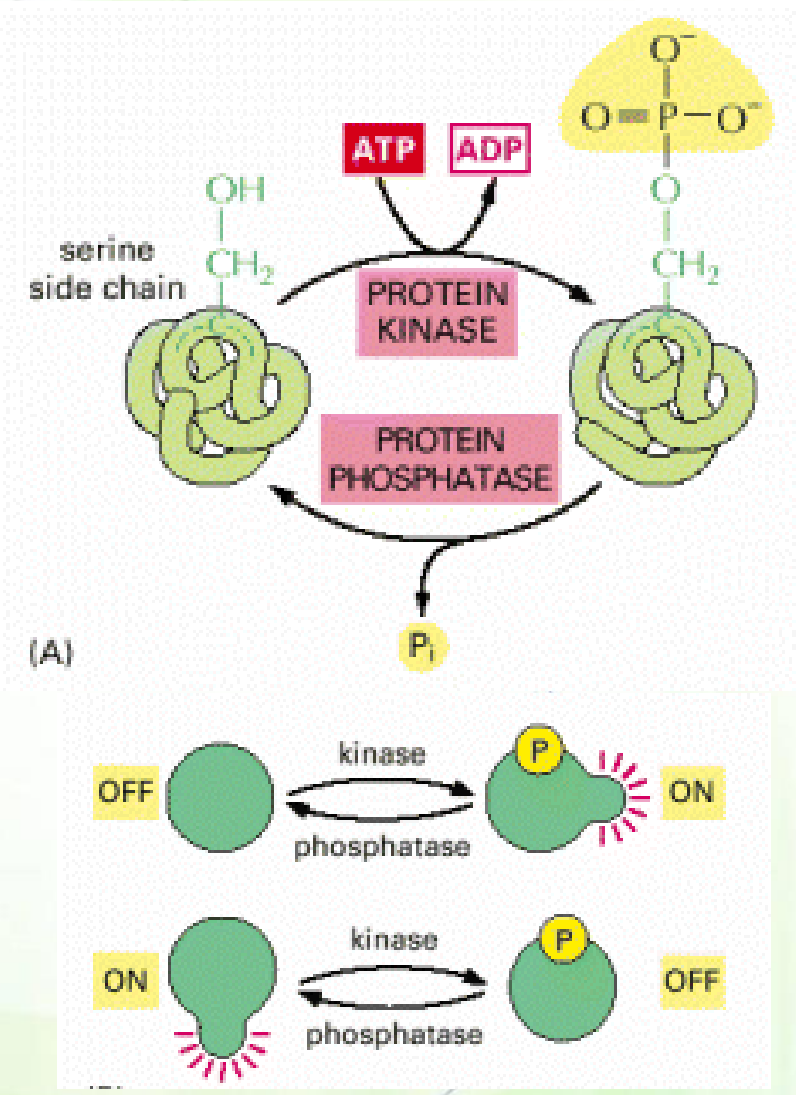
Advantage

- Rapid and transient.
- A most common mechanism is enzyme phosphorylation (the covalent addition of a phosphate group to one of its amino acid side chains).
- Usually serine, threonine, and tyrosine.



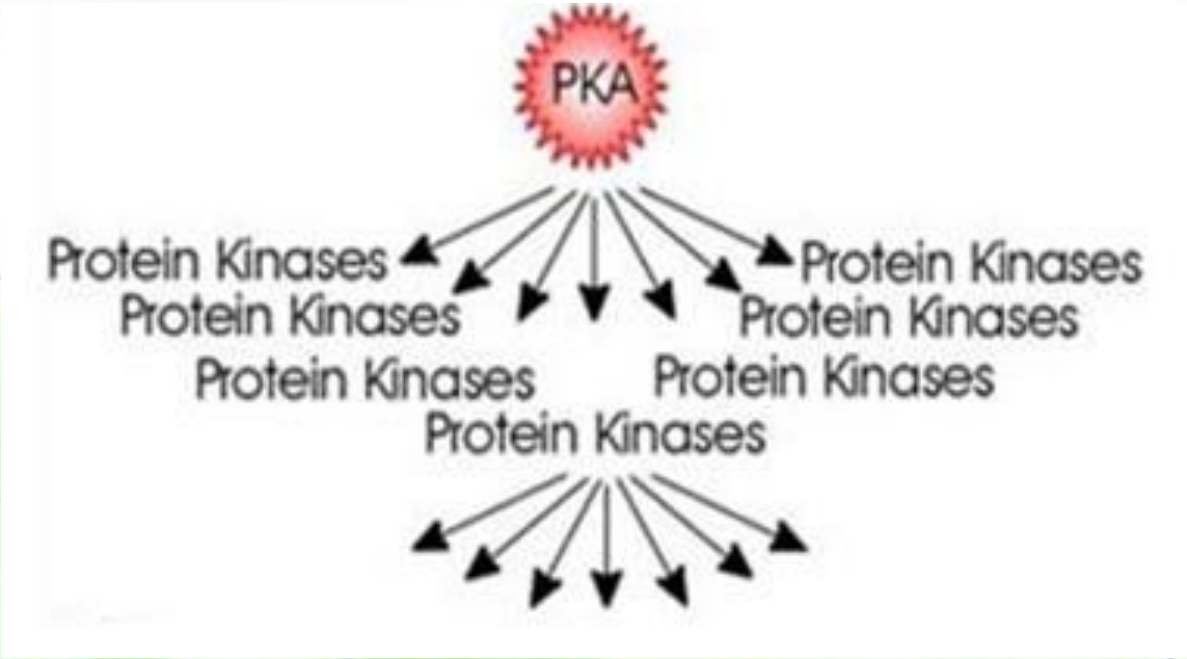
Enzymes

- ATP mostly is the phosphoryl donor in these reactions, which are catalyzed by protein kinases.
- The removal of phosphoryl groups (dephosphorylation) by hydrolysis is catalyzed by protein phosphatases.
- Note: dephosphorylation is not the reversal of phosphorylation.
- The addition or removal of a phosphate group to an enzyme may activate or inactivate these enzymes.



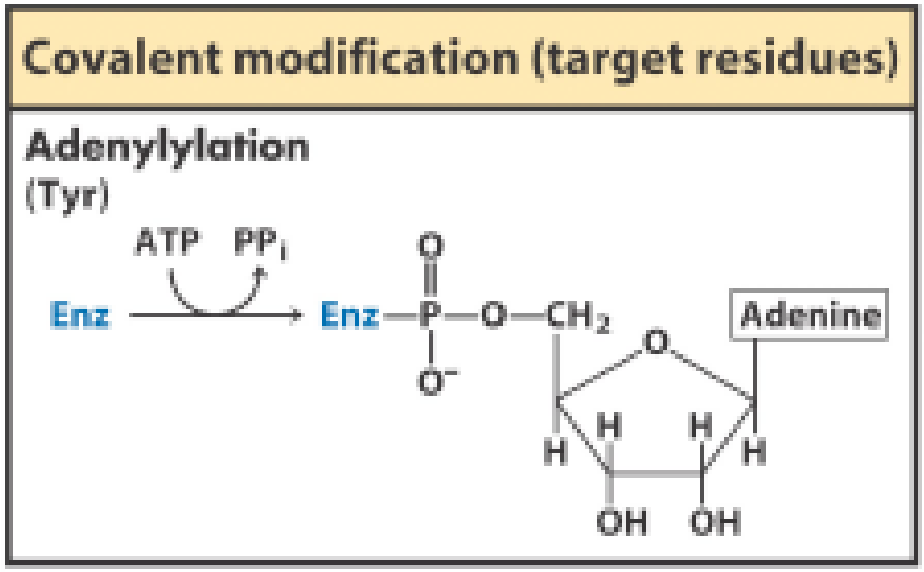
Why is it effective?

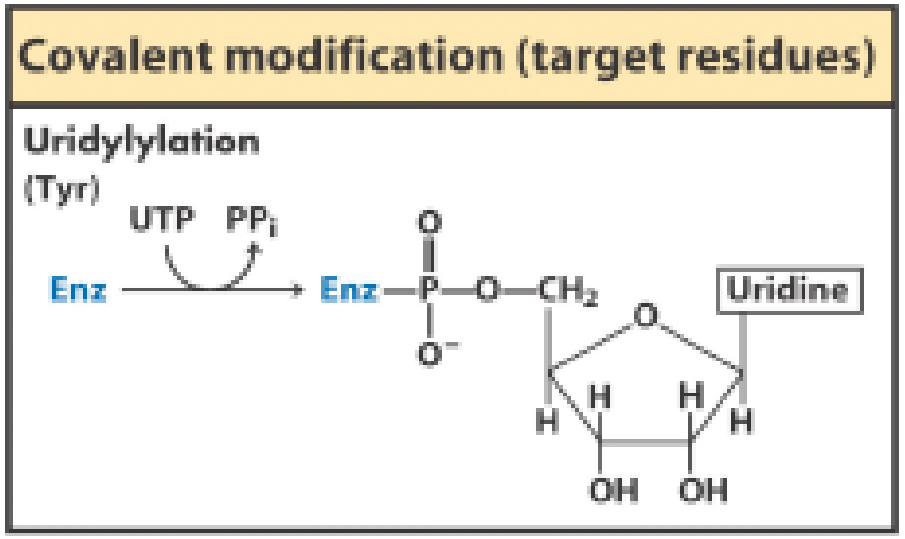
- Formation or removal of new electrostatic interactions and/or hydrogen bonds altering substrate binding and catalytic activity.
- It can happen in less than a second or over a span of hours.
- Phosphorylation often causes highly amplified effects.



Others

- Adenylylation (addition of adenylyl group). AMP is transferred to Tyr residues through phosphodiester linkage.
- The addition of bulky AMP inhibits cytosolic enzymes.
- Uridylylation (addition of uridylyl group).



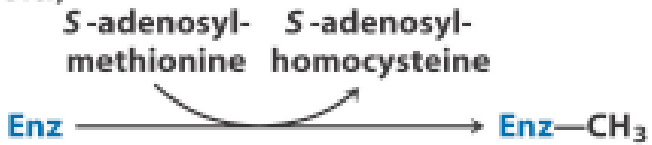


Others

- ADP-ribosylation (addition of adenosine diphosphate ribosyl group) inactivates enzymes.
- Methylation of carboxylate side chains masking negative charges.
- Acetylation (from acetyl Co) to lysine residues masking positive charges.

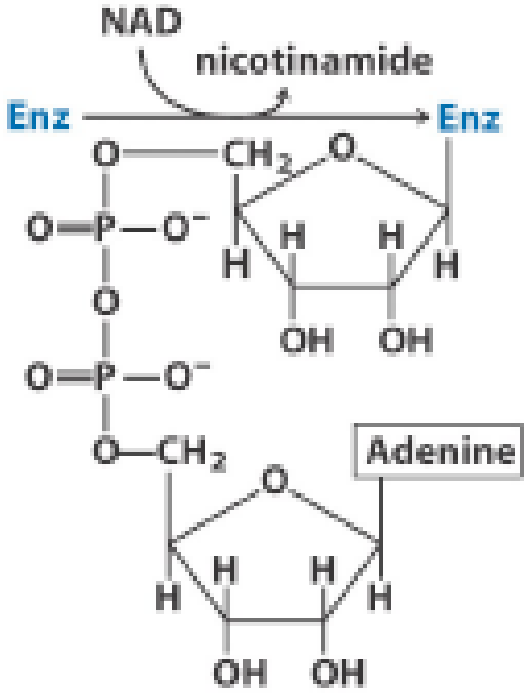
Covalent modification (target residues)

Methylation
(Glu)

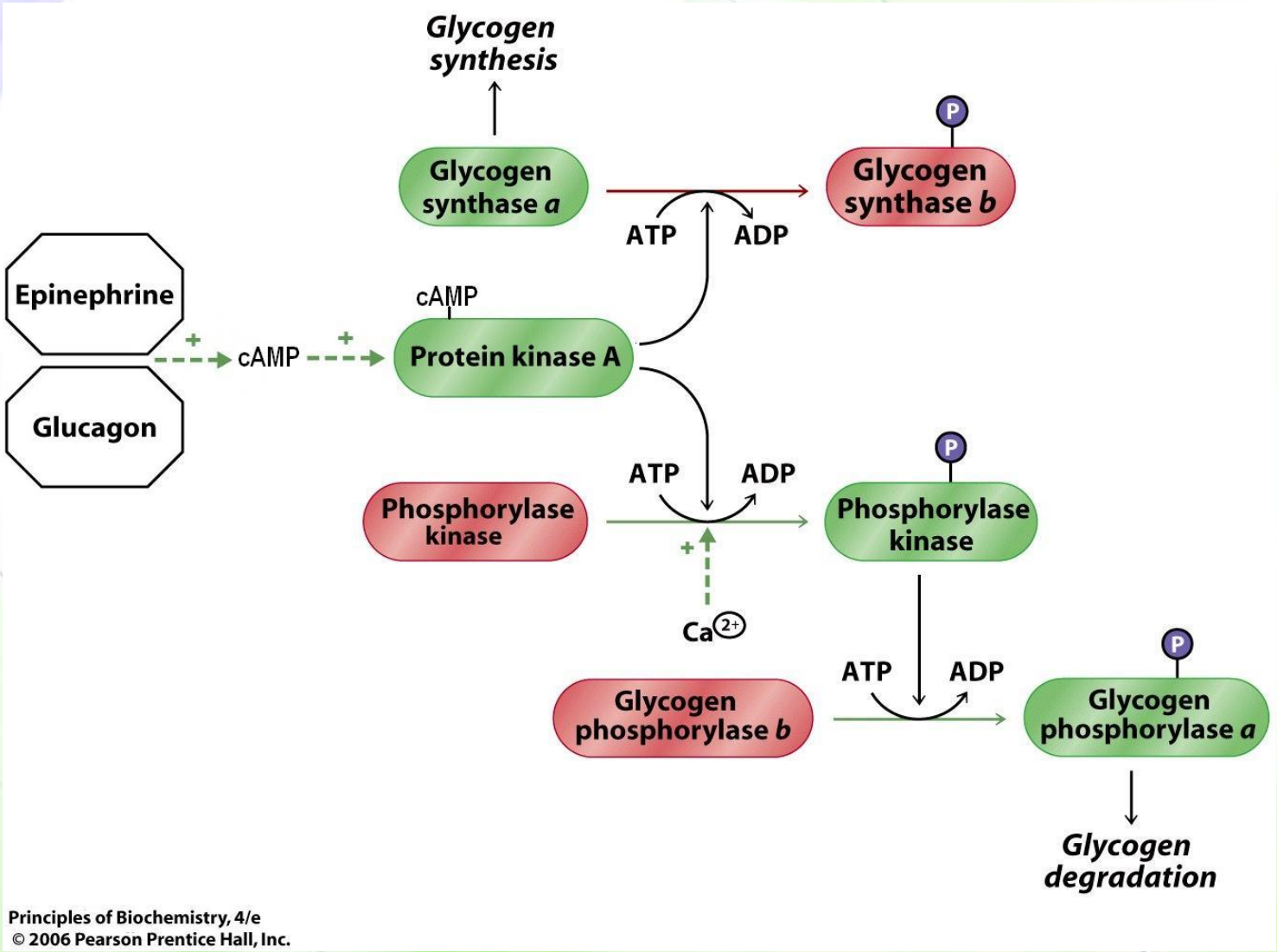


Covalent modification (target residues)

ADP-ribosylation
(Arg, Gln, Cys, diphthamide—a modified His)



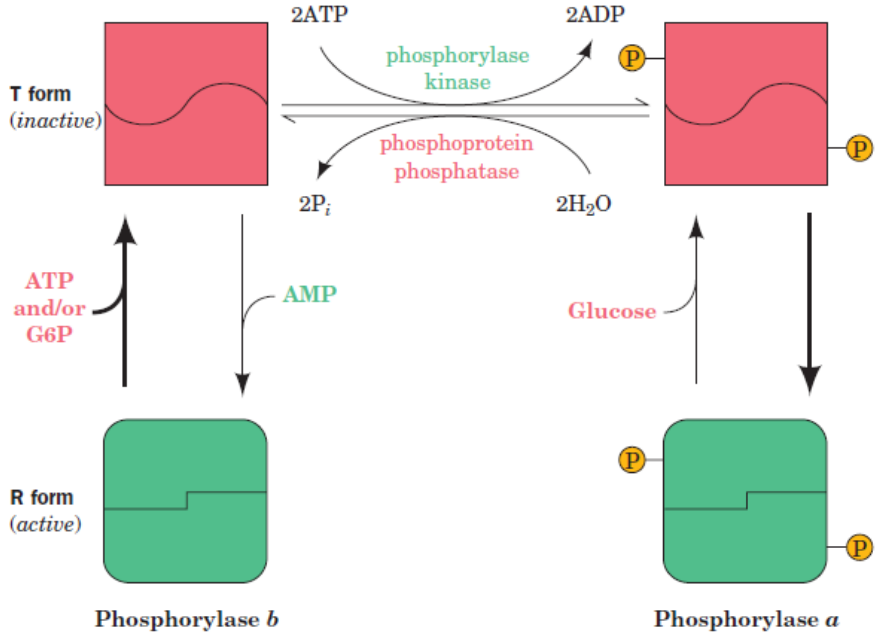
Phosphorylation cascade



Example: Glycogen phosphorylase

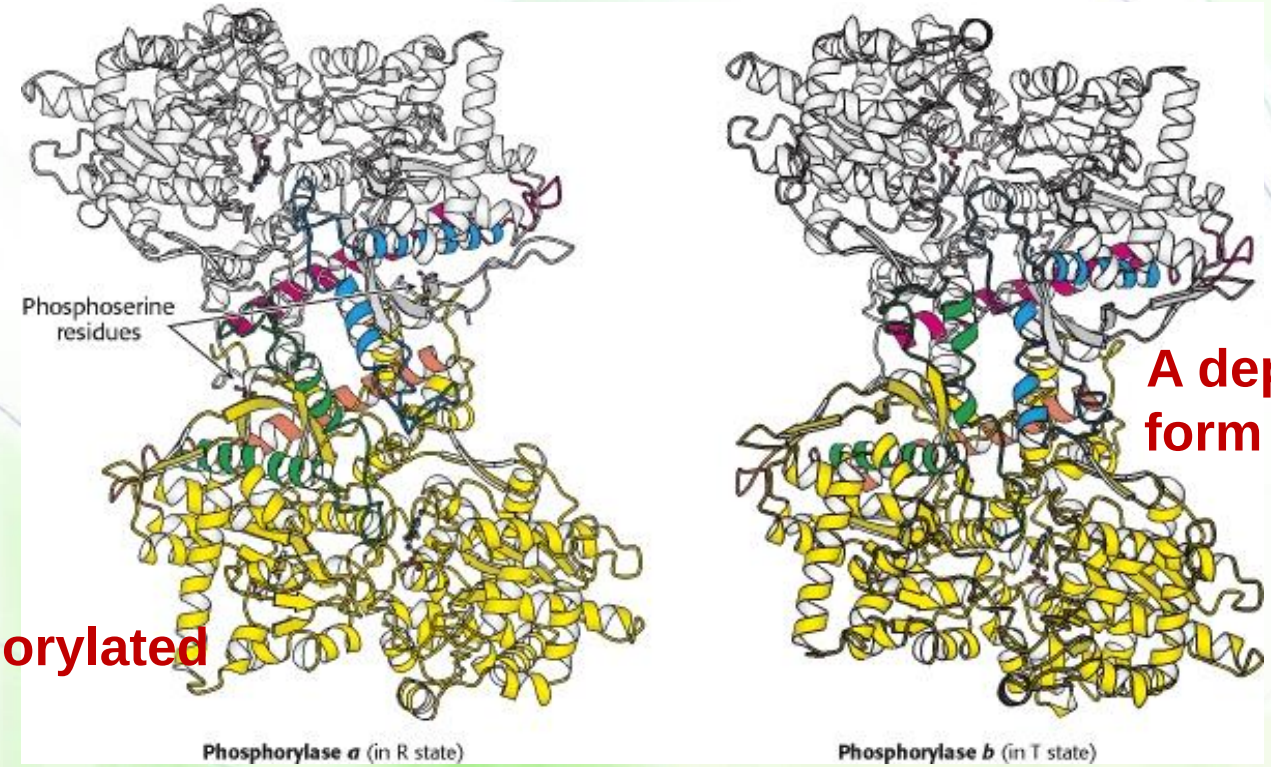
$$\text{glycogen}_{(n\ \text{residues})} + P_i \longrightarrow \text{glycogen}_{(n-1\ \text{residues})} + \text{glucose 1-P}$$

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



Phosphorylation

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



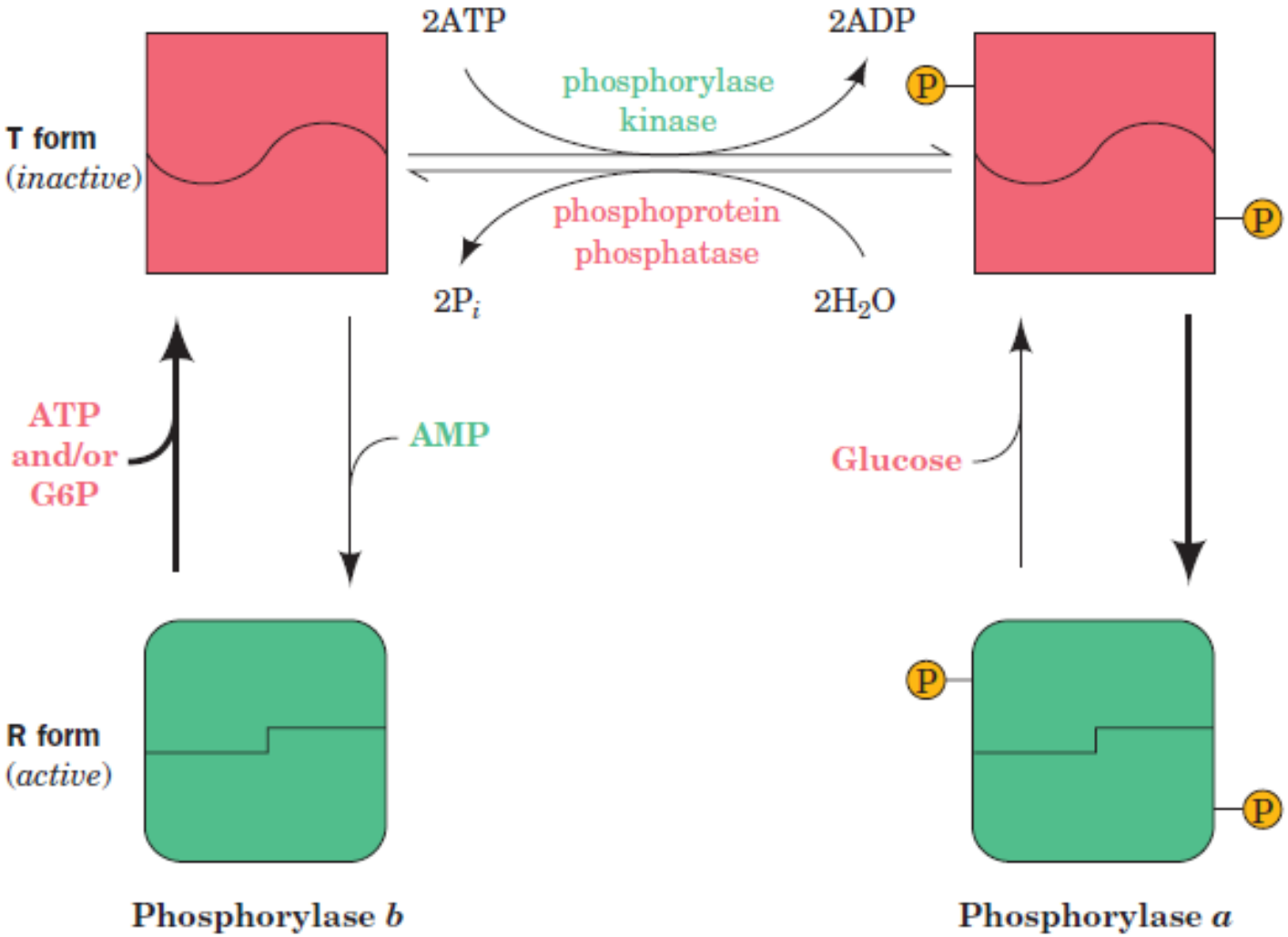
A phosphorylated form "a"

A dephosphorylated form "b"

The two forms of each form

- Both phosphorylase *b* and phosphorylase *a* exist as equilibria 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.

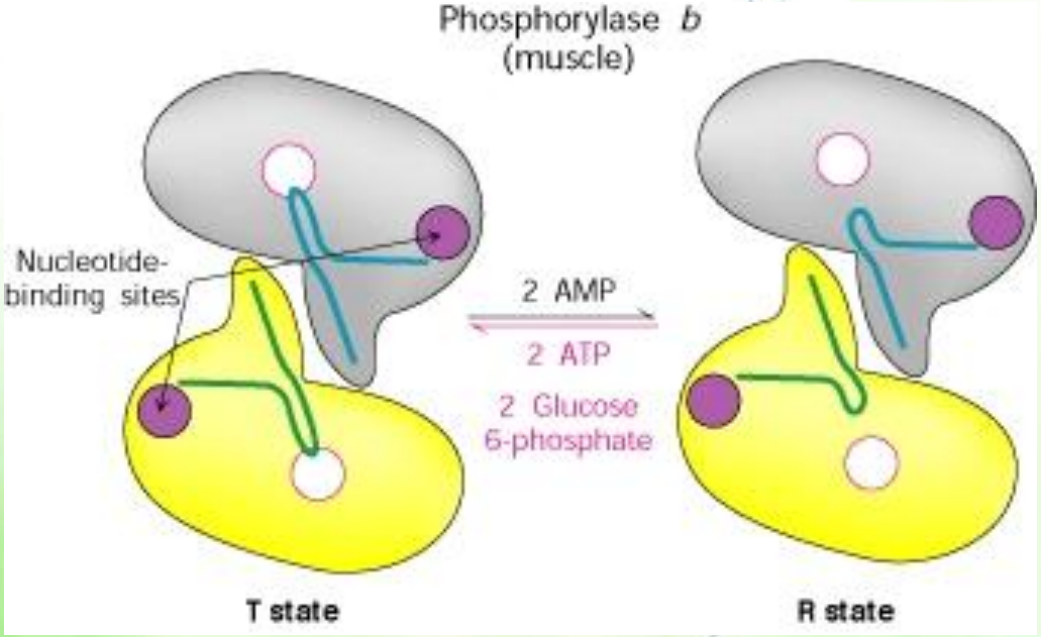
The transition of phosphorylase b between the T and the R state is controlled by the energy charge (ATP and AMP) of the muscle cell.

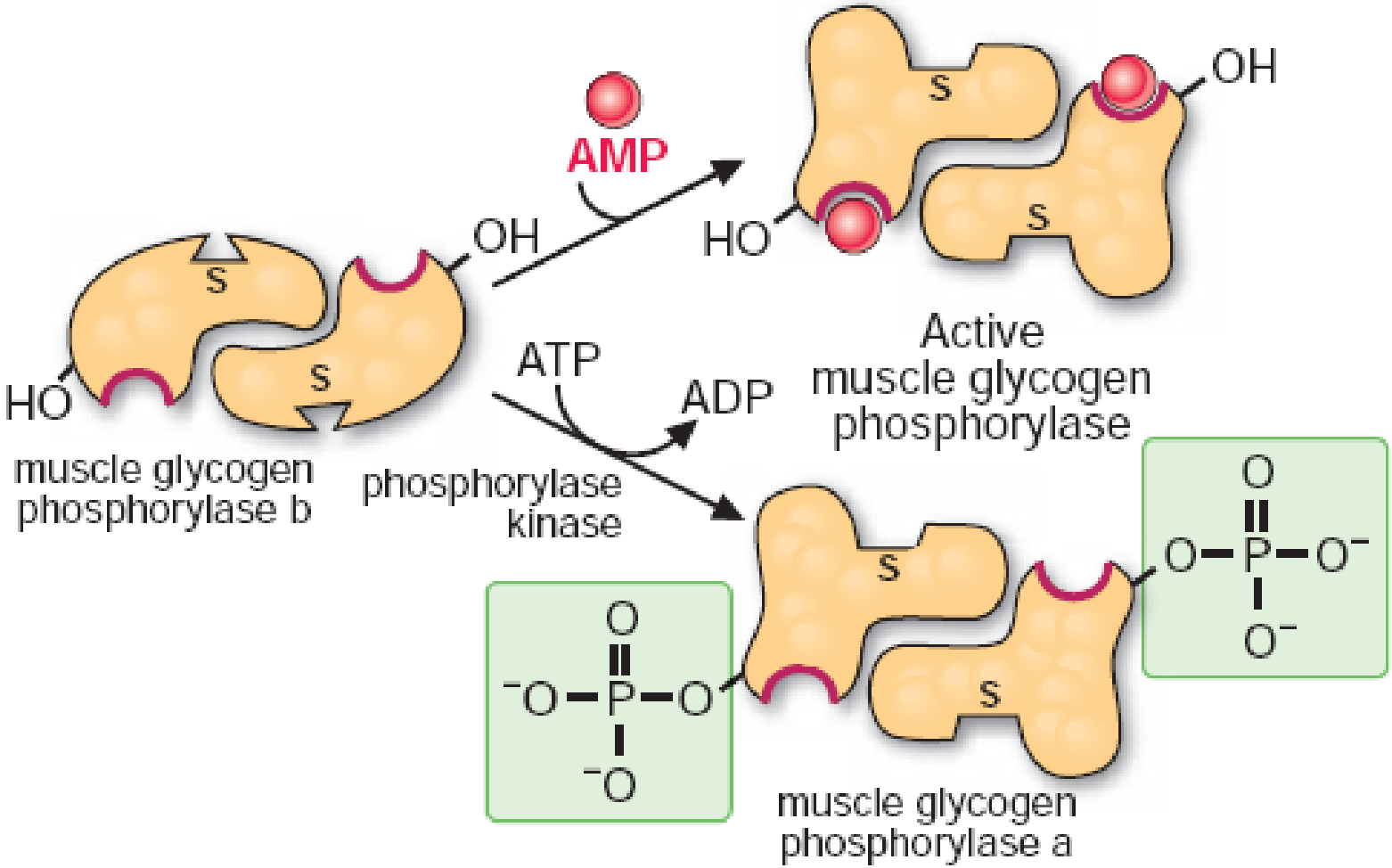

2ATP
2ADP
phosphorylase kinase
T form (inactive)
P
P
phosphoprotein phosphatase
$2P_i$
$2H_2O$
ATP and/or G6P
AMP
Glucose
R form (active)
P
P
Phosphorylase b
Phosphorylase a

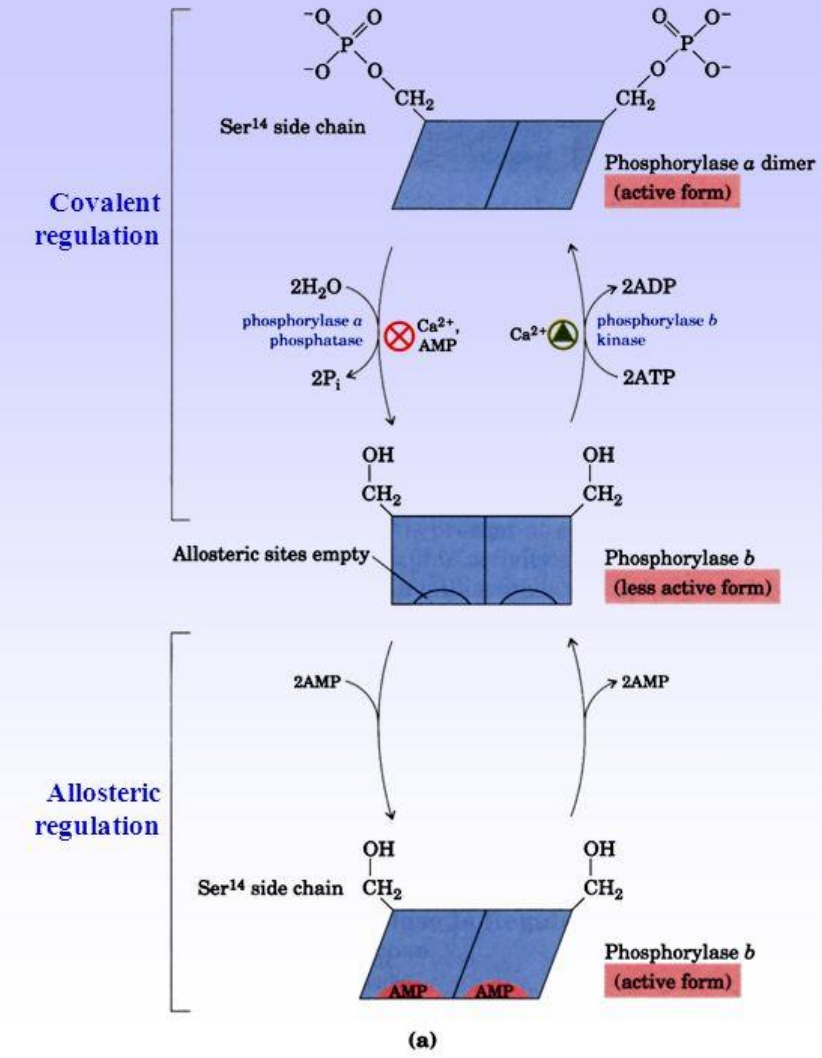
What do ATP and AMP do?

- 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.




AMP
OH
HO
S
S
Active
muscle glycogen
phosphorylase
ATP
ADP
muscle glycogen
phosphorylase b
phosphorylase
kinase
O
⁻O
P
O
⁻O
muscle glycogen
phosphorylase a
O⁻



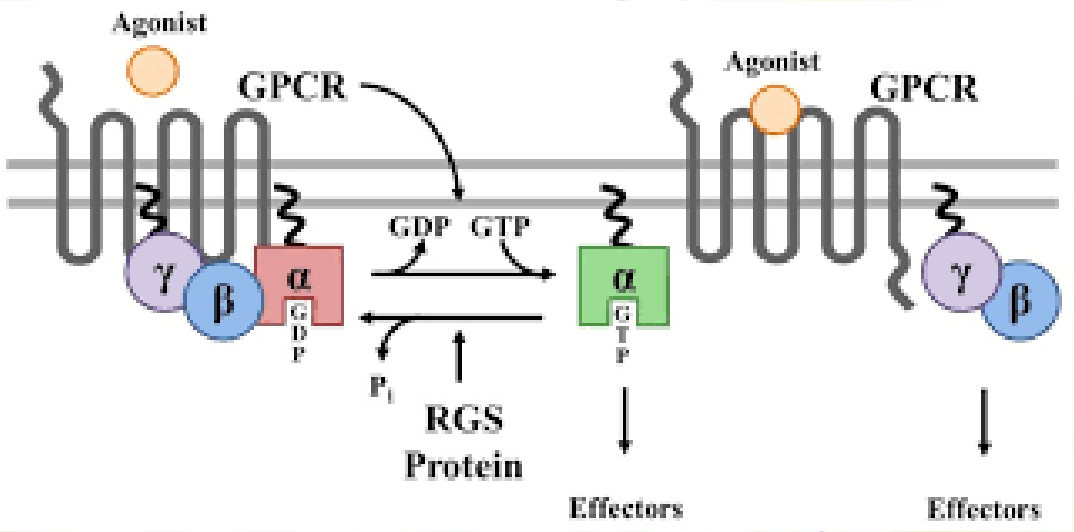
Covalent and allosteric regulation of glycogen phosphorylase in muscle.

(a) The enzyme has two identical subunits, each of which can be phosphorylated by phosphorylase *b* kinase at Ser^{14} to give phosphorylase *a*, a reaction promoted by Ca^{2+}. Phosphorylase a phosphatase, also called phosphoprotein phosphatase-1, removes these phosphate groups, inactivating the enzyme. Phosphorylase *b* can also be activated by noncovalent binding of AMP at its alosteric sites. Conformational changes in the enzyme are indicated schematically. Liver glycogen phosphorylase undergoes similar *a* and *b* interconversions, but has different regulatory mechanisms.

Regulation - Large regulatory molecules

- G protein: a family of trans-membrane proteins causing changes inside the cell. They communicate signals from hormones, neurotransmitters, and other signaling factors

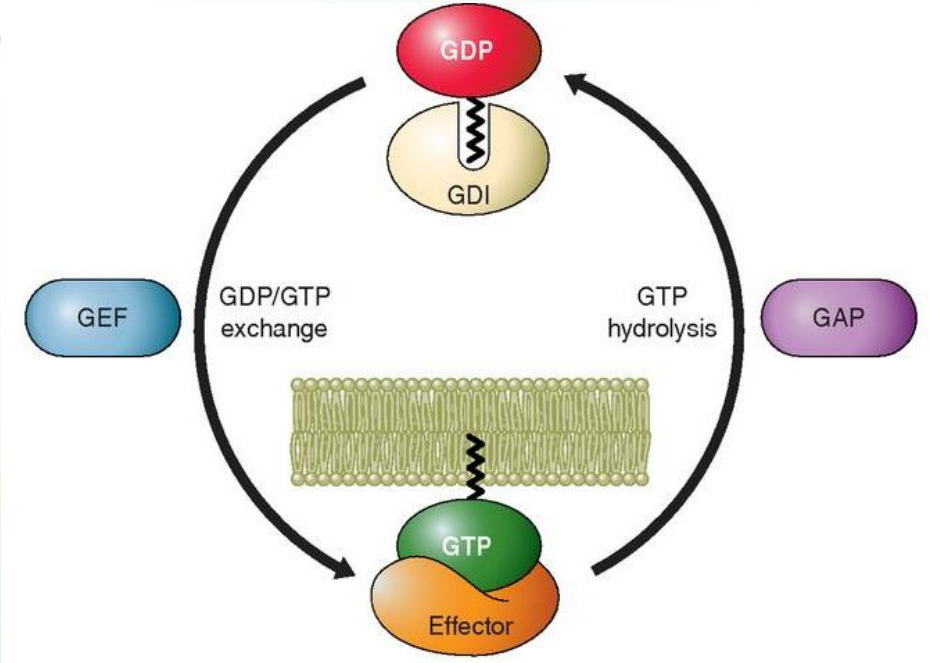
- **When they bind guanosine triphosphate (GTP), they are 'on', and, when they bind guanosine diphosphate (GDP), they are 'off'.**



- **The α subunit can be stimulatory or inhibitory.**

Monomeric G proteins

- When GTP is bound, the conformation of the G protein allows it to bind target proteins, which are then activated or inhibited.
- The 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 G protein.



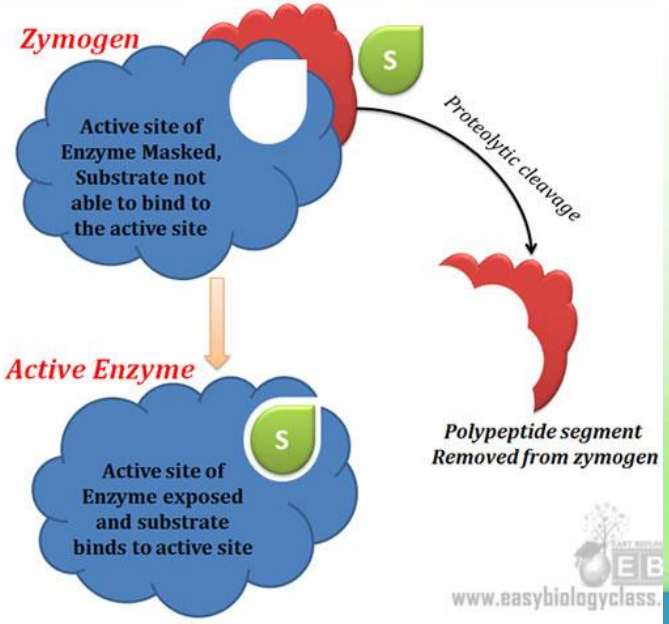
The activity of many G proteins is regulated by

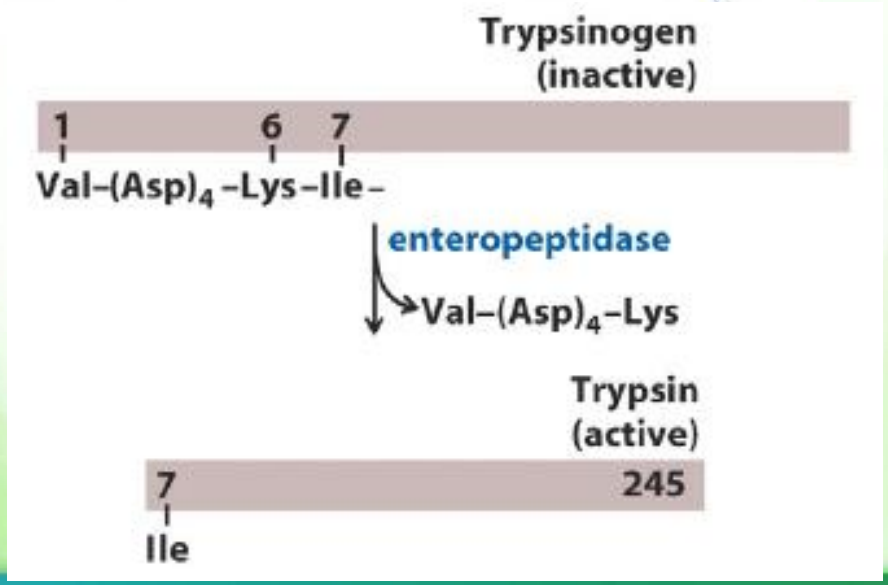
1. **GAPs [*GTPase-activating proteins*]**
2. ***GEFs [guanine nucleotide exchange factors]***
3. **GDIs [*GDP dissociation inhibitors*]**

Irreversible covalent modification (proteolytic activation)

Zymogens

- 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.





Nonspecific Inhibitors

Regulation of enzyme amount

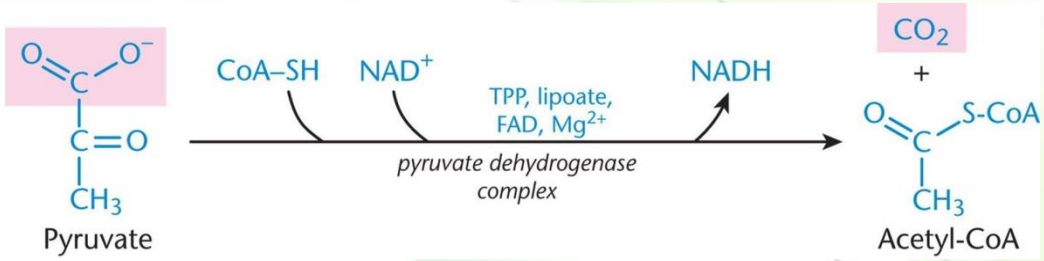
- Three mechanisms:
  - Synthesis of isozymes
  - Enzyme synthesis at the gene level
  - Enzyme degradation by proteases

- They are comparatively slow mechanisms for regulating enzyme concentration (hours-weeks).

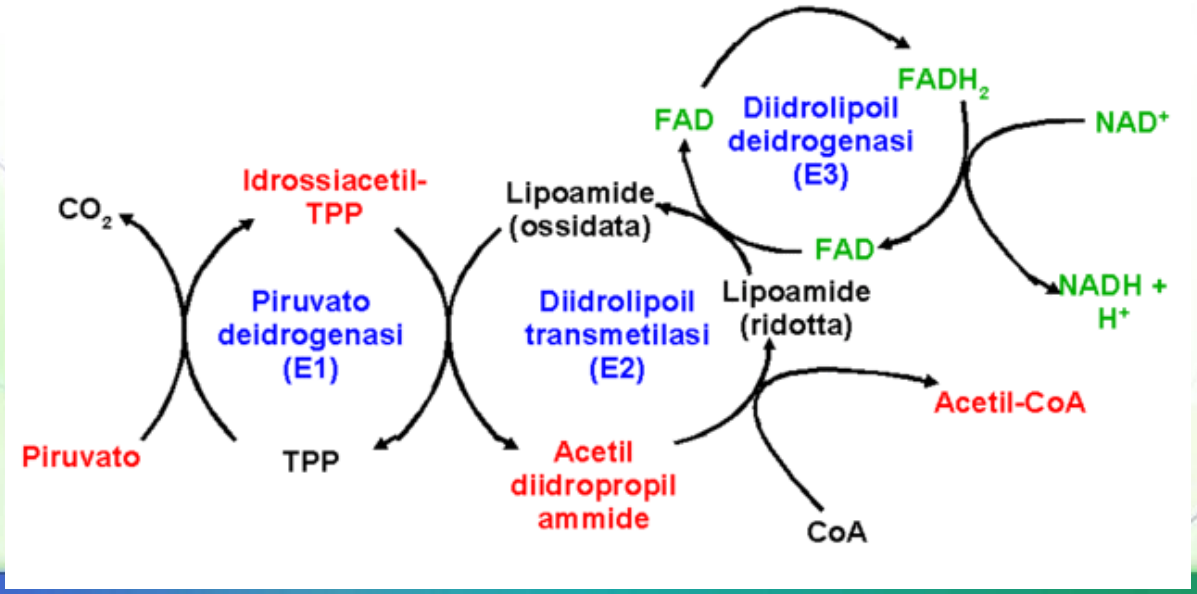
Compartmentalization

- Compartmentalization reduces the area of diffusion of both enzyme and substrate increasing the probability that they collide.
  - Example 1: lysosomal enzymes
  - Example 2: fatty acid metabolism
    - Synthesis occurs in cytosol, whereas break-sown is mitochondrial.

Enzyme complexing

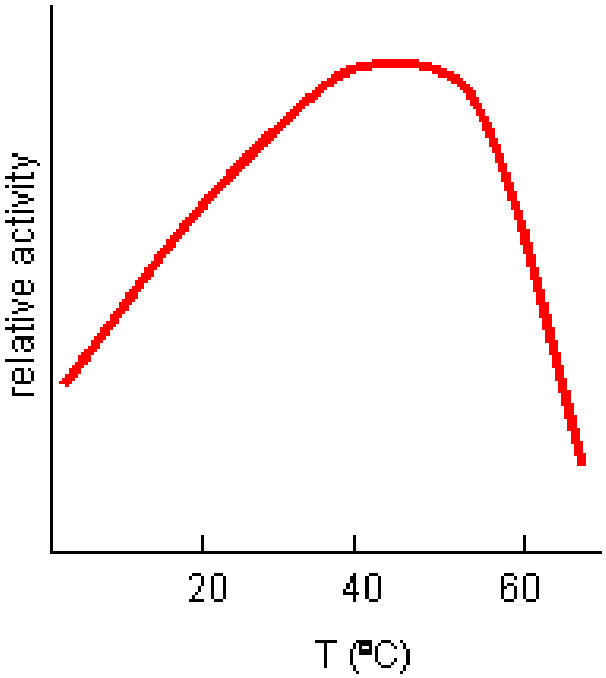


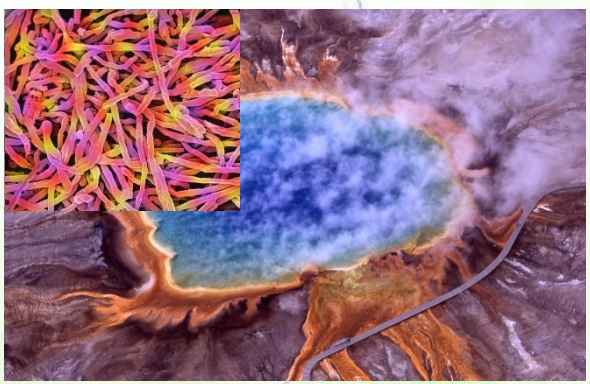
- Formation of a complex of multiple enzymes also reduces diffusion.
- Example: Pyruvate dehydrogenase (mitochondria) is composed of 3 enzymes: decarboxylation, oxidation, & transfer of the acyl group to CoA.



Temperature

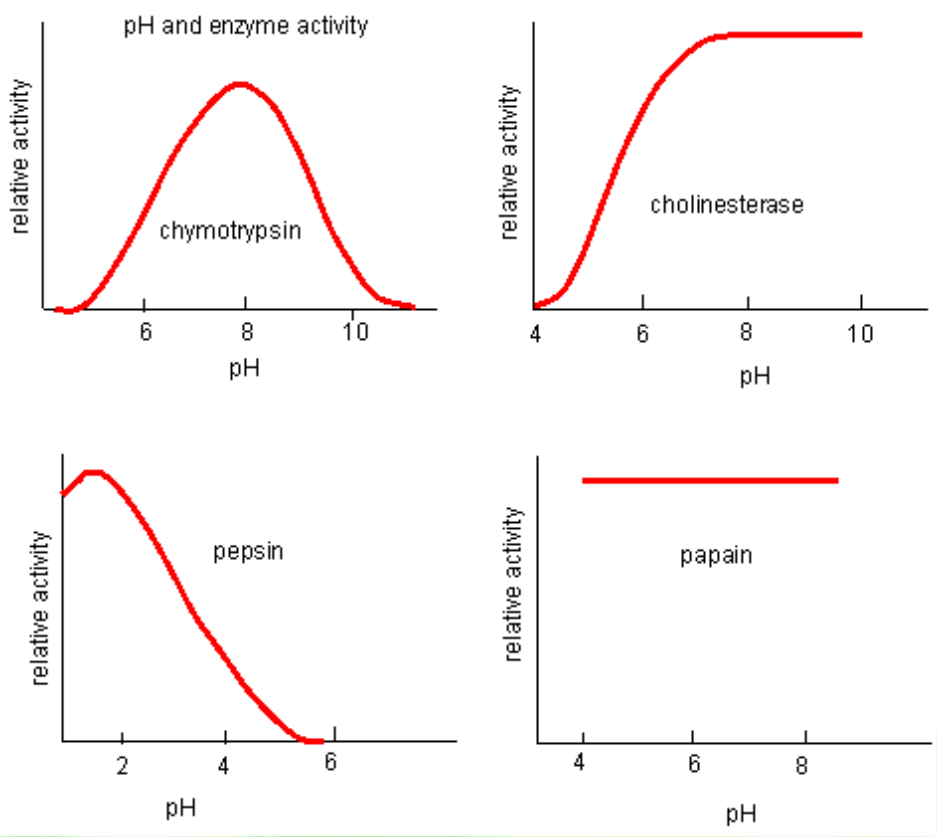
- Reaction rates increase with temperature due to increased kinetic energy of the molecules resulting in more collisions between enzymes and substrates.
- However, high temperatures lead to protein denaturation.
- Each enzyme has an optimal temperature.
- For thermophilic bacteria, the optimal temperature is as high as 65°C.





pH

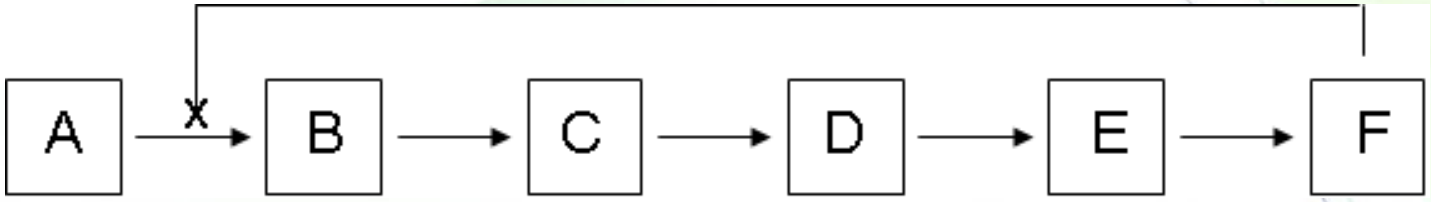
- pH alters the protonation state of the substrate and/or the enzyme and, hence, their binding.
- The effect of pH is enzyme-dependent.

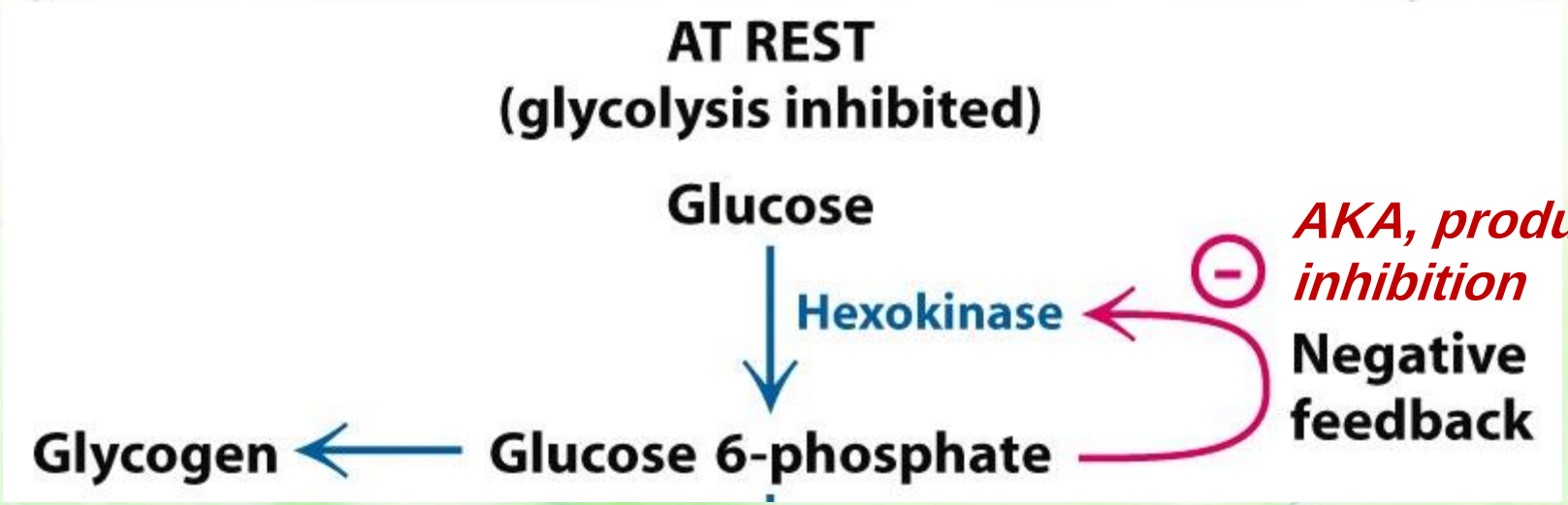


Modes of regulation

Feedback inhibition

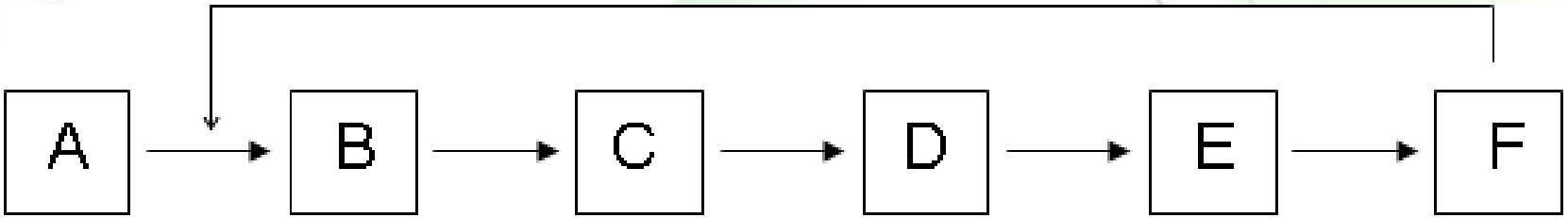
- Feedback inhibition or negative feedback regulation: an enzyme present early in a biochemical pathway is inhibited by a late product of pathway.

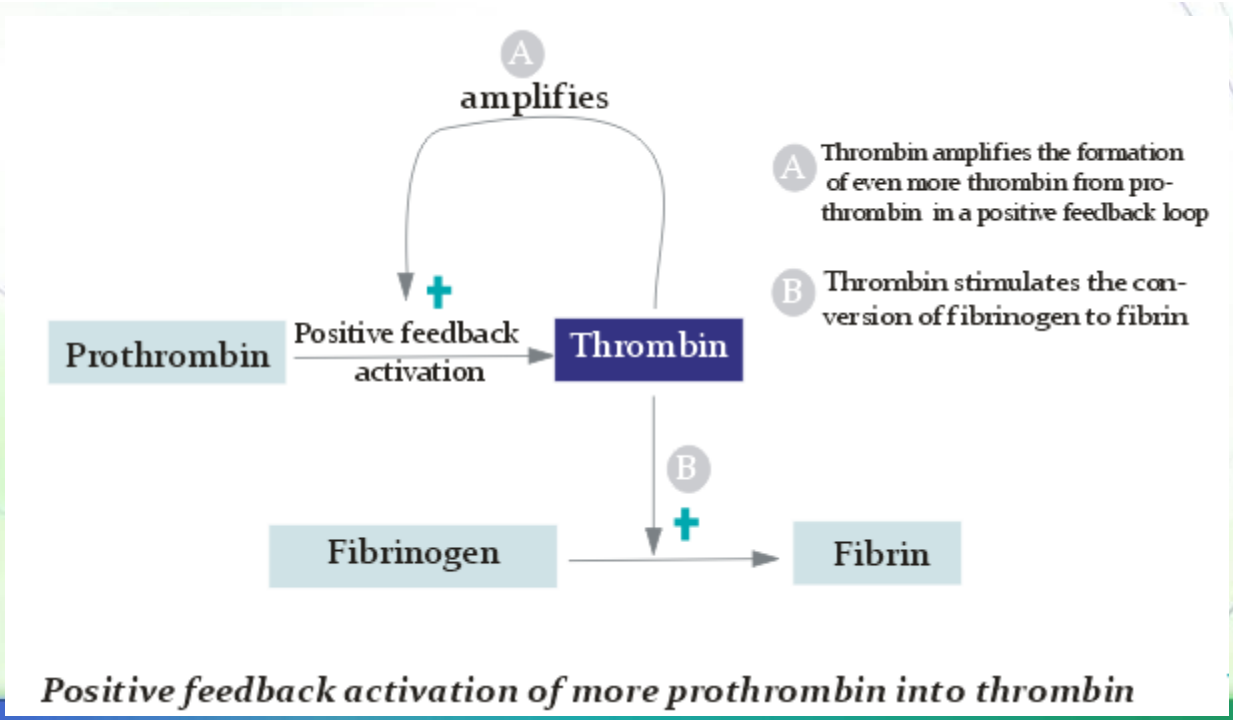




Feedback activation

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

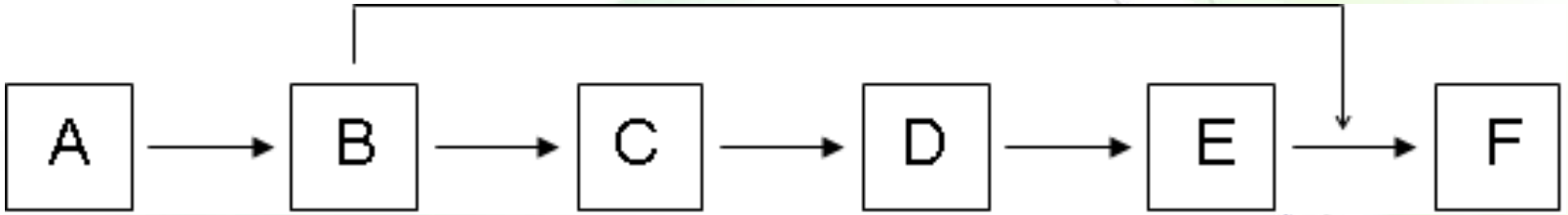




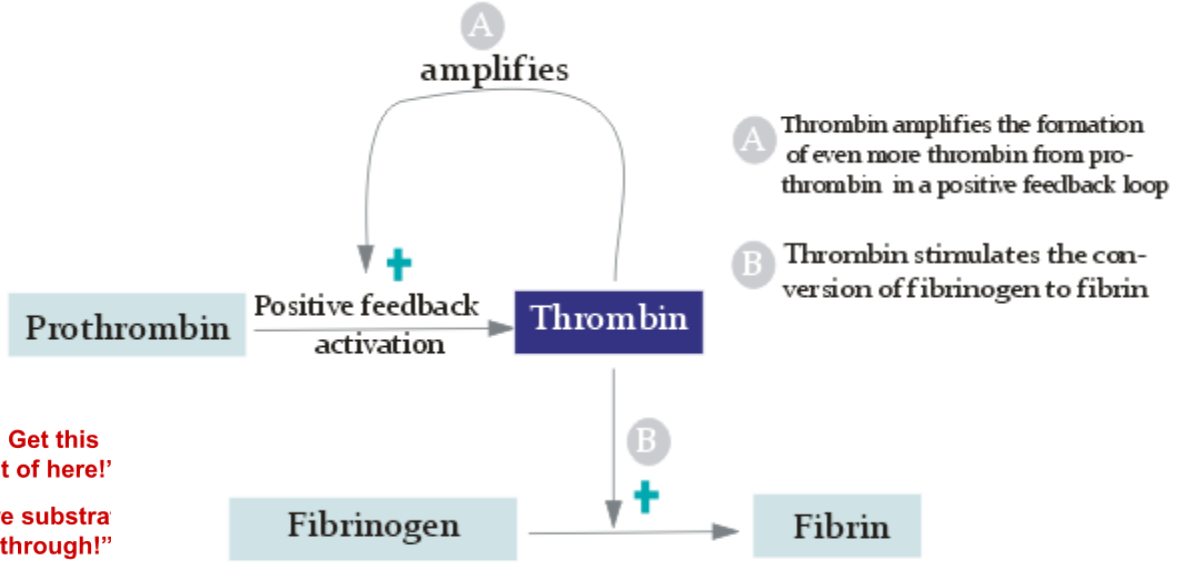
Positive feedback activation of more prothrombin into thrombin

Feed-forward regulation

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



- Blood clotting
- Poisoning



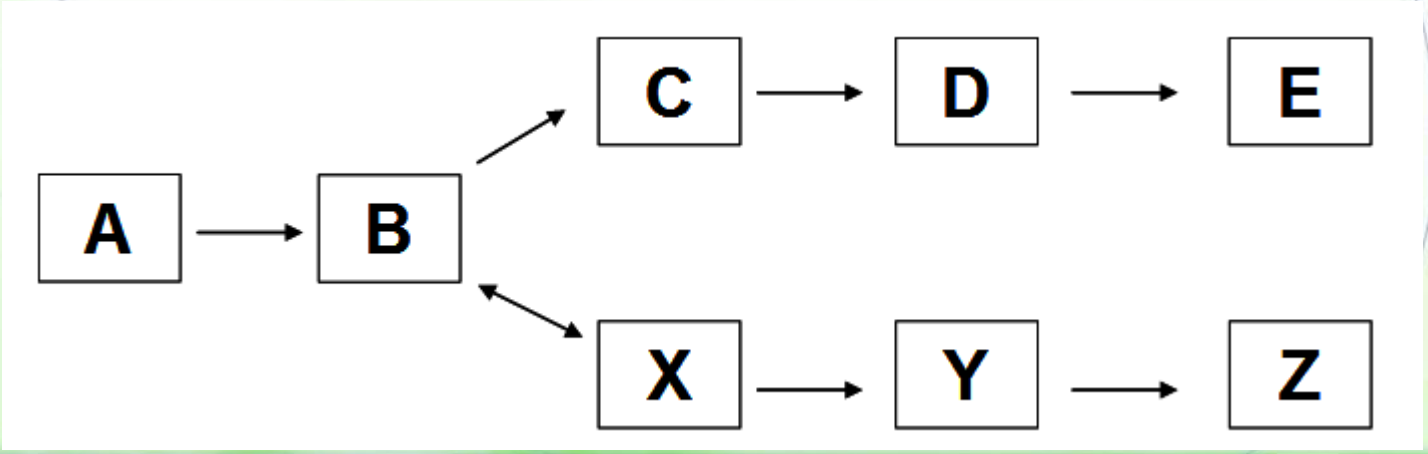
"Oh no! Get this poison out of here!'

"Alot more substra coming through!"

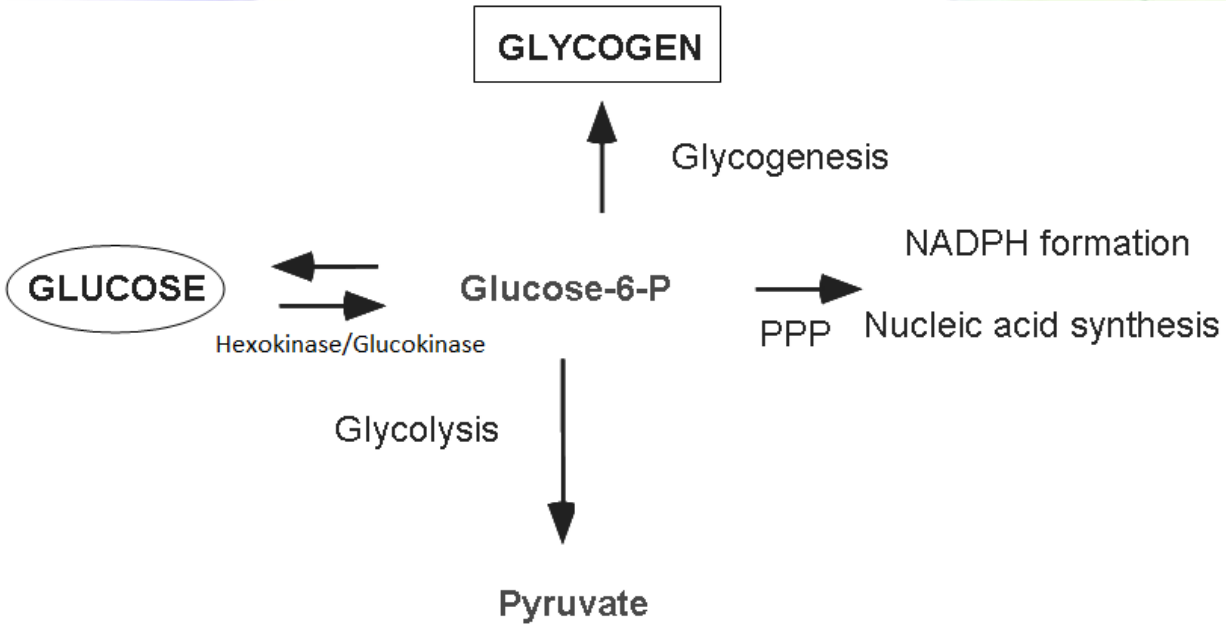
Positive feedback activation of more prothrombin into thrombin

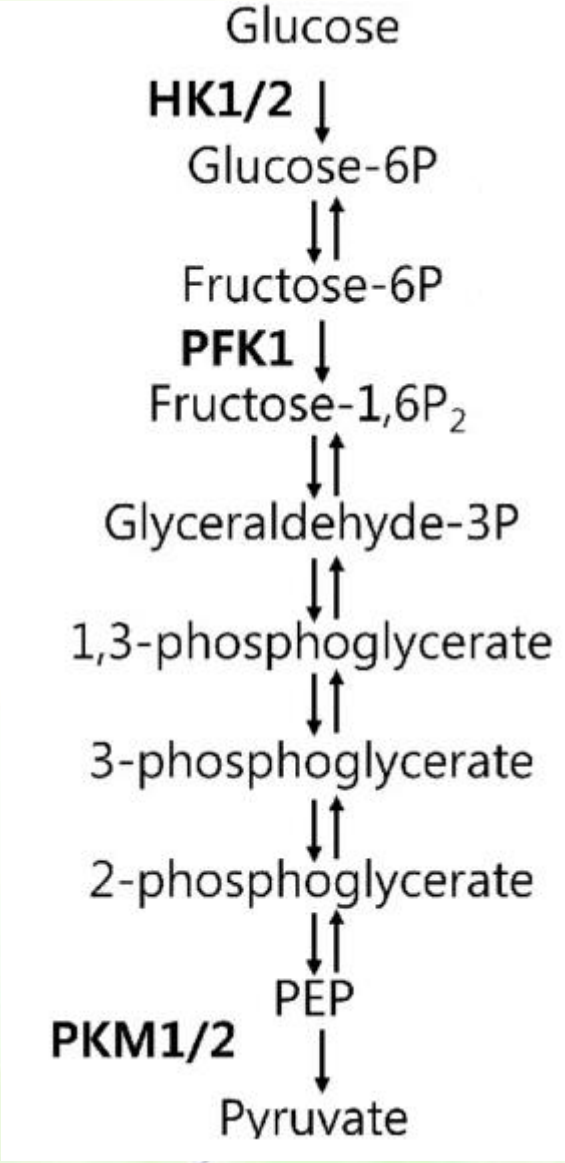
A committed step

- 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)



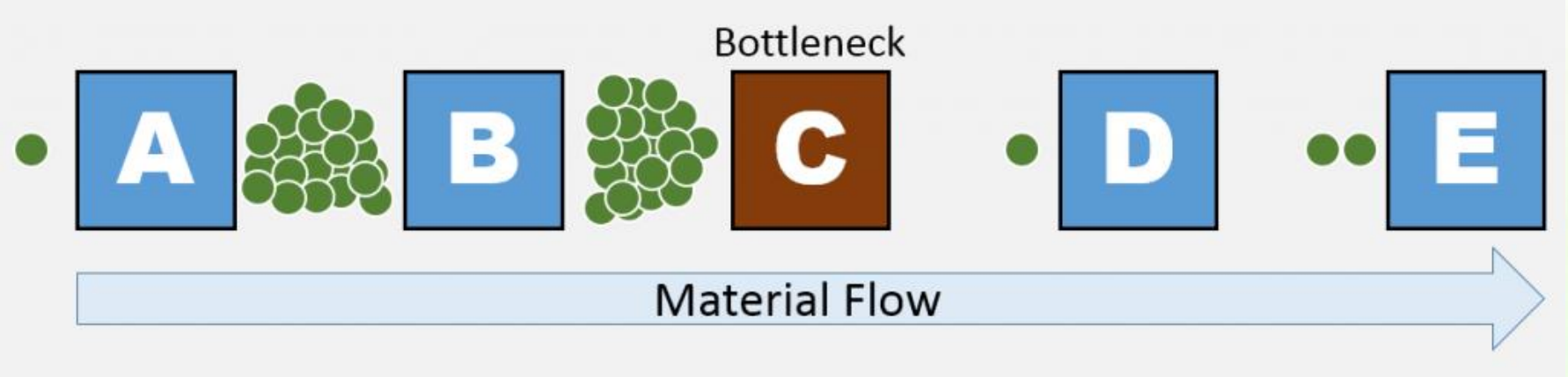
PFK, not HK/GK, is committed step





Rate-limiting reactions

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



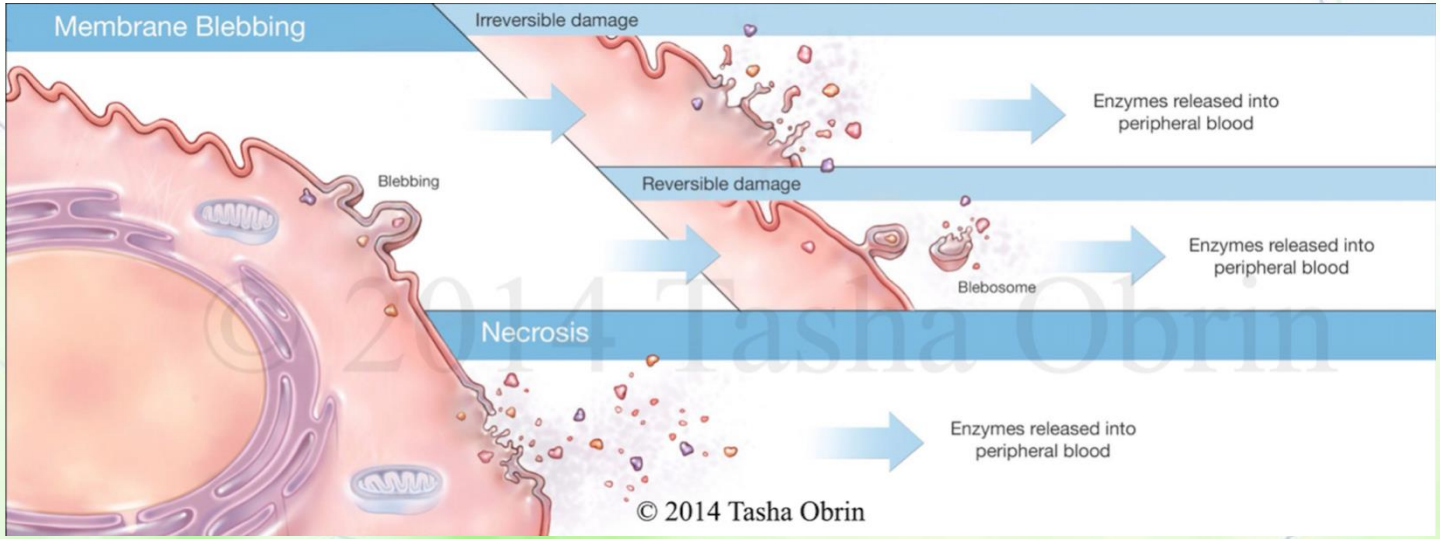
Enzymes in disease diagnosis

Concept

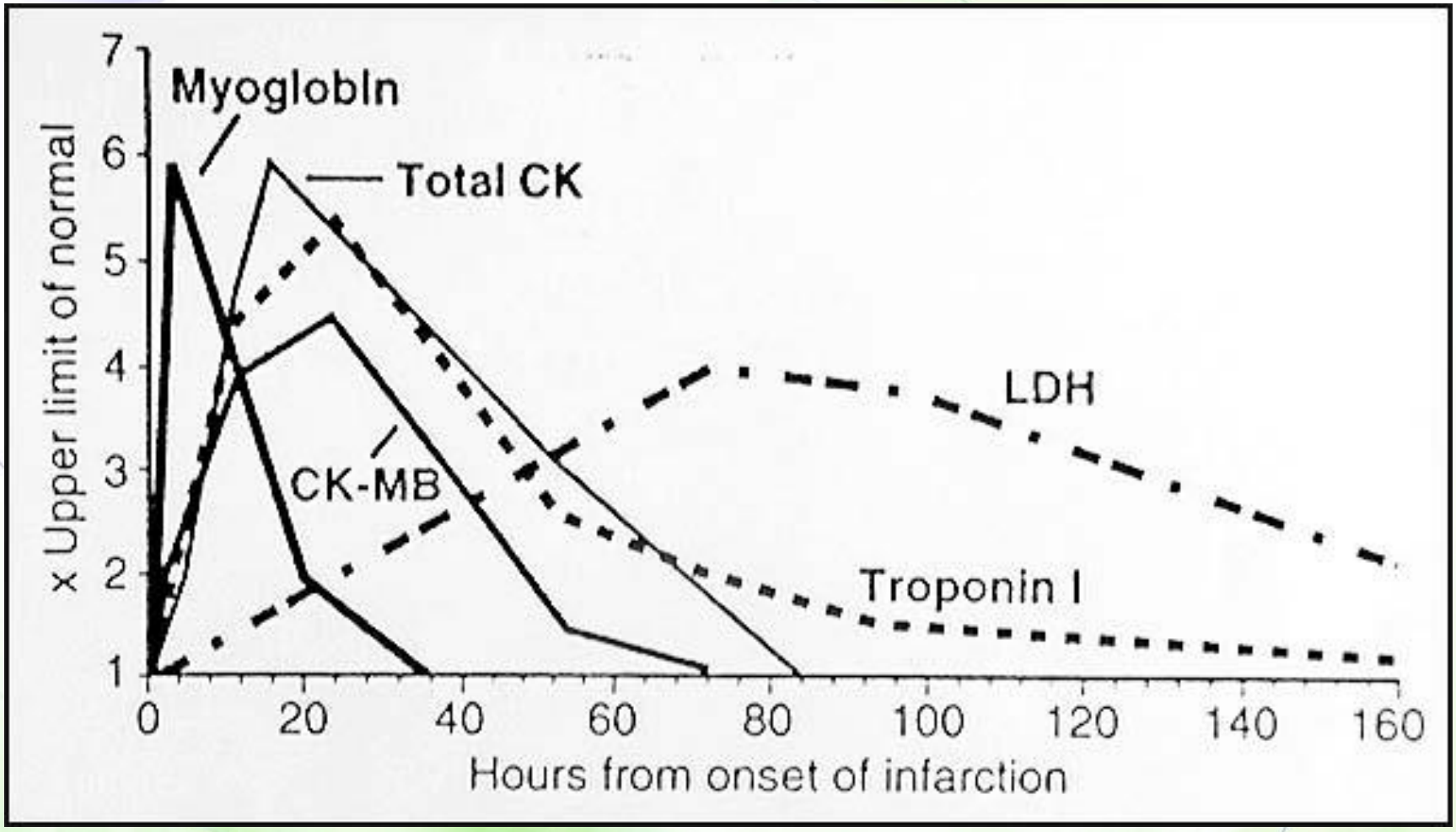
- The presence of enzymes in serum indicates that tissue or cellular damage.
- The measurement enzyme amount in serum is of diagnostic significance.
- Examples:
  - The amino transferases: alanine transaminase, ALT and aspartate aminotransferase, AST
  - Lactate dehydrogenase, LDH
  - Creatine kinase, CK (also called creatine phosphokinase, CPK)

AST and ALT

- The typical liver enzymes measured are AST and ALT.
- ALT is predominantly in hepatocytes.
- The ratio of ALT/AST is diagnostic.
  - Liver disease/damage (not of viral origin) < 1.
  - Viral hepatitis > 1.

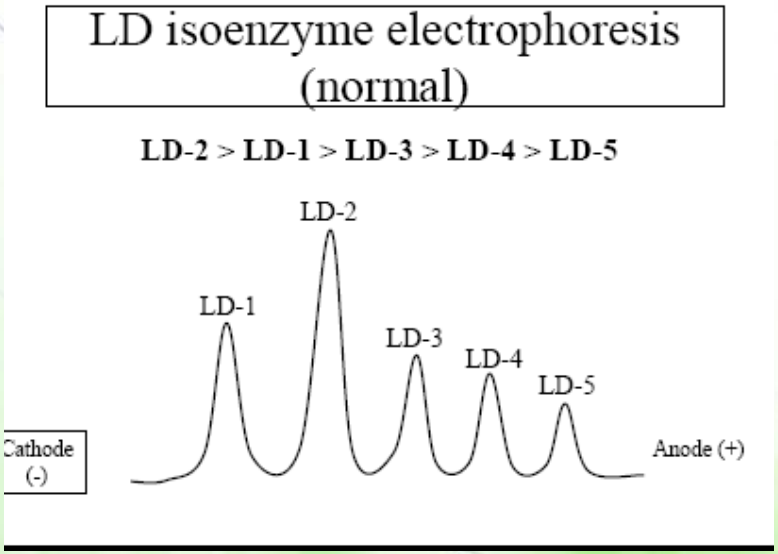


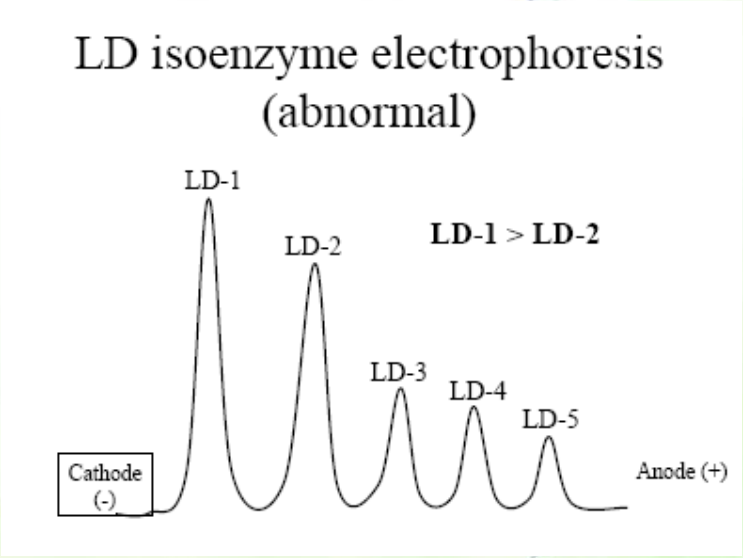
Protein profile in myocardial infarction



LDH

- A comparison of serum levels of LDH-1/LDH-2 ratio is diagnostic for myocardial infarction (heart attacks).
- Normally, this ratio is less than 1.
- Following an acute myocardial infarct, the LDH ratio will be more than 1.





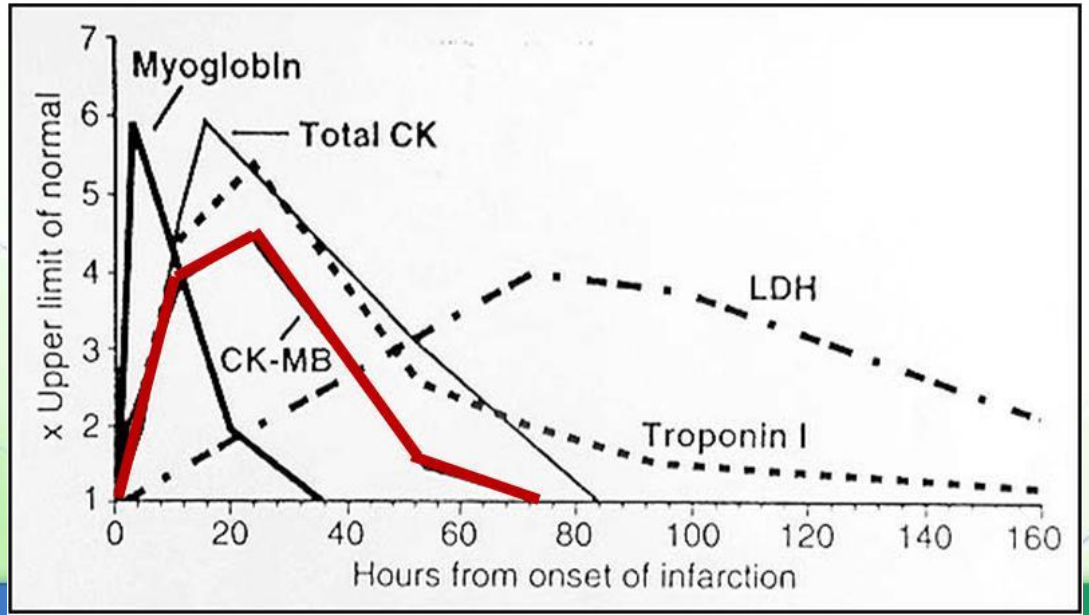
CPK

- 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)

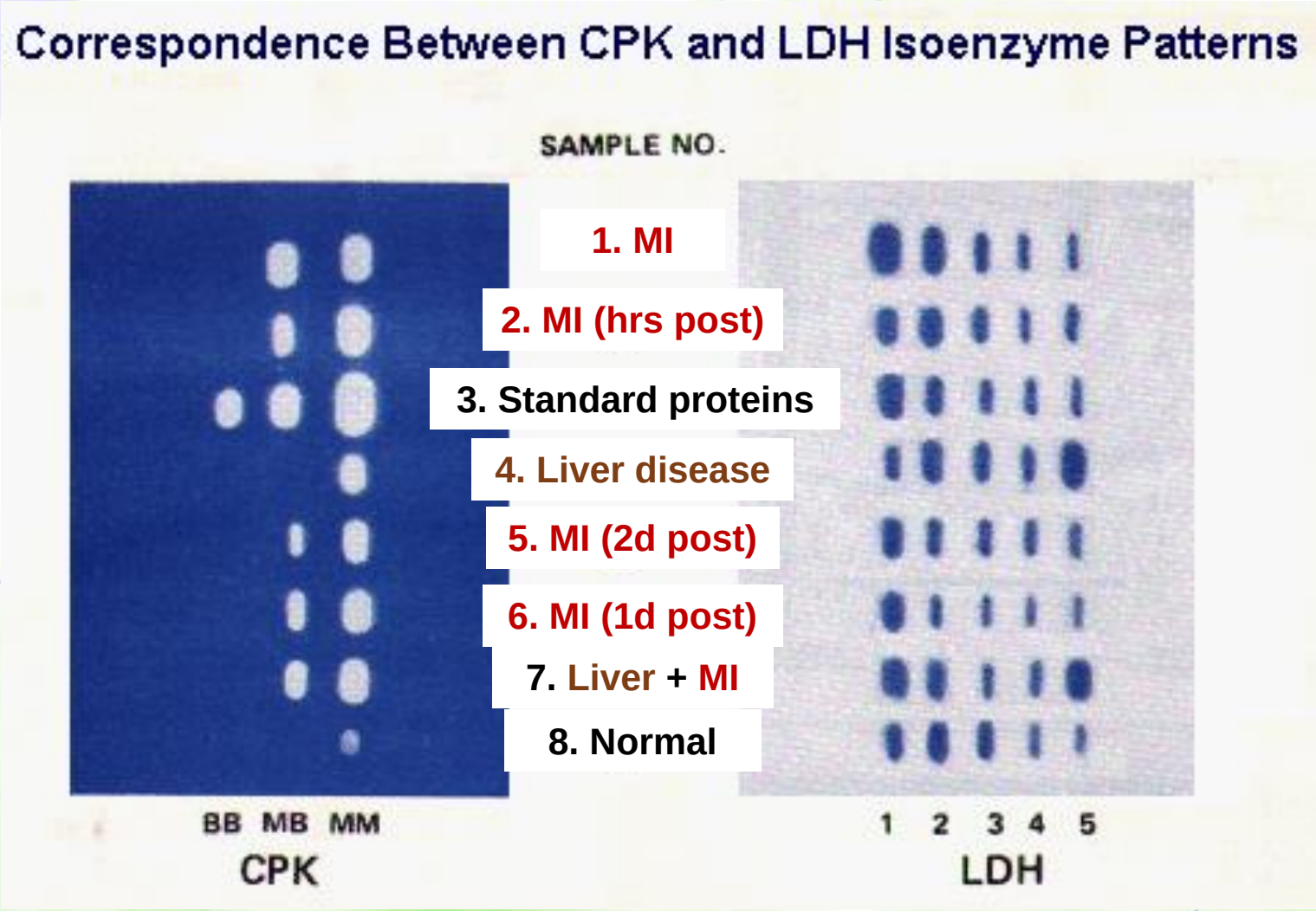
Serum	Skeletal Muscle	Cardiac Muscle	Brain
0 trace BB <6% MB >94% MM	0 trace BB 1% MB 99% MM	0% BB **20% MB** **80% MM**	97% BB 3% MB 0%MM

CPK and myocardial infarction

- A significant amount of CPK-MB is released after MI leading to increased CPK-MB/total CPK ratio (diagnostic of an acute infarction).
  - An increase of total CPK in itself may not.
- CPK-MB disappears in 1-3 days, so another elevation is indicative of another event (reinfarction).



Example


Correspondence Between CPK and LDH Isoenzyme Patterns
SAMPLE NO.
1. MI
2. MI (hrs post)
3. Standard proteins
4. Liver disease
5. MI (2d post)
6. MI (1d post)
7. Liver + MI
8. Normal
BB MB MM
CPK
1 2 3 4 5
LDH

Interpretation

- Sample #3 represents results for standard proteins.
- Sample #8 results are from a normal specimen.
- Sample# 1 MI patient. The specimen was collected at a time when the activity of both LDH and CK were elevated. Note the LDH flip and the high relative activity of the MB isoenzyme.
- Sample# 2 MI patient who experienced chest pain only several hours previously. Total CK is significantly elevated.
- Sample# 6 MI patient (the 1st day post MI); CK level is elevated with a high relative MB isoenzyme activity and the LDH flip is evident.
- Sample# 5 MI patient (2 days post MI) is like sample #6, but lower CK levels.
- Sample# 7 MI patient with passive liver congestion or the patient was involved in an accident as a consequence of the MI, and suffered a crushing muscle injury.
- Sample# 4 a patient with liver disease.

Troponins in MI

- Troponin levels rise within four to six hours after the beginning of chest pain or heart damage, and stay elevated for at least one week.
- This long elevation allows detection of a myocardial infarction that occurred days earlier, but prevents detection of a second infarction if it occurred only days after the first.

An exception to enzymes: *Ribozymes*

This is extra to types of enzymes

- Ribozymes are enzymes made of both protein and RNA part (only a few).
- For some, catalysis is performed by RNA.
- Example include those involved in RNA splicing reactions in those responsible for protein synthesis in ribosomes.
- The catalytic efficiency of RNAs is less than that of protein enzymes, but can be enhanced and stabilized by the presence of protein subunits.