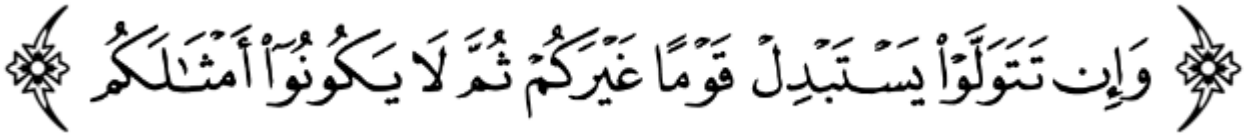
بسم الله الرحمن الرحيم

BIOCHEMISTRY



اللهم استعملنا لنصرة دينك

Lecture 28

Enzyme Regulation 2

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Edited by:

Laith Joudeh

Substrate and transition-State analogs

Suicide inhibitors

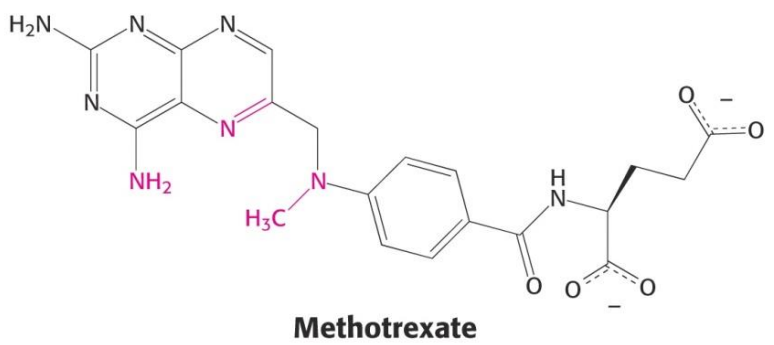
- They bind to the enzyme and are not released; they are stuck there.
- They modify the whole enzyme, unlike aspirin which only adds an acetyl group to an amino acid.
- They look like transition state molecules.
- They bind more tightly than substrates. Tight binding is not necessarily covalent.
- The enzyme cannot differentiate between the transition-state analog (suicide inhibitor) and the transition state of the substrate, so the enzyme will start the reaction causing the change of the position of some groups, protonation states and/or non-covalent interactions which causes the enzyme to unfold, losing its catalytic function.
- Drugs cannot be designed that precisely mimic the transition state! (highly unstable structure).

Methotrexate

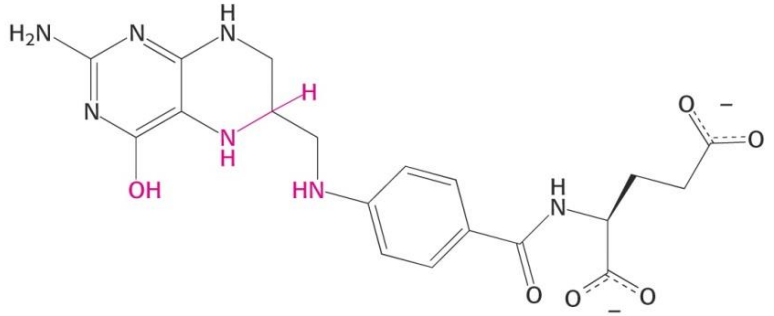
+ Rheumatoid arthritis (e.g.).

- Methotrexate is a synthetic inhibitor used to treat **cancer**
- It is a **structural analog of folate**, a substrate for the enzyme dihydrofolate reductase, and a coenzyme for thymidylate kinase, both of which are responsible for the synthesis of nucleotides.
- It binds to dihydrofolate reductase 1000-fold **more tightly** than the natural substrate and inhibits nucleotide base synthesis.

↳ Non-covalent but enough to cause permanent inactivation

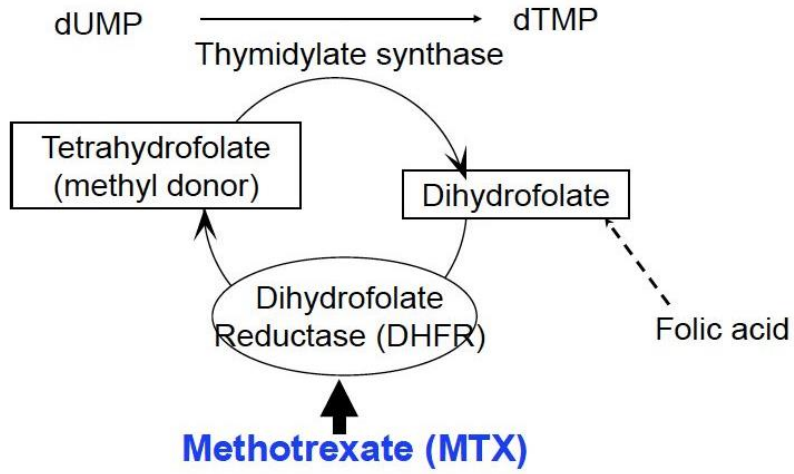


Methotrexate



Tetrahydrofolate

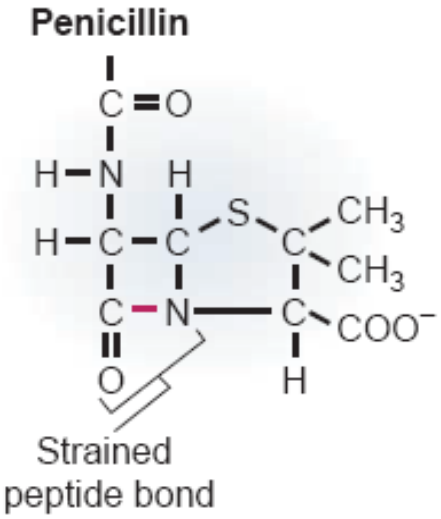
- Folate molecules produce deoxy TMP which is used in the synthesis of DNA.
- Methotrexate inhibits the enzyme that is responsible for the synthesis of deoxy TMP, preventing the DNA synthesis, affecting cancer cells and other actively dividing cells.
- Cancer patients often experience hair loss and the external layer of the gastrointestinal tract, which are non-renewable tissues. This can make it difficult for them to eat or drink easily. Additionally, immune cells and red blood cells are also affected.
- Rheumatoid arthritis is an autoimmune disease where the immune system mistakenly attacks and damages its own tissues. To reduce inflammation, we need to block the proliferation of active immune cells.

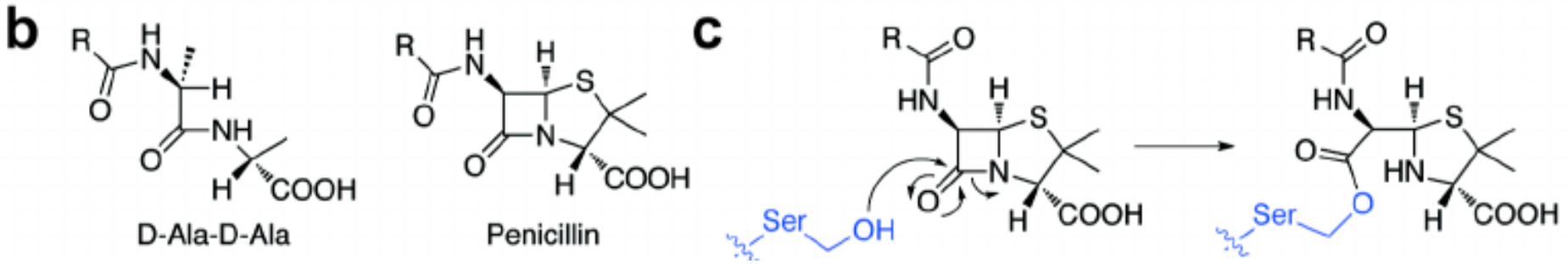


Penicillin

The first antibiotic to be discovered.

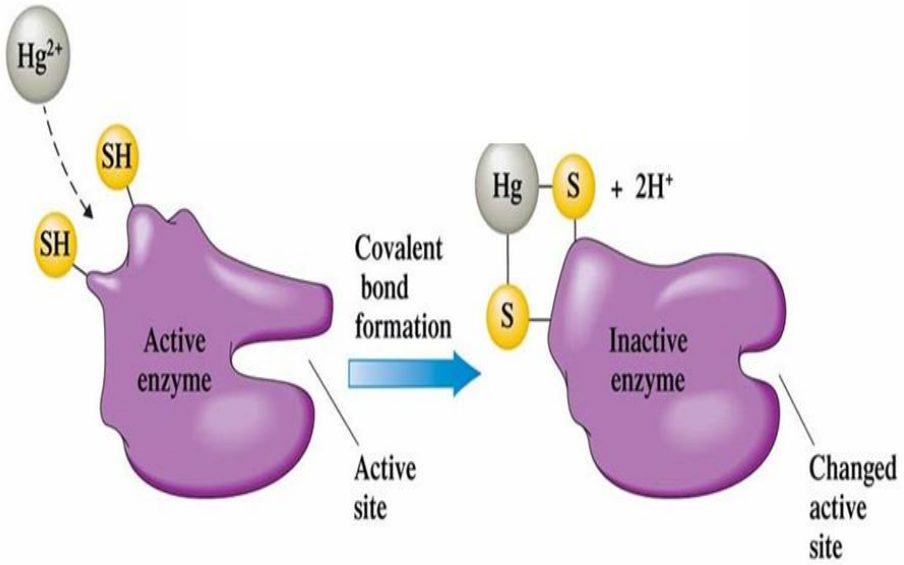
- Penicillin looks like the substrate alanyl-alanine.
- Penicillin inhibits glycopeptidyl transpeptidase
- It is a transition-state analog to glycopeptidyl transpeptidase, which is required for synthesis of the bacteria cell wall.
- The peptide bond in the β-lactam ring of penicillin looks like the natural transition-state complex.
- The active site serine attacks the highly strained β -lactam ring resulting in opening of the lactam. This reaction leads to irreversible covalent modification of the enzyme.





Heavy Metals They are toxic.

- Mercury (Hg), lead (Pb), aluminum (Al), or iron (Fe) result in tight binding to a functional group in an enzyme .
  - Nonspecific inhibition at high doses.
- Mercury binds to reactive sulfhydryl groups away from the active site and affect the binding of substrates.
  - Unknown enzymes in mercury toxicity.

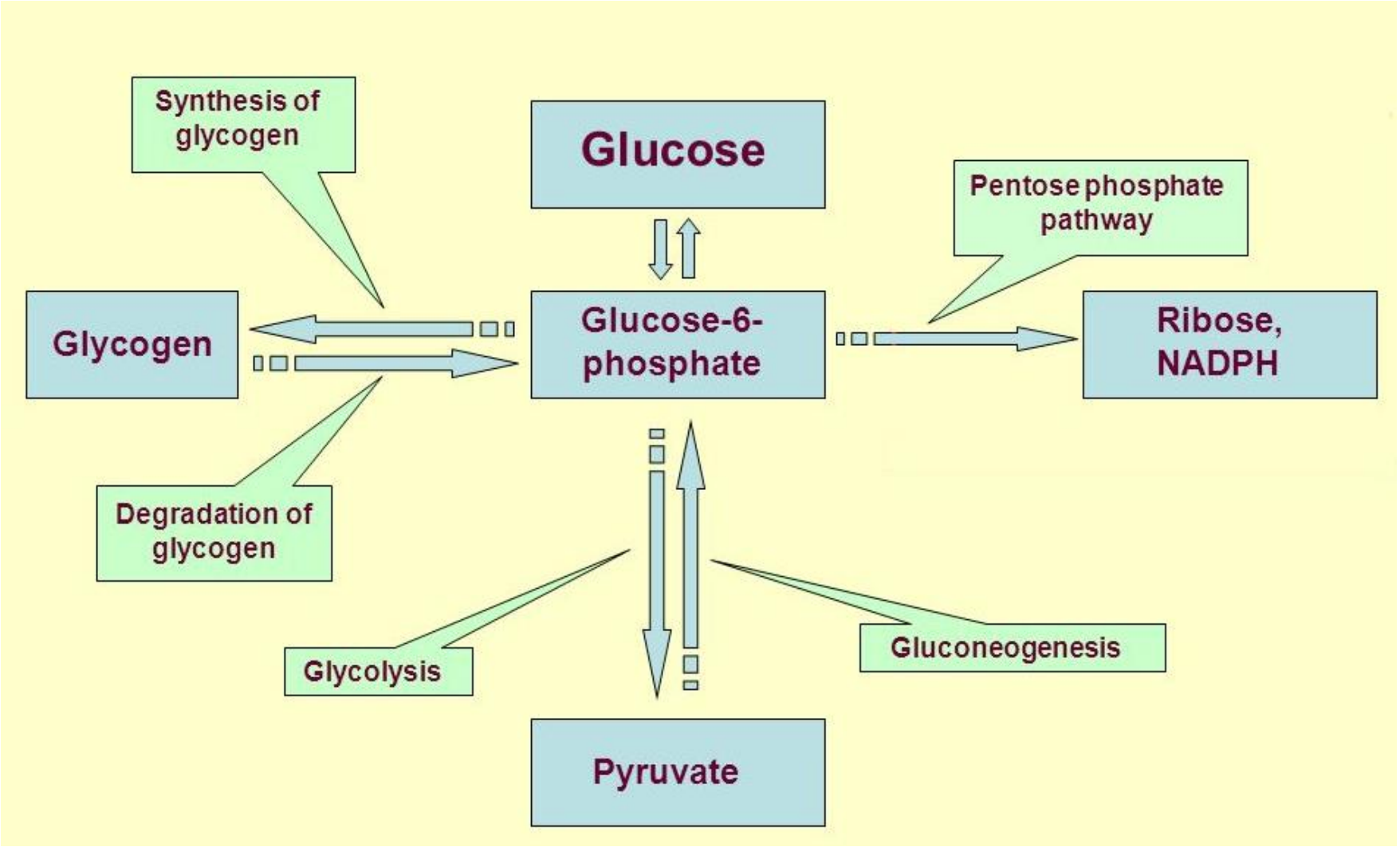


- Lead replaces the normal functional metal in an enzyme such as calcium, iron, or zinc by an irreversible mechanism.
  - Its developmental & neurologic toxicity may be caused by its ability to replace Ca+2 in several regulatory proteins that are important in the central nervous system and other tissues.

Regulation through conformational changes

through changing of the enzyme structure.

The different fates of glucose



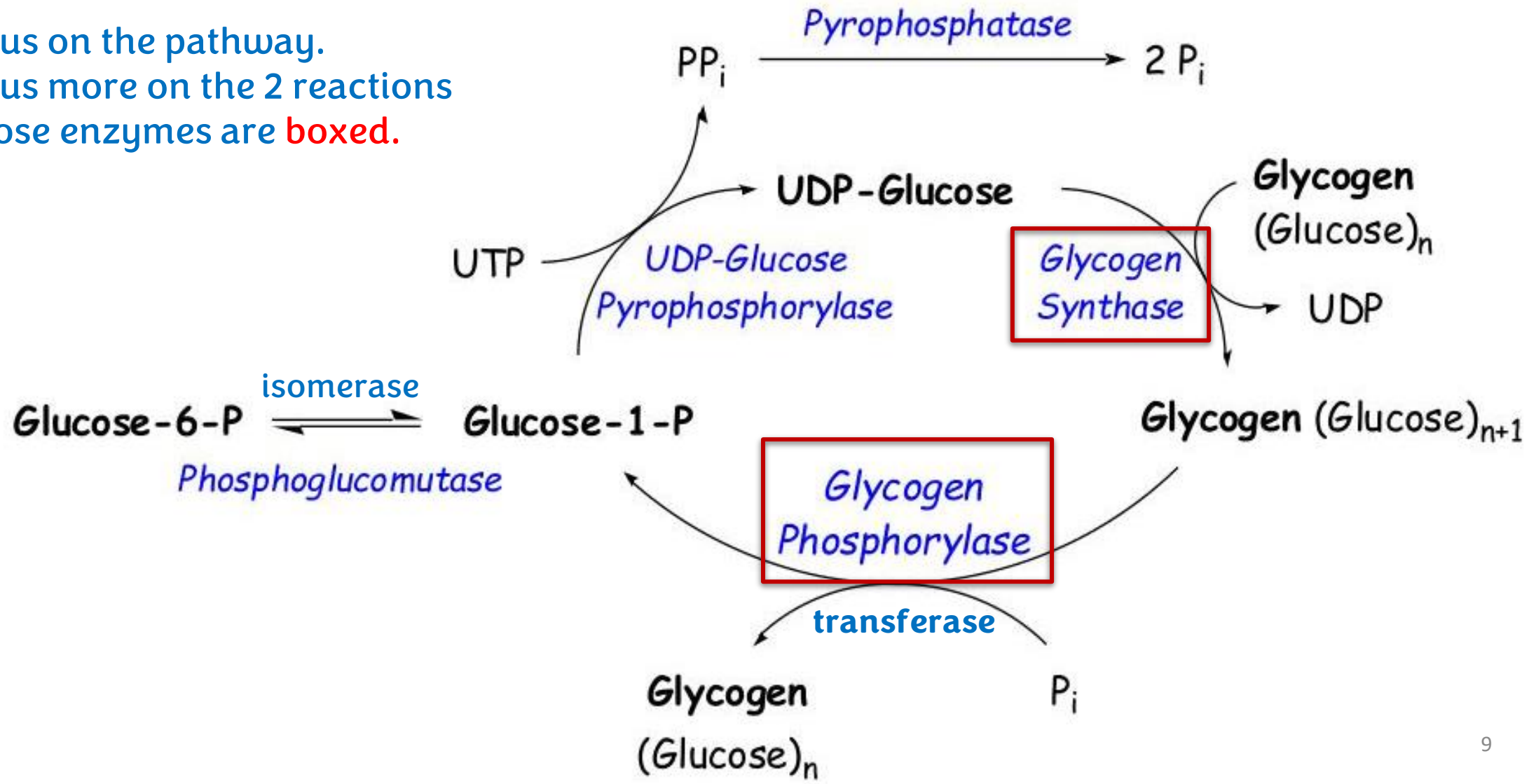
Notes on the previous slide

- 1- The first step is the phosphorylation of glucose to glucose-6-phosphate.

- 2- The liver converts glucose-6-phosphate to glycogen, muscles convert glucose-6-phosphate to pyruvate (glycolysis), dividing cells convert glucose-6-phosphate to ribose and NADPH for DNA synthesis.

Metabolism of glycogen **(occurs in the cytosol)**

Focus on the pathway.
Focus more on the 2 reactions whose enzymes are boxed.

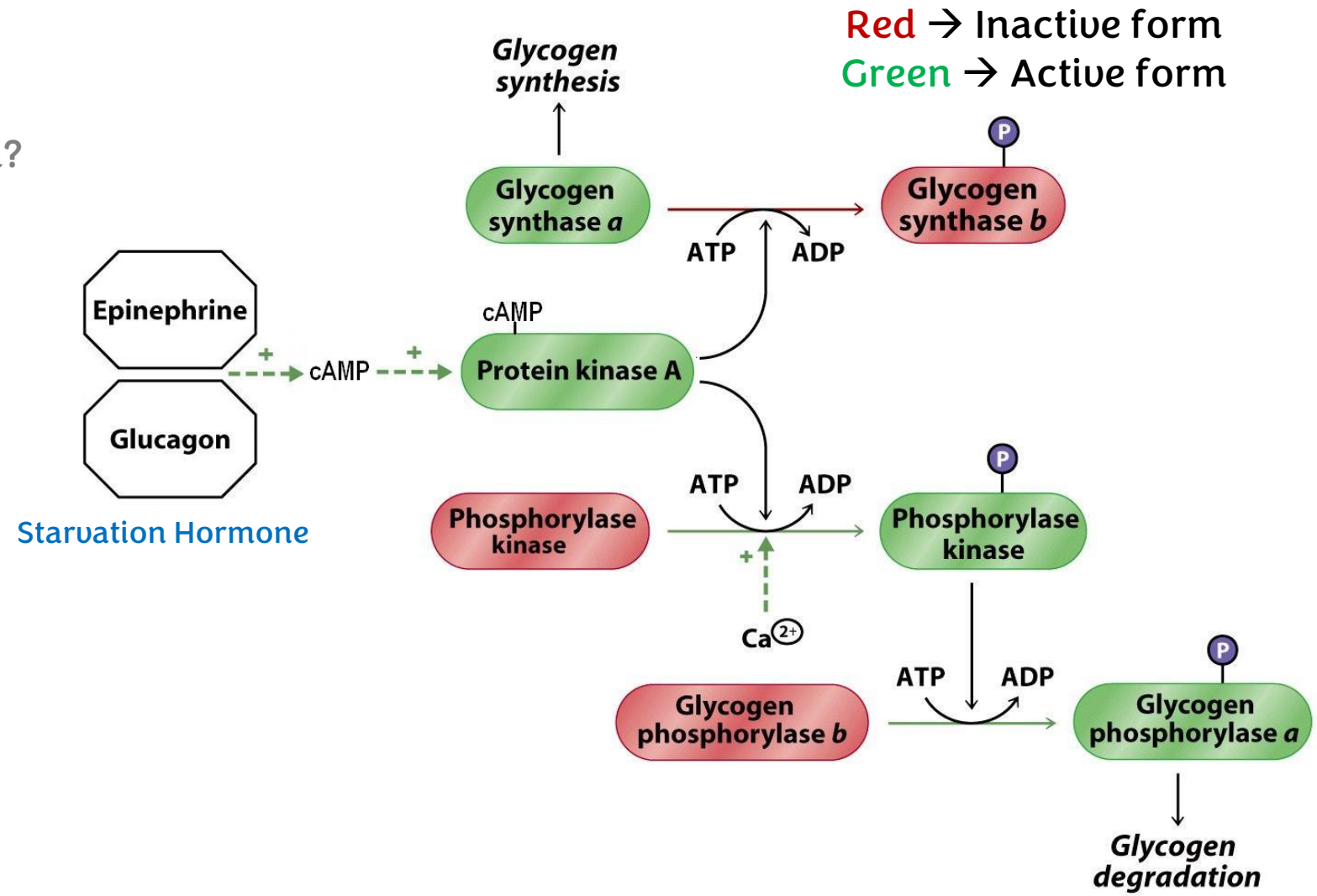


Regulation by phosphorylation

This regulation pathway occurs in a single shot

Focus on

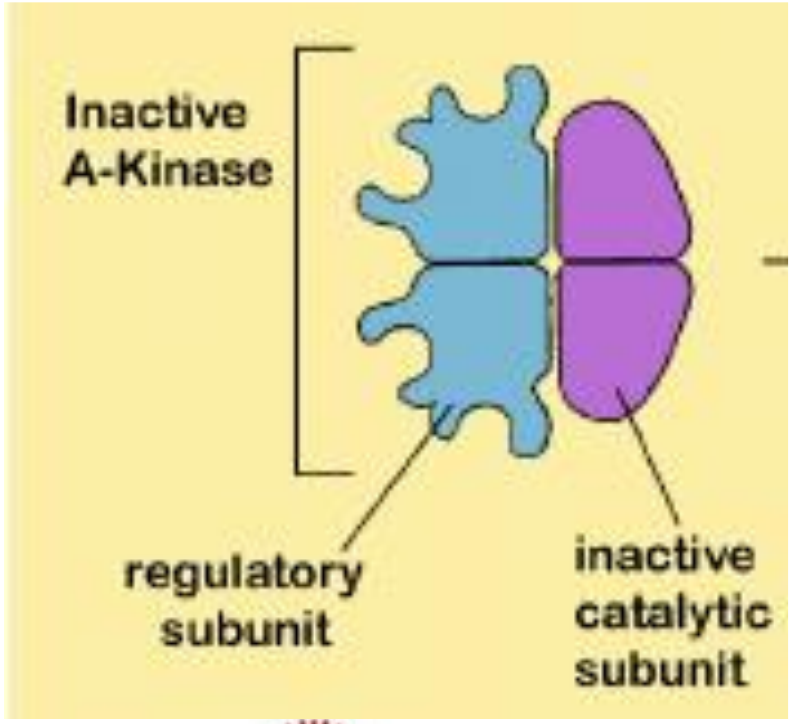
Which enzyme acts on which?
What is the effect?
Active/Inactive forms.
The physiological response.



PKA-structure and regulation

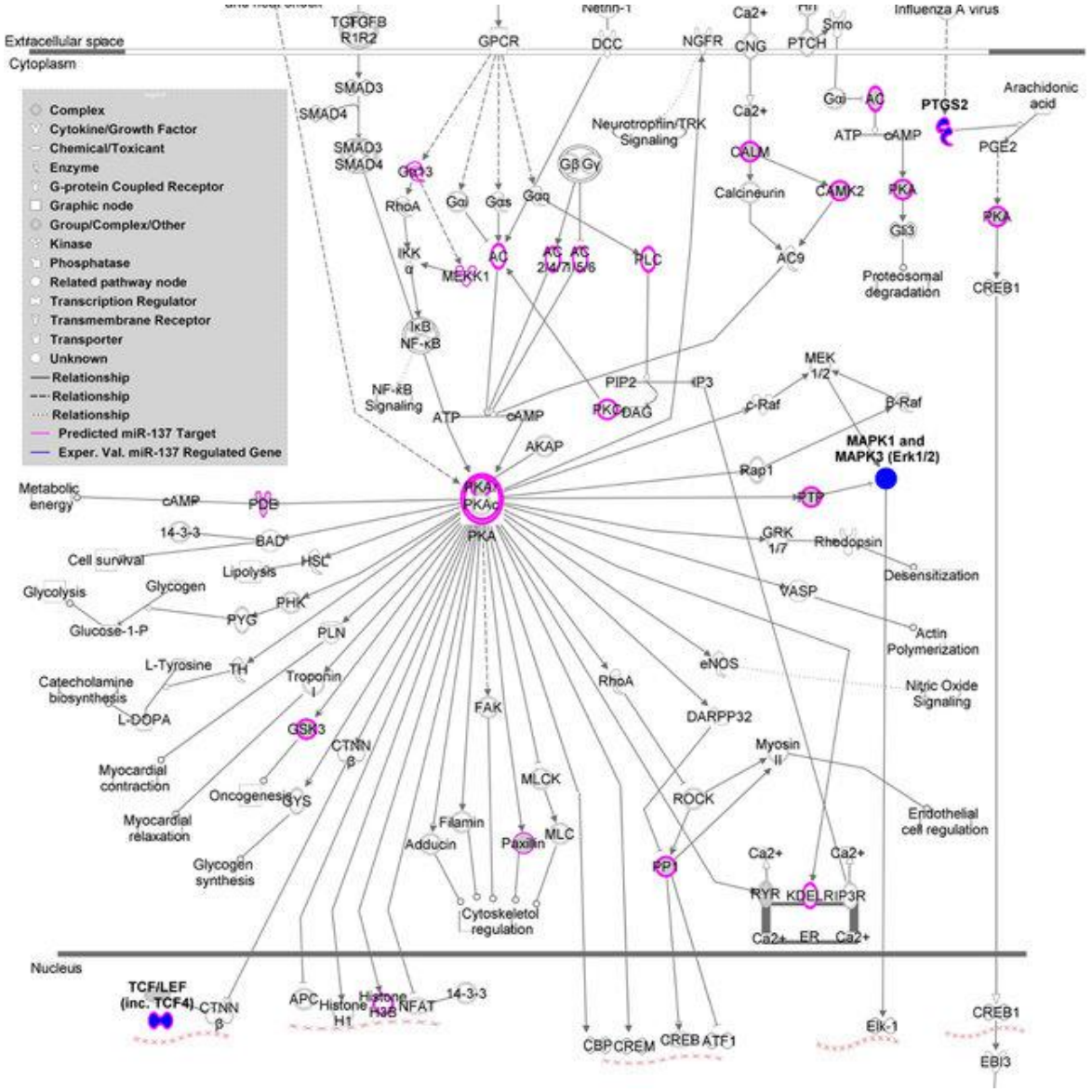
One advantage of enzyme activation is amplification, this means that a single PKA molecule could affect 1 million molecules in total.

- 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



An example of an amplified response **(NOT FOR MEMORIZATION)**:
One receptor activates 100 PKA molecules, each of which activates 1,000 phosphorylase kinases. This leads to the formation of 100,000 phosphorylase kinase molecules, each of which activates a thousand phosphorylases. Therefore, the total number of phosphorylase molecules activated is 100,000 * 1,000 = 100,000,000. Activating a single receptor can lead to the release of 10^8 molecules!

The many targets of PKA



cAMP and protein kinase A (PKA)

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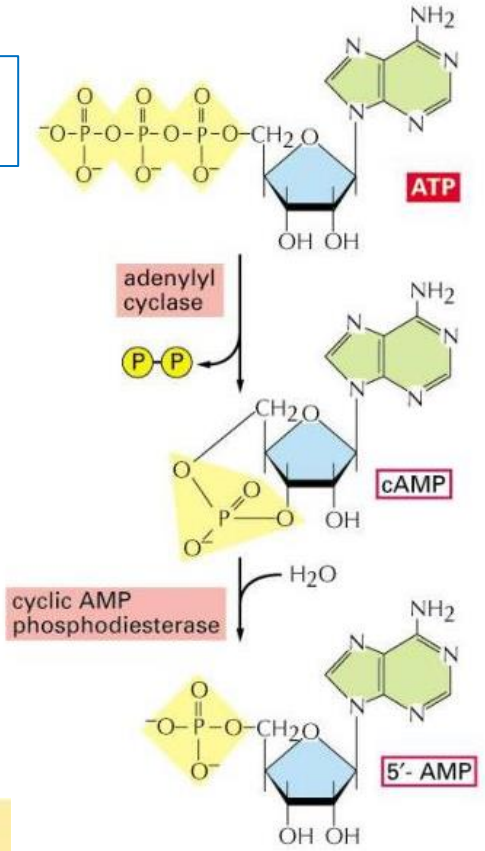
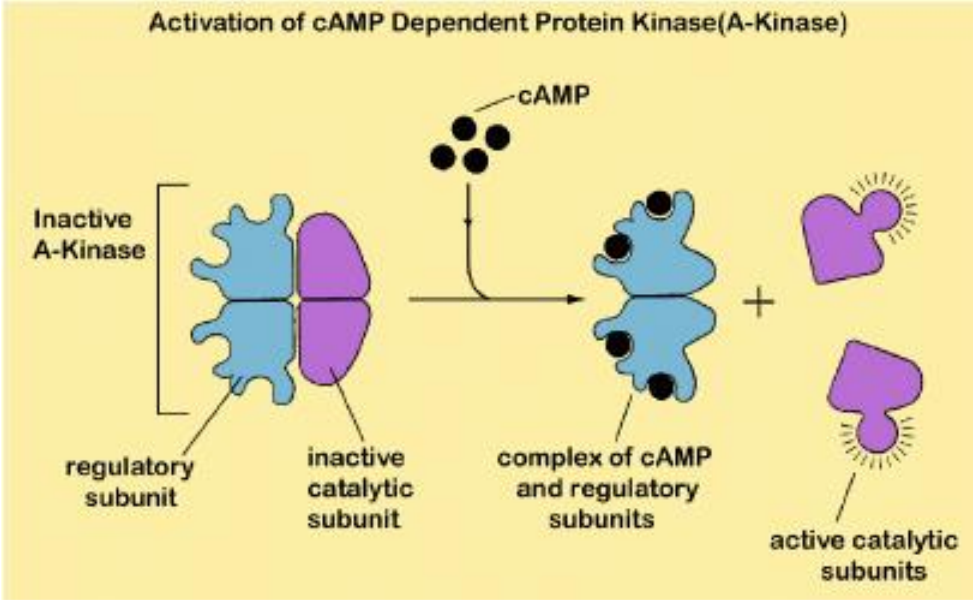


- Small-molecule modulators can have dramatic effects on enzymes.
- For example, cAMP, which is structurally modified AMP, can activate protein kinase A (PKA).

- The binding of two molecules of cAMP to each regulatory subunit leads to the dissociation of R2C2 into an R2 subunit and two active C subunits. (4 cAMP molecules are required to activate PKA)

The regulatory subunits of the enzyme function to inhibit the catalytic subunits from phosphorylating other proteins when the enzyme is in the R2C2 form.



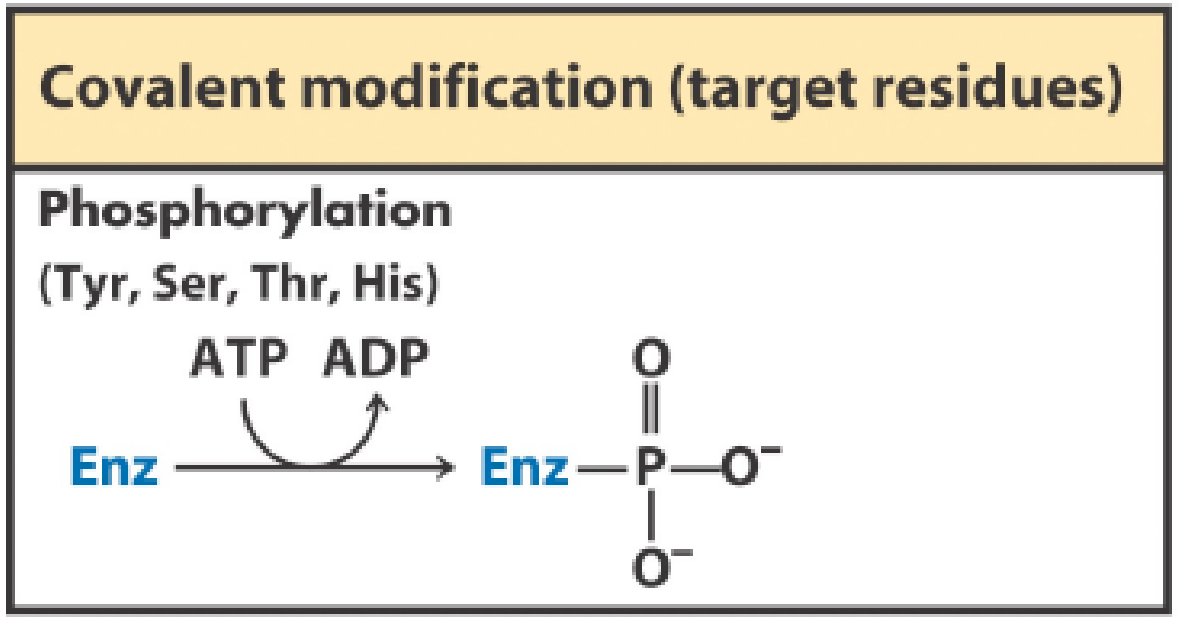
When a hormone attaches to a receptor on the cell surface, the receptor triggers the activation of an enzyme called “adenylyl cyclase.” This enzyme then converts adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP).

Reversible covalent modification

The enzymes which phosphorylate molecules are classified as kinases which are transferases

Advantage

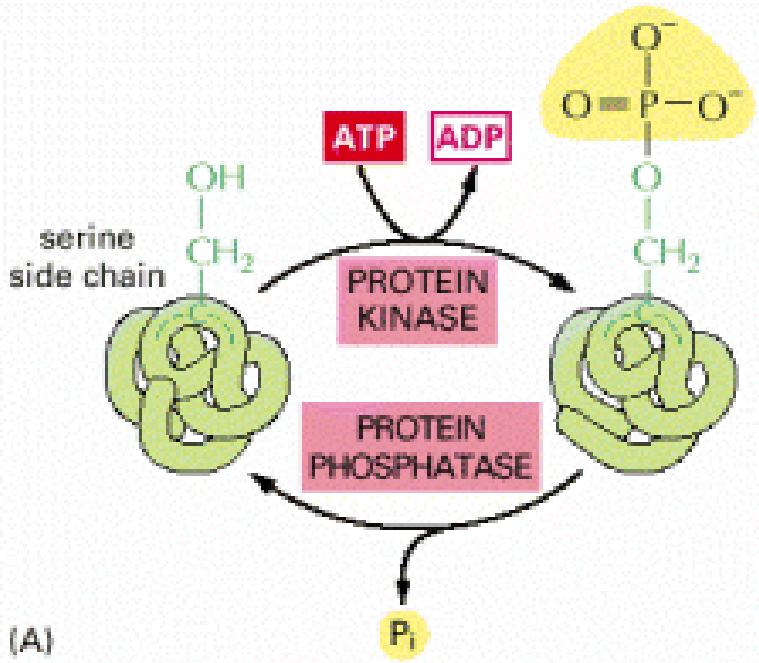
- Rapid and transient
- The most common mechanism is enzyme phosphorylation (the covalent addition of a phosphate group to one of its amino acid side chains).
  - Usually serine, threonine, and tyrosine



Despite its small size, the phosphate group's negative charge influences electrostatic interactions and hydrogen bonds between amino acids, leading to changes in the enzyme's structure.

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. Depends on the enzyme.



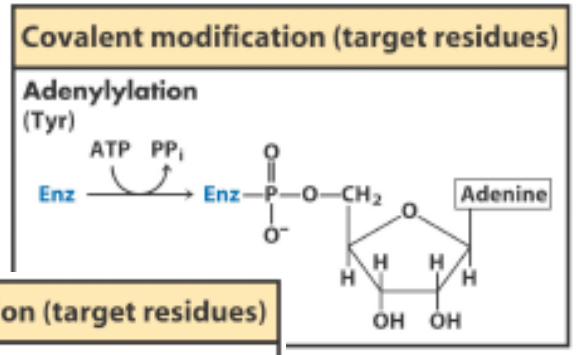
Why is phosphorylation effective?

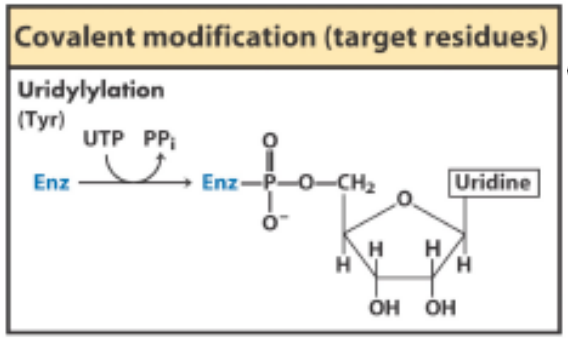
And why is it important?

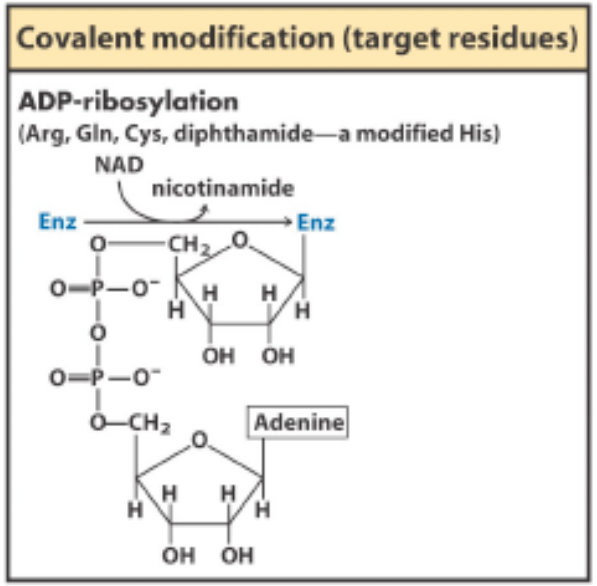
- Formation or removal of new electrostatic interactions and/or hydrogen bonds altering substrate binding and catalytic activity.
- It can happen in less than a second or over a span of hours.
- Phosphorylation often causes highly amplified effects.

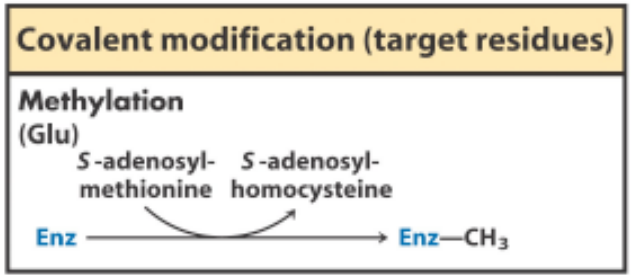
Other reversible covalent modifications

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



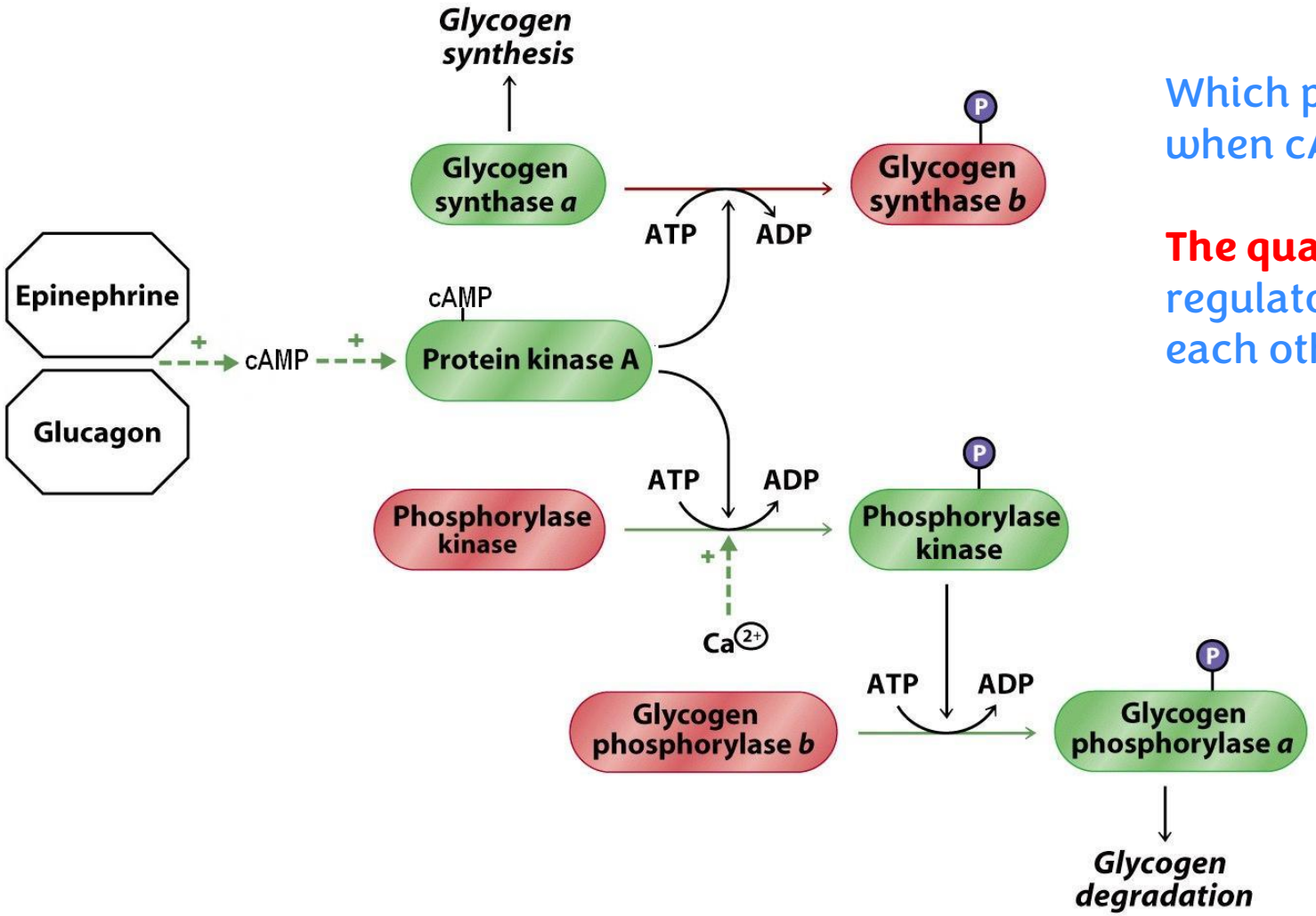






You don't have to memorize the structures.

Phosphorylation cascade



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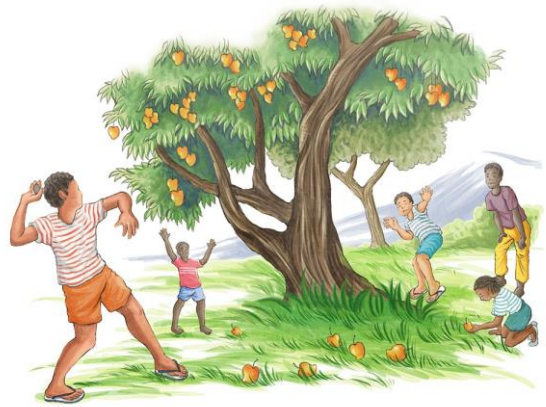
Which protein structural level of PKA is affected when cAMP activates it?

The quaternary structure. As the 4 subunits [2 regulatory (R) and 2 catalytic (C)] separate from each other when PKA is activated by cAMP.

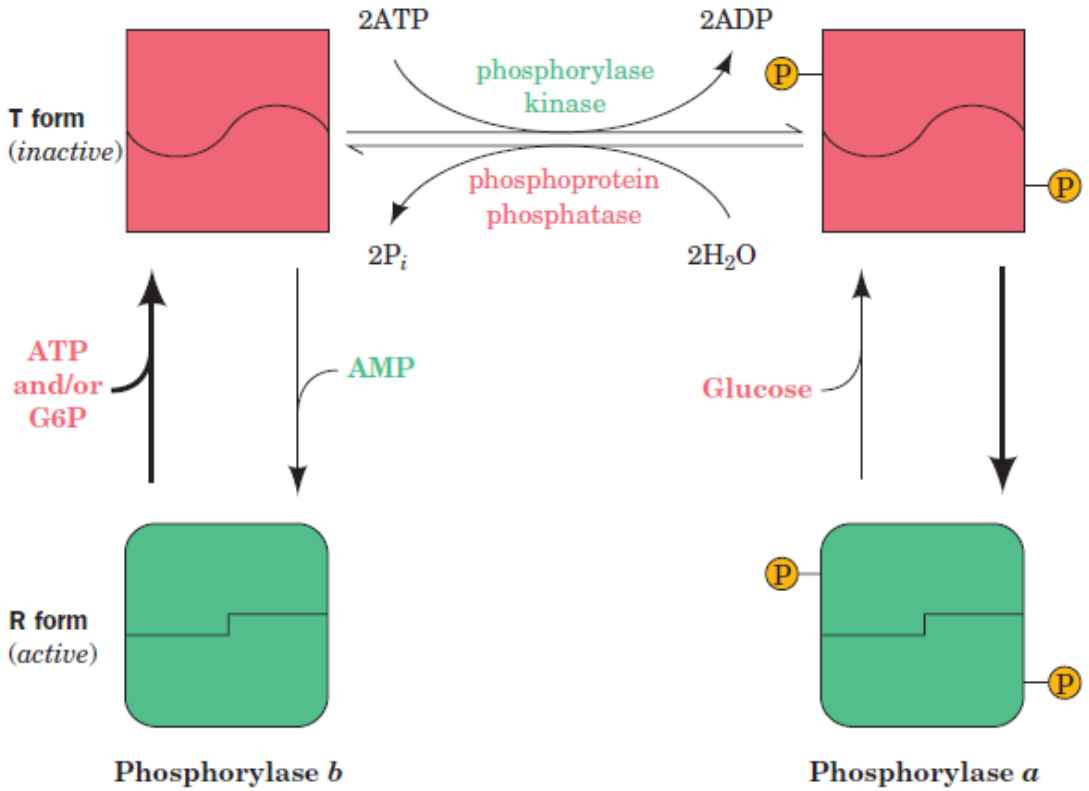
Example: Glycogen phosphorylase (allosteric)



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

- GP (glycogen phosphorylase) 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)

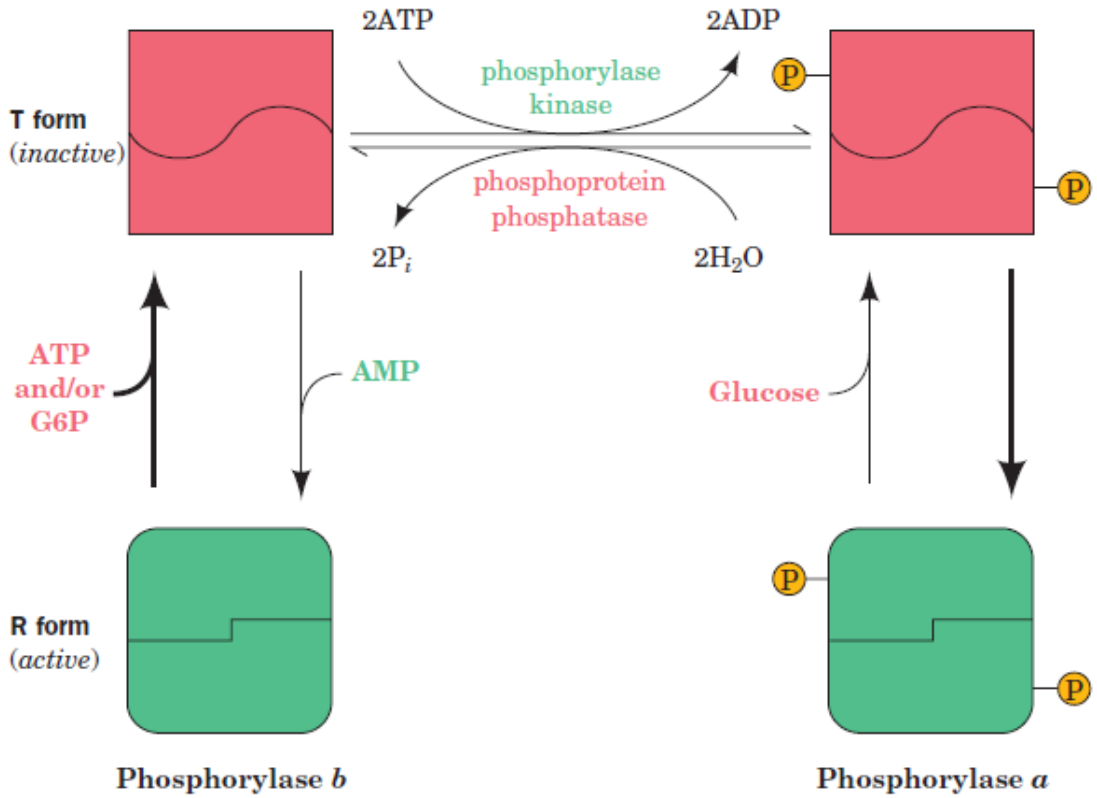
The enzyme must be in the R form to become active; phosphorylation alone is not enough.



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

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, but it can also exist in the R state.
- Phosphorylase a is usually active because the equilibrium favors the R state, but it can also exist in the T state.

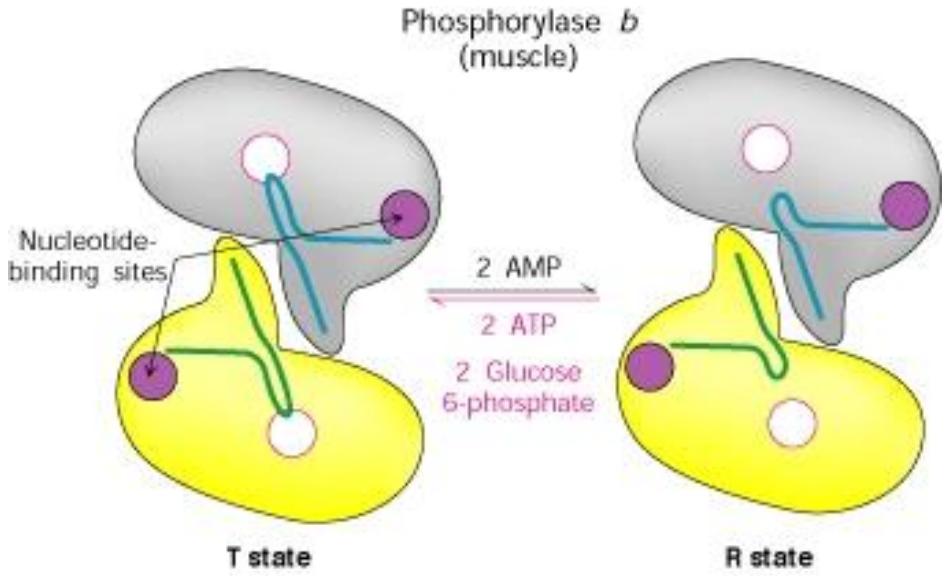


The transition of phosphorylase b between the T and the R state is controlled by the energy charge (ATP and AMP) of the muscle cell and availability of glucose-6-phosphate.

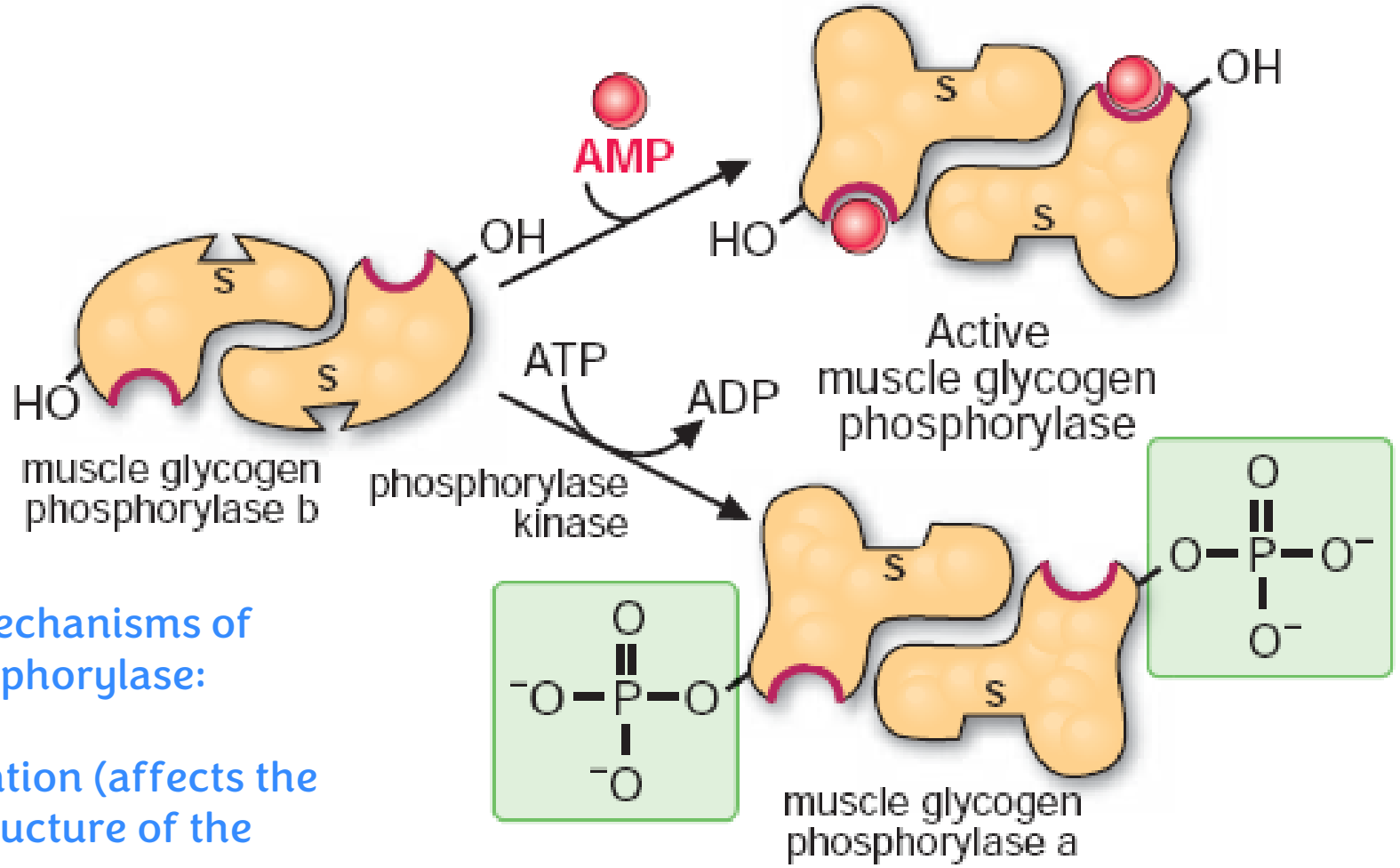
What do ATP and AMP do?

- 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.



Or phosphorylate glycogen of phosphorylase b



We have two mechanisms of regulating phosphorylase:

1. Phosphorylation (affects the allosteric structure of the protein)
2. Allosteric regulation (by G6P/AMP/ATP)

Notes on previous slides

Glycogen phosphorylase has 4 forms:

1. T-form phosphorylated
2. R-form phosphorylated
3. T-form unphosphorylated
4. R-form unphosphorylated

Other molecules can regulate glycogen phosphorylase b, such as:

1. G6P(glucose 6-phosphate): High concentrations of glucose hinder the glycogen phosphorylase b enzyme by shifting the equilibrium in favor of the T-form. This is because we have a lot of glucose available, so there's no need to release more of it.

2. ATP: High ATP concentrations transform glycogen phosphorylase b into the inactive T form because we have a lot of energy, so we don't need to release glucose. (ATP acts as a negative allosteric effector.)

3. AMP: High AMP concentrations indicate a low energy level. They activate glycogen phosphorylase b by shifting the equilibrium in favor of the R-form. This releases glucose, which we use to produce more energy through metabolism. AMP acts as a positive allosteric effector.

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

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V1 → V2	9; glycogen phosphorylase	hydrolase	transferase
V2 → V3	13; bottom left	AMP → cAMP	ATP → cAMP

Additional Resources Used:

رسالة من الفريق العلمي:

إن الله لغني عن العالمين
لا غالب إلا الله