Enzymes
Part III: regulation II

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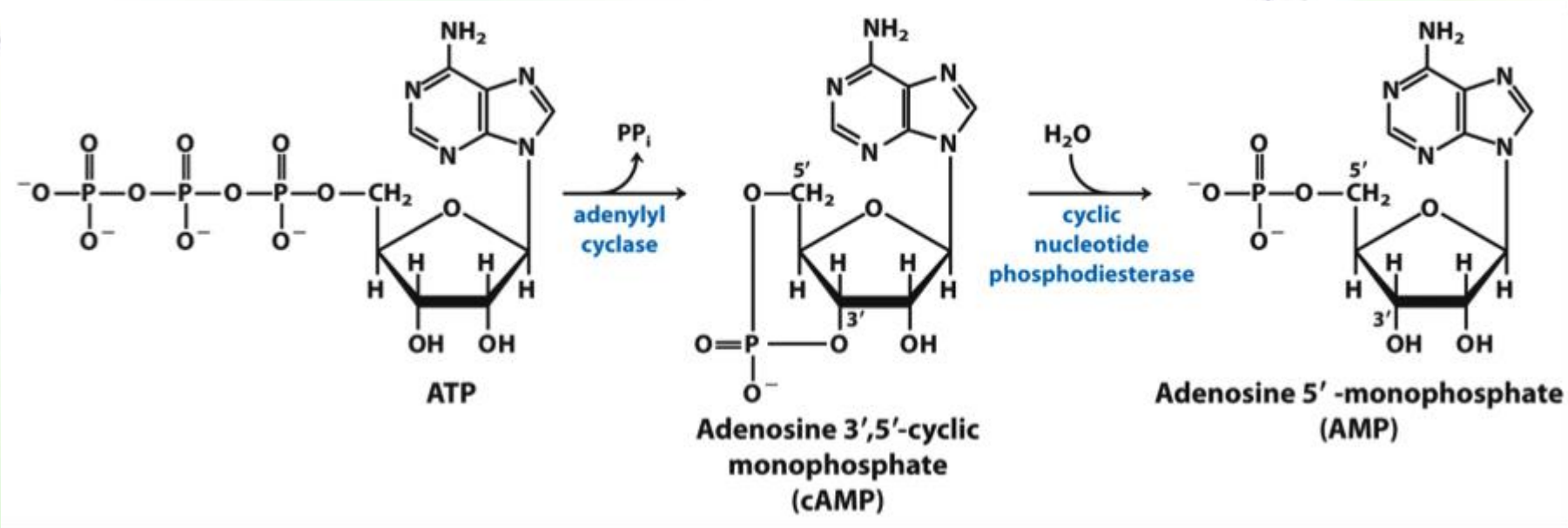
Regulation via modulators

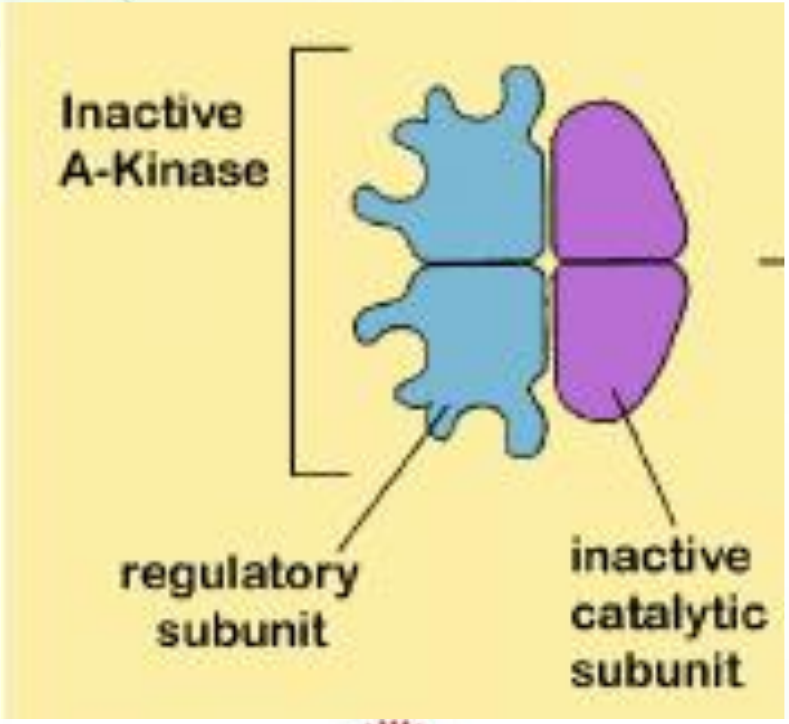
cAMP and protein kinase A (PKA)

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



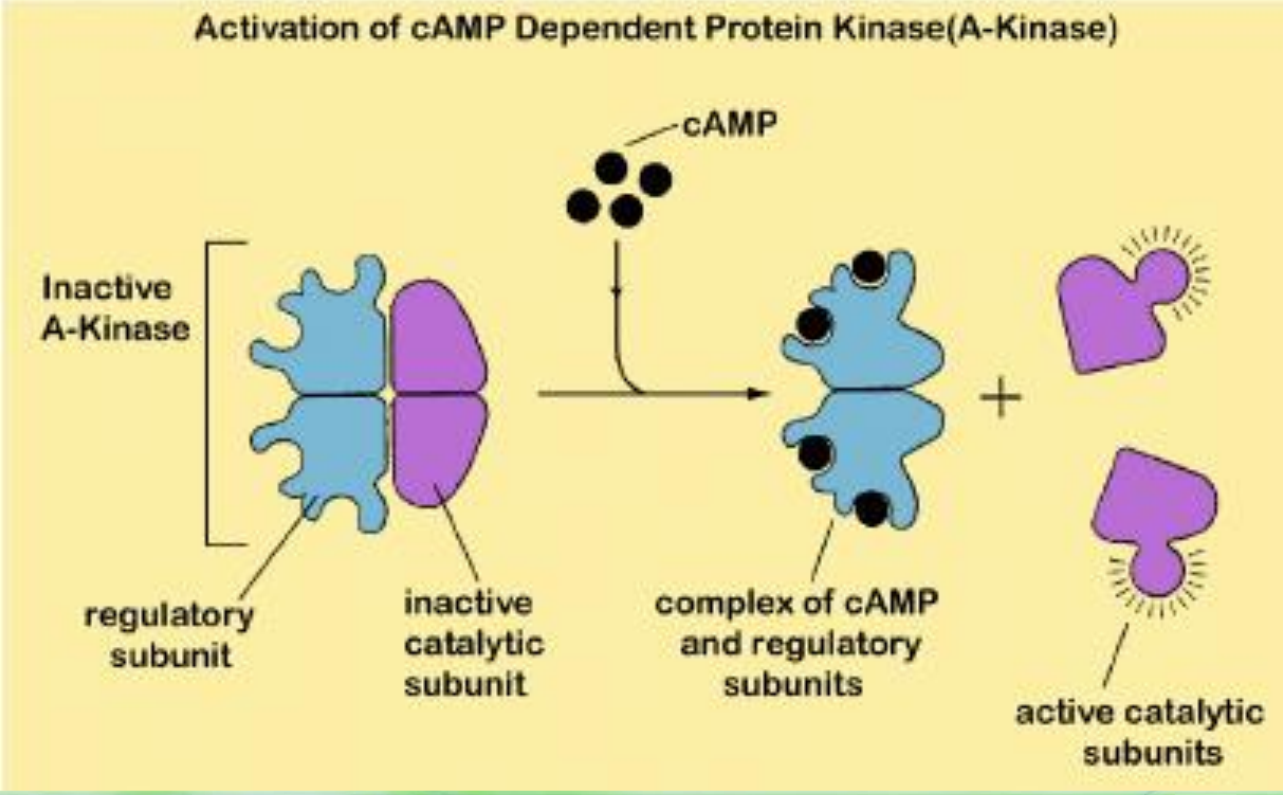
PKA-structure and regulation

- 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

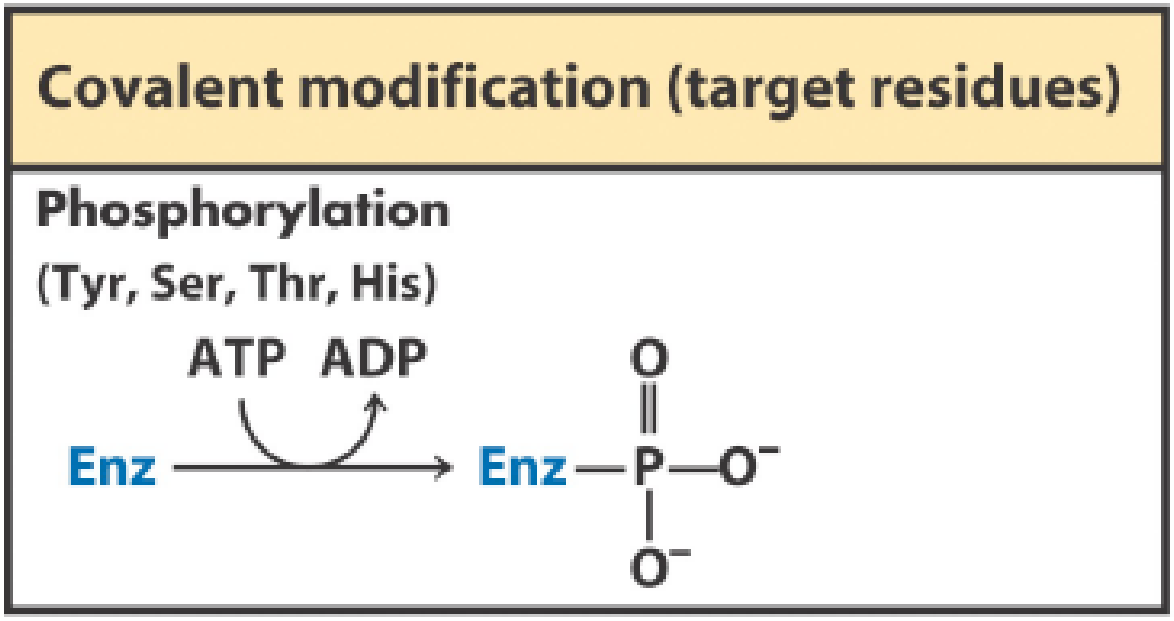
- The binding of two molecules of cAMP to the regulatory subunits leads to the dissociation of R2C2 into an R2 subunit and two active C subunits.



Reversible covalent modification

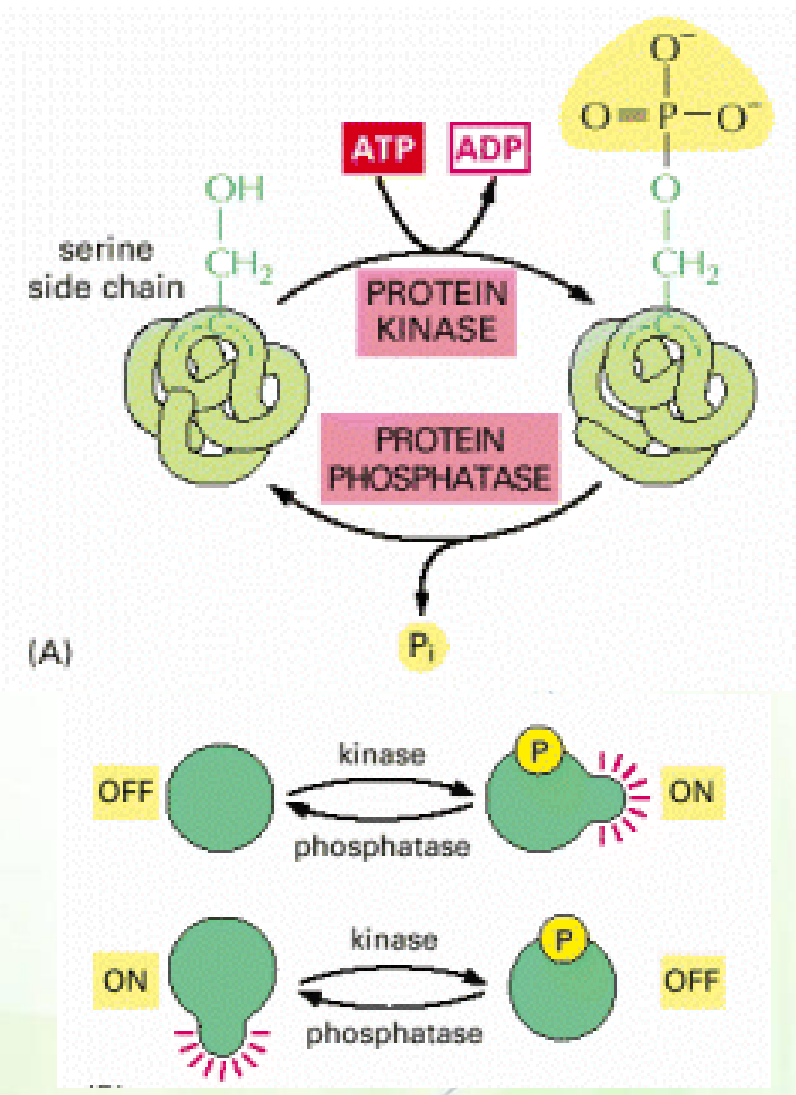
Advantage

- 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.



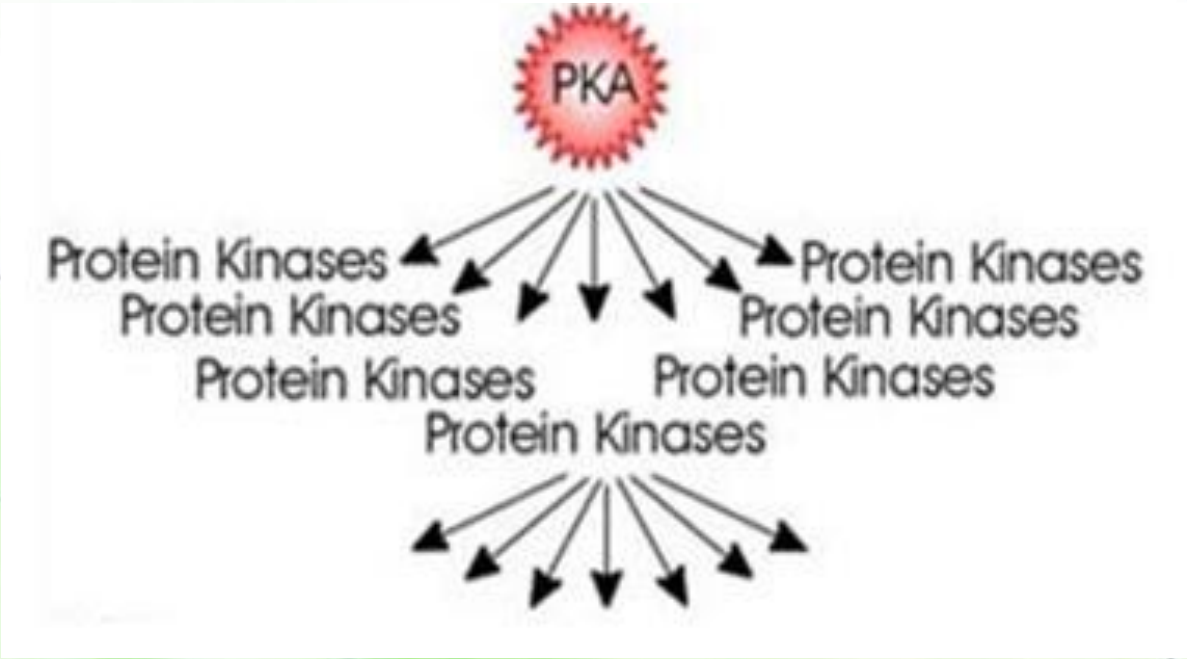
Enzymes

- 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.



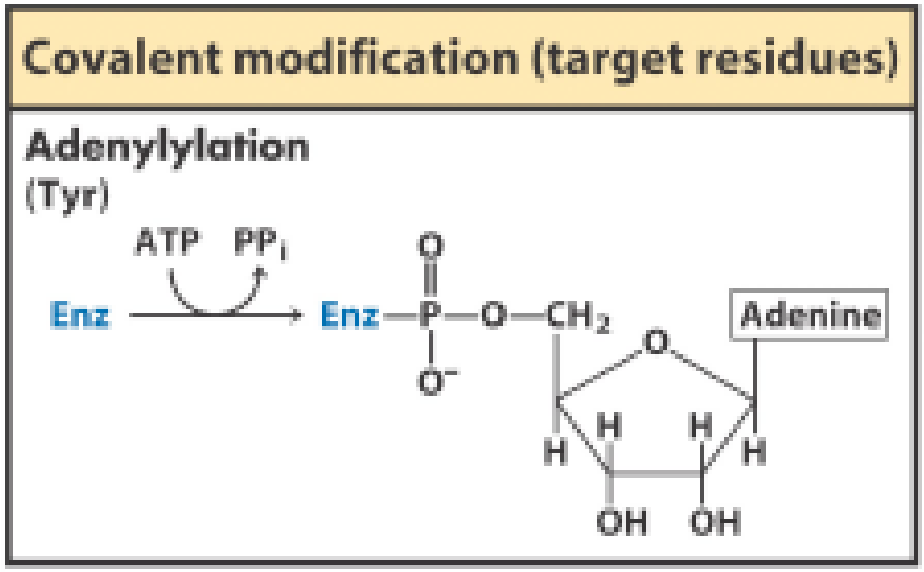
Why is it effective?

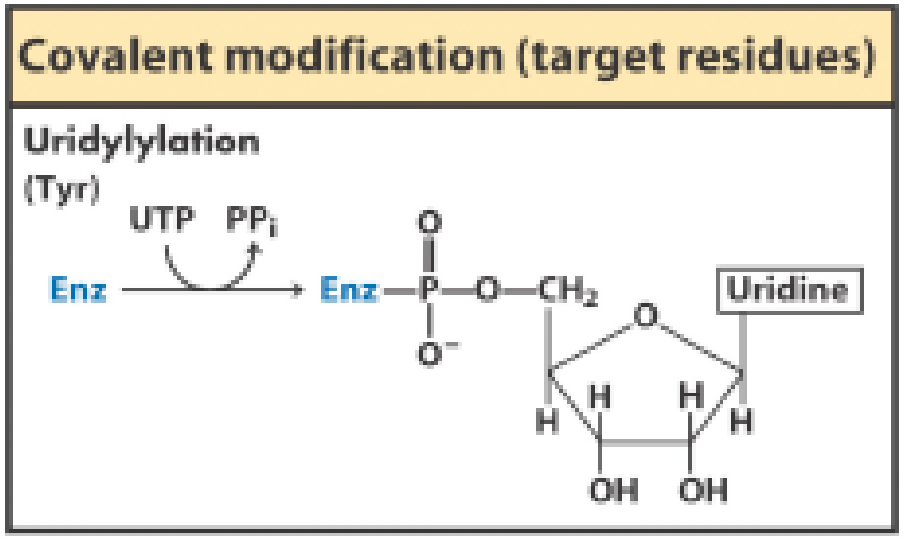
- Formation or removal of new electrostatic interactions and/or hydrogen bonds altering substrate binding and catalytic activity.
- It can happen in less than a second or over a span of hours.
- Phosphorylation often causes highly amplified effects.



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).



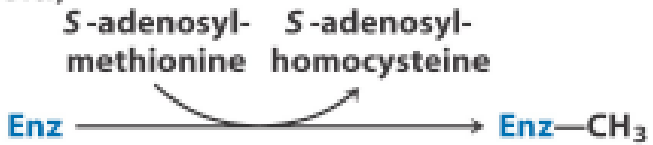


Others

- 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.

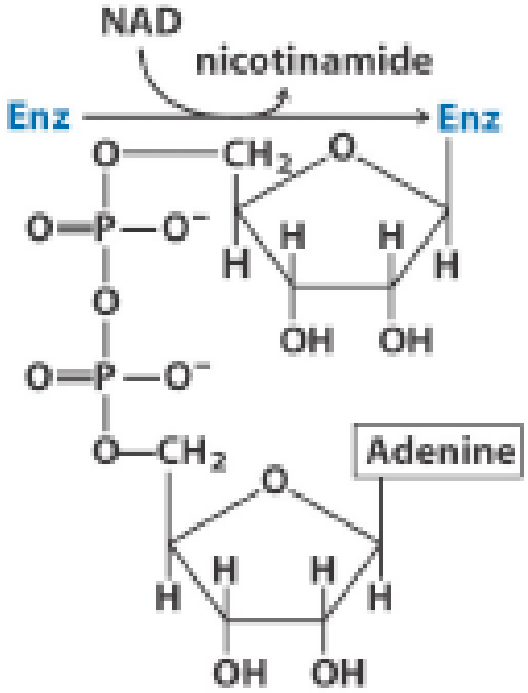
Covalent modification (target residues)

Methylation
(Glu)



Covalent modification (target residues)

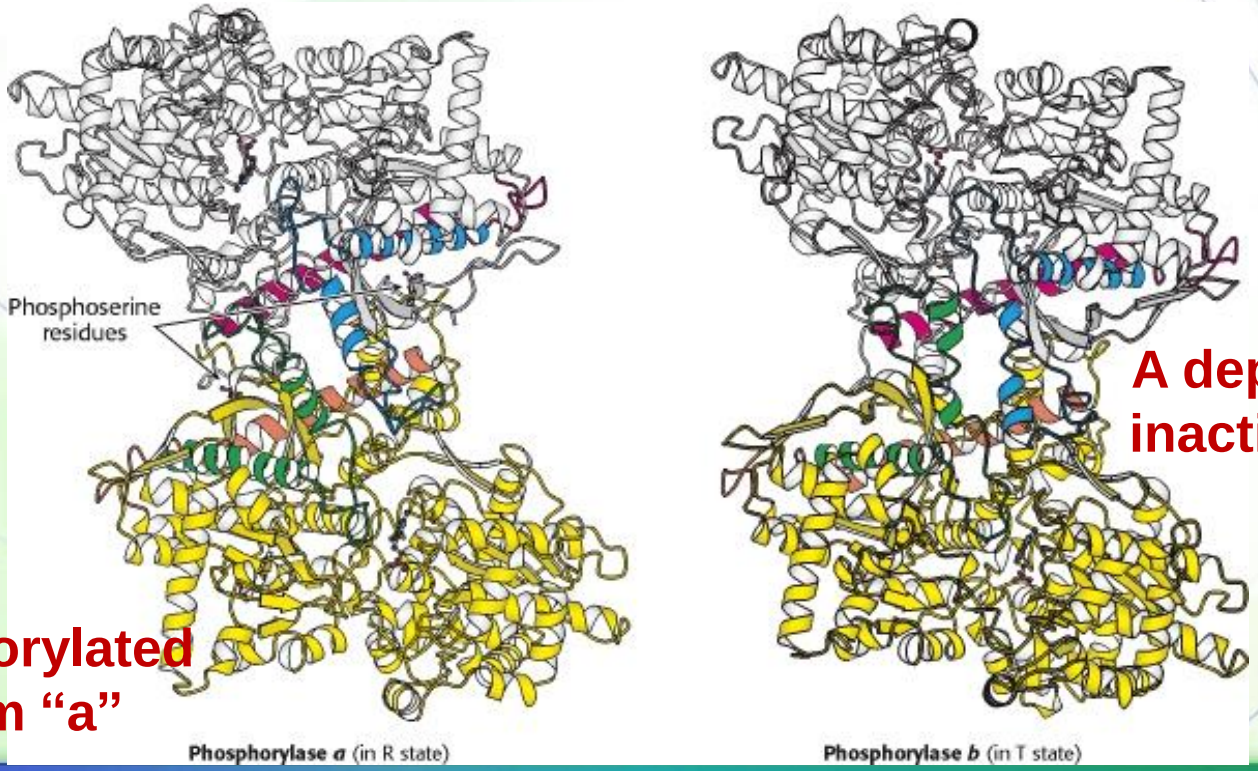
ADP-ribosylation
(Arg, Gln, Cys, diphthamide—a modified His)



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 two forms:



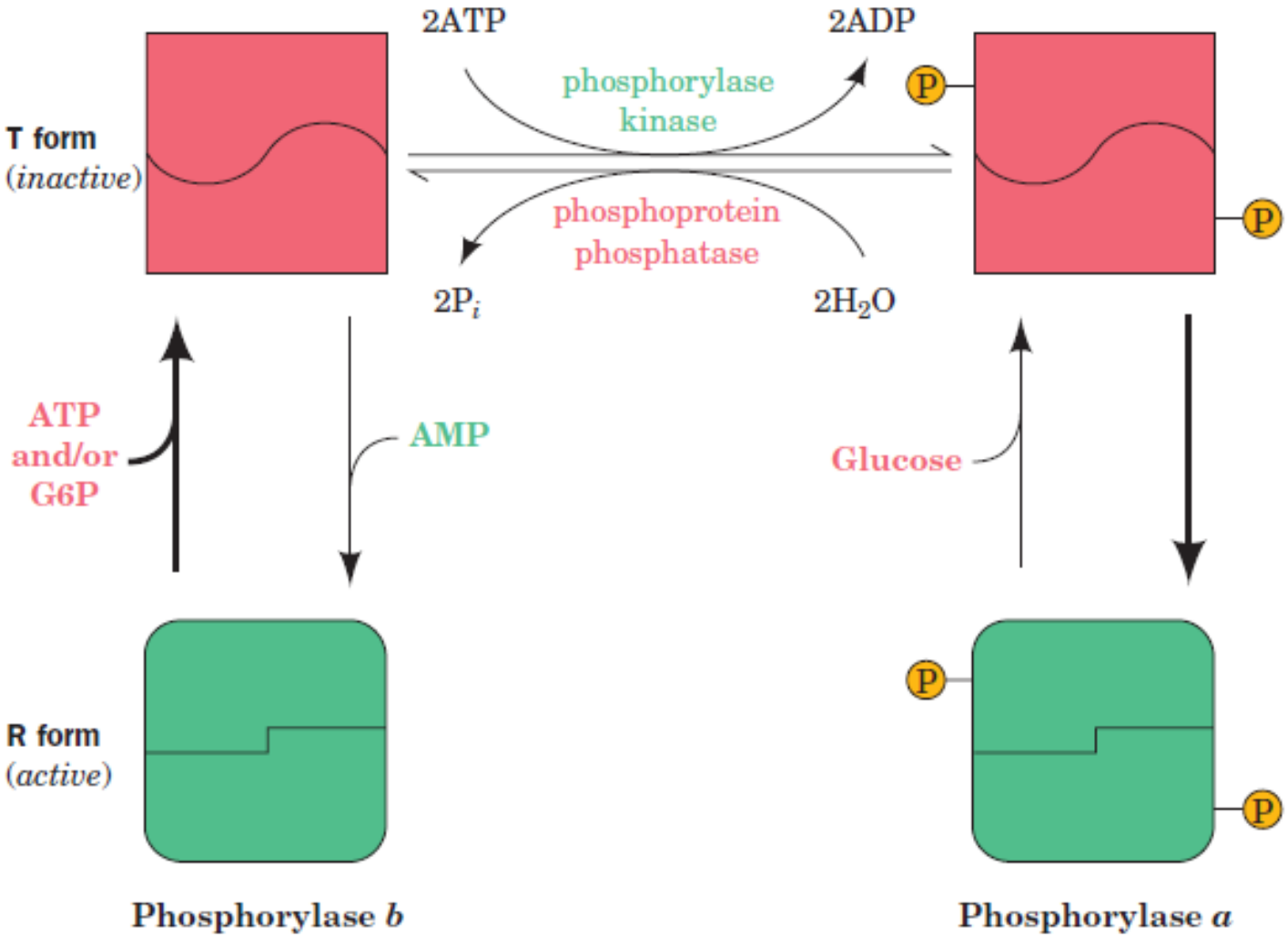
A phosphorylated active form “a”

A dephosphorylated inactive form “b”

The two forms of the enzyme

- 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.

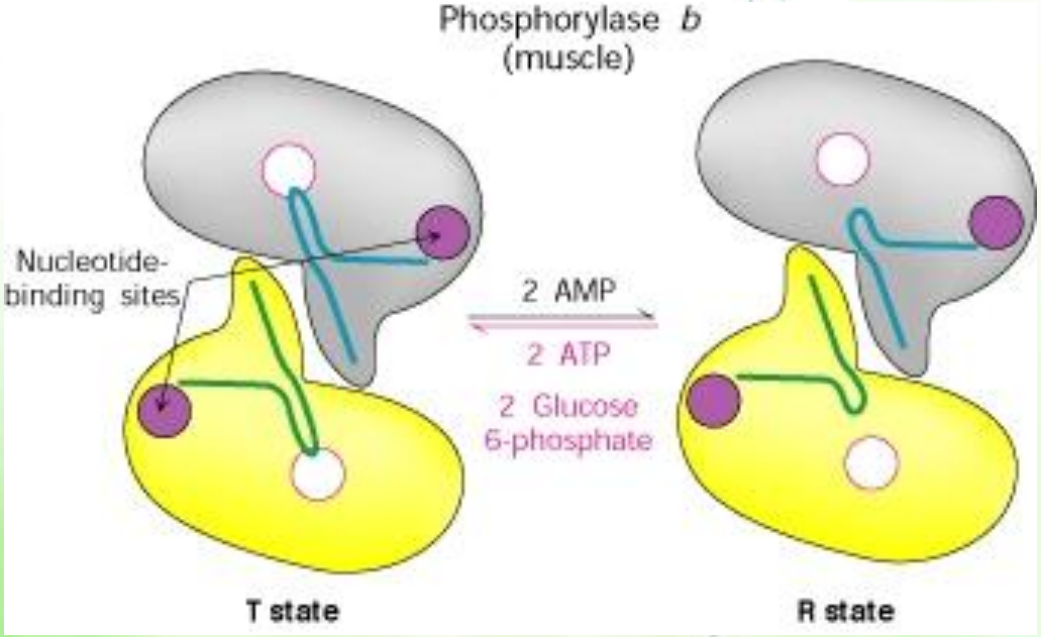
The transition of phosphorylase b between the T and the R state is controlled by the energy charge (ATP and AMP) of the muscle cell.

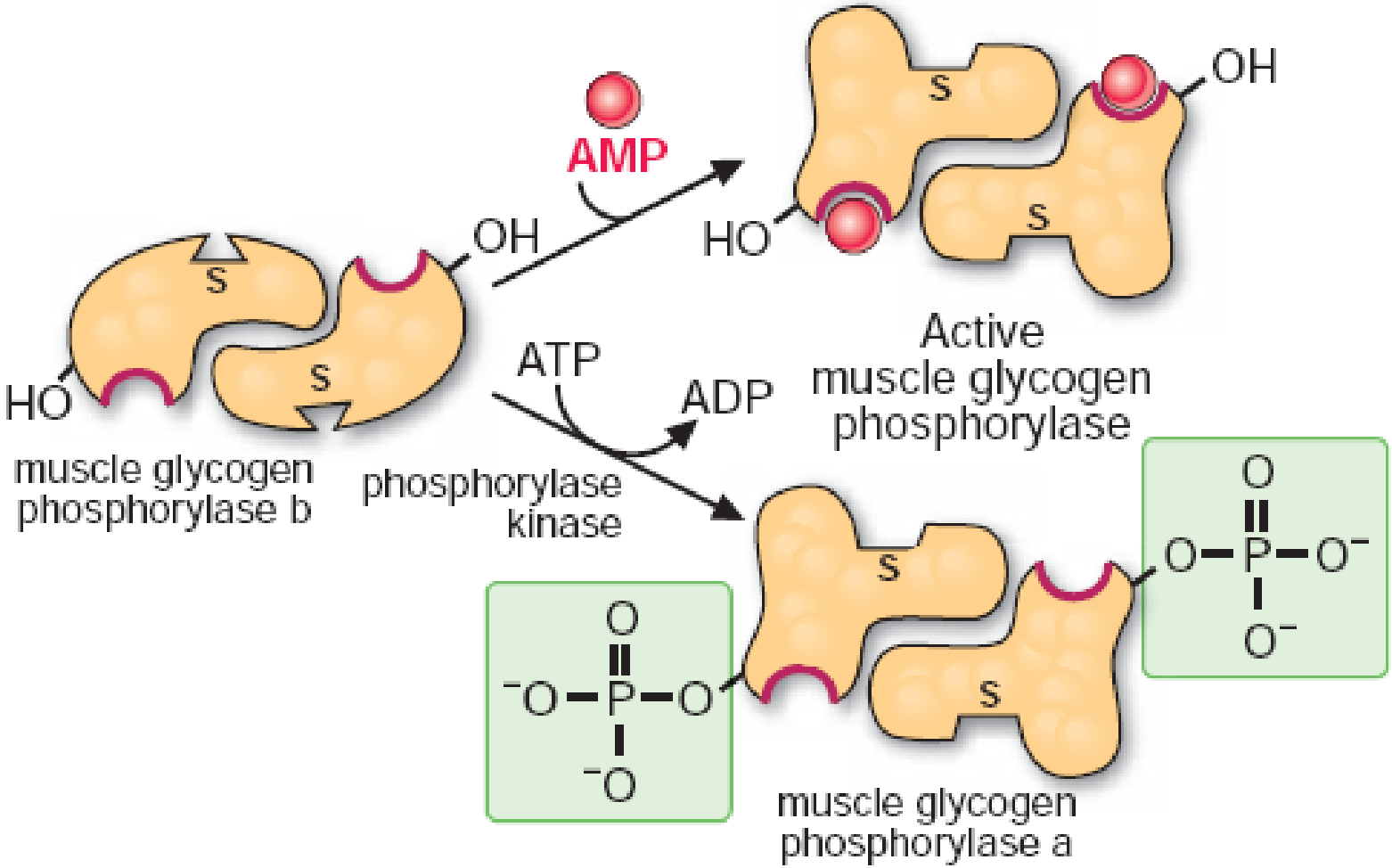

2ATP
2ADP
phosphorylase kinase
T form (inactive)
P
P
phosphoprotein phosphatase
$2P_i$
$2H_2O$
ATP and/or G6P
AMP
Glucose
R form (active)
P
P
Phosphorylase b
Phosphorylase a

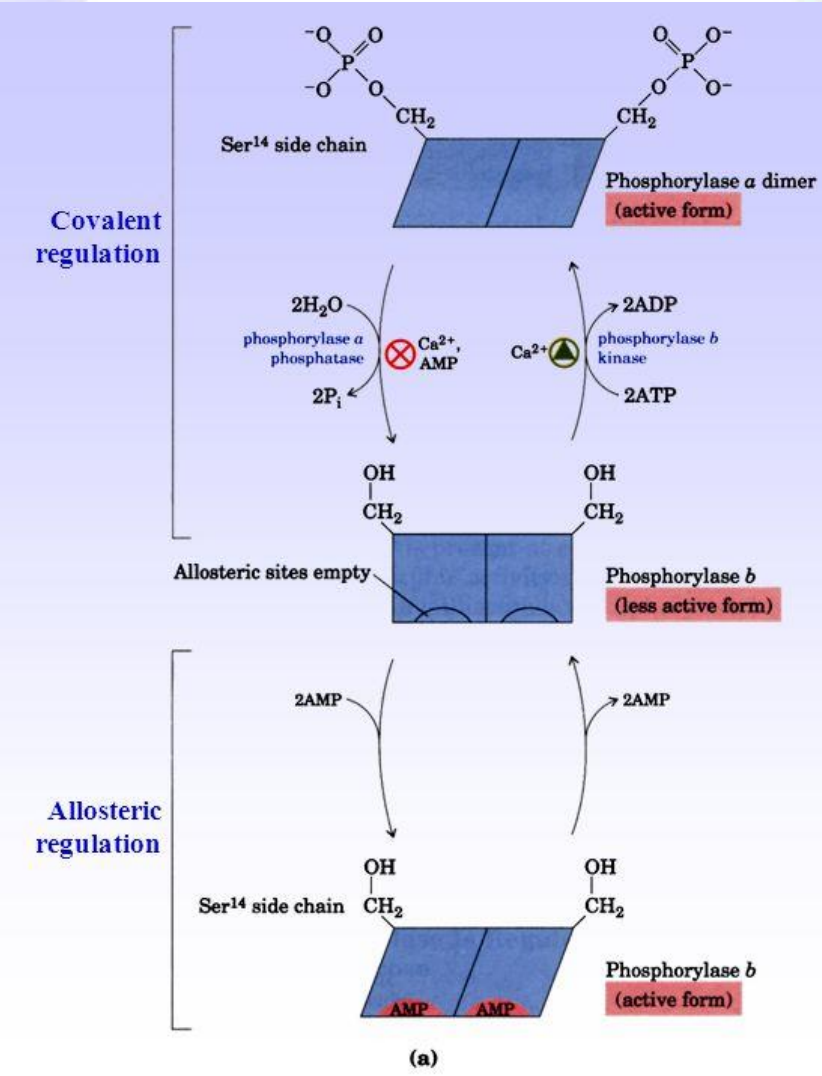
What do ATP and AMP do?

- 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.



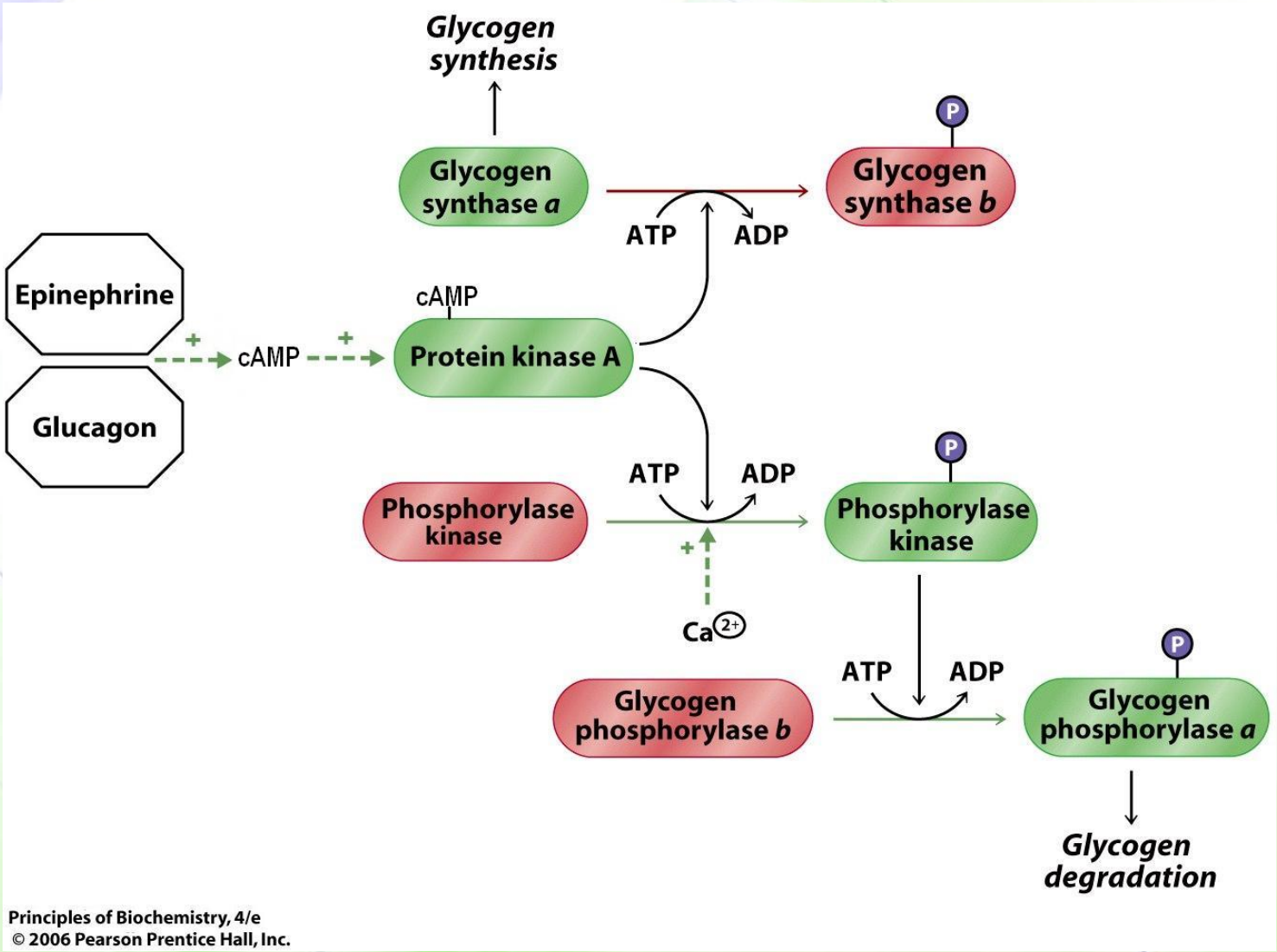

AMP
OH
HO
S
S
Active
muscle glycogen
phosphorylase
ATP
ADP
muscle glycogen
phosphorylase b
phosphorylase
kinase
O
⁻O
P
O
⁻O
muscle glycogen
phosphorylase a
O⁻



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.

Phosphorylation cascade

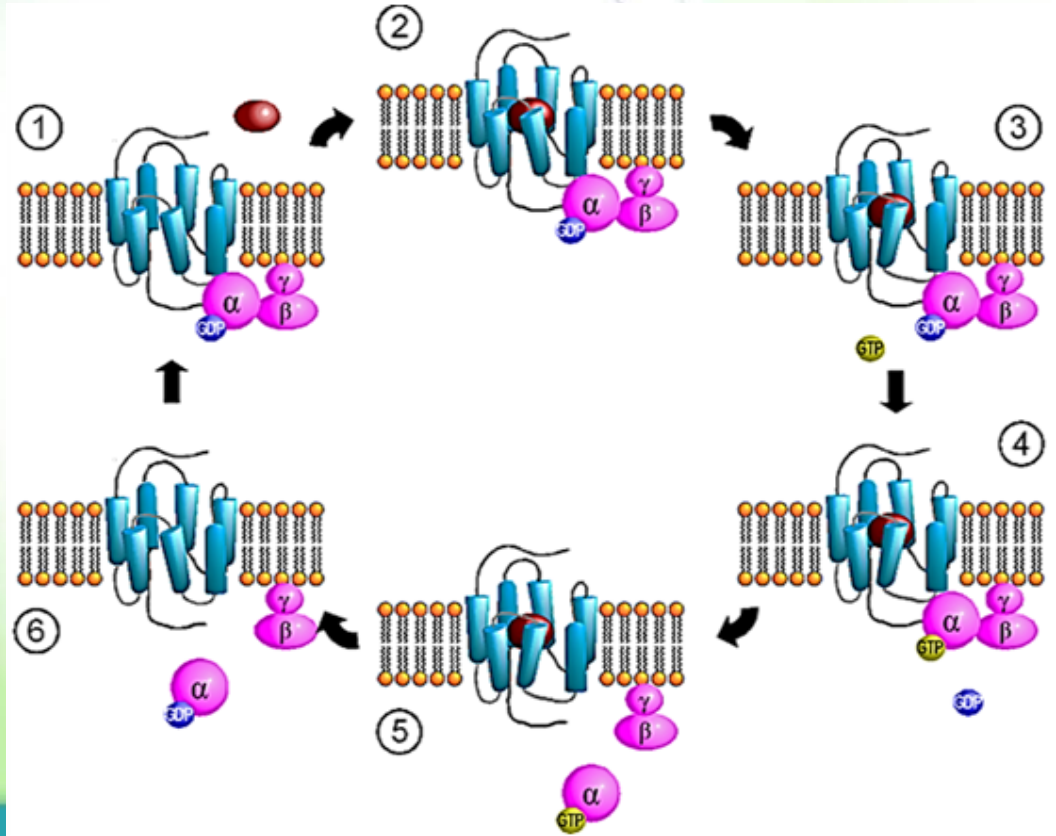


Regulation - Large regulatory molecules

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

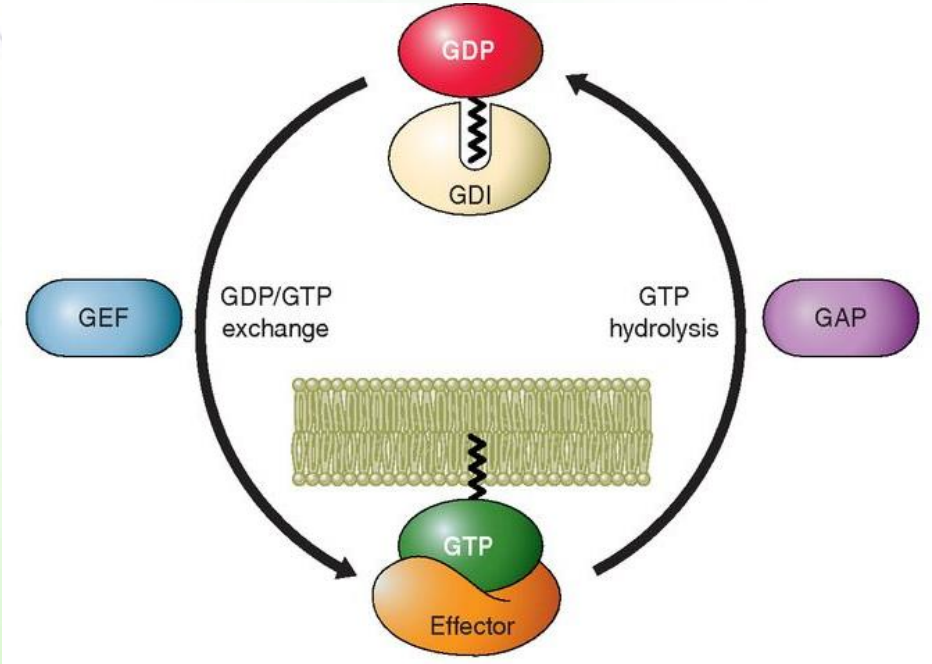
- **When they bind guanosine triphosphate (GTP), they are 'on', and, when they bind guanosine diphosphate (GDP), they are 'off'.**

- **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*]**