# CC 07

# REVERSIBLE AND IRREVERSIBLE COVALENT MODIFICATION OF ENZYME

LECTURE NOTE BY
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## 5- Covalent modification

- It means modification of enzyme activity through **formation of covalent bonds** e.g.
  - **Methylation** (addition of methyl group).
  - **Hydroxylation** (addition of hydroxyl group).
  - **Adenylation** (addition of adenylic acid).
  - **Phosphorylation** (addition of phosphate group).

# Reversible covalent modification

## What's covalently modulated enzymes?

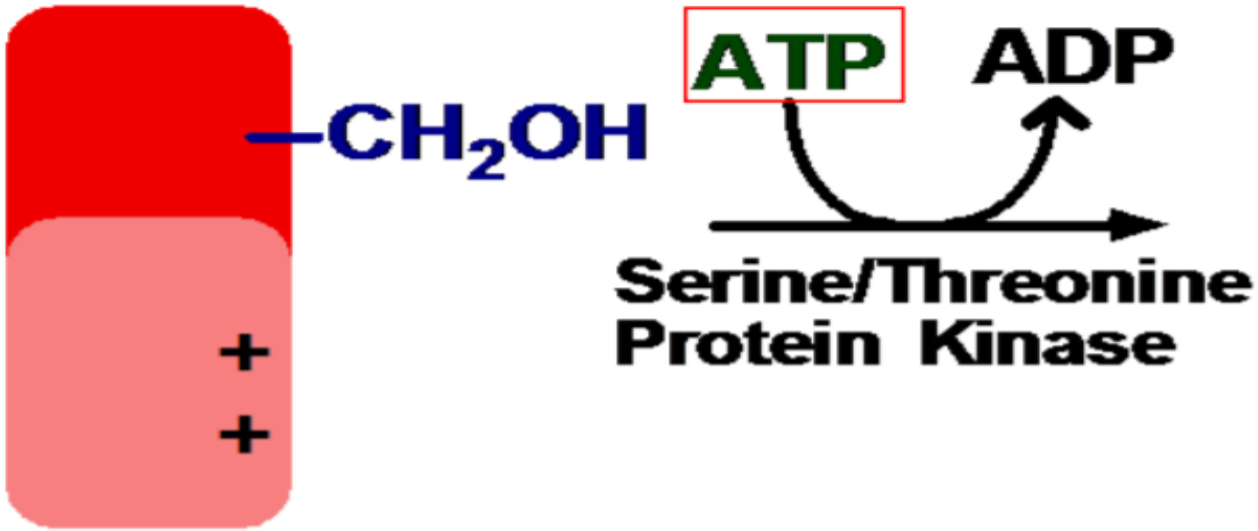
- Activity is modulated by covalent modification of one or more of its amino acid residues in the enzyme molecule.

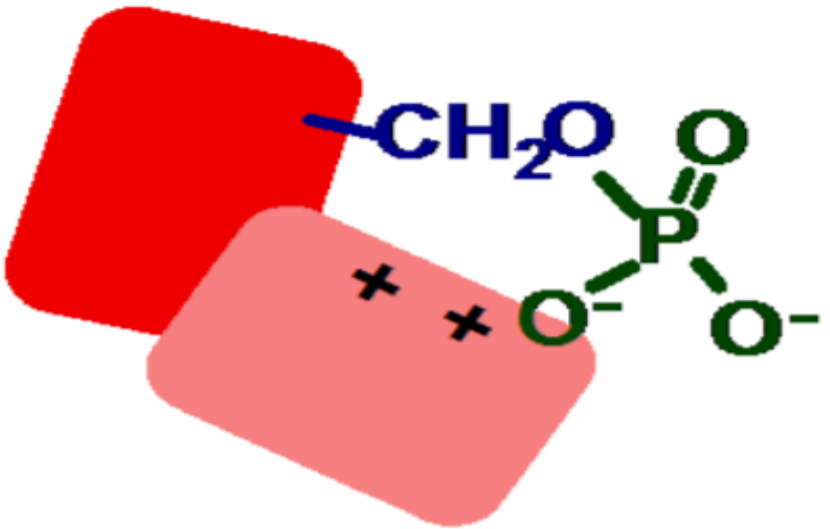
- Common modifying groups include: phosphoryl, adenylyl, methyl and hydroxyl.

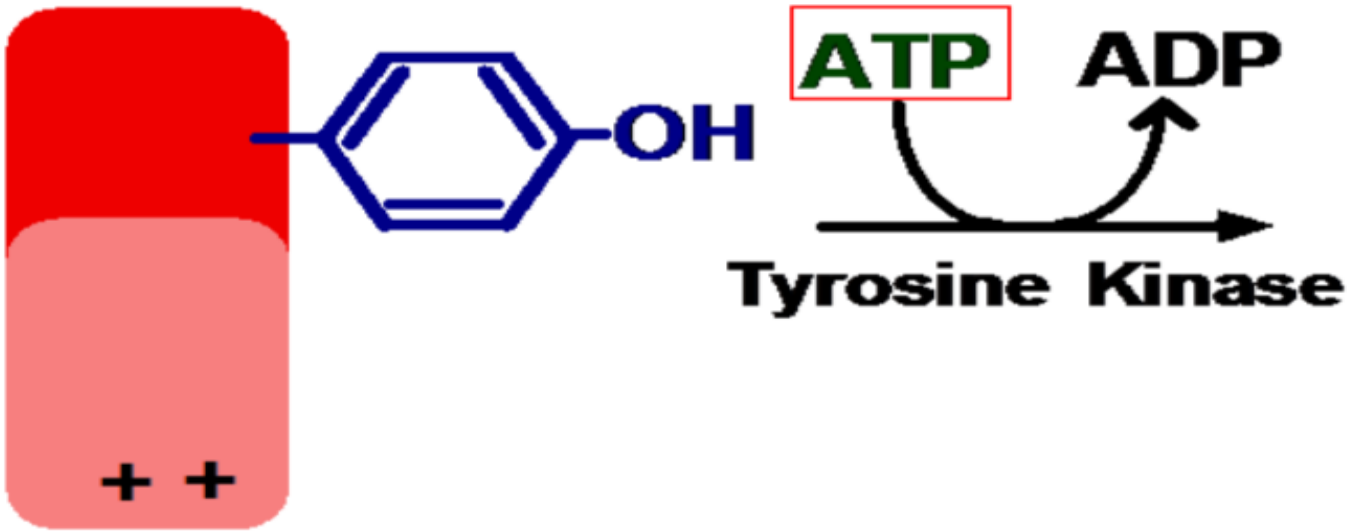
- These groups are generally linked to and removed from the regulatory enzyme by separate enzymes.

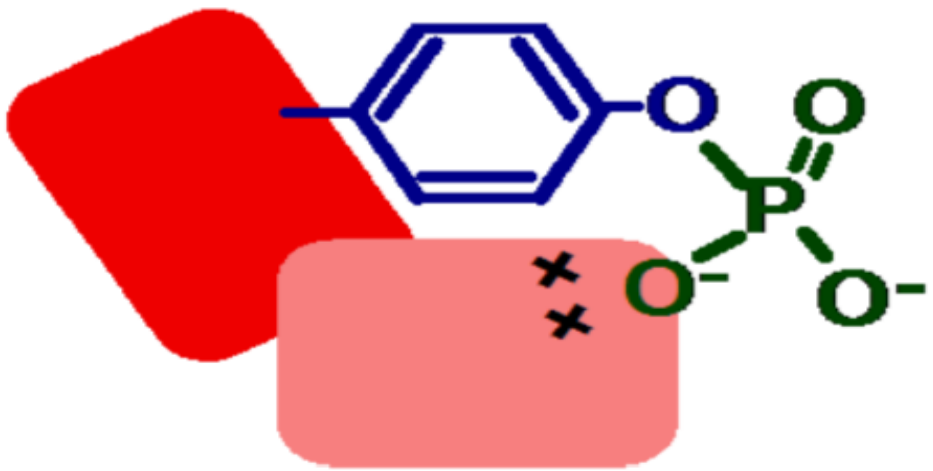
- **Phosphorylation** is the **most covalent modification** used to regulate enzyme activity.
- Phosphorylation of enzyme occurs by **addition of phosphate group** to the enzyme at the **hydroxyl group of serine, threonine or tyrosine.**
- This occurs by **protein kinase** enzyme.

# Protein kinases catalyze the phosphorylation of proteins



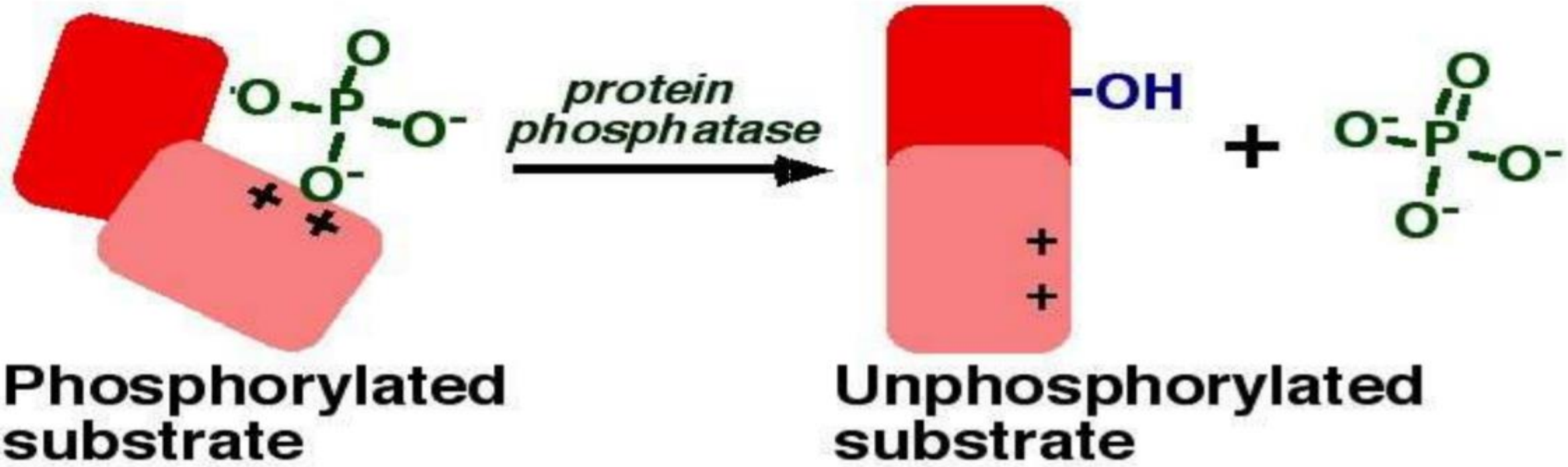



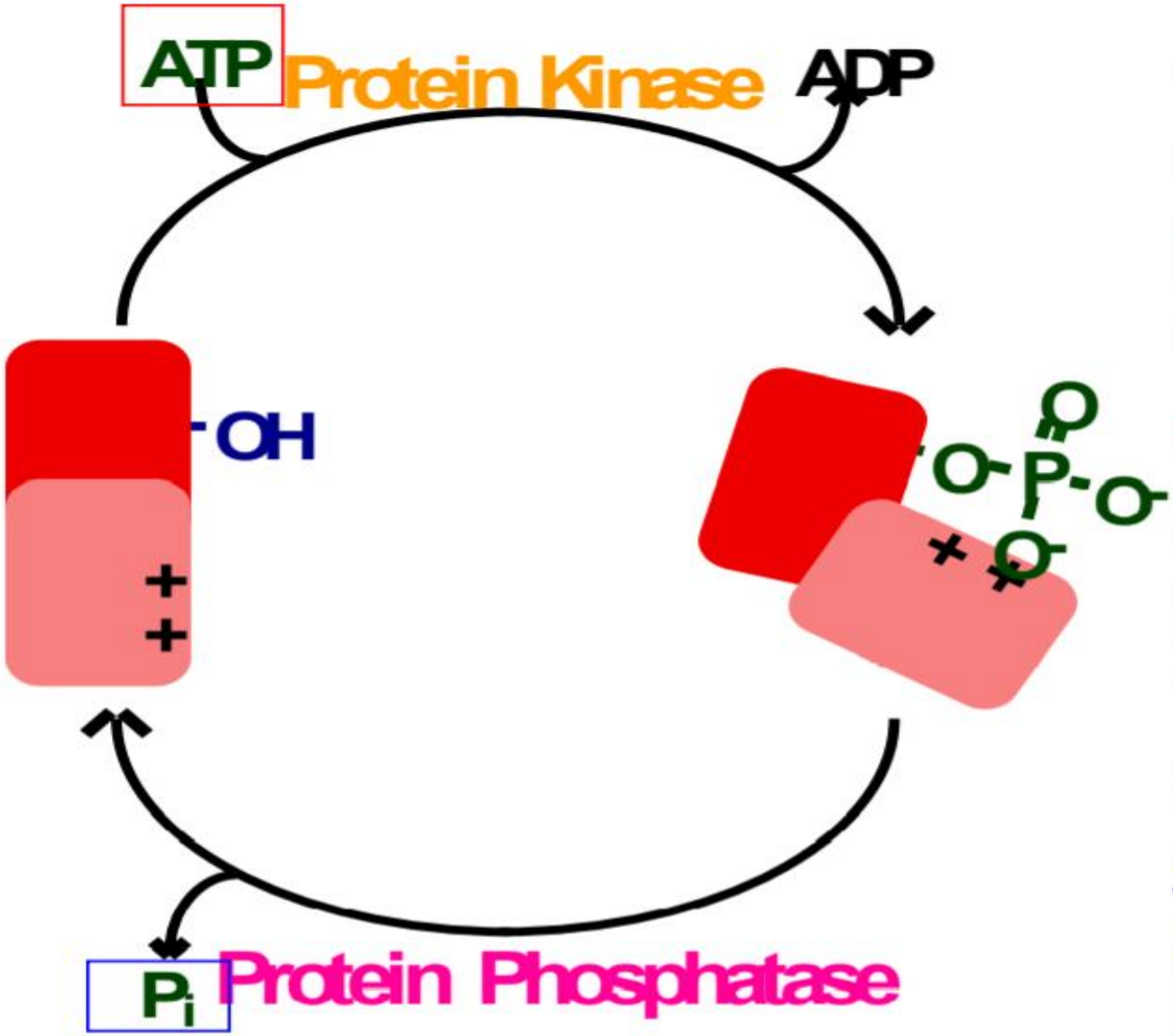



- **Dephosphorylation** of the enzyme occurs by **removal of phosphate group from the hydroxyl group of serine, threonine or tyrosine.**
- This occurs by **phosphatase** enzyme.

# Protein phosphatases remove phosphate groups from phosphorylated proteins



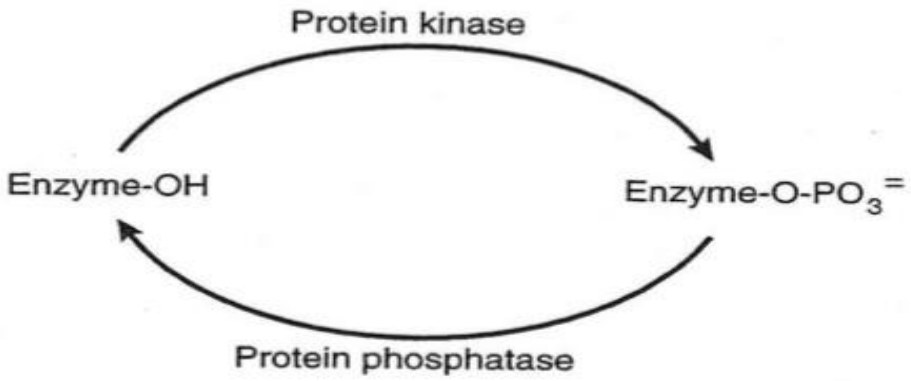



- Phosphorylation and dephosphorylation are **not the reverse** of one another.
- **The rate of cycling** between the phosphorylated and the dephosphorylated states ***depends on the relative activities of kinases and phosphatases.***

➢The **phosphorylated from** is the **active** form in some enzymes, while the **dephosphorylated form** is the **active form** in other enzymes.

# Covalent Regulation of Enzyme Activity
## **Phosphorylation and Dephosphorylation**

- Addition or deletion of phosphate groups to particular serine, threonine, or tyrosine residues alter the enzymes activity



# COVALENT ENZYME REGULATION

- Both reversible and irreversible covalent modification of enzymes play important roles in regulation of enzyme function

1. **Reversible covalent modification**

- The modulation of enzyme activity by the attachment or release of small groups plays a very important role in metabolic control

- Probably the most universal, and certainly the most well understood, is the phosphorylation of specific serine, threonine or tyrosine groups

## 2) Irreversible covalent modification

- Proteolytic cleavage of specific peptide bonds is often used to activate enzymes
- proteolysis is essentially irreversible, turning the activity off requires another mechanism, often binding of inhibitory proteins
- Examples of enzymes activated by proteolytic cleavage and the role of enzyme cascades

## 1) Reversible covalent modifications.

- Reversible covalent modifications require expenditure of energy and are often used in signaling from extracellular messages
- In contrast, noncovalent interactions are reversible with no metabolic energy expended and sense conditions within a cell

- Reversible covalent modifications that are known to alter enzyme activity include:
- a) Phosphorylation of serine, threonine or tyrosine and less frequently aspartate and histidine residues
- b) Acetylation of lysine or amino terminal groups

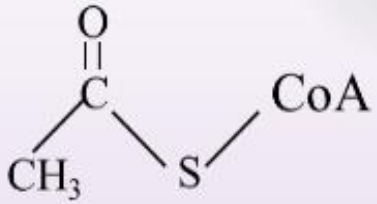
Cont…..

- c) Methylation of glutamate or aspartate residues
- d) Nucleotidylation of tyrosine residues
- e) ADP ribosylation primarily of arginine residues
- Most well understood of these reactions, and probably the most ubiquitous in eukaryotic cells, is the phosphorylation reaction
- which is based on the simple addition and removal of inorganic phosphate

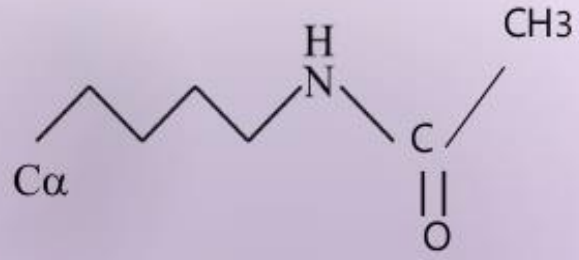
# Lysine Acetylation

- Acetylation of lysines in histones is important in regulation of gene expression
- Addition of the acetyl group to a lysine removes its positive charge, weakening the binding of histones to the negatively charged DNA
- Which, apparently, results in a conformation more favorable for transcription

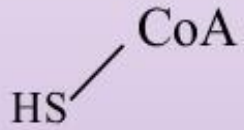

Lysine




Acetyl Coenzyme A




Acetylated Lysine

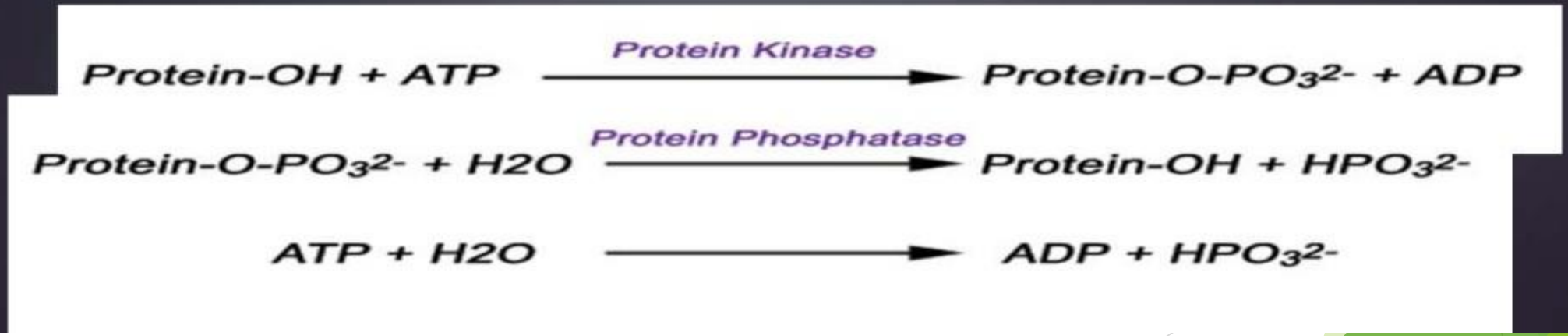



Coenzyme A

# Protein Phosphorylation

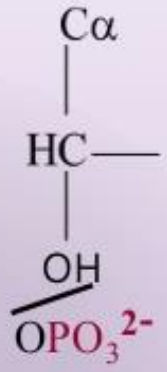
Enzymes catalyzing the transfer of a phosphate from ATP to a protein are known as kinases

Those catalyzing the hydrolytic removal of the phosphate group are known as phosphatases



- Kinases and Phosphatases come in two major classes - those that act specifically on serine and threonine residues and those that act on tyrosine residues


Serine




Threonine


Tyrosine

## Regulation of Protein Kinases

- Regulation by phosphorylation requires that the kinases and phophatases must, in turn, be regulatedRegulation of kinases, and probably phosphatases as well, most often involves one or more of three regulatory strategies:

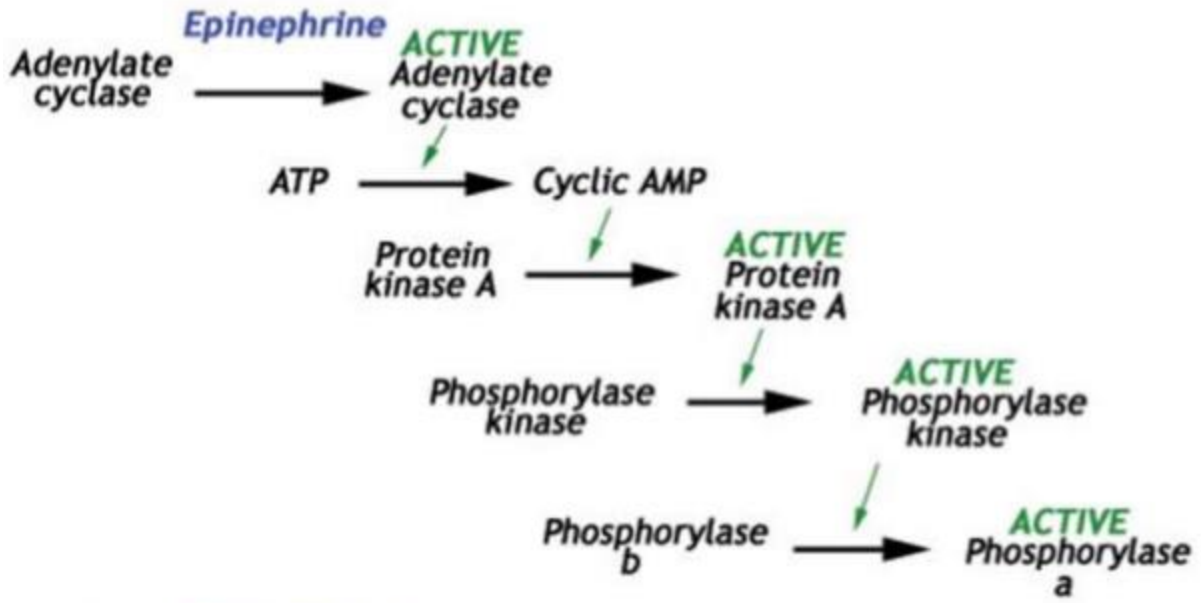
- a) interaction with peptides or subunits whose binding may depend upon chemical messengers such as calcium or cyclic AMP

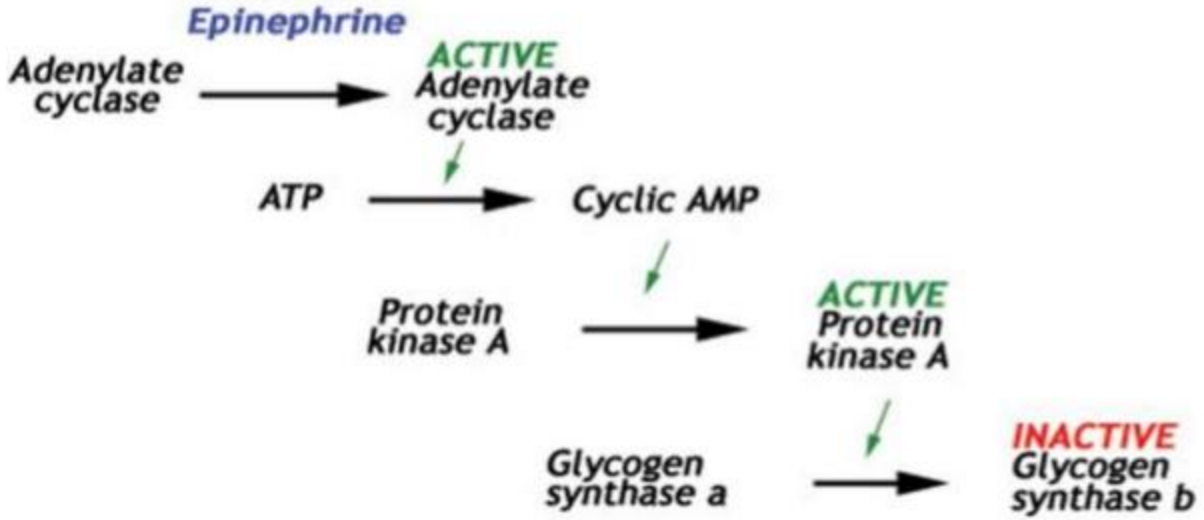
- b) phosphorylation itself is a very common mechanism for regulation of protein kinases (an enzyme that catalyzes this reaction would be known as a kinase kinase)

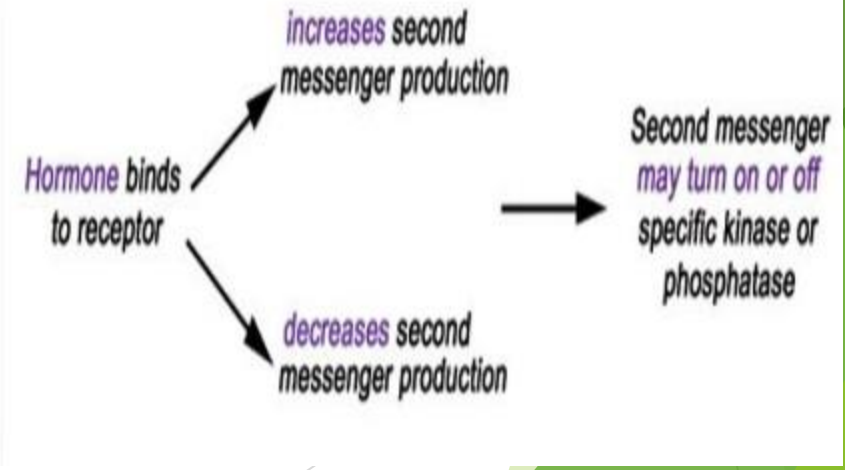
- c) localization to particular cellular components.

# Phosphorylation in the regulation of glycogen metabolism

- The role of reversible covalent modification was first shown to be important in the control of glycogen metabolism
- Glycogen is used by the body as a readily mobilized storage of glucose
- Glycogen synthase catalyses synthesis of glycogen
- While Glycogen phosphorylase catalyses the stepwise removal of glucose units from glycogen


Epinephrine
Adenylate cyclase
ACTIVE Adenylate cyclase
ATP
Cyclic AMP
Protein kinase A
ACTIVE Protein kinase A
Phosphorylase kinase
ACTIVE Phosphorylase kinase
Phosphorylase b
ACTIVE Phosphorylase a


Epinephrine
Adenylate cyclase
ACTIVE Adenylate cyclase
ATP
Cyclic AMP
Protein kinase A
ACTIVE Protein kinase A
Glycogen synthase a
INACTIVE Glycogen synthase b


Hormone binds to receptor
increases second messenger production
decreases second messenger production
Second messenger may turn on or off specific kinase or phosphatase

## II) Irreversible covalent modification (limited proteolysis)

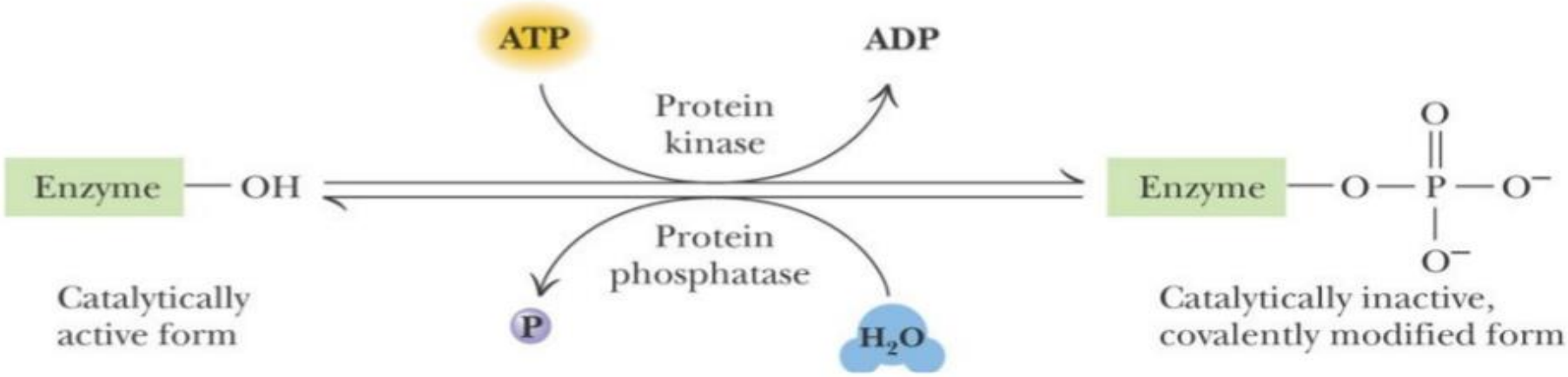
- In a number of cases, it is necessary to synthesize an enzyme in an inactive state
- And activate it later by selective cleavage of one or more peptide bonds
- The inactive precursors are termed **zymogens or proenzymes**
- Proteolytic cleavage generally occurs at surface loops, rather than secondary structural elements
- Generally, the proteolytic site is amino terminal relative to the active site of the protein
- Since the polypeptide is synthesized in the amino to carboxy direction, the protein can, thus, avoid gaining catalytic activity as it folds during synthesis

# A) Digestive enzymes

- The digestive enzymes are classic examples for which activation of enzyme activity occurs by selective cleavage
- Synthesis as inactive zymogens permits export to the digestive tract before the destructive catalytic powers of these enzymes are unleashed on the synthesizing cells

**Gastric and pancreatic digestive enzymes**

| Site of synthesis | Zymogen | Active Enzyme |
|---|---|---|
| Stomach | Pepsinogen | Pepsin |
| Pancreas | Chymotrypsinogen | Chymotrypsin* |
| Pancreas | Trypsinogen | Trypsin* |
| Pancreas | Procarboxypeptidase | Carboxypeptidase |
| Pancreas | Proelastase | Elastase* |



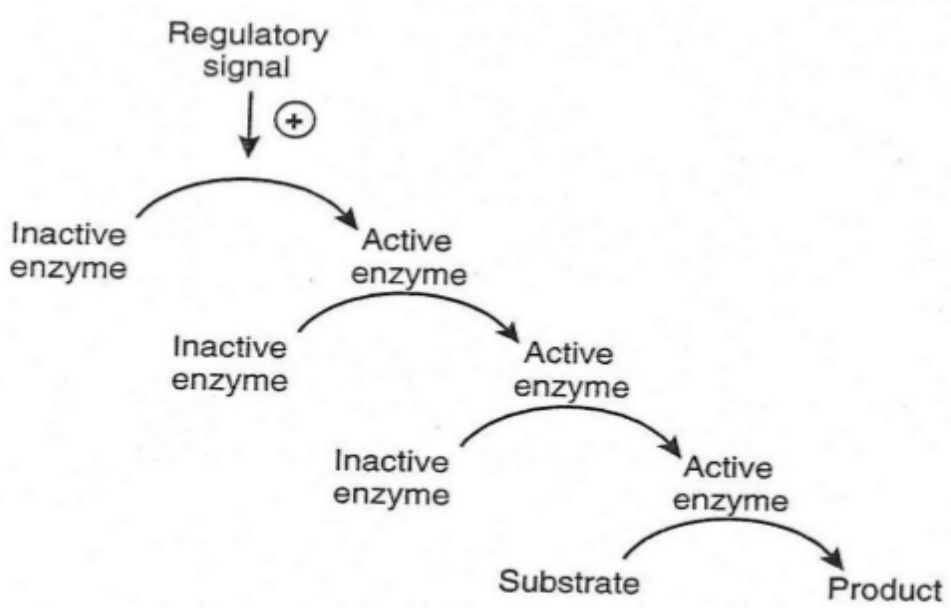

Enzymes regulated by covalent modification are called **interconvertible enzymes.**

The enzymes (*protein kinase* and *protein phosphatase*) catalyzing the conversion of the interconvertible enzyme between its two forms are called **converter enzymes.** In this example, the free enzyme form is catalytically active, whereas the phosphoryl-enzyme form represents an inactive state.

The -OH on the interconvertible enzyme represents an -OH group on a specific amino acid side chain in the protein (for example, a particular Ser residue) capable of accepting the phosphoryl group.

# Covalent Regulation of Enzyme Activity
## **Enzyme Cascades**

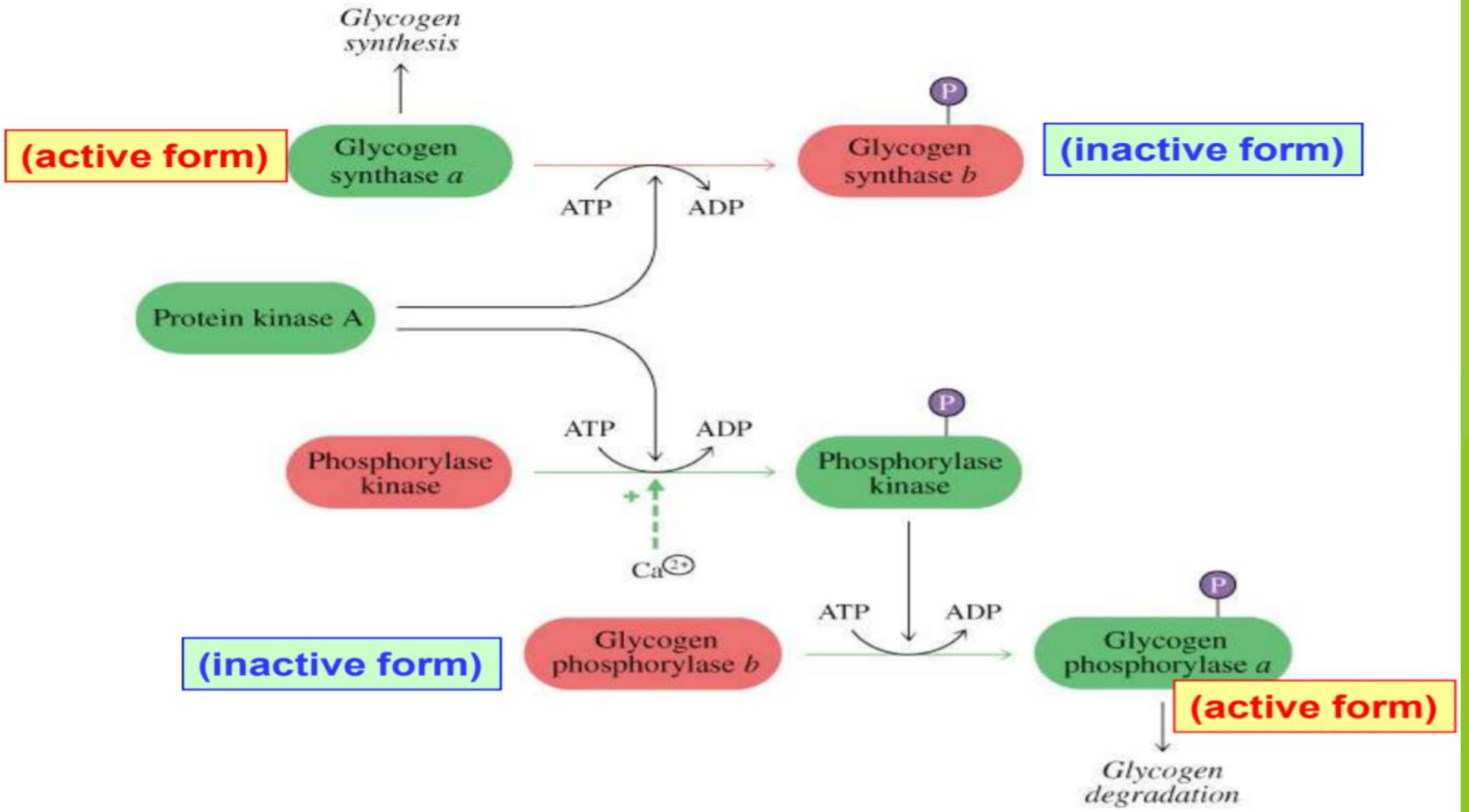
- Enzymes activating enzymes allows for amplification of a small regulatory signal



## Enzymes inactivated by phosphorylation:

- These are enzymes of **biosynthetic reactions**

1. **Glycogen Synthetase**, which catalyzes biosynthesis of glycogen.
2. **Acetyl CoA carboxylase**, an enzyme in fatty acid biosynthesis.
3. **HMG CoA reductase**, an enzyme in cholesterol biosynthesis.


Glycogen synthesis
(active form)
Glycogen synthase a
ATP
ADP
P
Glycogen synthase b
(inactive form)
Protein kinase A
Phosphorylase kinase
ATP
ADP
P
Phosphorylase kinase
Ca2+
(inactive form)
Glycogen phosphorylase b
ATP
ADP
P
Glycogen phosphorylase a
(active form)
Glycogen degradation

## Enzymes inactivated by phosphorylation:

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3. **HMG CoA reductase**, an enzyme in cholesterol biosynthesis.

# Significance of Covalent Modifications

Conversion of enzyme from one form to another is enzyme catalysed.

- Rapid change in the amount of active enzymes.
- Large amplification of the initial signal

Reversible modifications permit controlled responses of metabolic signals.

- System is always poised for activation or inactivation.
- System can be rapidly activated, since it is reversible in nature.
- When stimulus removed, system rapidly converted back to resting state.

# THANK YOU