CONCEPT OF METABOLISM

CC-12
UNIT-1

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Unit 1: Concept of metabolism

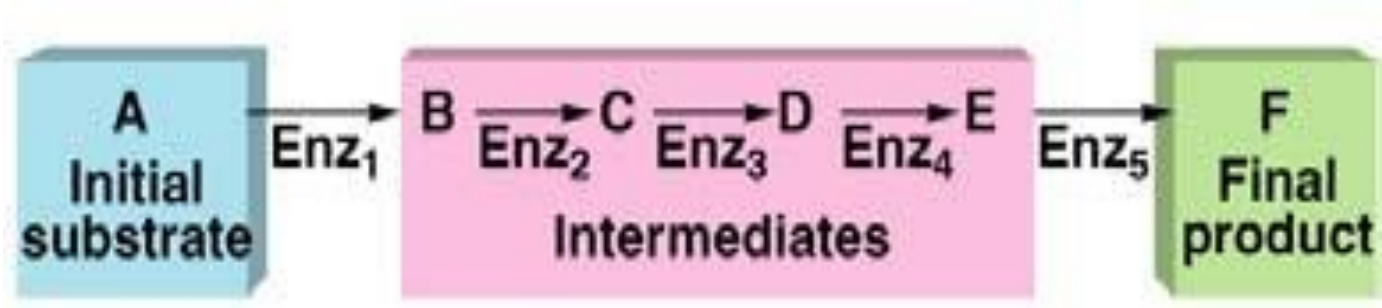
Introduction, anabolic and catabolic pathways, regulation of metabolism, role of regulatory enzymes (allosteric ,covalent modulation and Isozymes).

Metabolism

- Metabolism is a term that is used to describe all chemical reactions involved in maintaining the living state of the cells and the organism.
- Metabolism can be conveniently divided into two categories:
  - Catabolism - the breakdown of molecules to obtain energy
  - Anabolism - the synthesis of all compounds needed by the cells
- Metabolism is closely linked to nutrition and the availability of nutrients.
- Bioenergetics is a term which describes the biochemical or metabolic pathways by which the cell ultimately obtains energy.
- Energy formation is one of the vital components of metabolism.

Metabolism

- Metabolism is a network of metabolic /biochemical reactions.
- Carried out in living cells.
- In a well organized, integrated and regulated manner.
- Related to various biomolecules viz
  - Carbohydrates
  - Lipids
  - Proteins
  - Nucleoproteins
  - Metabolite precursors are transformed to end products via many specific intermediates.



Metabolism

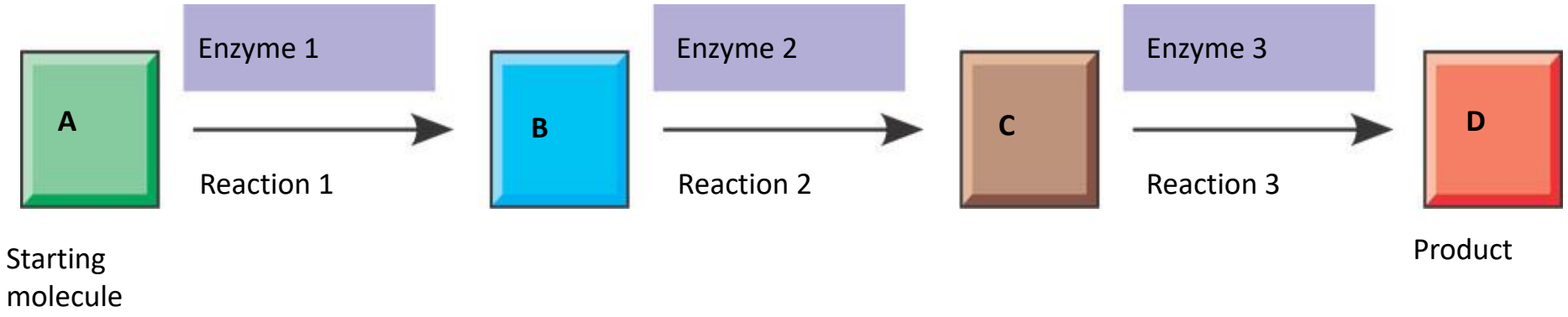
- **Metabolism is the sum of the chemical changes that convert:**
  - **Nutrients into energy.**
  - **Chemically complex substances of cells into simpler forms.**
  - **Chemically simple substances into functional complex biomolecules.**

The Metabolic Map

Different pathways can intersect forming an integrated and purposeful network of chemical reactions - Pathways that regenerate a component are called cycles.

Metabolic pathways

- The chemical reactions of metabolism are organized into metabolic pathways. These allow the basic chemicals from nutrition to be transformed through a series of steps into another chemical, by a sequence of enzymes.
- Enzymes are crucial to metabolism because they allow organisms to drive desirable reactions that require energy. These reactions also are coupled with those that release energy.
- As enzymes act as catalysts they allow these reactions to proceed quickly and efficiently.
- Enzymes also allow the regulation of metabolic pathways in response to changes in the cell's environment or signals from other cells.

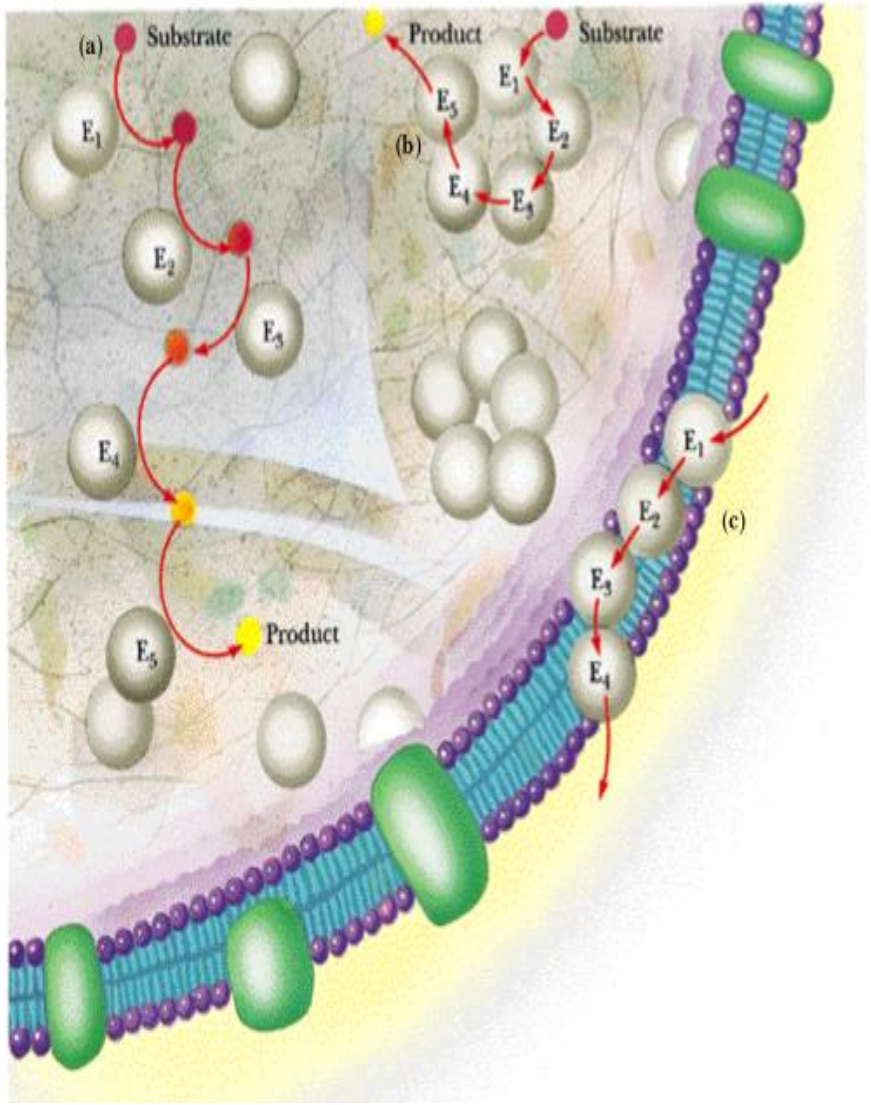


Principal characteristics of metabolic pathways

1. Metabolic pathways are irreversible.
2. Catabolic and anabolic pathways must differ.
3. Every metabolic pathway has a first committed step.
4. All metabolic pathways are regulated.
5. Metabolic pathway in eukaryotic cells occur in specific subcellular compartments.

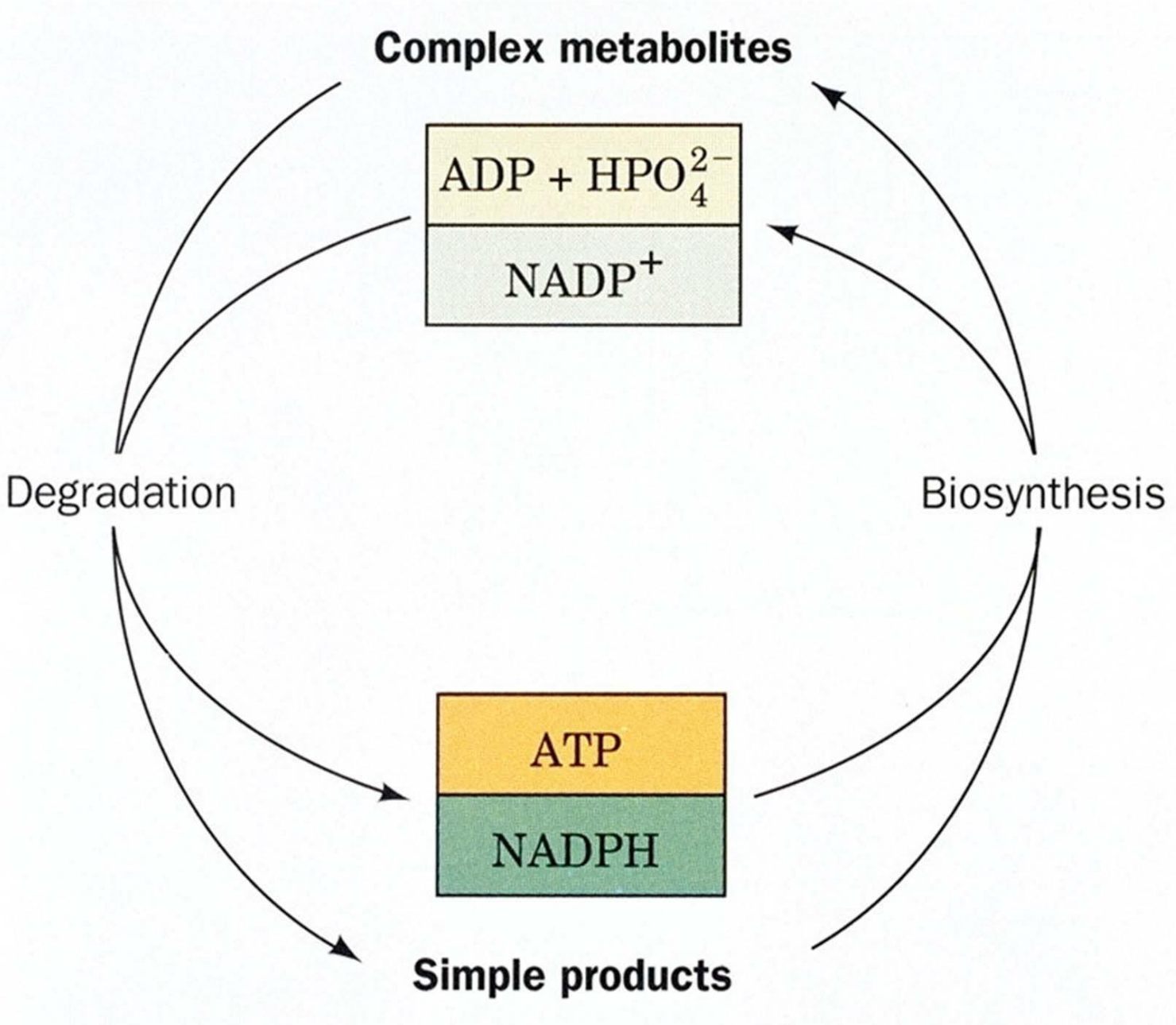
Organization of Metabolic Pathways

- Pathways consist of sequential steps.
- The enzymes may be separate.
- May form a multienzyme complex.
- May be a membrane-bound system.
- New research indicates that multienzyme complexes are more common than once thought.



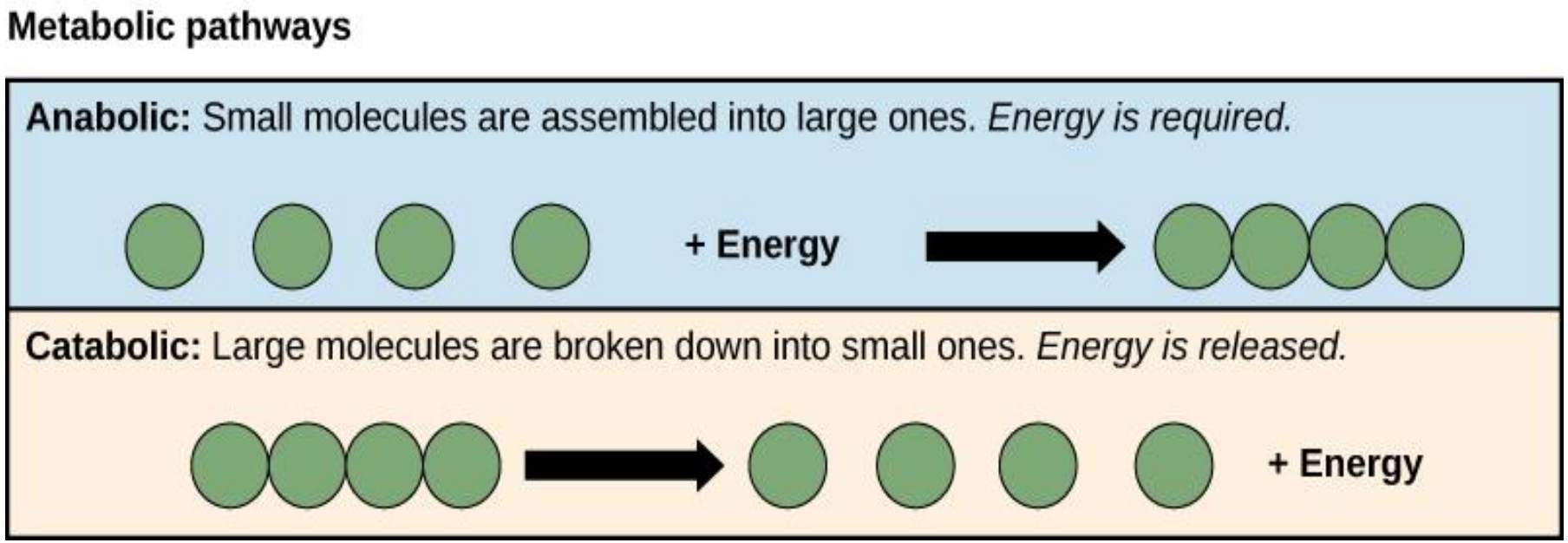
Metabolic principle:

Degradation is coupled to the formation of ATP (energy store) and NADPH (reduction equivalents), that represent sources of free energy for biosynthetic reactions.



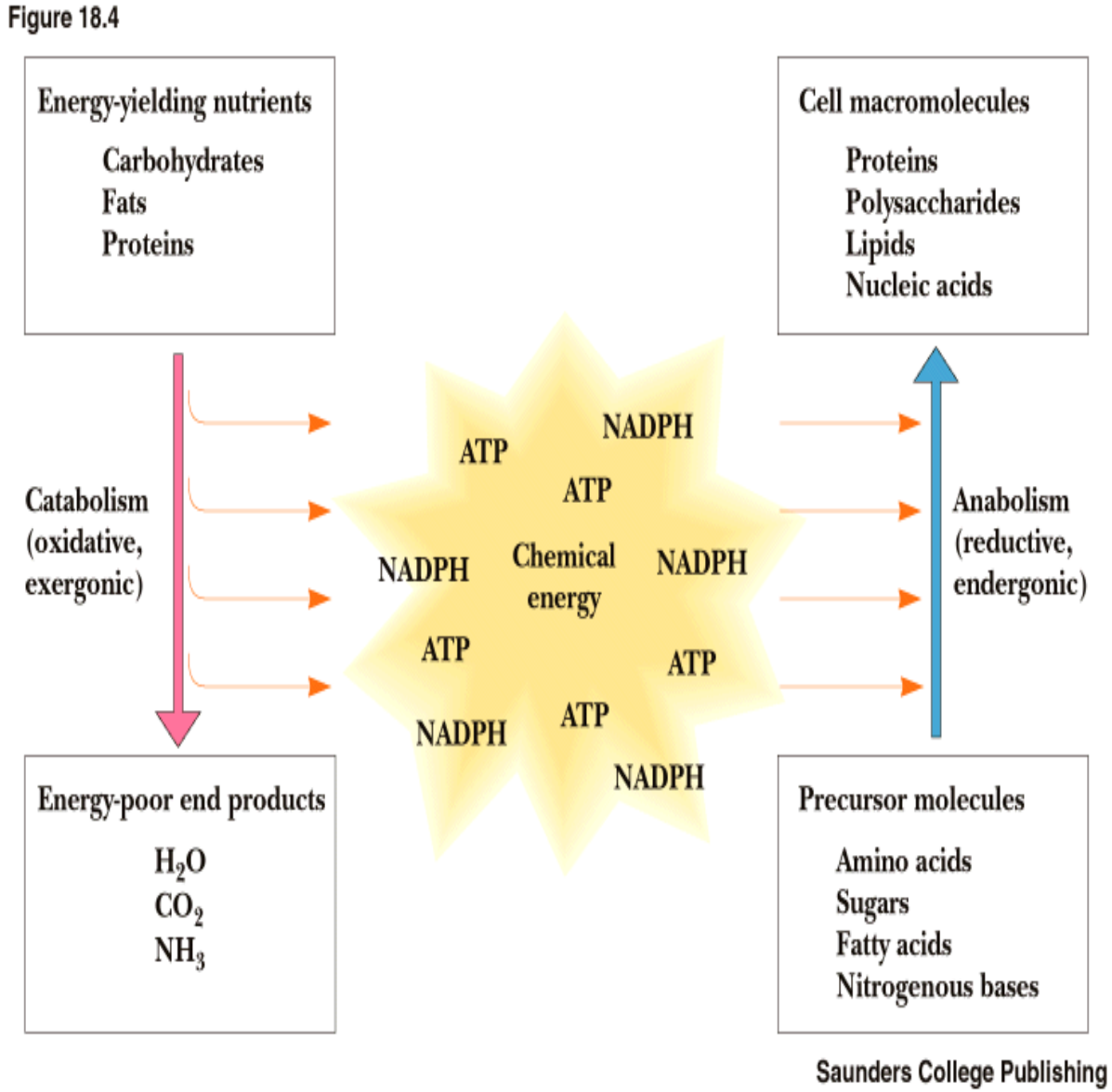
Types Of Metabolic Pathway

- **Catabolic/Degradative /Energy Generating/ATP producing Pathways/Exothermic.**
- **Anabolic/Synthetic/Energy Utilizing/ ATP Using Pathways/Endothermic.**



Types Of Metabolic Pathway

- Catabolic pathways involve oxidative reactions producing reducing equivalents- $NADH+H^+$ and $FADH_2$.
- Catabolic pathways converge to few end products.
- Anabolic pathways diverge to synthesize many biomolecules.
- Some pathways serve both in catabolism and anabolism ,those are Amphibolic pathways.

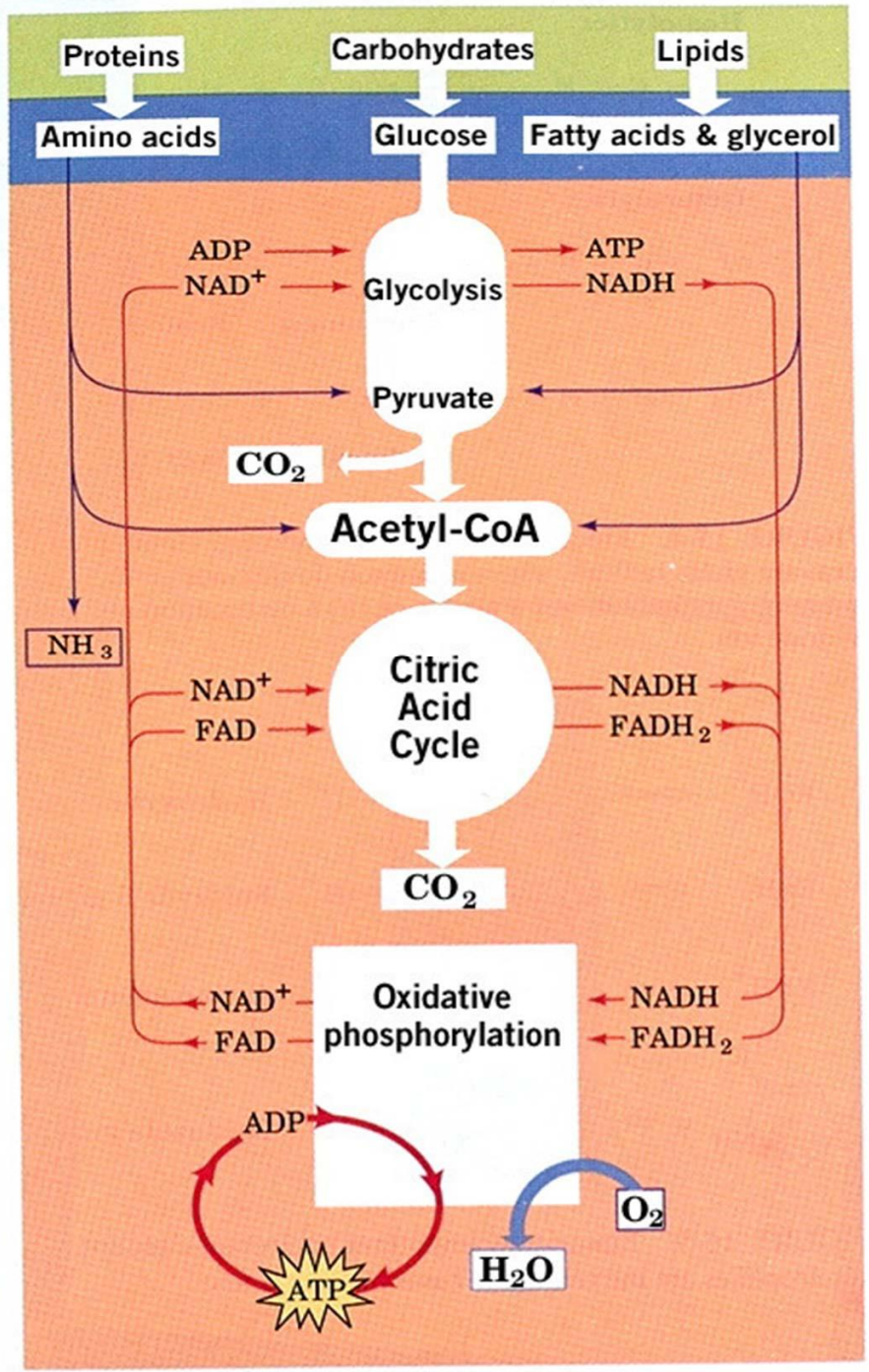


Overview of catabolism

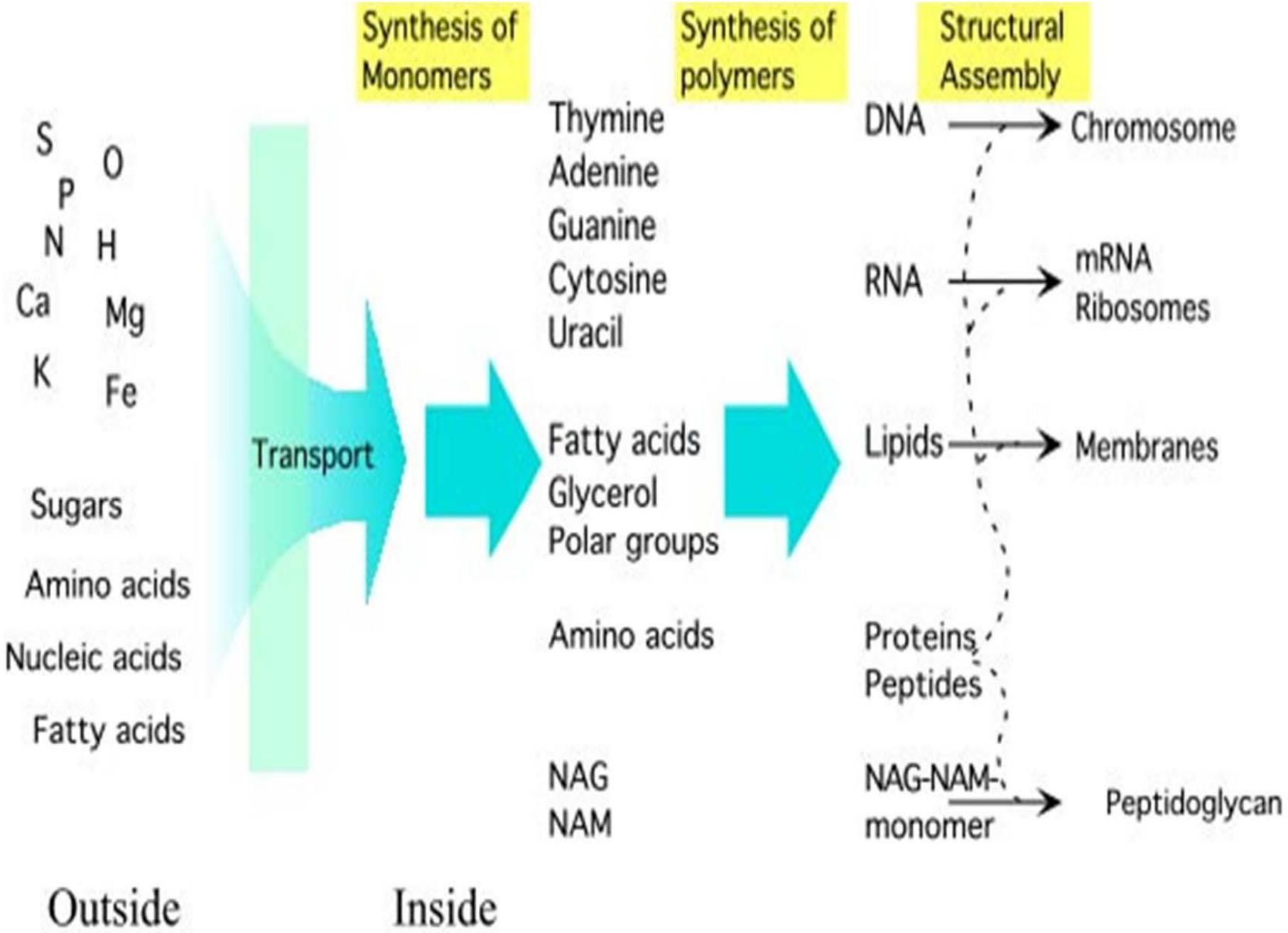
Stage 1: Proteins, polysaccharides and lipids are broken down into their component building blocks.

Stage 2: The building blocks are degraded into the common product, generally the acetyl groups of acetyl-CoA.

Stage 3: Catabolism converges to three principal end products:
water, carbon dioxide, and ammonia.



Anabolism


Synthesis of Monomers
Synthesis of polymers
Structural Assembly
S
O
P
N
H
Ca
Mg
K
Fe
Sugars
Amino acids
Nucleic acids
Fatty acids
Transport
Thymine
Adenine
Guanine
Cytosine
Uracil
Fatty acids
Glycerol
Polar groups
Amino acids
NAG
NAM
DNA
Chromosome
RNA
mRNA
Ribosomes
Lipids
Membranes
Proteins
Peptides
NAG-NAM-
monomer
Peptidoglycan
Outside
Inside

Intrinsic regulation Vs Extrinsic control

Intrinsic regulation

For intrinsic regulation of metabolic pathways the reactions self-regulate to respond to changes in the levels of substrates or products. For example, a decrease in the amount of product can increase the metabolic pathway. This is called a feedback mechanism.

Extrinsic control

- Extrinsic control involves a cell in a multicellular organism changing its metabolism in response to signals from other cells.
- The signals approach the pathways via soluble messengers such as hormones and growth factors. These act by being detected by specific receptors on the cell surface.
- These signals are then transmitted inside the cell by second messenger systems that often involved the phosphorylation of proteins.

For example, the hormone insulin from the beta cells of the pancreas is produced in response to rises in blood glucose levels. Binding of the hormone to insulin receptors on cells then activates a cascade of protein kinases that cause the cells to take up glucose and convert it into storage molecules such as fatty acids and glycogen.

Mode of Metabolic Regulation

Metabolism is regulated by controlling -

- **Catalytic activity of enzymes:-** allosteric and cooperative effects, reversible covalent modification, substrate concentration
- **The amount of enzymes:-** synthesis of adaptable enzymes
- **The accessibility of substrates:-** compartmentalization segregates biosynthetic and degradative pathways, the flux of substrates depends on controlled transfer from one compartment of a cell to another
- **The energy status of the cell:-** of which the energy charge or the phosphorylation potential are used as indexes
- **Communication between cells:-** hormones, neurotransmitters, and other extracellular molecular signals often regulate the reversible modification of key enzymes

Mechanism of Regulation

The metabolic pathways, in general, are controlled by four different mechanisms:

1. **The availability of substrates**
2. **Covalent modification of enzymes**
3. **Allosteric regulation**
4. **Regulation of enzyme synthesis.**

Regulation By substrates, products and cofactor

(1) Intracellular concentrations of its substrates, products and cofactor.

(a) Substrate availability – Any metabolic pathway could in theory at least, be regulated very simply by the availability of substrate. A reduction insubstrate conc. will decrease the activity of the enzyme (provided it is not saturated with substrate) and this could result in a decreased flux through the pathway. Similarly, an increase in (S) could stimulate the pathway.

(b) Cofactor availability – Some what similar to control by substrate availability. However, substantial inhibition of enzyme activity (and therefore the rate of the metabolic pathway) could be achieved only if the conc. of the cofactor was reduced to very low levels.

e.g. regulation of e-transport and oxidative phosphonylation in the mitochondria by adenine nucleotides.

Regulation By substrates, products and cofactor

(c) Product removal – If substrate is converted to the product by a series of reactions, the removal of the product could control the rate of its formation from the substrate. Minor pathways or perhaps specific portions of metabolic pathways may be controlled by such a mechanism.

A typical example is the conversion of pyruvate to lactate in muscle catalysed by lactate DH and the movement of lactate from the muscle to the blood. An increased blood flow through the muscle will increase the rate of lactate removal from the muscle which could therefore increase the rate of conversion of pyruvate to lactate.

Regulation By regulatory enzymes

(2) The second level of control of metabolic pathways is through the action of regulatory enzymes. There are 2 major types of regulatory enzymes:

(a) Allosteric enzymes: These are enzymes whose catalytic activity is modulated through the non-covalent binding of a specific metabolite at a site on the protein other than the catalytic site;

(b) Covalently modulated enzymes: These are enzymes that are inter- converted between active and inactive forms by the action of other enzymes. They also respond to non-covalent allosteric modulators.

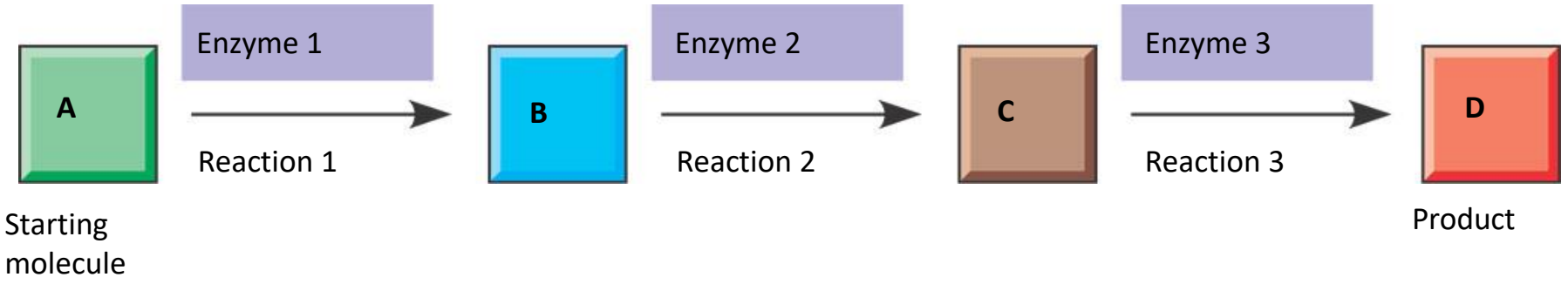
The 2 types of regulatory enzymes respond to alterations in the metabolic state of a cell or tissue on a relatively short time scale –allosteric enzymes within seconds and covalently regulated enzymes within minutes.

Allosteric enzymes

Allosteric regulation acts to modulate enzymes situated at key steps in metabolic pathways.

In metabolic pathways, the end product of the reaction sequence may inhibit an enzyme at or near the beginning of the sequence; such that the rate of the entire pathway is determined by the steady-state concentration of the end-product.

Consider the reaction sequence:



Allosteric enzymes

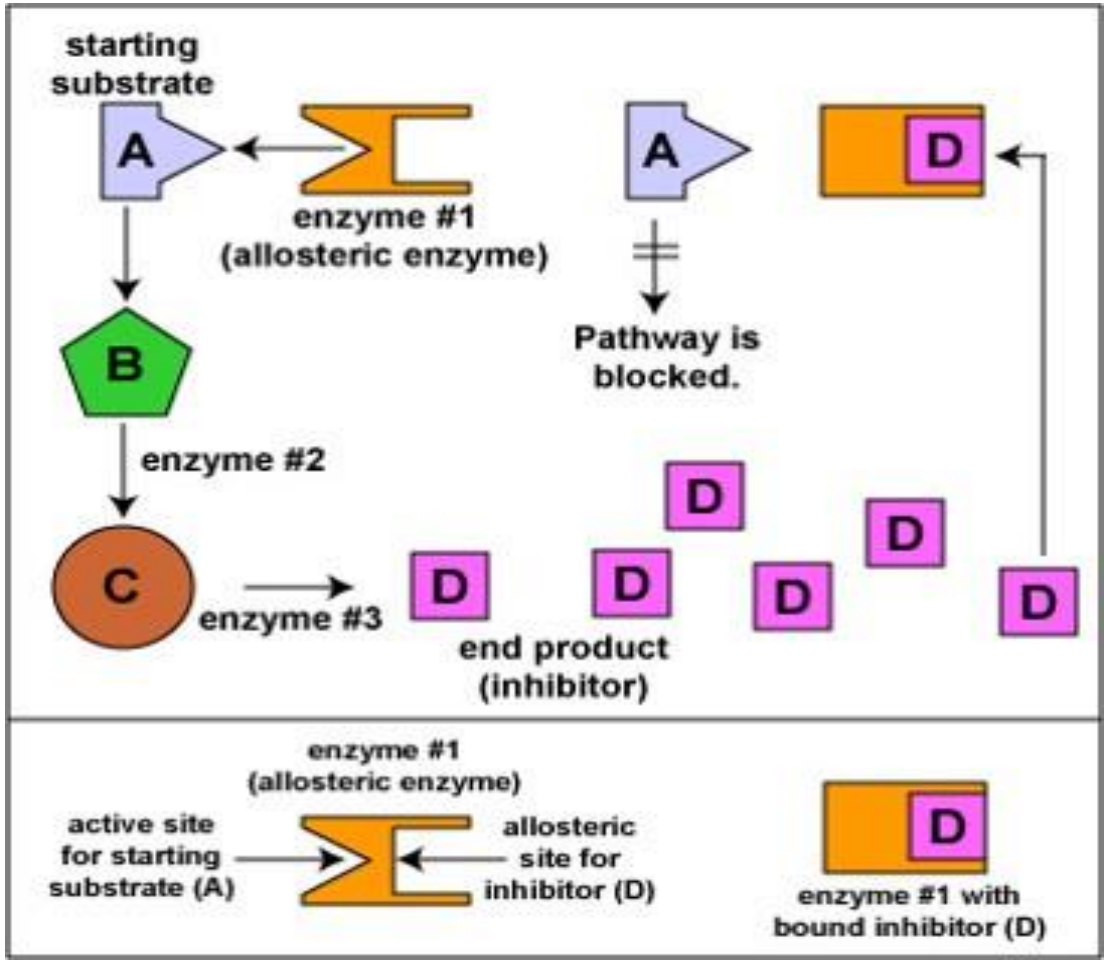
In this scheme, D represents an essential metabolite (lipid, protein, nucleotide).

Here, D, the end-product inhibits the 1st step in the reaction sequence catalysed by E1. Therefore, when sufficient D is synthesized, it blocks further synthesis of itself.

This phenomenon whereby product of a reaction sequence inhibits the activity of an enzyme early in the biosynthetic pathway is referred to as feedback inhibition or feedback regulation or end-product inhibition.

The 1st enzyme in this sequence that is inhibited by the end product is called an Allosteric enzyme.

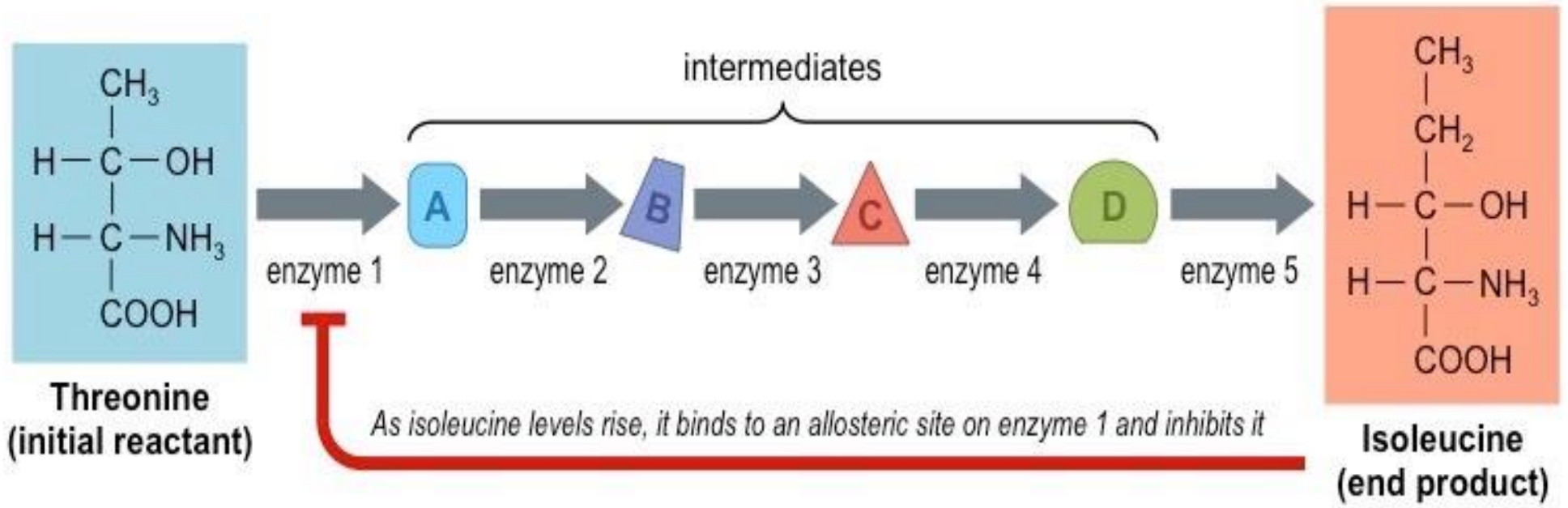
The reaction catalysed by the allosteric enzyme is usually irreversible under intra-cellular conditions. It is often called the committing reaction or the rate-limiting step; once it occurs all the ensuing reactions of the sequence will take place.



Allosteric enzymes

Commonly found in regulation of biosynthesis of amino acids and purines in microorganisms.

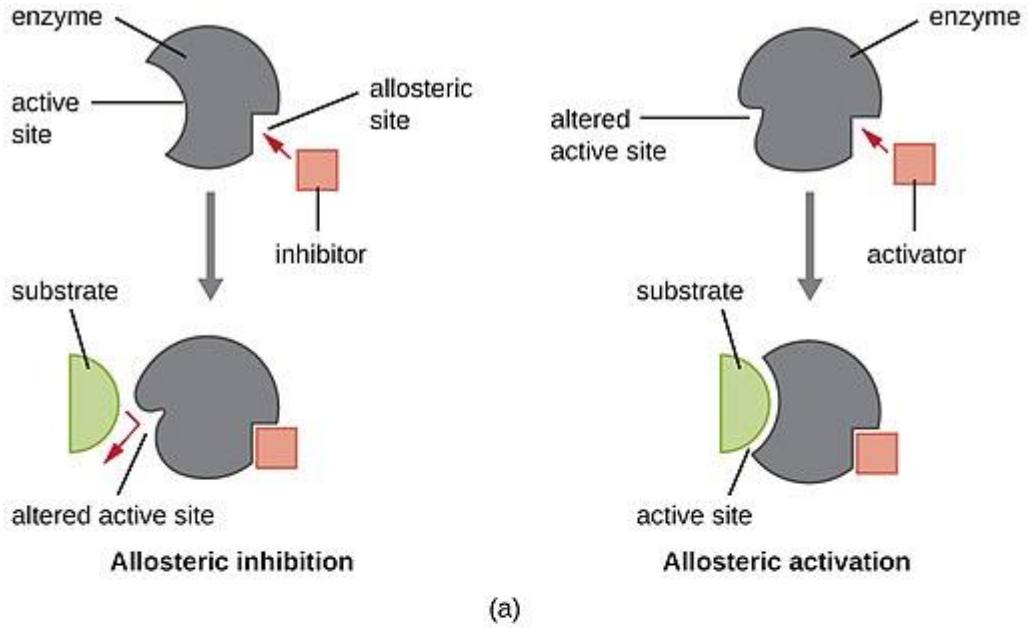
In the synthesis of L-isoleucune from L-threonine, ile inhibits the 1st enzyme in the pathway, threonine deaminase

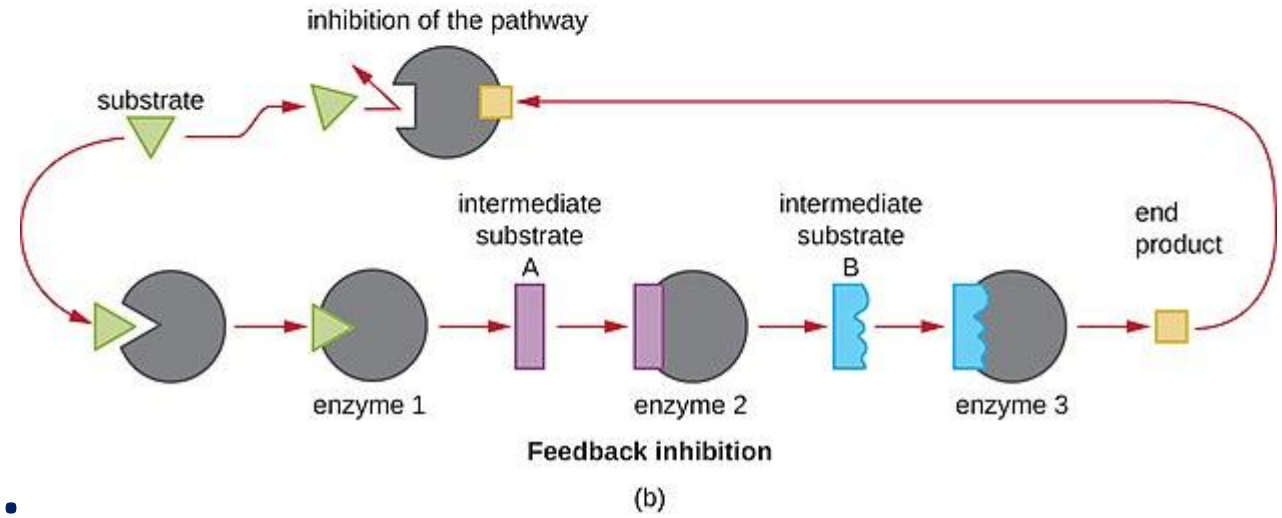


Properties of Allosteric Enzymes

(1) An allosteric enzyme possesses at least 2 spatially distinct binding sites on the protein molecules the active or the catalytic site and the regulator or the allosteric site.

The metabolic regulator molecule binds at the allosteric site and produces a change in the conformational structure of the enzyme, so that the geometrical relationship of the amino acid residues in the catalytic site is modified. Consequently, the enzyme activity either increases (activation) or decreases (inhibition).





Properties of Allosteric Enzymes

(2) Allosteric enzymes show 2 different types of control – heterotropic and homotrophic depending on the nature of the modulating molecule. Heterotrophic enzymes are stimulated or inhibited by an effector or modulator molecule other than their substrates, e.g. threonine deaminasethe substrate is threonine and the modulator is isoleucine.

In homotropic enzymes, the substrate also functions as the modulator. Homotropic enzymes contain 2 or more binding sites for the substrate modulation depends on how many of the substrate sites are bound.

(3) Their Kinetics do not obey the Michaelis – Mention equation.

Properties of Allosteric Enzymes

(4) Inhibition of a regulatory enzyme does not conform to any normal inhibition pattern and the inhibitor does not bear any obvious structural relationship to the substrate.

(5) Allosteric enzymes have an oligomeric organization. They are composed of more than one polypeptide chain and have more than one S-binding site per enzyme molecule.

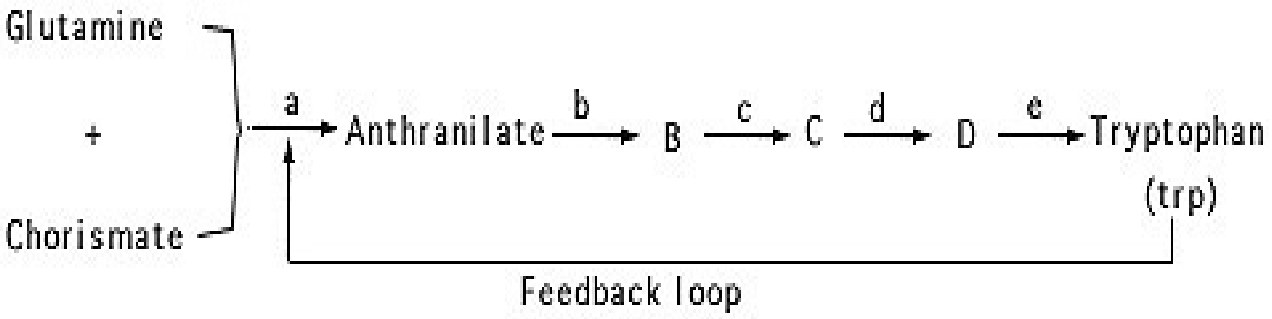
(6) Treatment of the allosteric enzyme with agents or conditions that exert a mild denaturing effect can result in loss of sensitivity to the effects of the regulatory molecule without changing the catalytic activity.

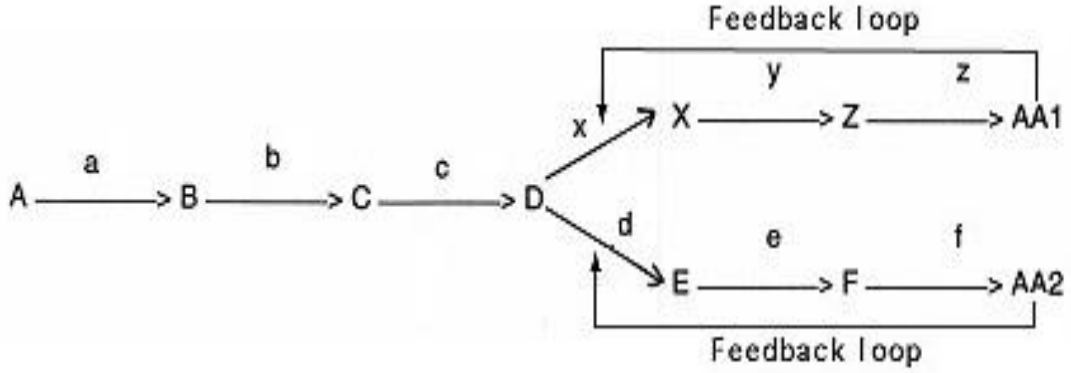
Pattern of Allosteric Regulation

Regulated in two major patterns-linear and branched patterns.

In linear pathways, the end-product usually inhibits the 1st enzyme in the sequence. Sometimes, the 1st substrate or the precursor may act as a +ve stimulator and stimulate the 1st reaction.

In branched pathways the metabolite at the branch point is often the feed-back inhibitor of the 1st enzyme, whereas the 2 end-products of the branches (P1 & P2) often act as feedback, inhibitors of the 1st enzyme after the branch point.

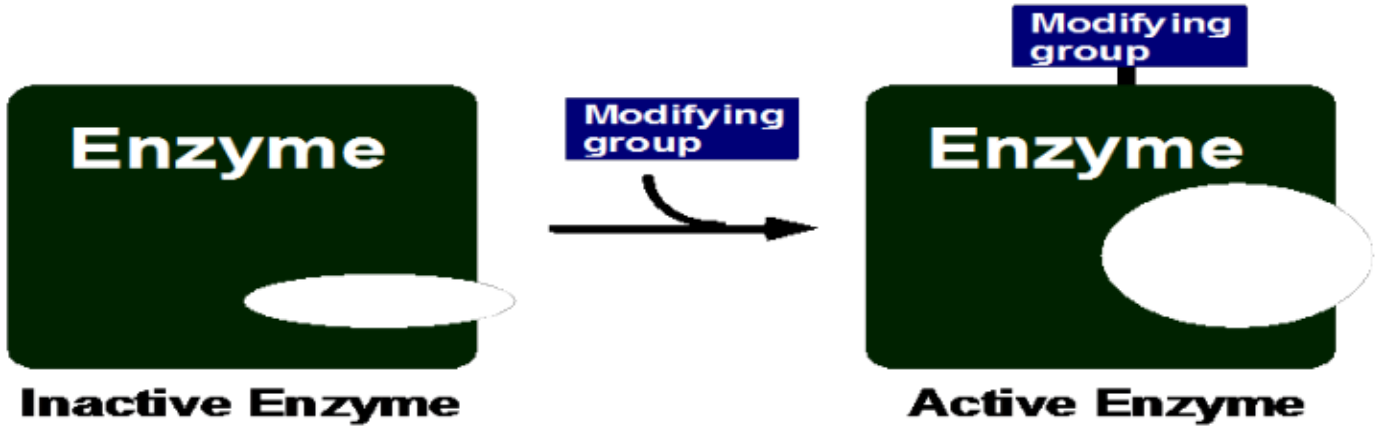




Covalently modulated enzymes

- This is a group of regulatory enzymes that are inter-converted between active and inactive forms by other enzymes by covalent modification of specific amino acid residues on the enzyme surface.
- Activity is modulated by covalent modification of one or more of its amino acid residues in the enzyme molecule.
- It means modification of enzyme activity through formation of covalent bonds with some specific group:

- Phosphorylation (addition of phosphate group at the hydroxyl group of serine, threonine or tyrosine)
- Methylation (addition of methyl group).
- Hydroxylation (addition of hydroxyl group).
- Adenylation (addition of adenylicacid) etc.



Covalently modulated enzymes

- 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

- **Phosphorylation is the most common kind of covalent modification in the enzymes. After covalent modification enzyme get either active or inactive depends on modification.**
- **Phosphorylation of enzyme occurs by addition of phosphate group to the enzyme at the hydroxyl group of serine, threonine or tyrosine.**

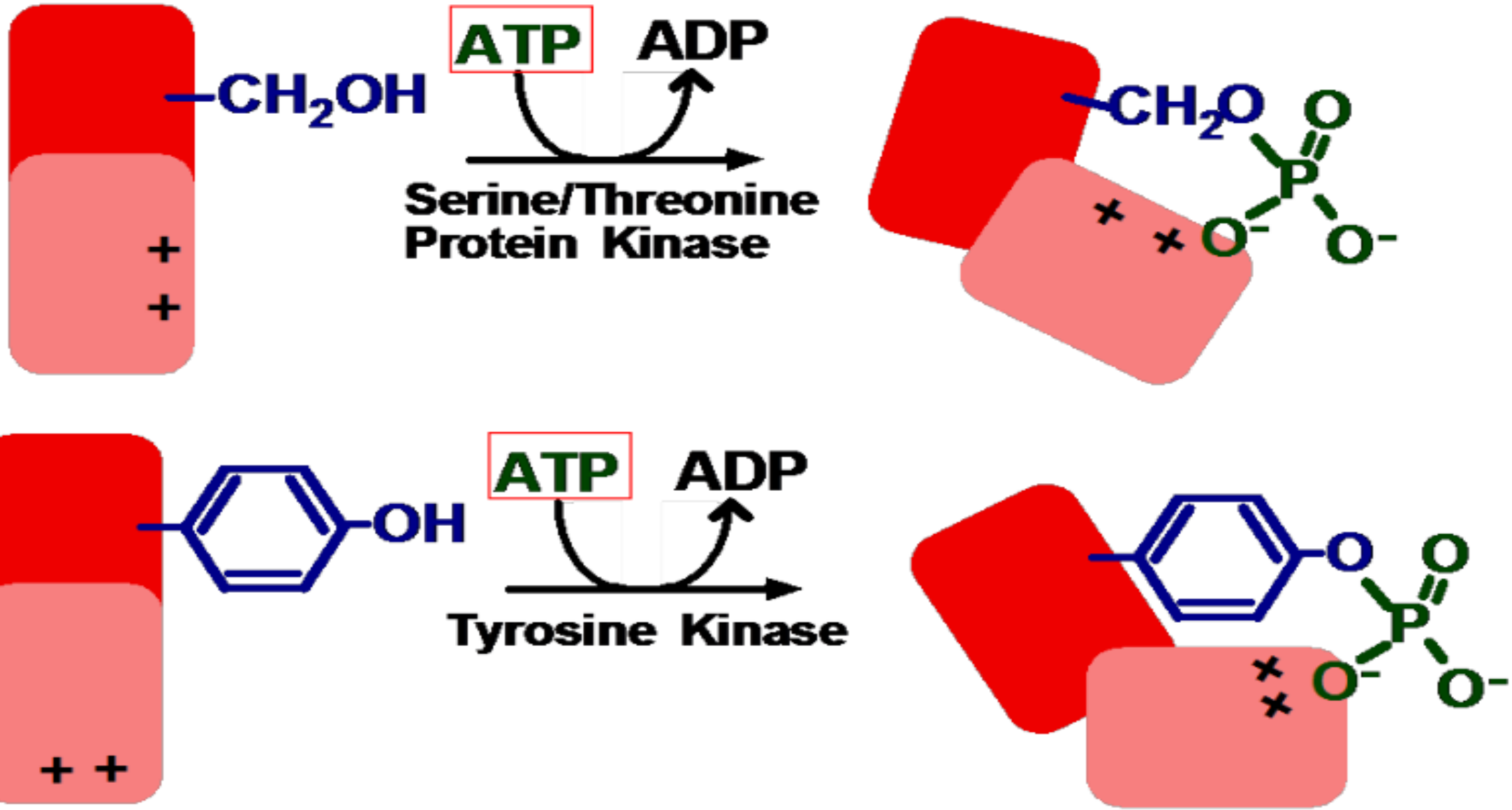
➢ **Examples of enzymes inactivated by phosphorylation:**

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

➢ **Examples of enzymes activated by phosphorylation:**

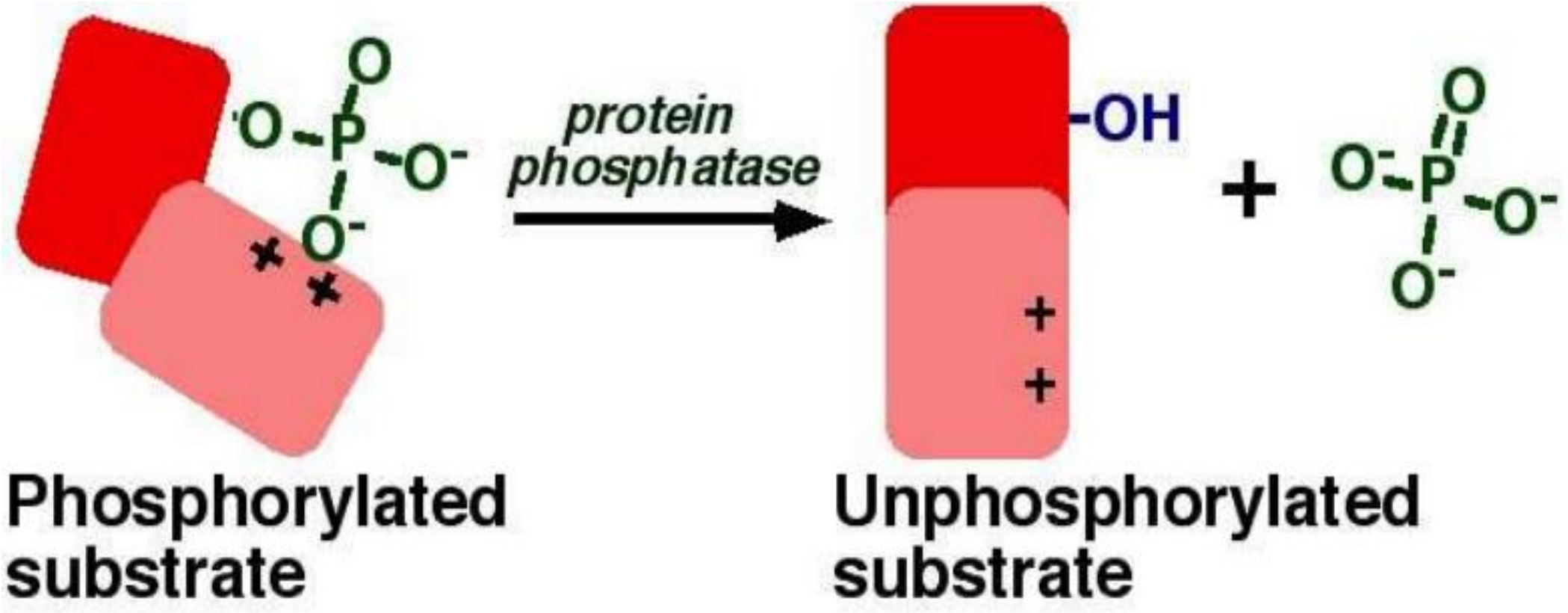
- **Glycogen phosphorylase that breaks down glycogen into glucose.**
- **Citrate lyase, which breaks down citrate.**
- **Lipase that hydrolyzes triglyceride into glycerol and 3 fatty acids.**

Protein kinases catalyze the phosphorylation of proteins



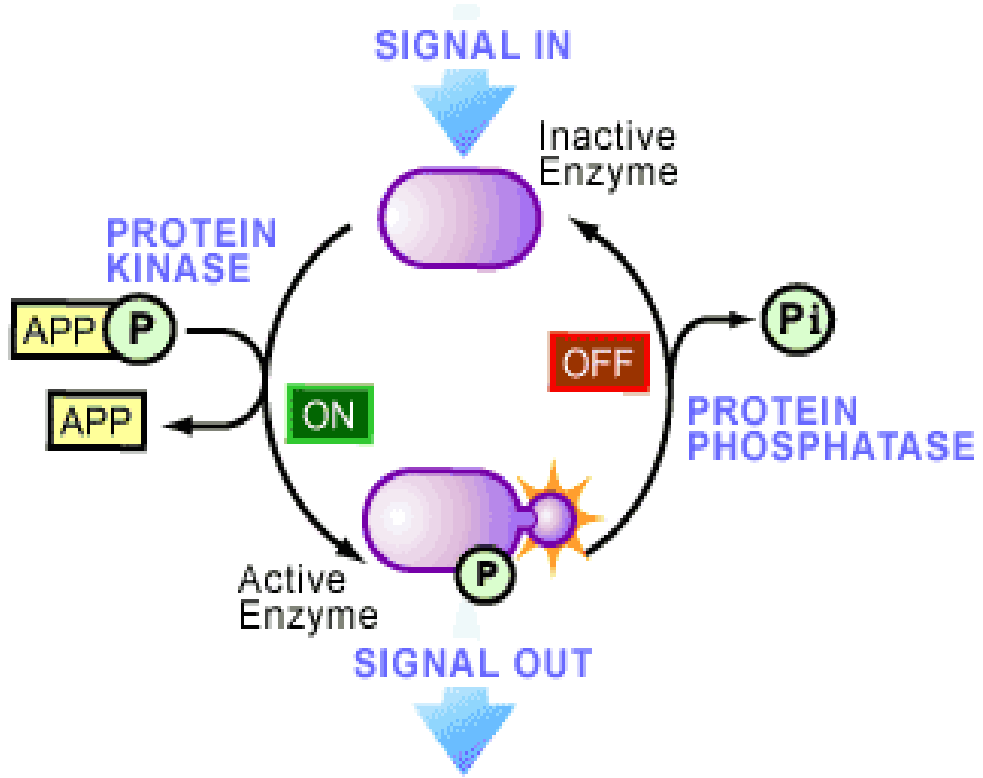
Dephosphorylation of the enzyme by Phosphatase

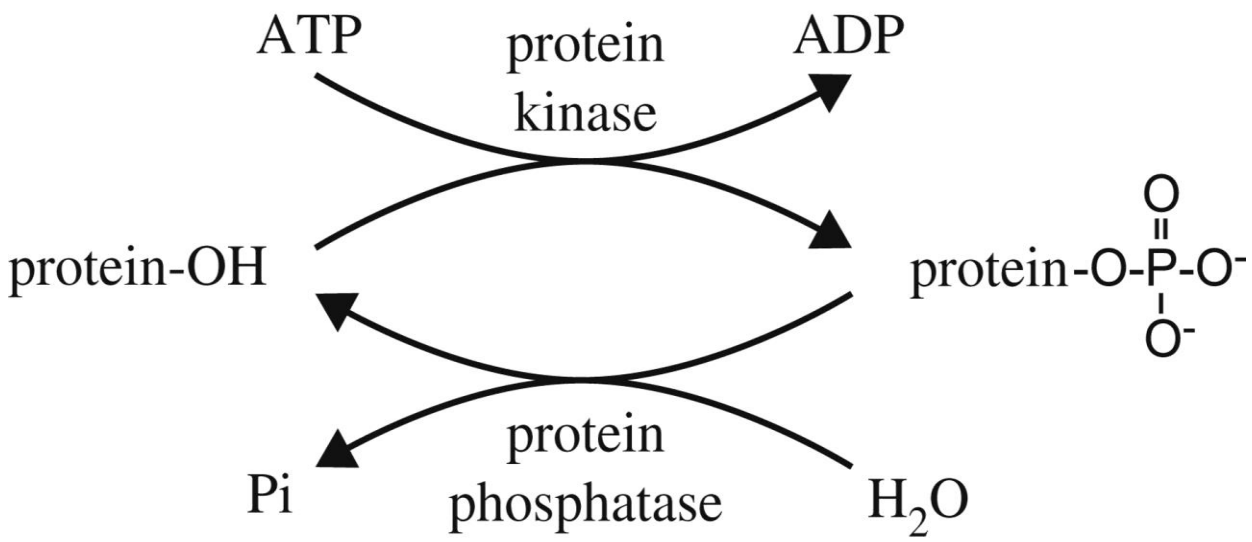
- Dephosphorylation of the enzyme occurs by removal of phosphate group from the hydroxyl group of serine, threonine or tyrosine.
- This occurs by phosphatase enzyme.



Phosphorylation and dephosphorylation

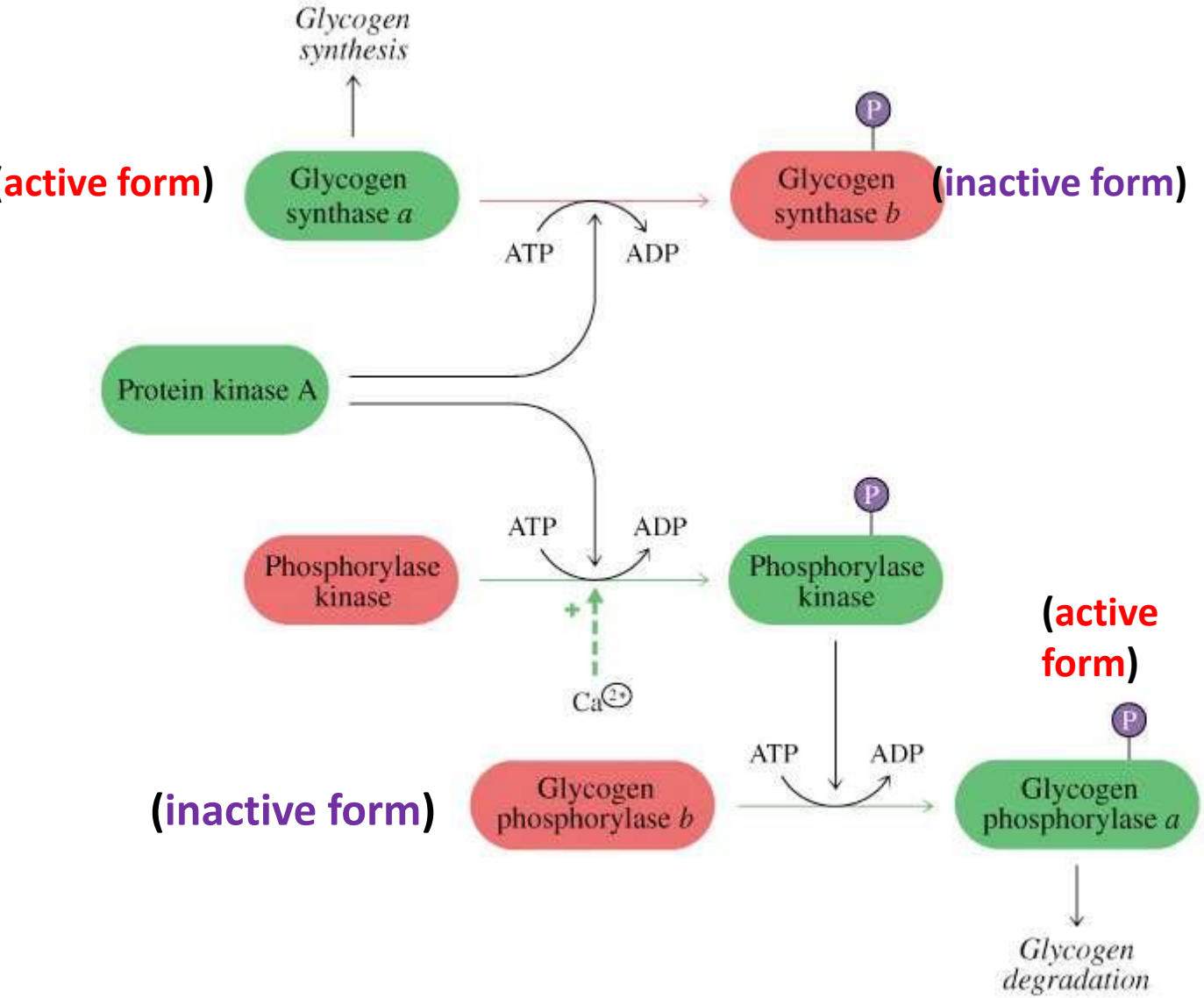
- Phosphorylation and dephosphorylation are not the reverse of one another.
- The rate of cycling between the phosphorylated and the dephosphorylated states depends on the relative activities of kinases and phosphatases.





Phosphorylation and dephosphorylation

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



Other ways of Regulating Metabolic pathways

(1) Covalent activation of zymogens:

- **Most proteins become fully active when their synthesis is completed and they spontaneously fold into their nature, 3-dimensional conformation/structures. Some proteins, however, are synthesized and secreted as in active precursors known as Pro-proteins.**
- **Unlike allosteric regulation or covalent modification, zymogen activation is an irreversible process.**

Other ways of Regulating Metabolic pathways

Characteristics of zymogens:

- **Zymogens are inactive precursors of enzymes, some enzymes secreted in this form, for example pepsinogen, trypsinogen, chymotrypsinogen, prothrombin,clotting factors and Insulin an important metabolic regulator.**
- **Zymogens or proenzymes acquire full activity only upon specific proteolytic cleavage of one or several of their peptide bond and it'sactivation is irreversibleprocess .**
- **Zymogen is inactive because it contains an additional polypeptide chain that masks (blocks) the active site of the enzyme.**

Other ways of Regulating Metabolic pathways

Examples:

Activation of zymogens can occur by one of the following methods:

- **Activation by HCl**

Pepsinogen ⟶ Pepsin

- **Activation by other enzymes:**

Enterokinase

Trypsinogen ⟶ Trypsin

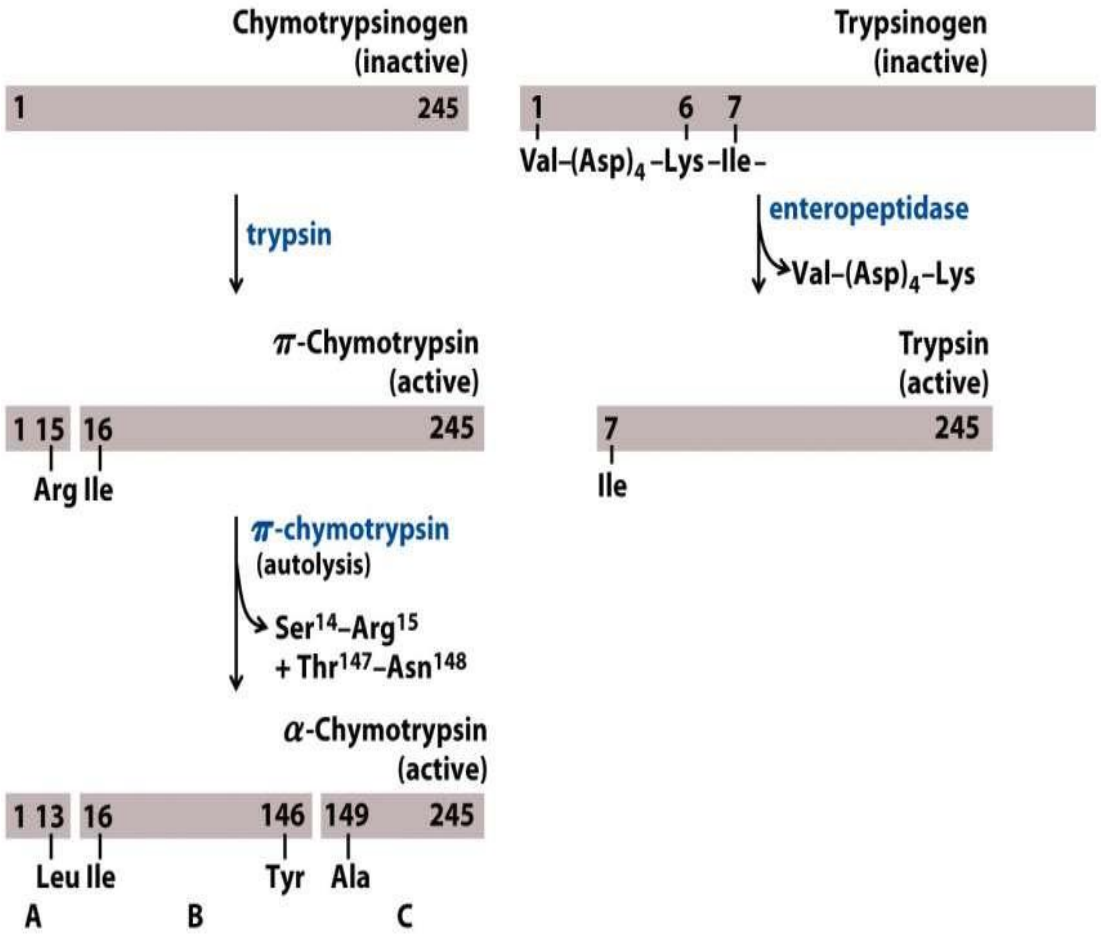
Thrombokinase + Ca++

Prothrombin ⟶ Thrombin

- **Auto activation i.e. the enzyme activates itself.**

Pepsin

Pepsinogen ⟶ Pepsin



Other ways of Regulating Metabolic pathways

2. Isozymes

Isozymes are multiple forms of the same enzyme that occur in a single species of organism or even in a single cell.

A classic, e.g. is mammalian lactate DH, which exists as 5 different isozymes in the tissues of rat and other vertebrates. They all catalyse the same overall reaction.

Lactate + NAD + Pyruvate + NADH + H^+

(1) All 5 isozymes have the same mut, about 134,000

(2) All contain 4 polypeptide chains eachof mut 33,500

(3) The 5 isozymes consist of 5 different combinations of 2 different kinds of polypeptide chains designated A and B. The isozyme predominating in skeletal muscle has 4 identical A chains and is designated A_4. Another which predominates in heart has 4 identical B chains and is designated B_4. The other 3 isozomes have the composition A_3B,A_2B_2 and AB_3.

Isozymes of lactate DH

- Although all catalyse the same reaction, they differ in their dependence on substrate conc., particularly pyruvate, as well as their Vmax values when pyruvate is the substrate.
- The isozyme A4, characteristic of skeletal muscle and embryonic tissues, reduces pyruvate to lactate at a relatively high rate.
- The B4 isozyme on the other hand, characteristic of the heart and other red muscles, reduces pyruvate at a relatively low rate.

Isozymes of lactate DH

- If compare these kinetic characteristics with the metabolic features of the tissues in which the A4 and B4 isozymes predominate, the function of LDH isozymes becomes clear.
- Skeletal muscle and embryonic tissue have anaerobic metabolism, thus can convert glucose to lactate via glycolysis. A4 isozyme is thus adapted for this role and has a high Vmax for pyruvate.
- The heart muscle on the other hand has aerobic metabolism and does not form lactate from glucose. Rather, it oxidizes pyruvate to C02 without intermediate formation of lactate.
- Heart muscle cells are rich in mitochondria whereas most skeletal muscles contain relatively few mitochondrin

Creatine kinase (CK)

- It is an enzyme that catalyzes phosphorylation of creatine.

Creatine Kinase

Creatine ----------→ Creatine phosphate

ATP ADP

- CK enzyme is a dimmer formed of 2 protein subunits (protomers), B (after brain) and M (after muscle).
- CK has 3 isoenzymes:

- CK BB which increases in brain tumors.
- CK MB which increases in heart diseases.
- CK MM which increases in skeletal muscle disease

Source of isoenzymes

- **Isoenzymes may be produced by the same gene but the subunits undergo different post-translation modifications in different organs.**
- **Isoenzymes may be produced by more than one gene; each gene produces one subunit.**