CDL Advances Chemical Studies (ACS)

Lecture

Metabolic Biochemistry 7 CFU = 6+1

Laboratory

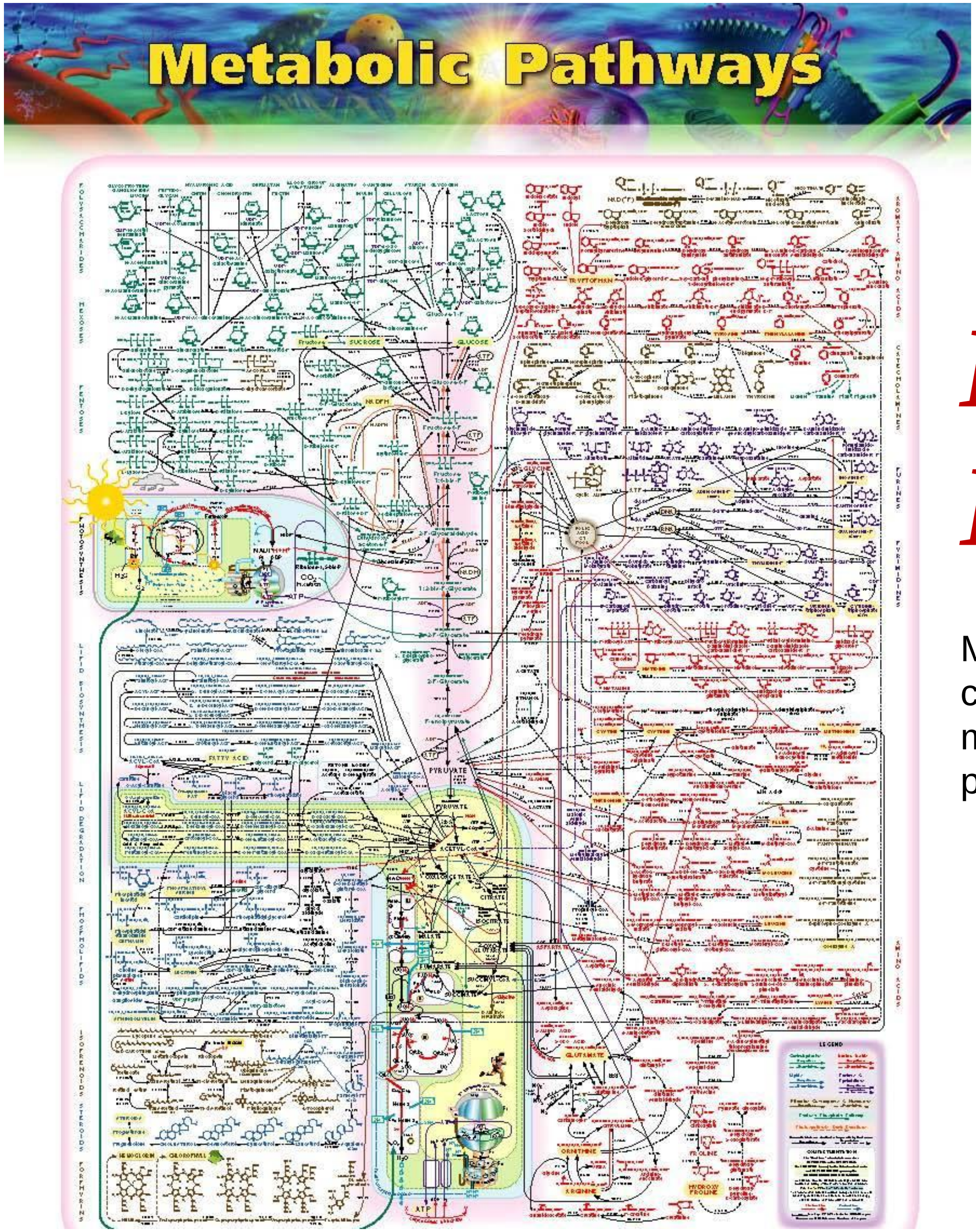
Prof.ssa Alessandra Olianas
Prof.ssa Francesca Pintus

Prof.ssa Alessandra Olianas

Department of Life and Enviromental Science
Biomedical Section (Biochemistry Laboratory)

Dip. Scienze della Vita e dell’Ambiente
Sezione Biomedica (laboratorio di Biochimica)
Tel. 0706754507
Students reception: by appointment

olianas@unica.it



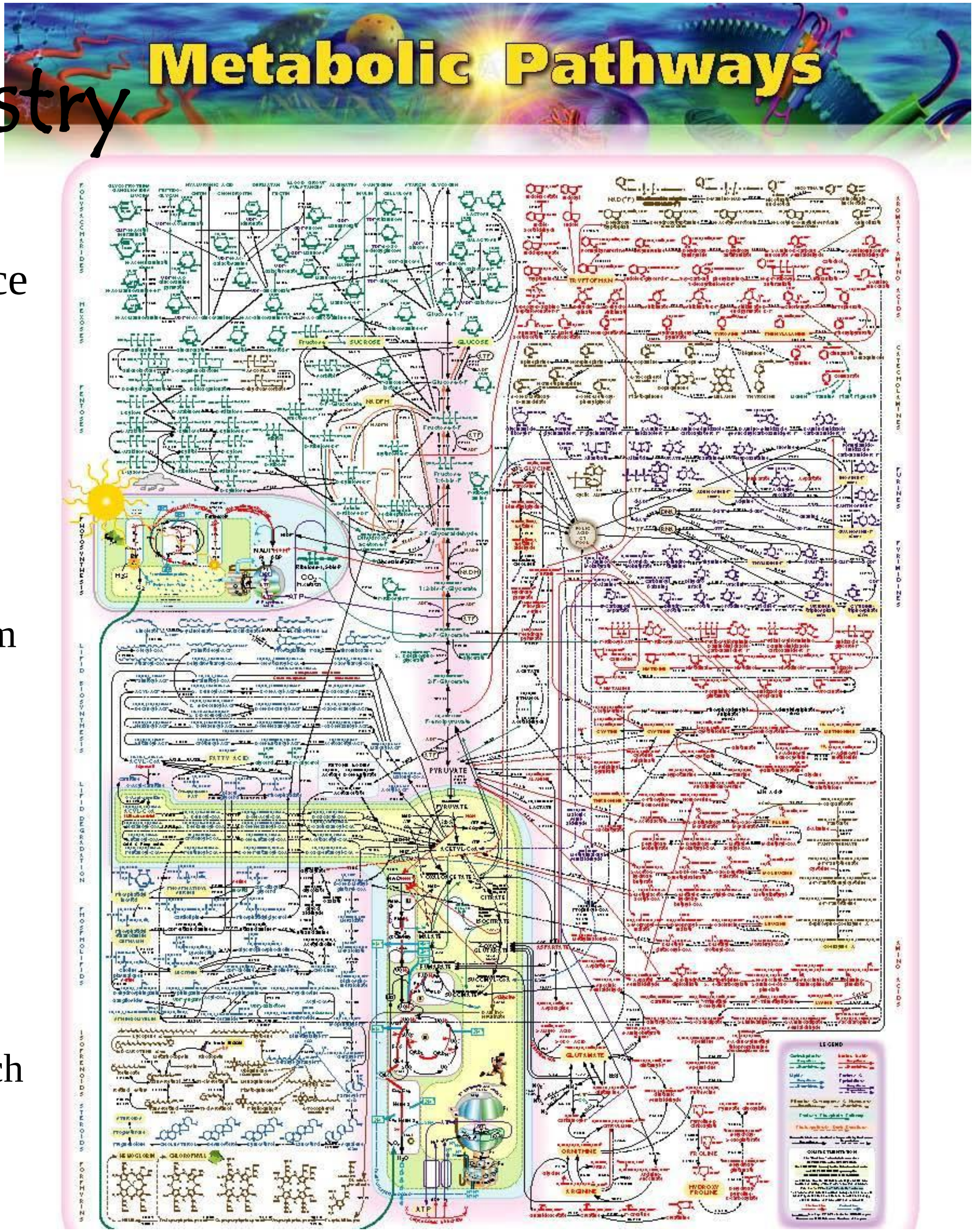
Metabolic Biochemistry

Metabolism is a highly coordinated cellular activity in which many multienzyme systems (metabolic pathways)

Metabolic Biochemistry

Set of biochemical reactions that take place in an organism, which give rise to the processes of synthesis or demolition of organic constituents

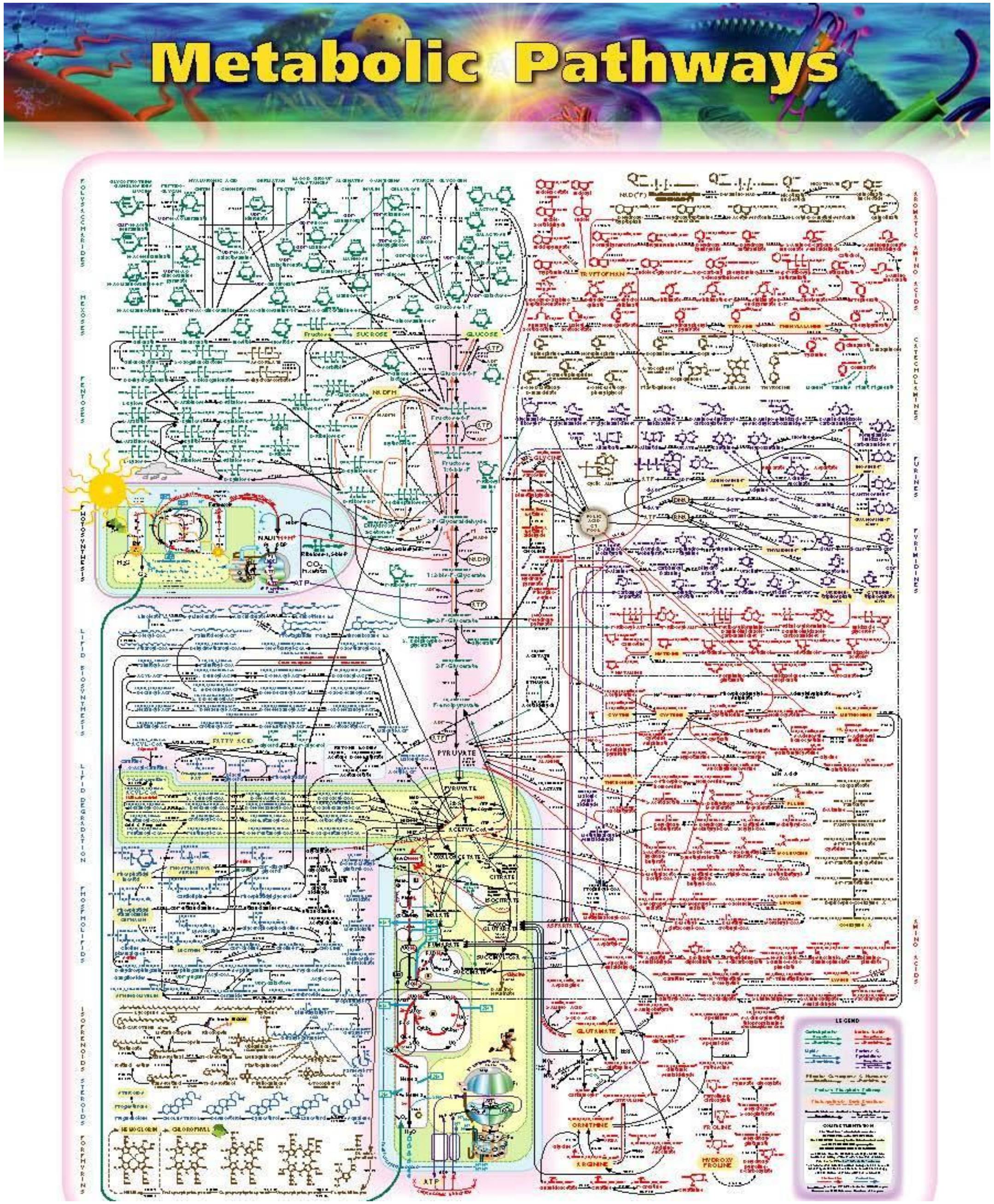
(1) obtain chemical energy by capturing solar energy or degrading energy-rich nutrients from the environment;
(2) convert nutrient molecules into the cell’s own characteristic molecules, including precursors of macromolecules;
(3) polymerize monomeric precursors into macromolecules: proteins, nucleic acids, and polysaccharides;
(4) synthesize and degrade biomolecules required for specialized cellular functions, such as membrane lipids, intracellularmessengers, and pigments.



The most complex chemical transformations do not occur in a single reaction but through several reactions.

Called

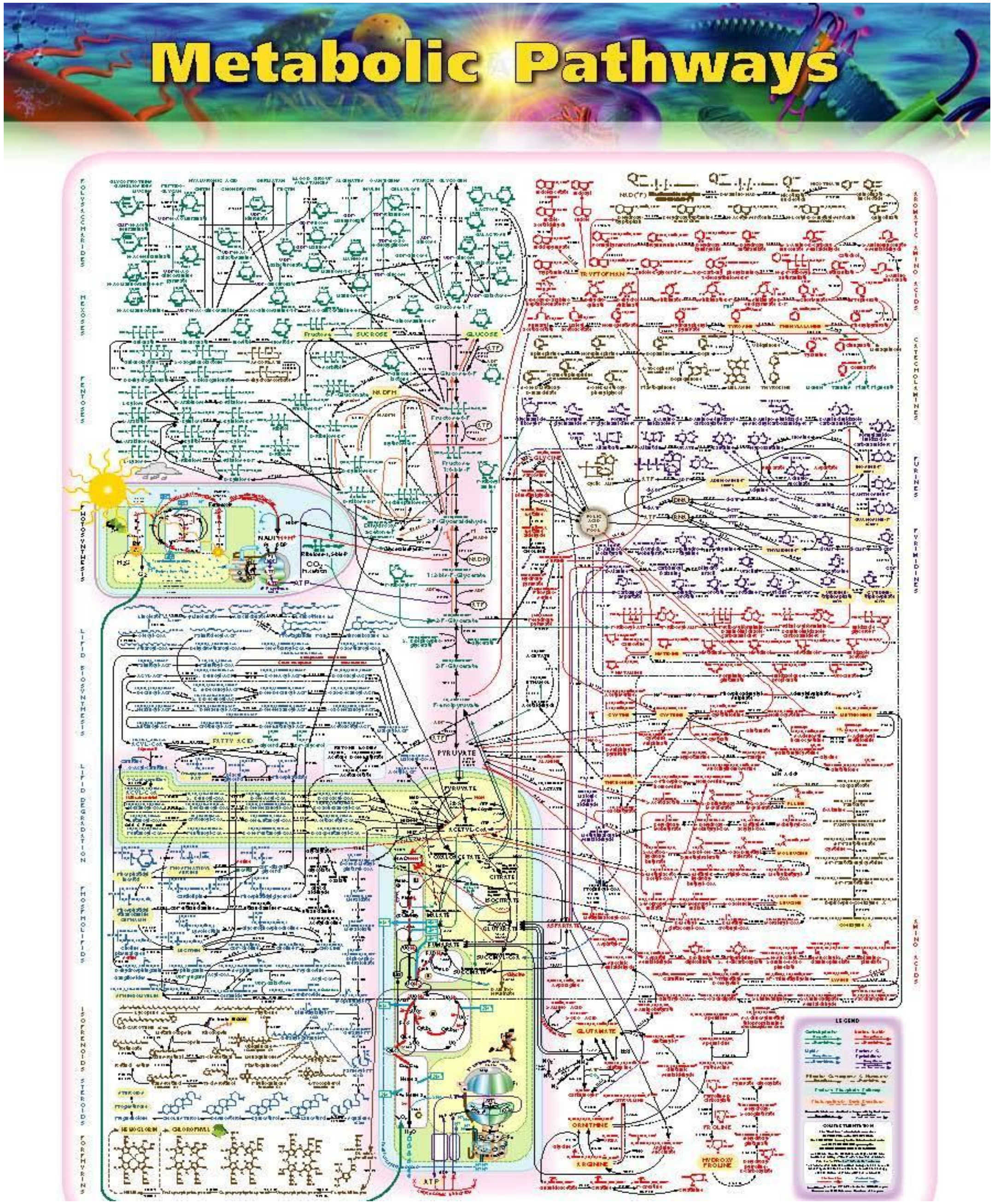
“Metabolic Pathway”



Metabolic Biochemistry

Each reaction is catalized by specific enzyme

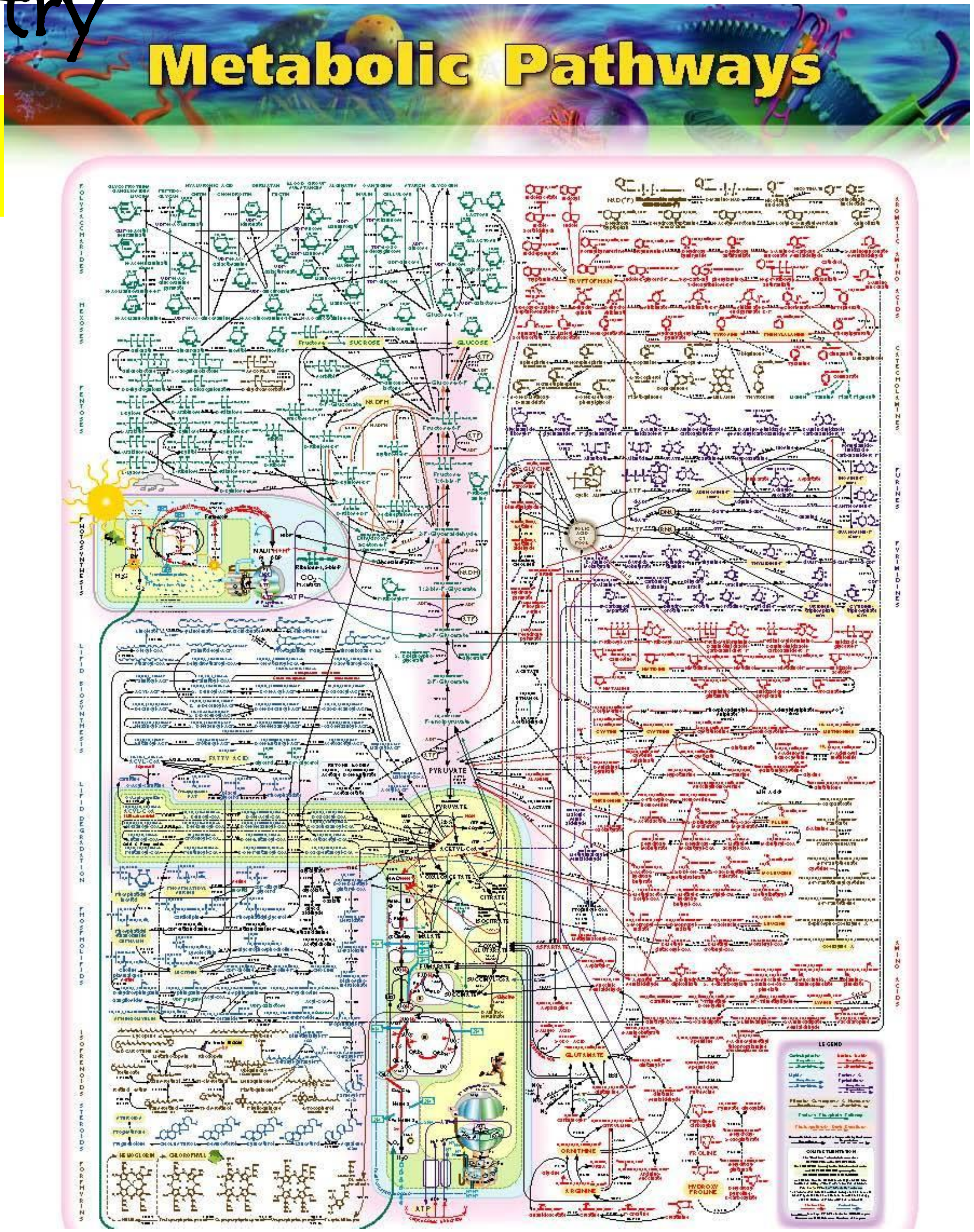
Many metabolic pathways are compartmentalized into organelles or tissues.



Metabolic Biochemistry

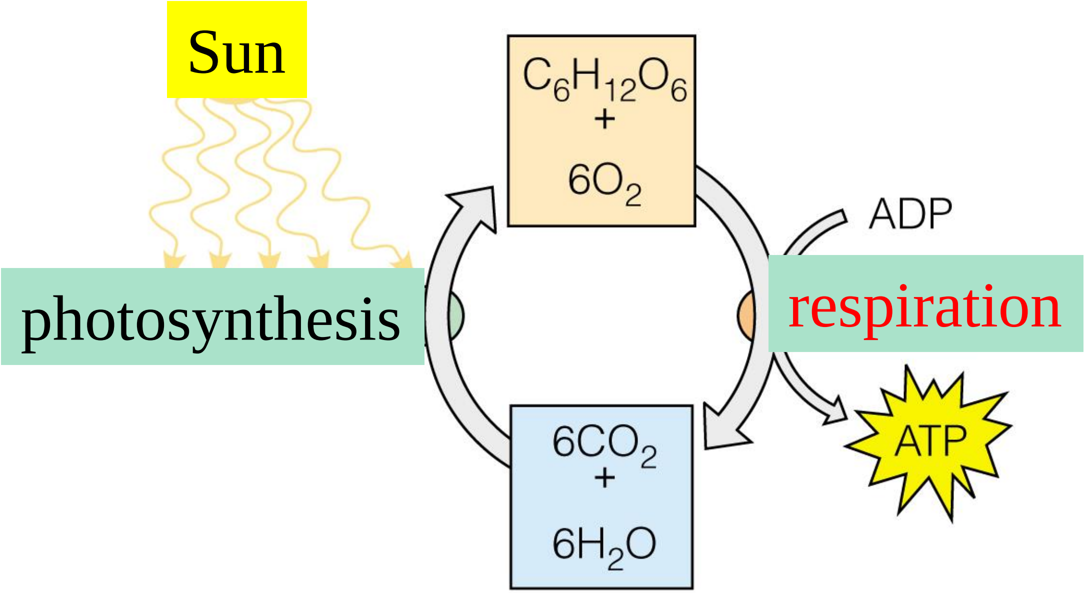
What are the purposes of the reactions?

- ☞ Converting exogenous molecules into endogenous molecules
- ☞ Form physiologically useful macromolecules
- ☞ Obtain chemical energy



Autotrophic organisms use light energy to produce food substance during photosynthesis

Both autotrophic and heterotrophic organisms obtain energy from food compounds produced by autotrophs.

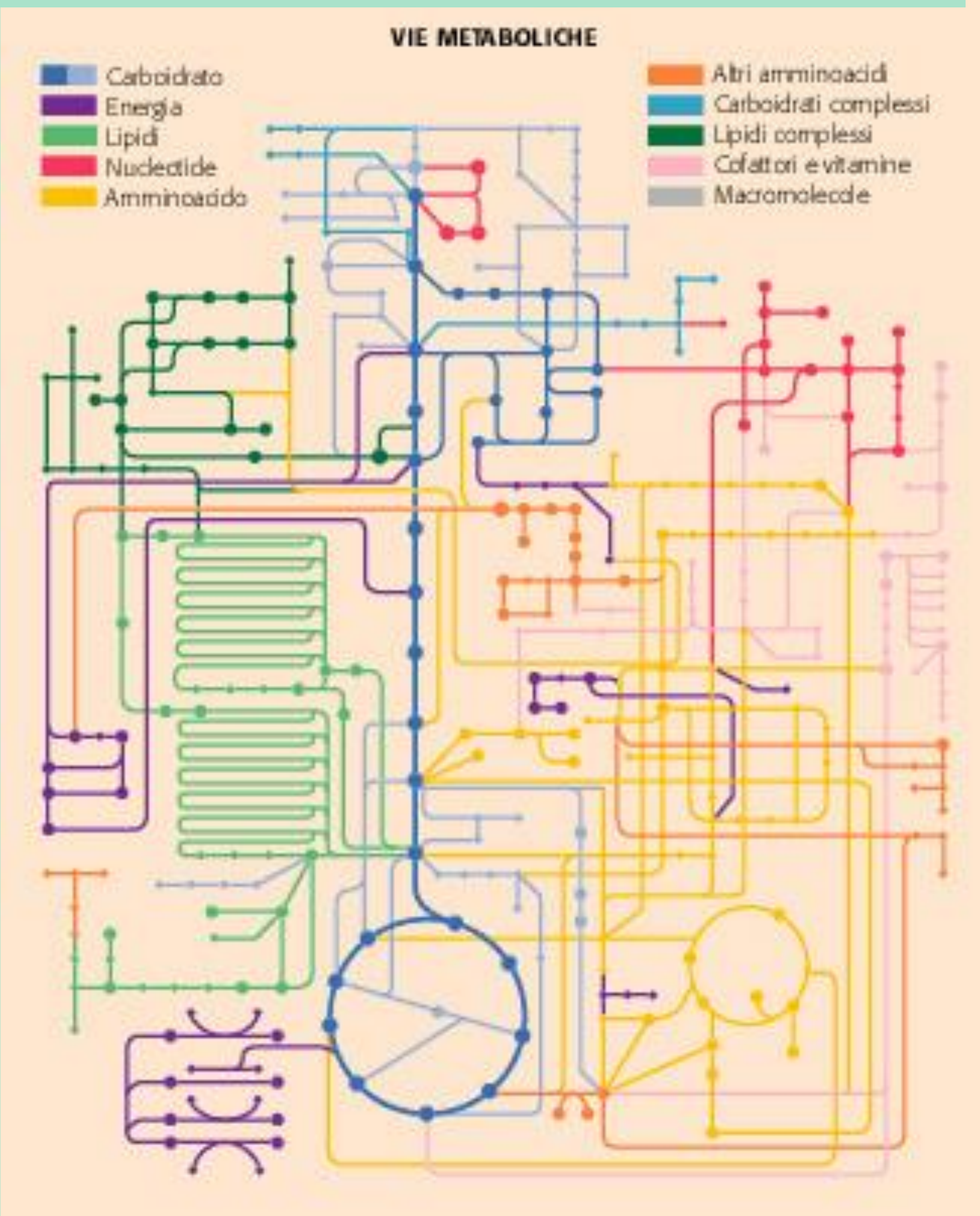


They convert these compounds into glucose which they metabolize through glycolysis and fermentation or cellular respiration

Cellular metabolism is organized to metabolic pathway

METABOLISM:

Different reactions through which biological molecules are synthesized and degraded, allow to obtain, accumulate and use energy

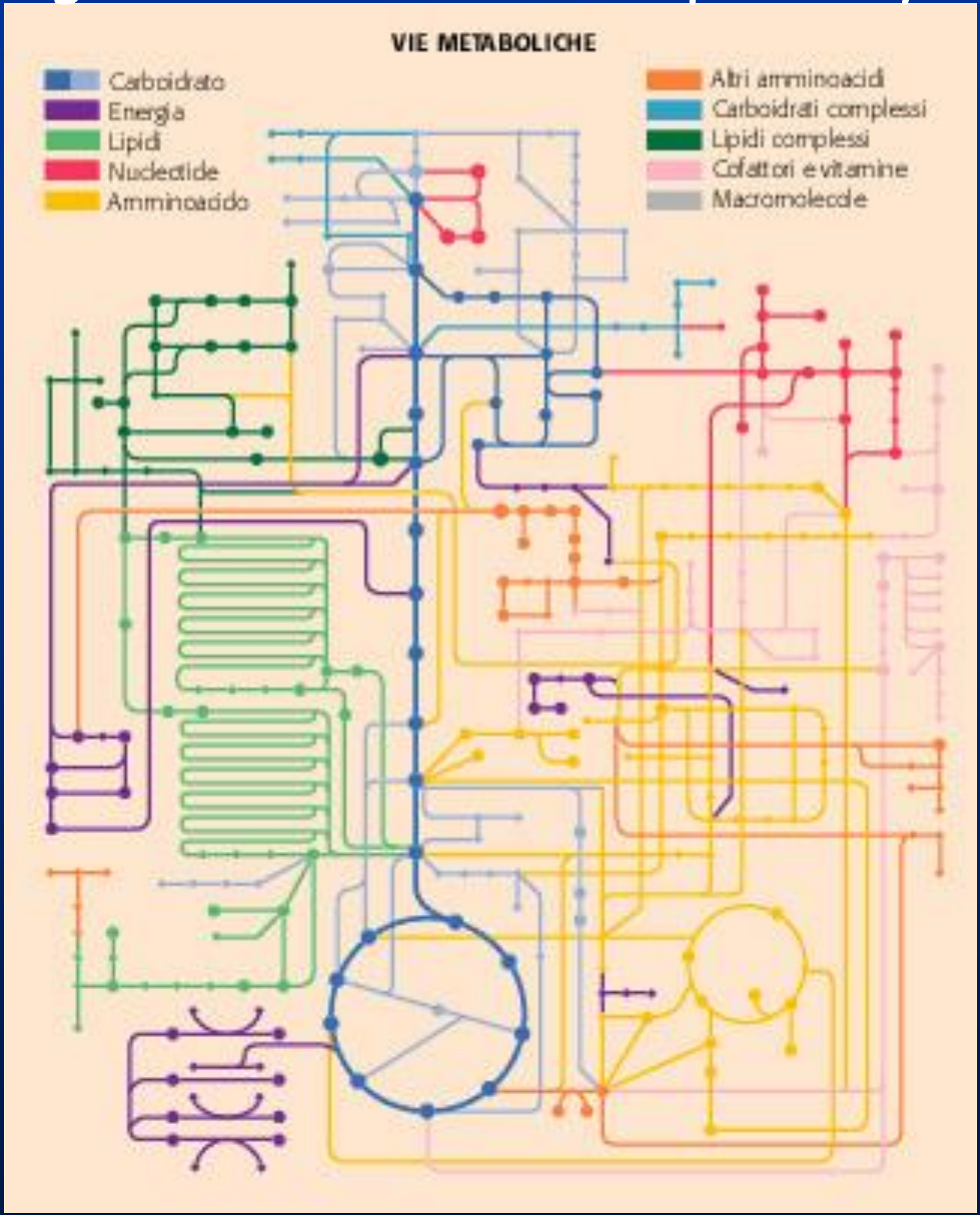


Cellular metabolism is organized to metabolic pathway

In cells, single enzymatic reaction including sequences with more stage called metabolic pathway

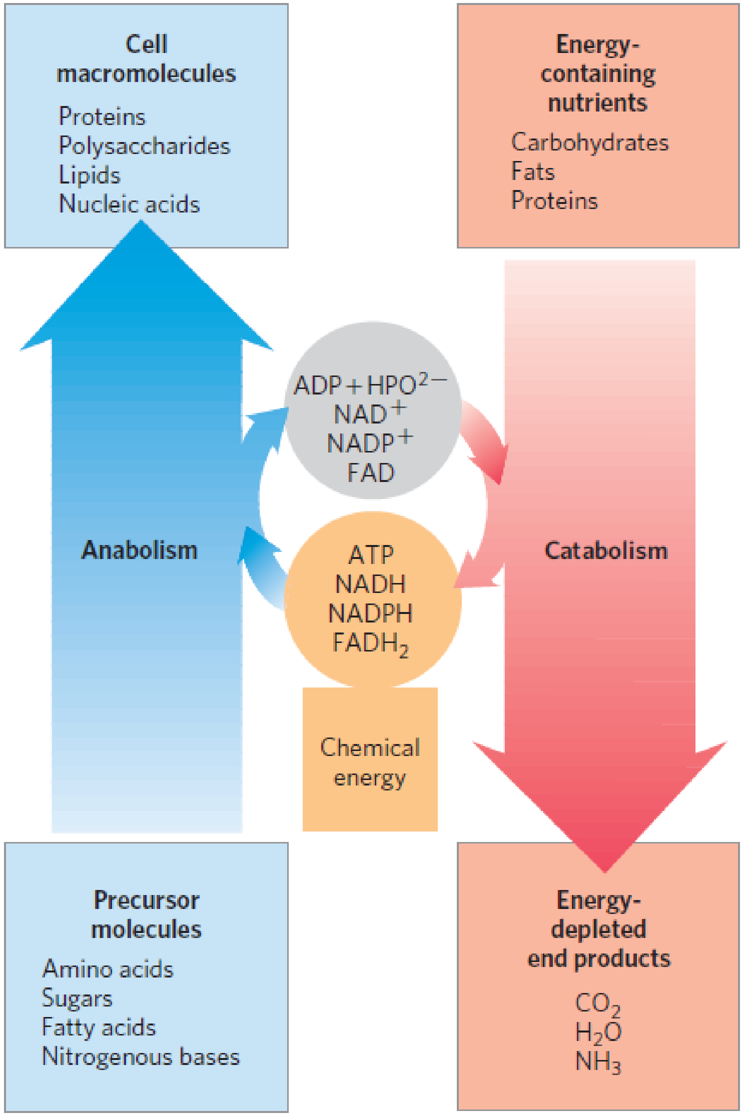
In a single way, the product of a reaction constitutes the substrates in the next reaction

Metabolism is the whole reactions



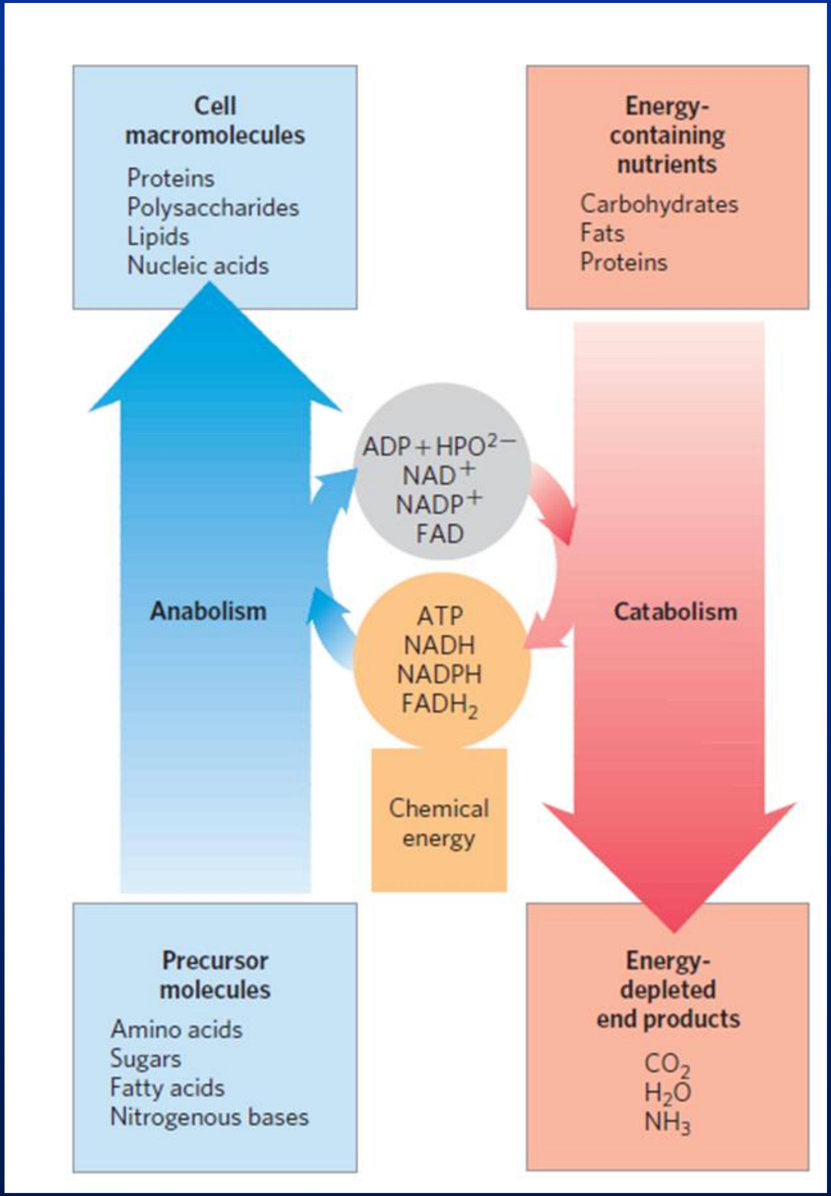
Energetic Relation between catabolic and anabolic pathway

Metabolism consists of two opposite processes, catabolism and anabolism which together constitute the chemical transformations that convert food into usable forms of energy and into complex biological molecules.



Energetic Relation between catabolic and anabolic pathway

Catabolism is responsible for the degradation of the introduced food or energy reserves in the form of carbohydrates, lipids and proteins, into usable or storable forms of energy.



Cell macromolecules

Proteins
Polysaccharides
Lipids
Nucleic acids

Energy-containing nutrients

Carbohydrates
Fats
Proteins

Anabolism

$ADP + HPO_4^{2-}$
NAD^+
$NADP^+$
FAD

ATP
NADH
NADPH
$FADH_2$

Chemical energy

Catabolism

Precursor molecules

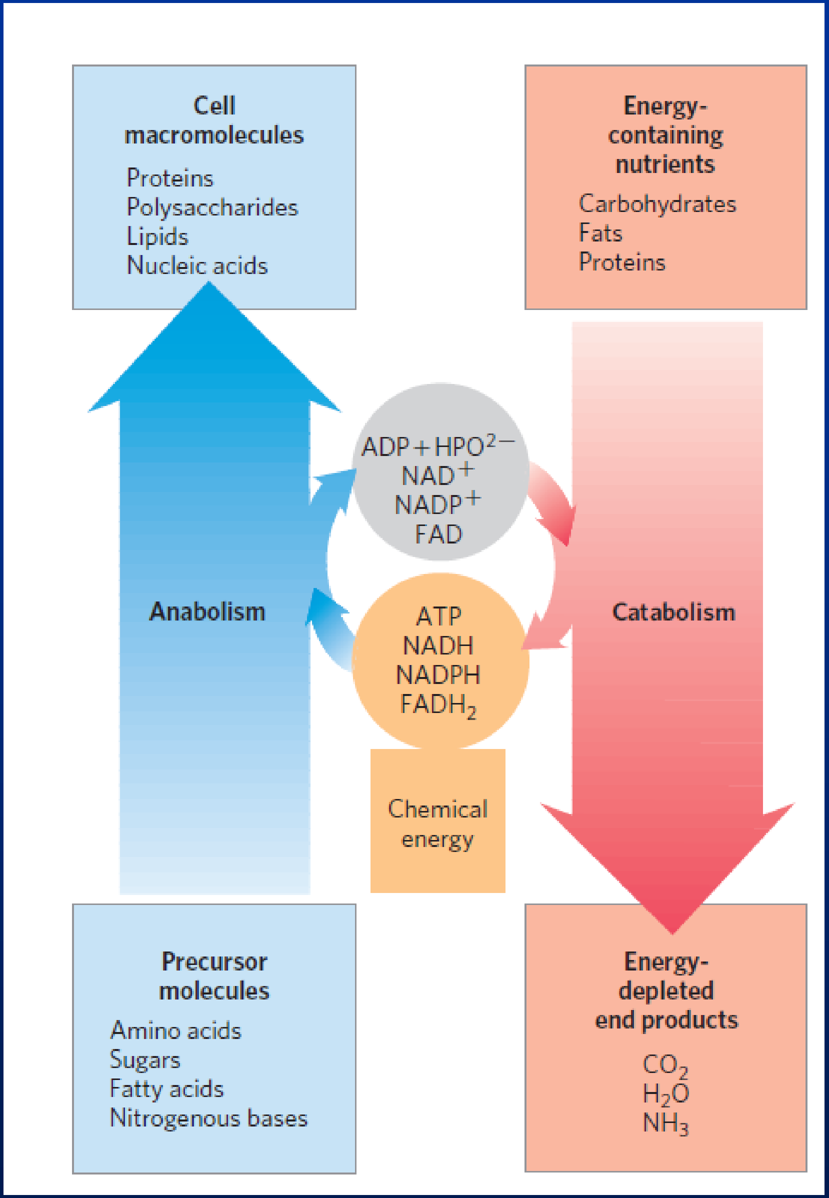
Amino acids
Sugars
Fatty acids
Nitrogenous bases

Energy-depleted end products

CO_2
H_2O
NH_3

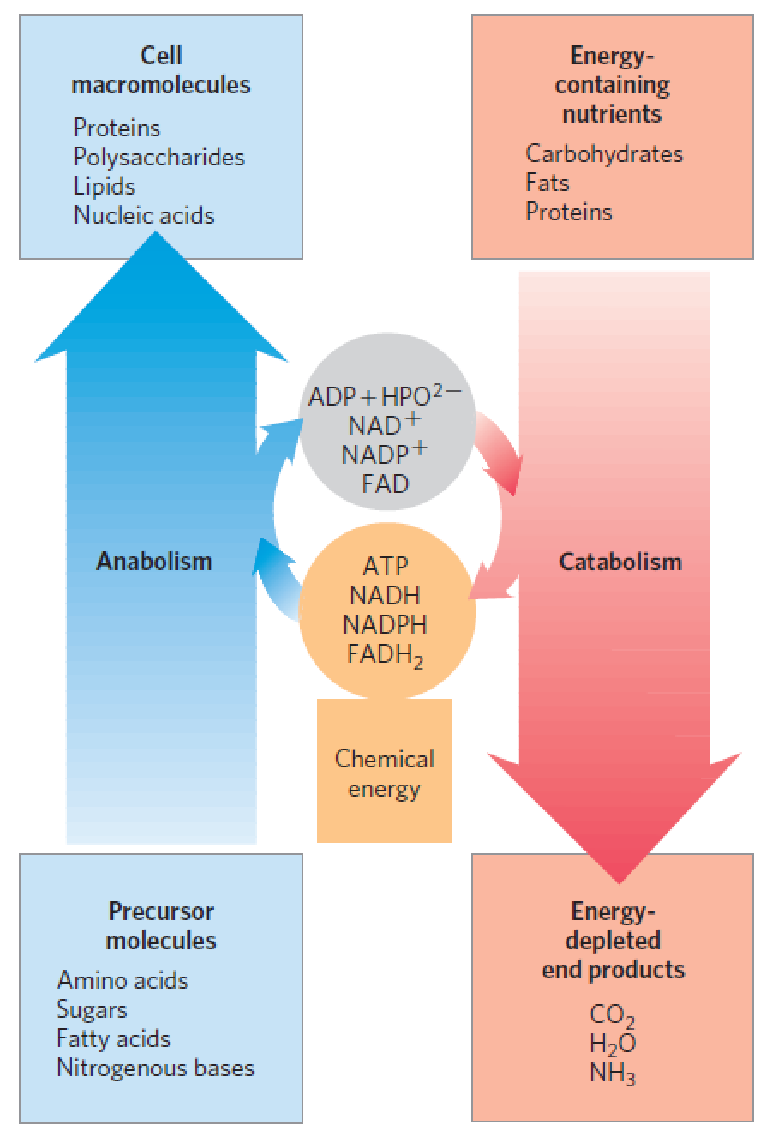
Energetic Relation between catabolic and anabolic pathway

Catabolic reactions generally consist in the conversion of large complex molecules into smaller molecules (CO_2 and H_2O) and in mammals, they require oxygen consumption



Energetic Relation between catabolic and anabolic pathway

Catabolic reactions are usually exergonic and the released energy is trapped in the formation of ATP



Cell macromolecules

Proteins
Polysaccharides
Lipids
Nucleic acids

Energy-containing nutrients

Carbohydrates
Fats
Proteins

Anabolism

ADP + HPO_4^{2-}
NAD^+
$NADP^+$
FAD

ATP
NADH
NADPH
$FADH_2$

Chemical energy

Catabolism

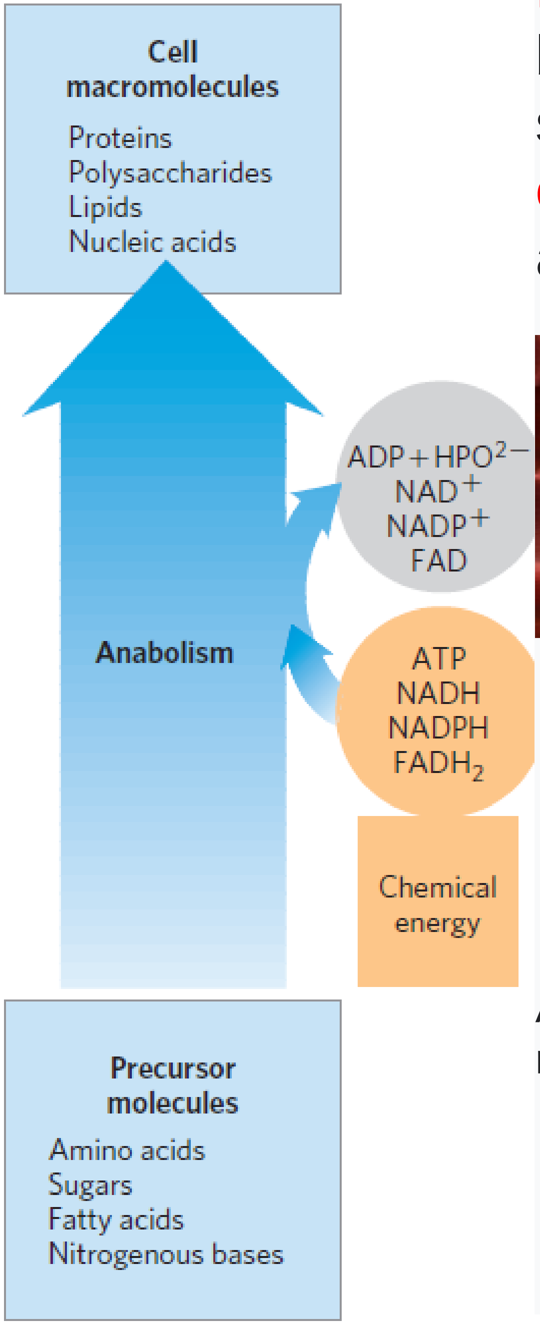
Precursor molecules

Amino acids
Sugars
Fatty acids
Nitrogenous bases

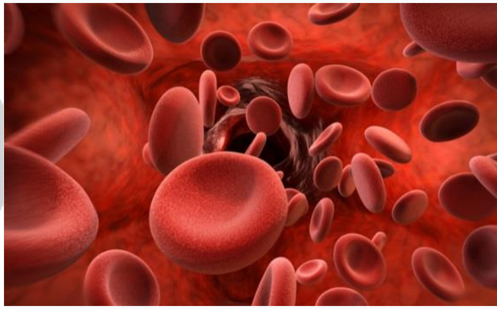
Energy-depleted end products

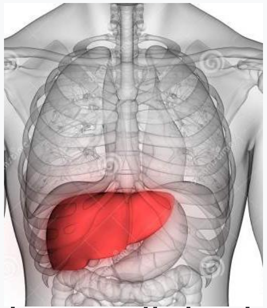
CO_2
H_2O
NH_3

The energy that comes from carbohydrates, lipids and proteins depends on the tissue and the nutritional and hormonal state.



Erythrocytes and brain in the feeding state only use carbohydrates as an energy source.

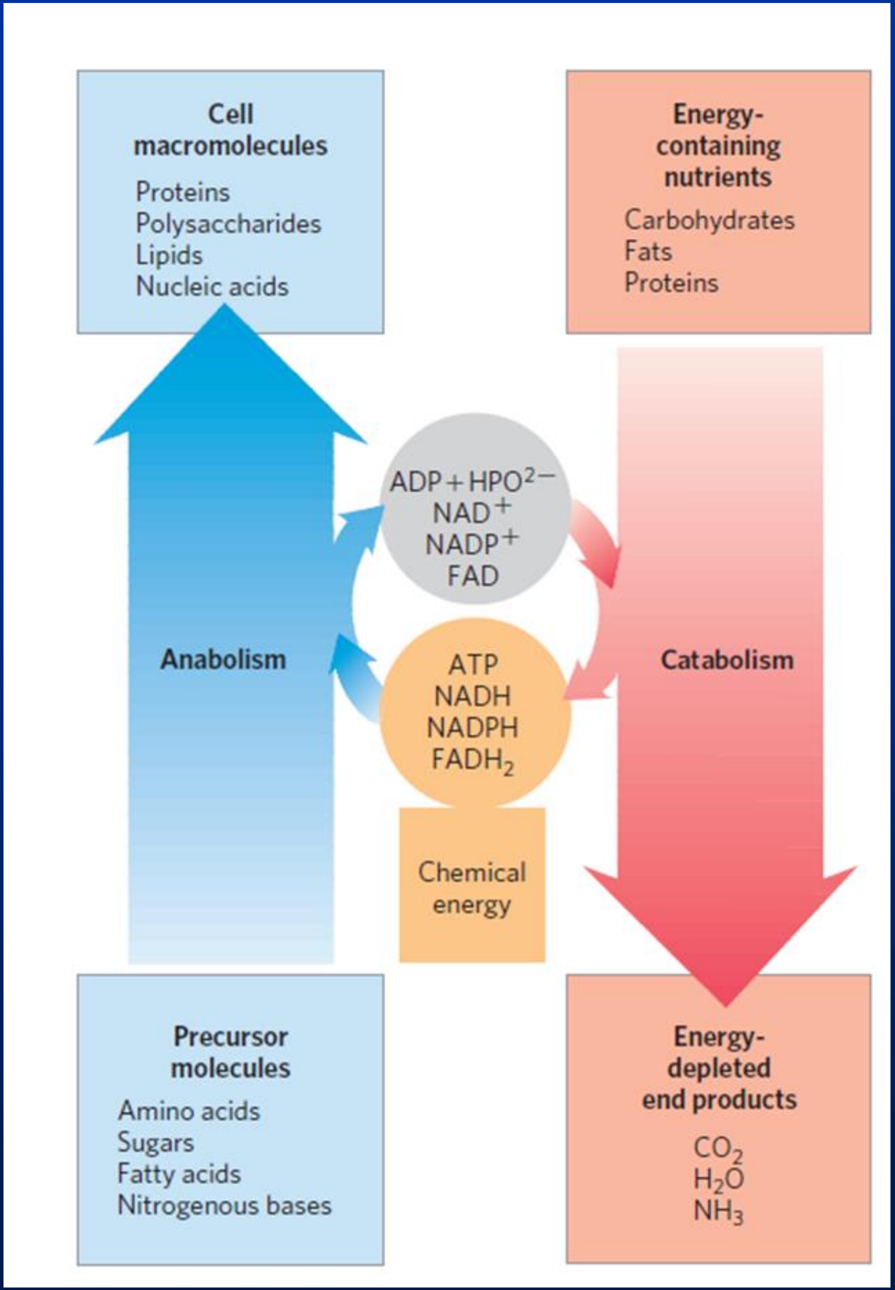




A fasting or diabetic's liver mainly metabolizes lipids

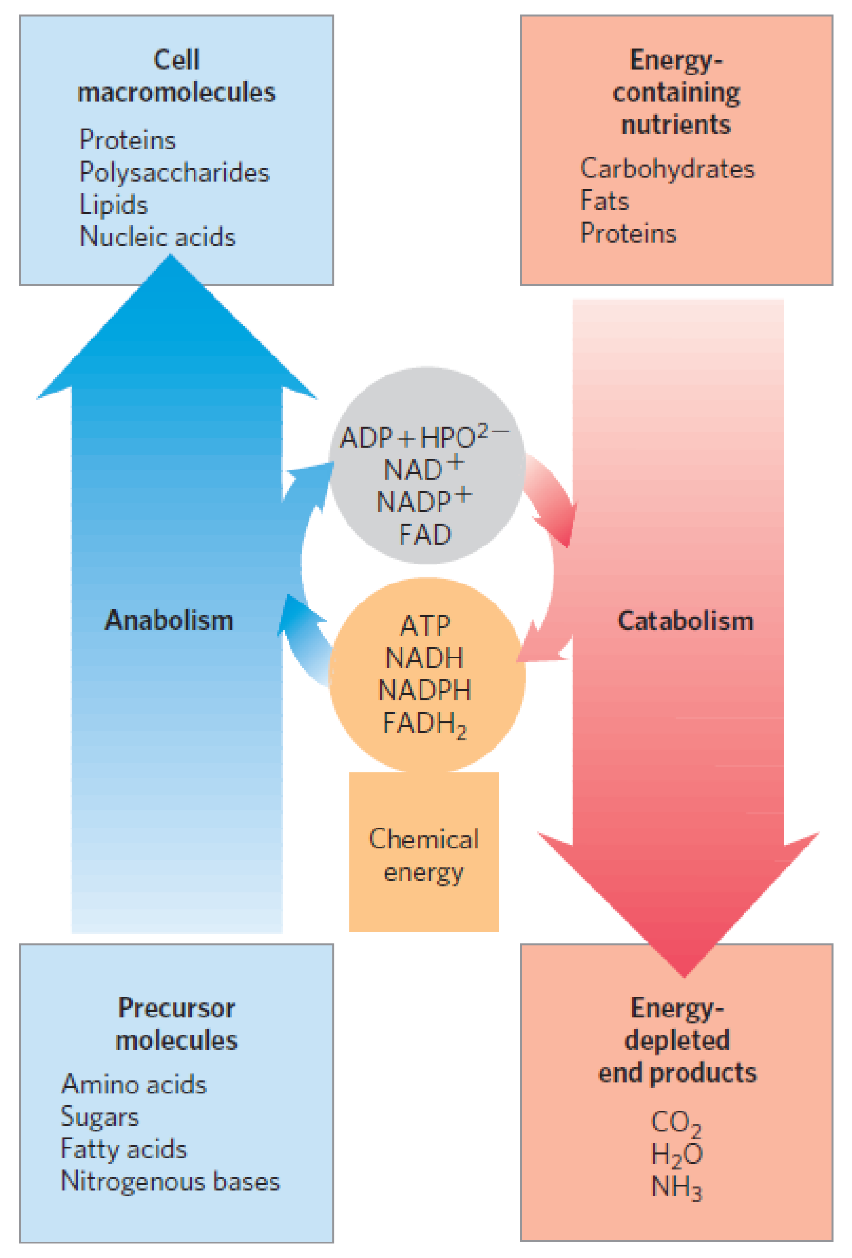
Energetic Relation between catabolic and anabolic pathway

Catabolic reactions transfer reducing equivalents to the NAD^+ and $NADP^+$ coenzymes to form NADH and NADPH.



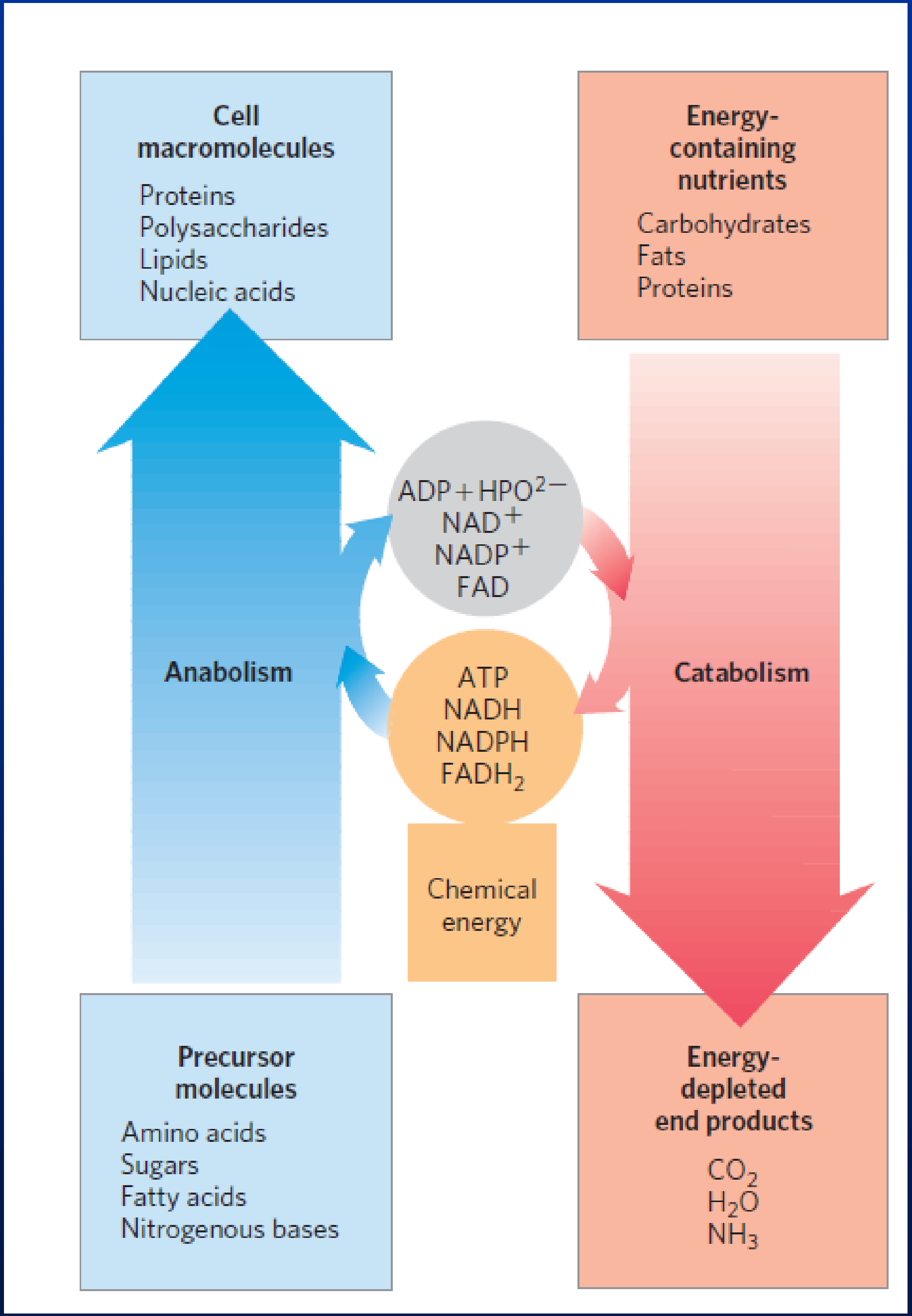
Energetic Relation between catabolic and anabolic pathway

Anabolic reactions are responsible for the biosynthesis of large molecules from smaller precursors



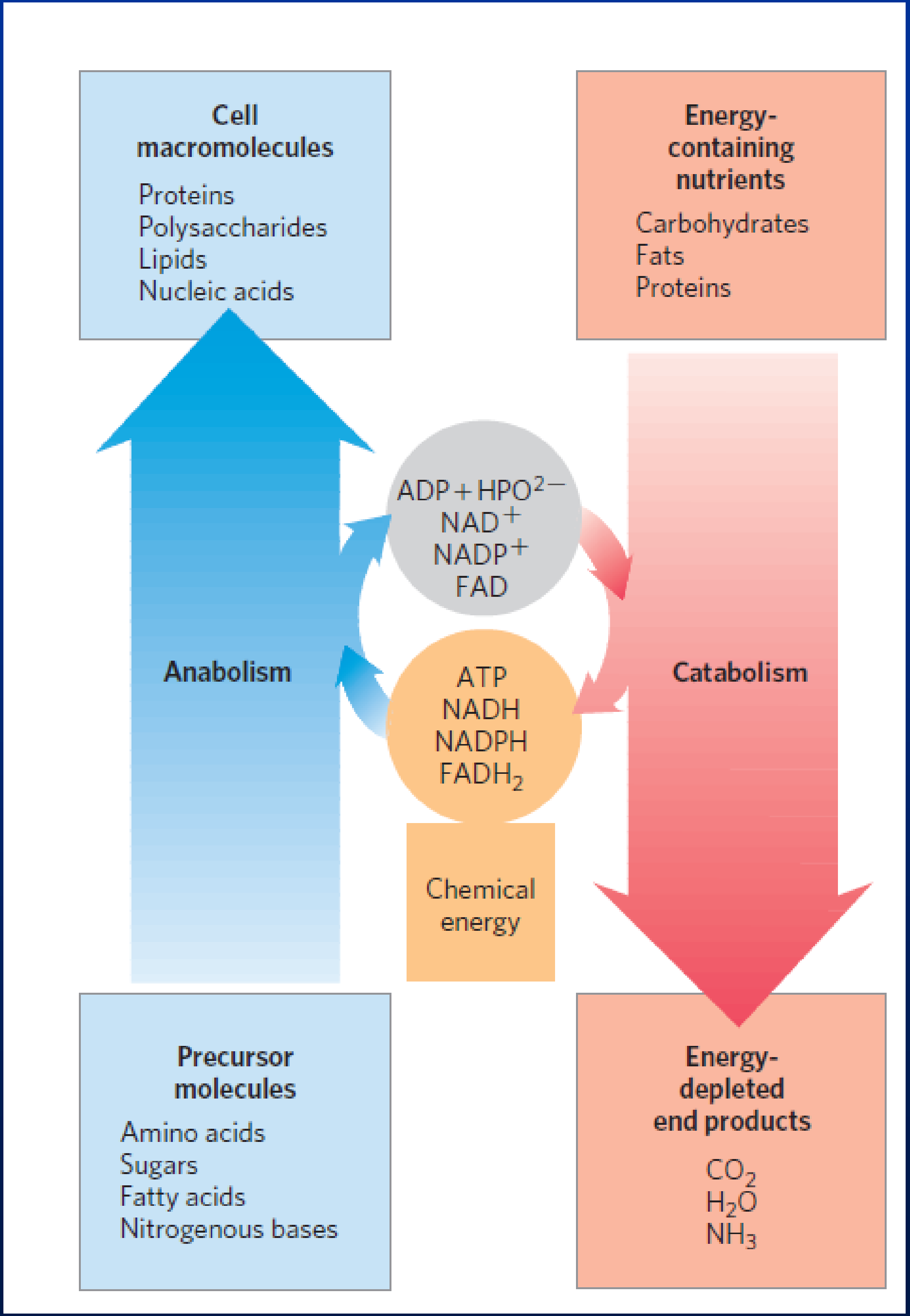
Energetic Relation between catabolic and anabolic pathway

Anabolic processes are endoergonic and use stored chemical energy such as ATP, NADH and NADPH



Energetic Relation between catabolic and anabolic pathway

Reactions that use energy perform various very often tissue-specific functions such as the conduction of nerve impulses, contraction, growth and cell division



Cell macromolecules

Proteins
Polysaccharides
Lipids
Nucleic acids

Energy-containing nutrients

Carbohydrates
Fats
Proteins

Anabolism

ADP + HPO^{2-}
NAD^+
$NADP^+$
FAD

Catabolism

ATP
NADH
NADPH
$FADH_2$

Chemical energy

Precursor molecules

Amino acids
Sugars
Fatty acids
Nitrogenous bases

Energy-depleted end products

CO_2
H_2O
NH_3

Catabolic reactions

☞are oxidative

produce reducing power and energy

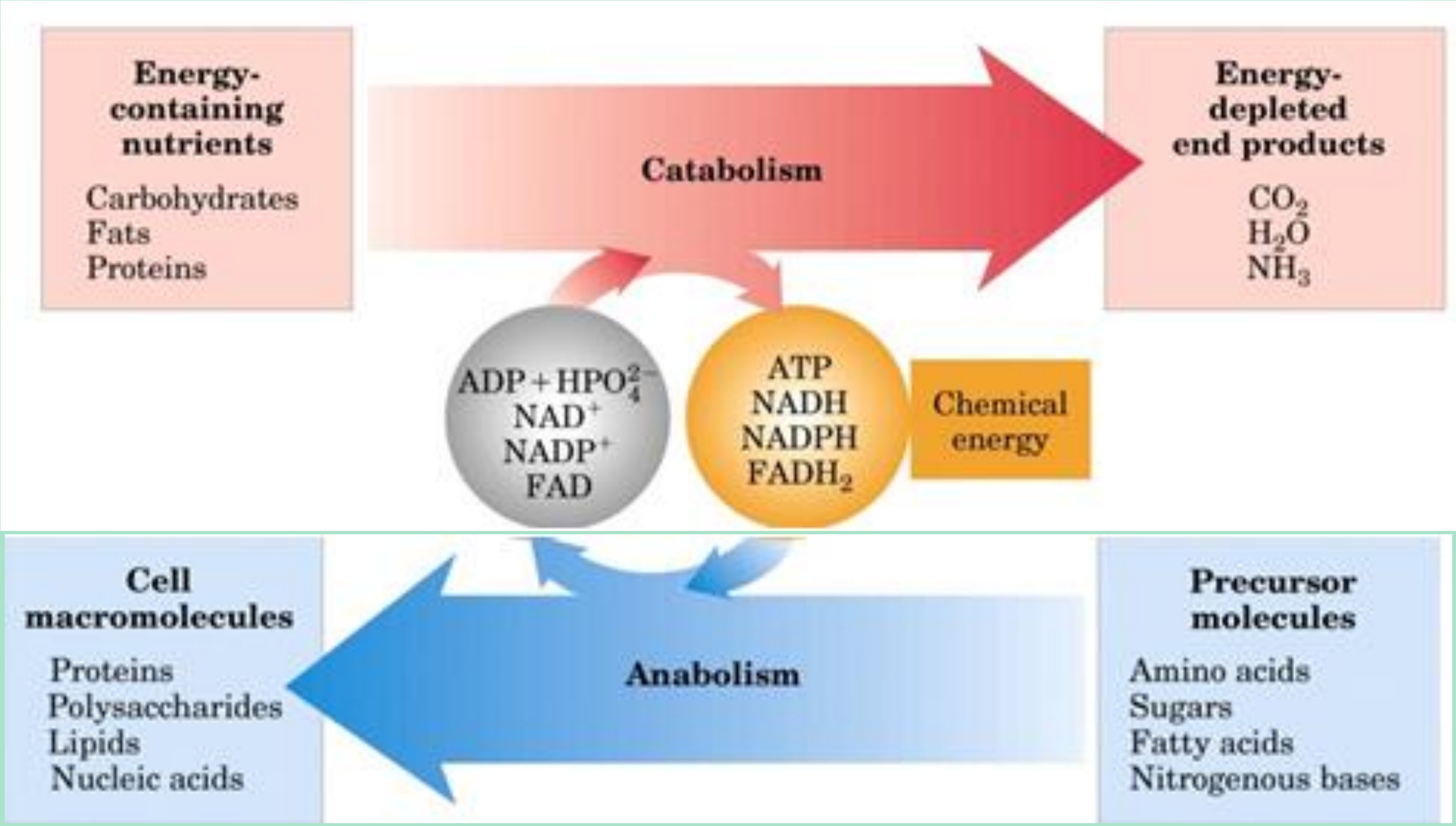
↓

Anabolic reactions

☞are reductive

↓

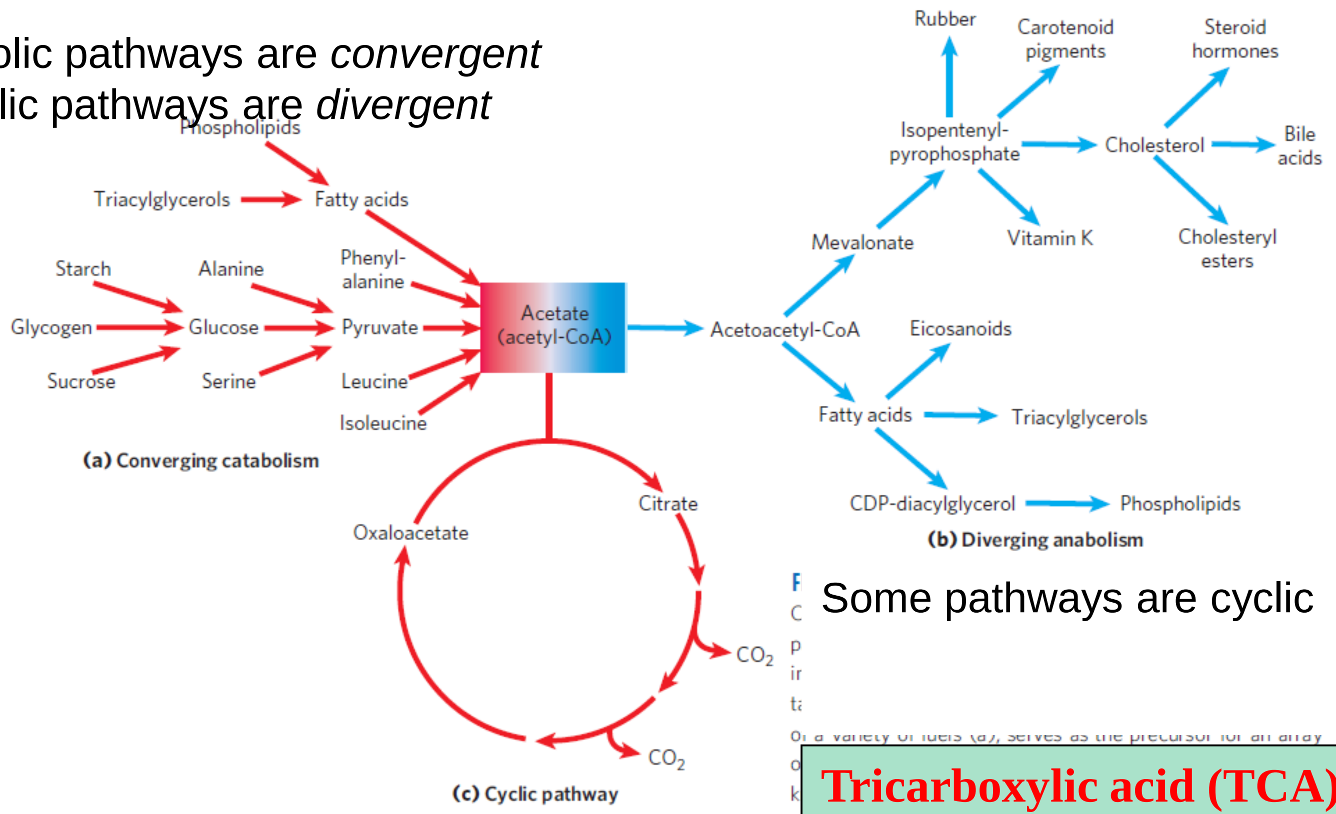
require reducing power and energy



Metabolic Pathways Are Not Necessarily Linear

Some are branched, yielding multiple useful end products from a single precursor or converting several starting materials into a single product.

Catabolic pathways are *convergent*
Anabolic pathways are *divergent*

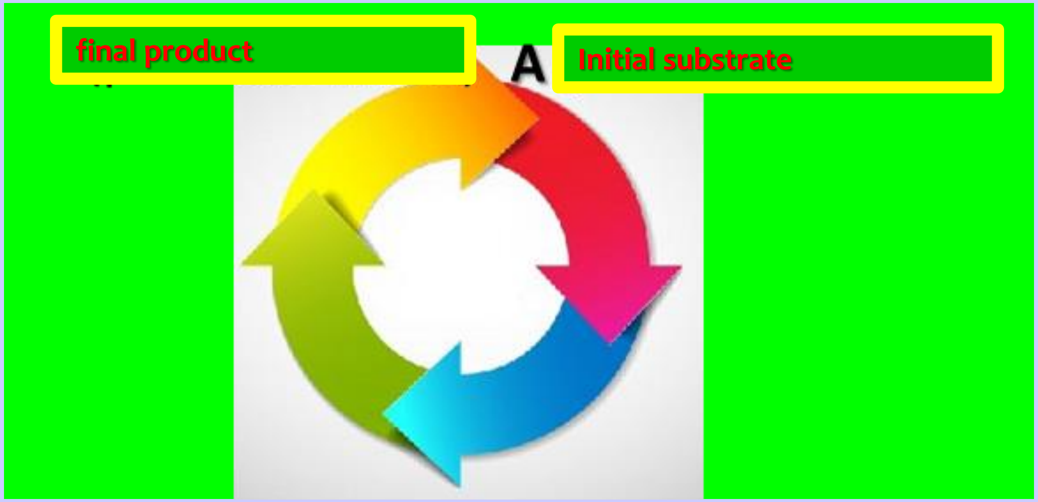


Some pathways are cyclic

Tricarboxylic acid (TCA) cycle and the urea cycle are circular pathways

Glycolysis Are Linear

Cyclical Metabolic Pathways



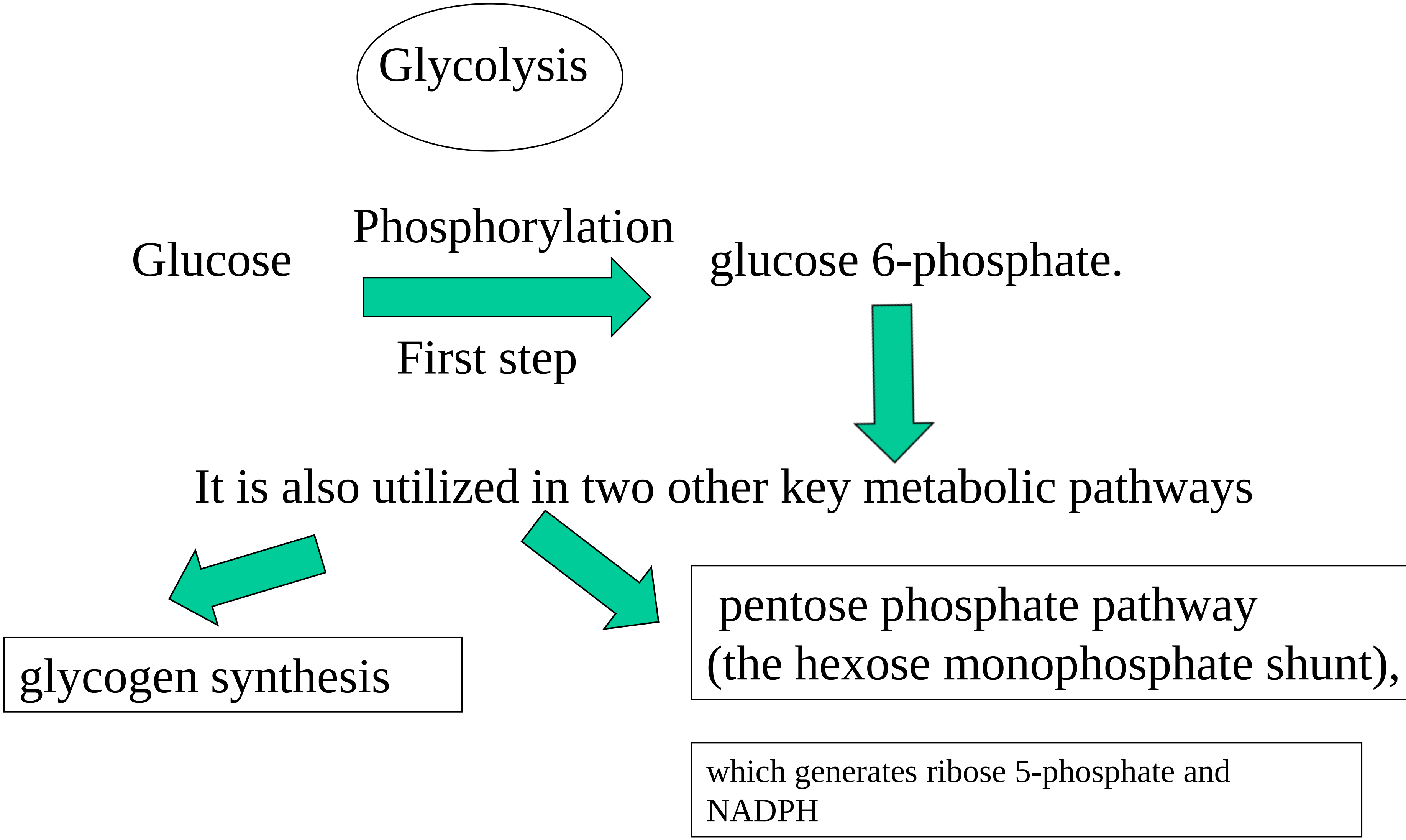
The pathway is initiated by addition of a small molecule to a key metabolic intermediate:

TCA cycle
(Oxaloacetate)

Urea cycle
(Ornithine)

At the end of one cycle, the key intermediate is regenerated and available to participate in another turn of the cycle.

Metabolic Pathways Are Interconnected



Glycolysis

Glucose

Phosphorylation

First step

glucose 6-phosphate.

It is also utilized in two other key metabolic pathways

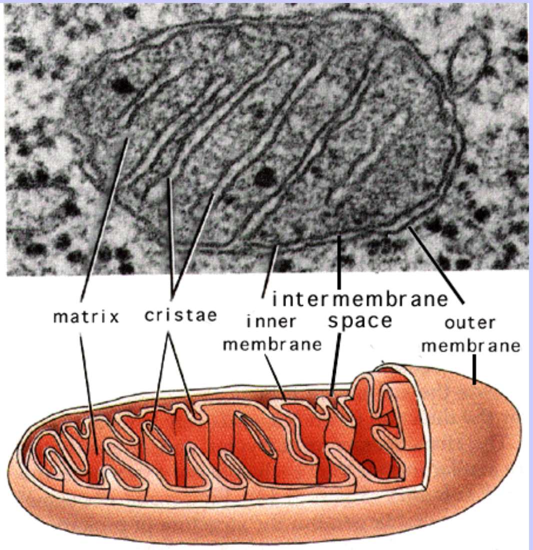
glycogen synthesis

pentose phosphate pathway
(the hexose monophosphate shunt),

which generates ribose 5-phosphate and NADPH

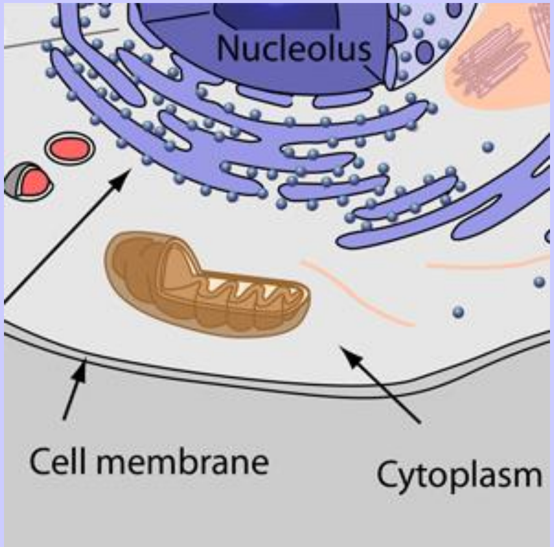
Metabolic Pathways Are Localized to Specific Compartments

Many metabolic pathways occur within the **mitochondria,** ,



Oxidation of fatty acid
TCA cycle,
Oxidative phosphorylations

Many metabolic pathways are cytosolic



Glycolysis
Pentose phosphate pathway
Fatty acid synthesis.

Others

Urea cycle
Heme synthesis

mitochondria and cytosol.

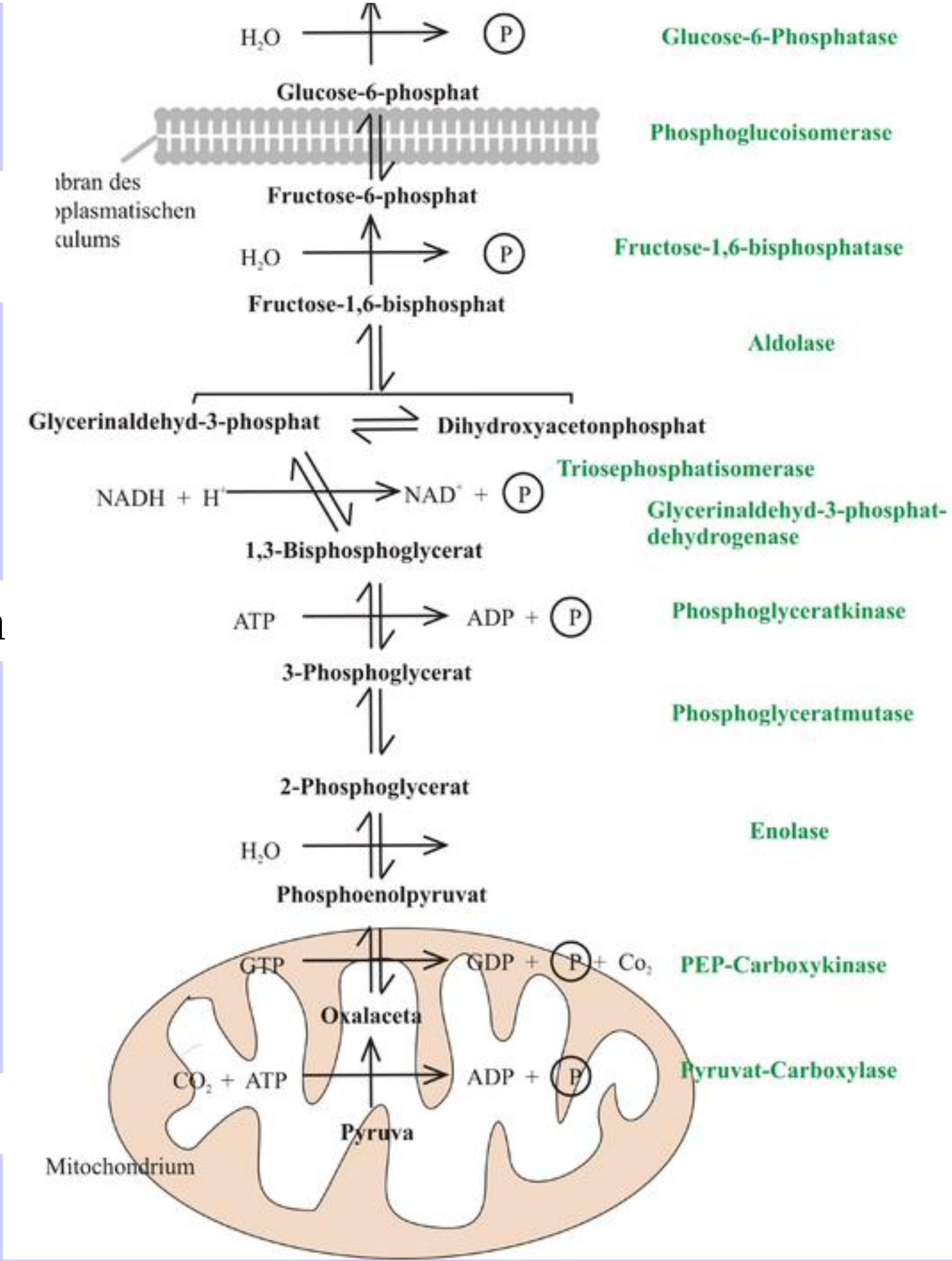
Metabolic Pathways Are Localized to Specific Compartments

Gluconeogenesis occurs in three different cellular locations

Endoplasmic Reticulum

Cytoplasm

Mitochondria



Metabolic Pathways Only Occur in Specific Tissue

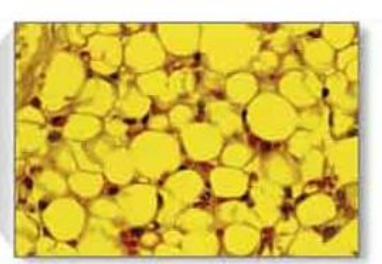
Fatty acid Synthesis

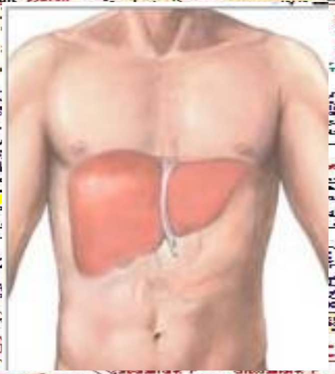
Fatty acid oxidation

Predominantly:

☞Liver

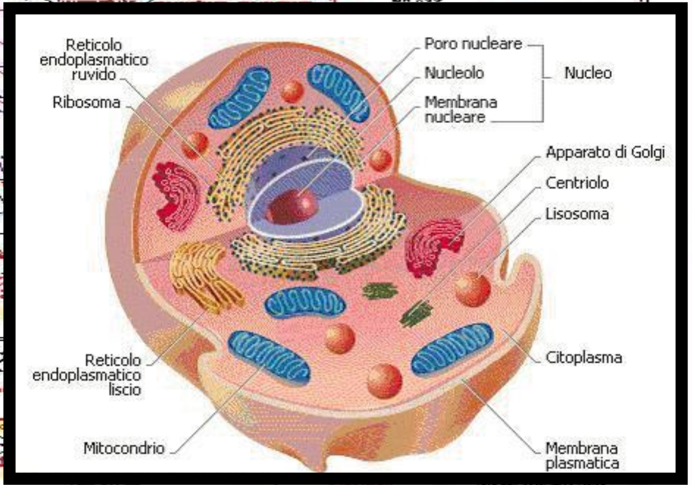
☞Adipose Tissue





~~Brain Tissue~~

~~Erythrocytes~~



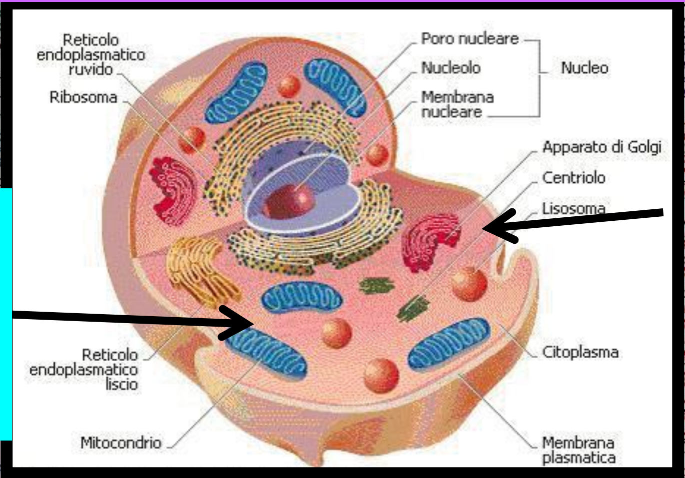
Metabolic Pathways

Some opposite reactions are compartmentalized

ADVANTAGE

☞**Separation of enzymes from antagonistic metabolic pathways**

☞ **Different distribution of allosteric effectors**



Biosynthesis of fatty acids (cytolasm hepatocytes and adipocytes)

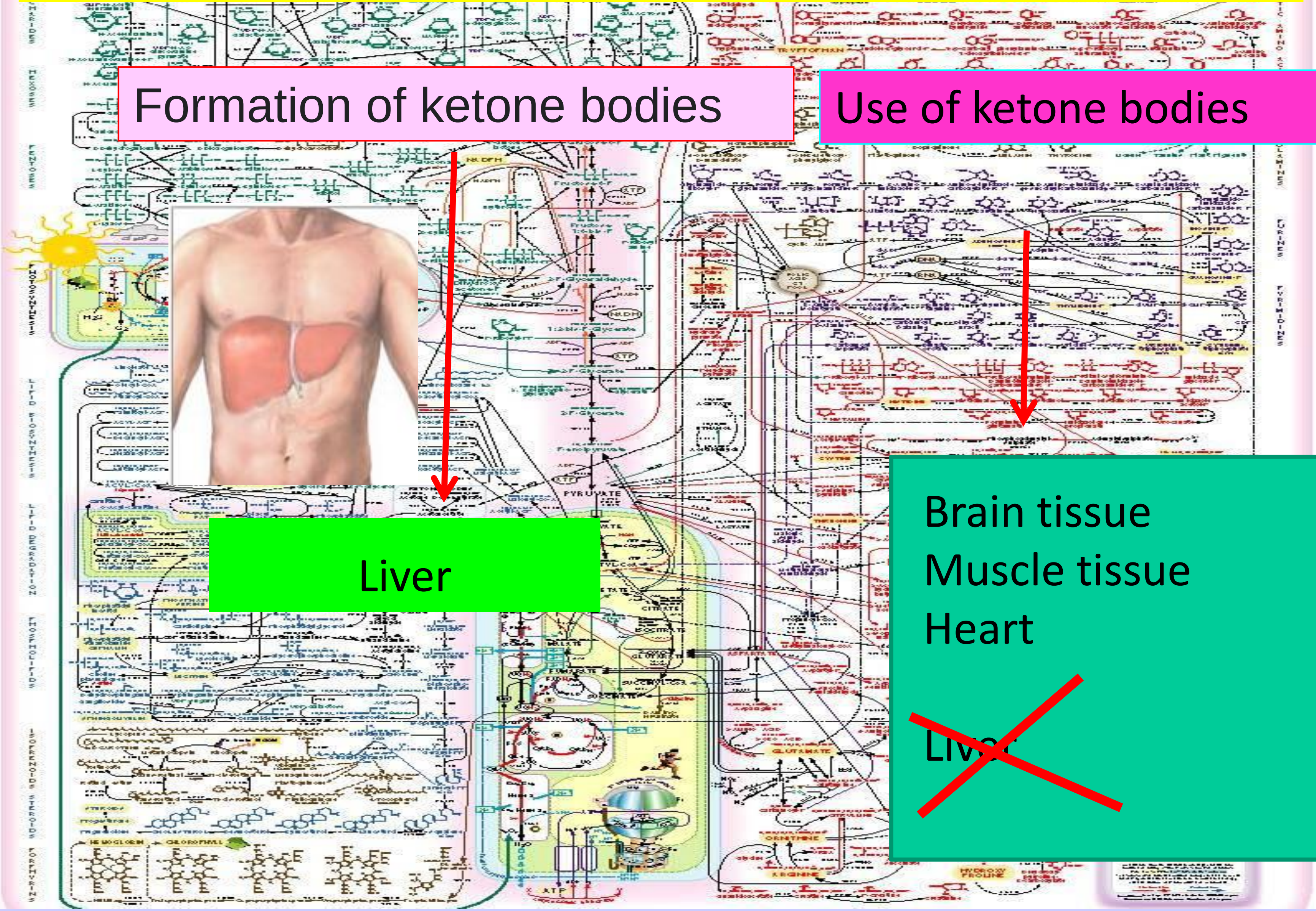
β-oxidation of fatty acids (mitochondria hepatocytes and adipocytes)

DISADVANTAGE

Need to carryer membrane

Metabolic Pathways Only Occur in Specific Tissue

Some opposite reactions are compartmentalized



Formation of ketone bodies

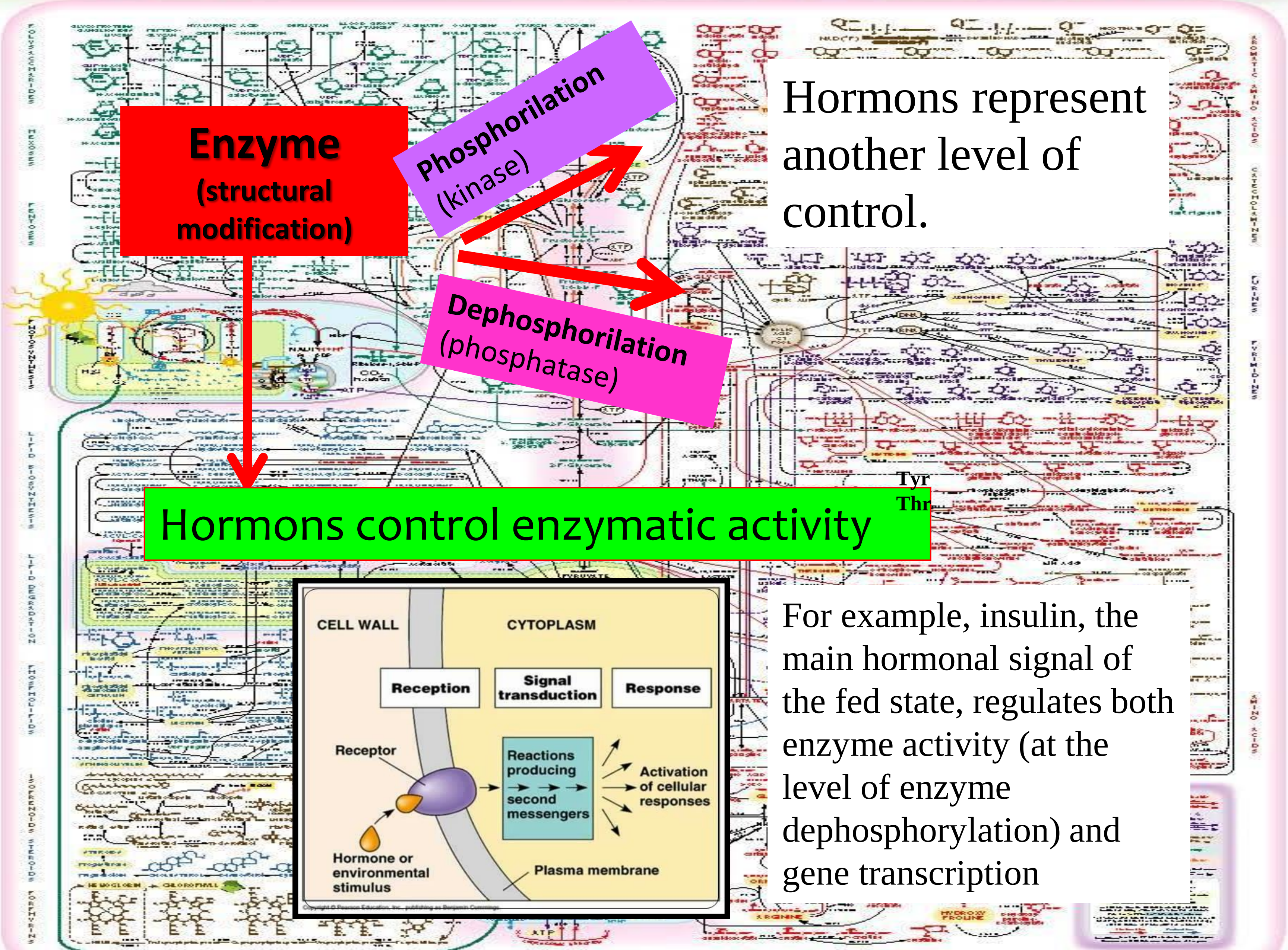
Use of ketone bodies

Liver

Brain tissue
Muscle tissue
Heart

~~Liver~~

Metabolic Pathways Are Hormonal Regulated



Enzyme
(structural modification)

Phosphorilation
(kinase)

Dephosphorilation
(phosphatase)

Tyr
Thr

Hormons control enzymatic activity

Hormons represent another level of control.

For example, insulin, the main hormonal signal of the fed state, regulates both enzyme activity (at the level of enzyme dephosphorylation) and gene transcription

REGOLATION

Separated regolation of catabolic and anabolic pathway at least a one step.

Compartmentalization (citosol or mithocondria)

Substrate-level

Concentrations of key metabolites activate or inhibit enzymatic reactions.

Citrate is a metabolite that regulate several pathways

- Inhibits glycolysis
- Activates the first step in the pathway of fatty acid synthesis

Dependent from substrate disponibility

The flow of metabolites is regulated by the concentrations of

substrates and products.

Allosteric regolation

The flow of metabolites through these reactions is controlled by modulating the enzymes that catalyze them.

Hormonal regolation

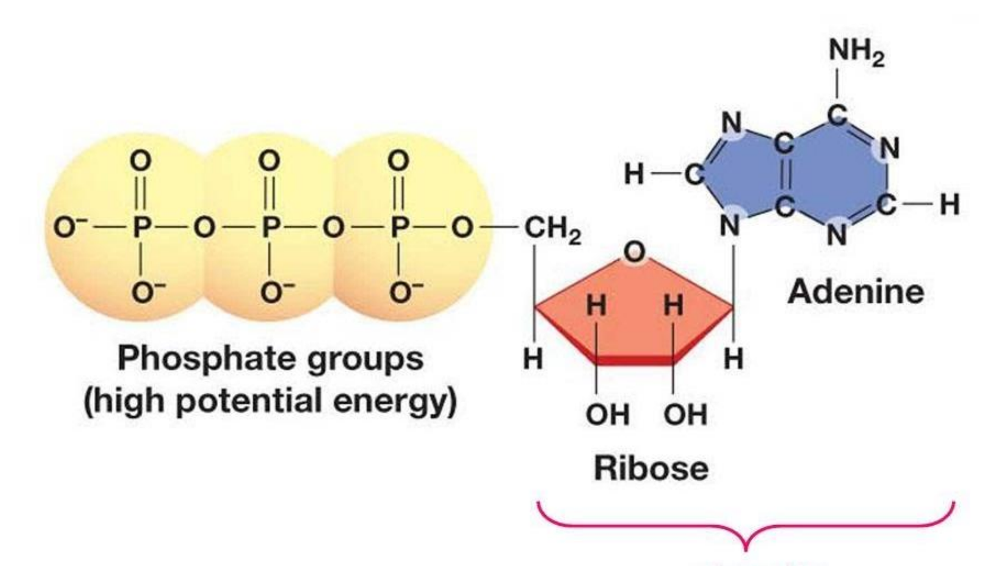
What is chemical energy?

The chemical energy that derives from carbohydrates, lipids and proteins during cellular respiration is used to generate ATP molecules

ATP acts as an accumulator

- stores energy obtained from food

- releases energy when needed



What is chemical energy used for?

To obtain the synthesis of complex molecules starting from simple precursors

To obtain gradients of concentration, movement, heat,

i.e: In skeletal muscle, the chemical energy of ATP is converted into mechanical energy during muscle contraction

BYOENERGETIC

Bioenergetics is a quantitative study of E**nergy Transductions**
—changes of one form of energy into another that occur in living cells and nature
and function of the chemical processes underlying these transductions.

All energy transformations that occur in nature follow the laws of thermodynamics

The laws of thermodynamics allow to predict whether a reaction will be spontaneus into the organism

Two fundamental laws of thermodynamics.

.

First law is the principle of the conservation of energy:
for any physical or chemical change,

The total amount of energy in the universe remains constant;

Energy may change form or transported from one region to another, but it cannot be created or destroyed.

.

Two fundamental laws of thermodynamics.

Second law of thermodynamics

The universe always tends toward increasing disorder: *in all natural processes, the entropy of the universe increases.*

Entropy (S) :

degree of disorder and randomnes of a system

Free energy (G) is used in biochemical processes

Gibbs free energy, (*G*),

The amount of energy capable of doing work during a reaction at constant temperature and pressure. →

When a reaction proceeds with the release of free energy
(when the system changes so as to possess less free energy),

ΔG -> a negative value and the reaction is called "exergonic"

.

When a reaction gains or absorbs free energy

ΔG -> a positive value and the reaction is called "endergonic"

The free energy (G) of a system is that portion of the total energy that is available for useful work.

It is defined by

$$\Delta G = \Delta H - T\Delta S$$

ΔG is the change in free energy

ΔH is the change of hentalpy or the heat content

T is the absolute temperature

ΔS is the change in entropy

Enthalpy (H)

The heat content of the reacting system.

Chemical reaction releases heat--→exothermic $(-\Delta H)$

The heat content of the products is less than that of the reactants

Reacting systems that take up heat----→ endothermic $(-\Delta H)$.

Free energy is correlated to Keq

Equilibrium

$$\boxed{\Delta G° = - R\ T\ \ln K_{eq}}$$

Pressure 1 atm

Temperature 25°C = 298 K

pH = 7.0

$$\frac{k_1}{k_{-1}} = \frac{[C_{eq}]\ [D_{eq}]}{[A_{eq}]\ [B_{eq}]} = K_{eq}$$

Equilibrium constant

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C + D$$

At Constant temperature

$$\frac{k_1}{k_{-1}} = \frac{[C_{eq}]\ [D_{eq}]}{[A_{eq}]\ [B_{eq}]} = K_{eq}$$

Direct reaction :

$v_{dir} = k_1\ [A]\ [B]$

Opposite reaction:

$v_{inv} = k_{-1}\ [C]\ [D]$

$v_{dir} = v_{inv}$

$k_1\ [A_{eq}]\ [B_{eq}] = k_{-1}\ [C_{eq}]\ [D_{eq}]$

Equilibrium reaction: Vdir=Vinv

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C + D$$

$$\frac{k_1}{k_{-1}} = \frac{[C_{eq}]\,[D_{eq}]}{[A_{eq}]\,[B_{eq}]} = K_{eq}$$

- K_{eq} is high ($K_{eq} >> 1$) equilibrium towards products
- [C] [D] is major to [A] [B])
  (k1 > k-1)

- K_{eq} is low ($K_{eq} << 1$) equilibrium towards reagents
- [A] [B] is major to [C] [D]
- (k-1 < k1)

- K_{eq} is equal to 1 (K_{eq} ~1) equilibrium not towards reagents and neither towards reagents
- (k-1= k1)

In living organism, the condition of reaction is not standard and the real variation of free energy depends from the ratio between reagents and products concentration presents in the cellular environmental .

$$A + B \rightleftarrows C + D$$

$$\Delta G'_{reaz} = \Delta G^{0'}_{reaz} + RT \ln \frac{[C]\,[D]}{[A]\,[B]}$$

R = universal constant of gas (8.315 J/K·mol)
T = temperature (Kelvin)

$$\frac{[C_{eq}]\,[D_{eq}]}{[A_{eq}]\,[B_{eq}]} = K_{eq}$$

$$\Delta G'_{reaz} = \Delta G^{0'}_{reaz} + RT \ln K_{eq}$$

$$\rightarrow \boldsymbol{\Delta G^{0'} = - RT \ln K_{eq}}$$

$\Delta G°' < 0$

Spontaneus reaction
(EXERGONIC)

$\Delta G°' > 0$

Not-spontaneus reaction
(ENDERGONIC)

$\Delta G°' = 0$

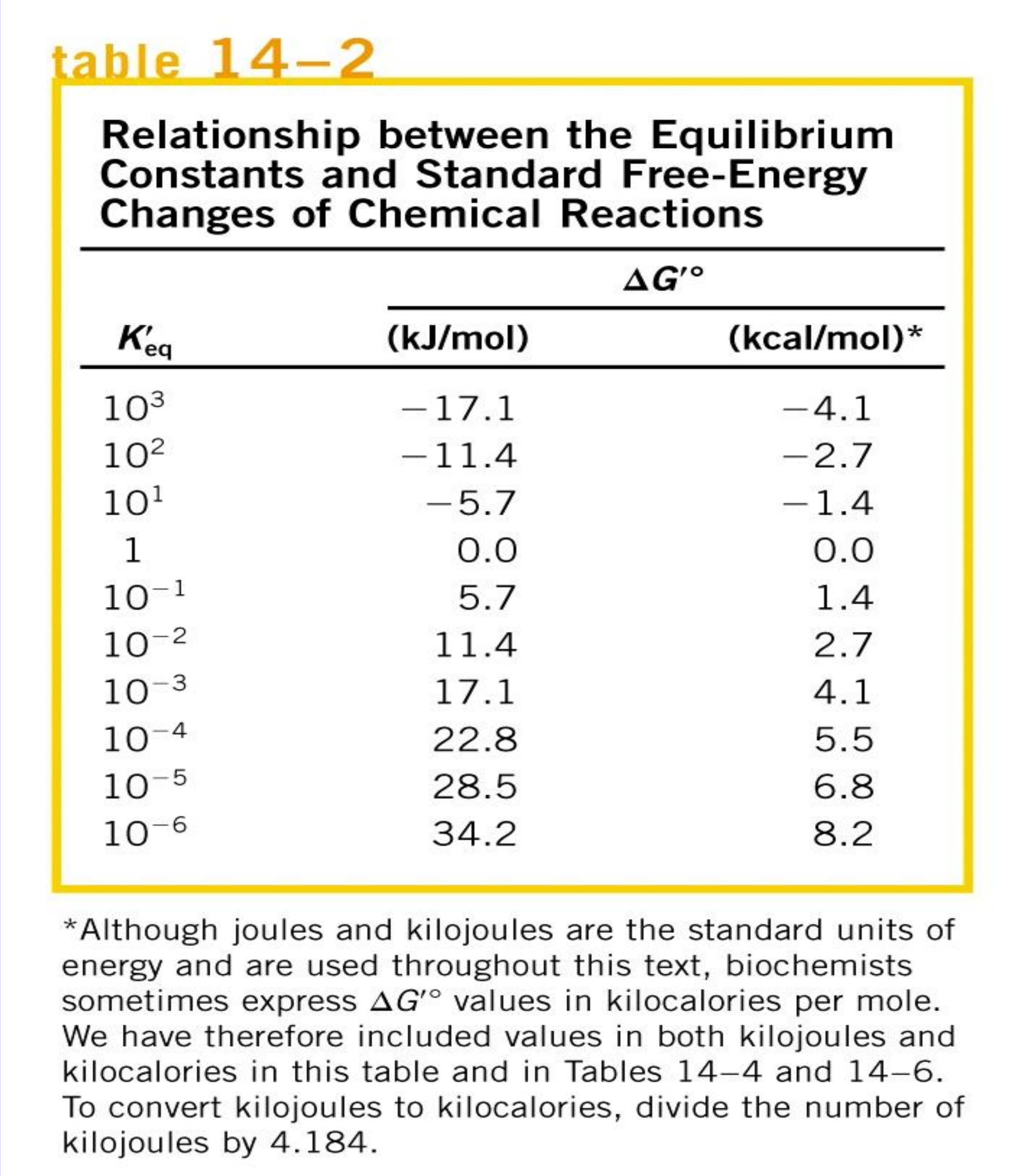


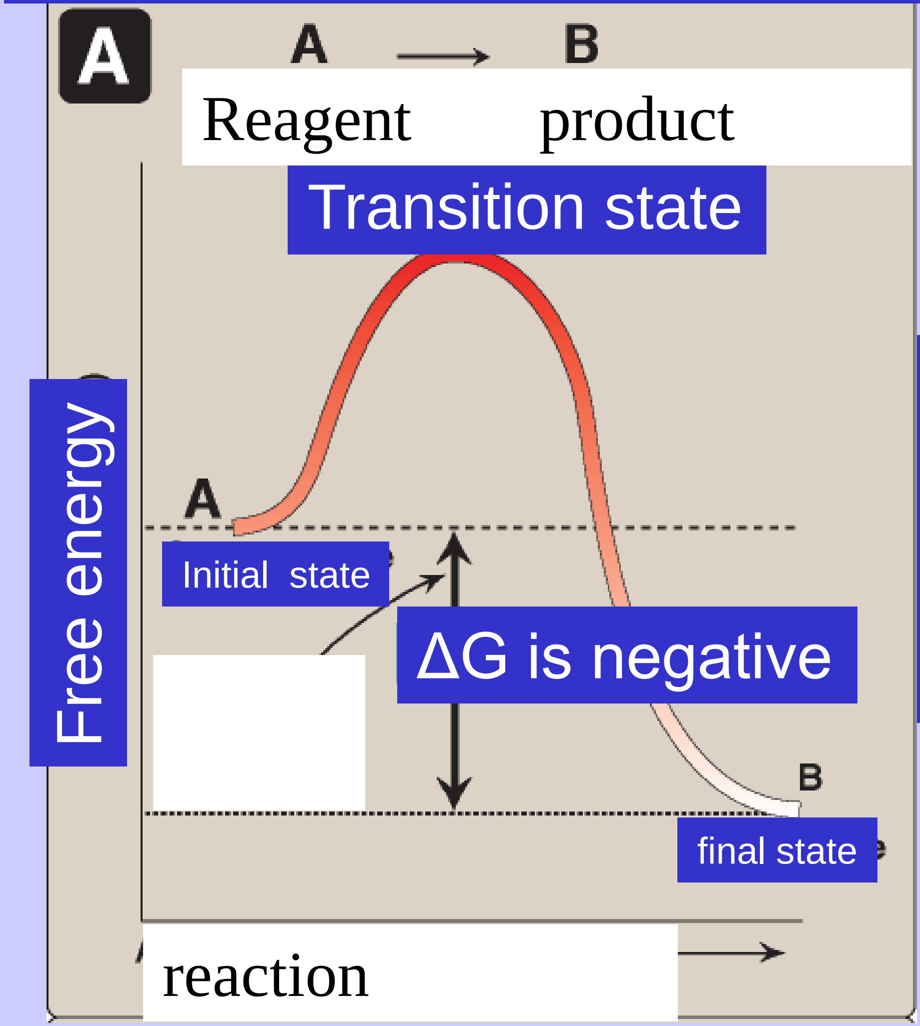
table 14–2

Relationship between the Equilibrium Constants and Standard Free-Energy Changes of Chemical Reactions

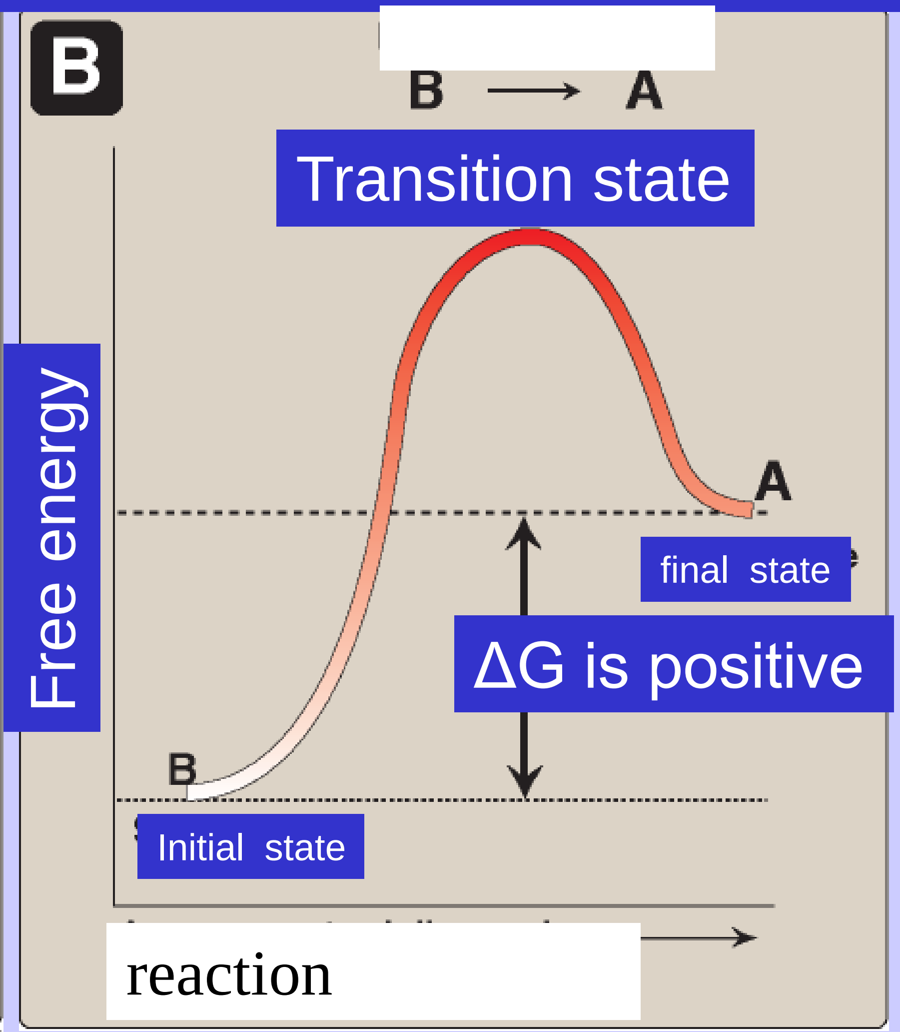
K'_{eq}	$\Delta G'°$ (kJ/mol)	$\Delta G'°$ (kcal/mol)*
10^{3}	−17.1	−4.1
10^{2}	−11.4	−2.7
10^{1}	−5.7	−1.4
1	0.0	0.0
10^{-1}	5.7	1.4
10^{-2}	11.4	2.7
10^{-3}	17.1	4.1
10^{-4}	22.8	5.5
10^{-5}	28.5	6.8
10^{-6}	34.2	8.2

*Although joules and kilojoules are the standard units of energy and are used throughout this text, biochemists sometimes express $\Delta G'°$ values in kilocalories per mole. We have therefore included values in both kilojoules and kilocalories in this table and in Tables 14–4 and 14–6. To convert kilojoules to kilocalories, divide the number of kilojoules by 4.184.

The spontaneity of the reaction is established by its ΔG



EXERGONIC REACTION
(SPONTANEUS)



ENDERGONIC
REACTION
(NOT SPONTANEUS)

In the metabolic process a unfavorable reaction termodynamically ($\Delta G^{0\prime} \geq 0$) can be favored:

1) If the product is quickly consumed in the next one reaction.

Reactions of a metabolic pathway are linked.

$[C]\,[D] < [A]\,[B]$

$$\Delta G'_{reaz} = \Delta G^{0\prime}_{reaz} + RT \ln \frac{[C]\,[D]}{[A]\,[B]}$$

$$\ln \frac{[C]\,[D]}{[A]\,[B]} < 0$$

2) Coupling it to a second sufficiently exergonic reaction, since the variations of free energy are additive

Free energy changes can be add:

Enzyme often couples two process (one endergonic and one exergonic),
The sum with a highly exergonic process produce a variation of total standard free energy

$$(1) \quad A \longrightarrow \cancel{B} \qquad \Delta G'^{\circ}_{1} = +20 \text{ kJ/mole}$$

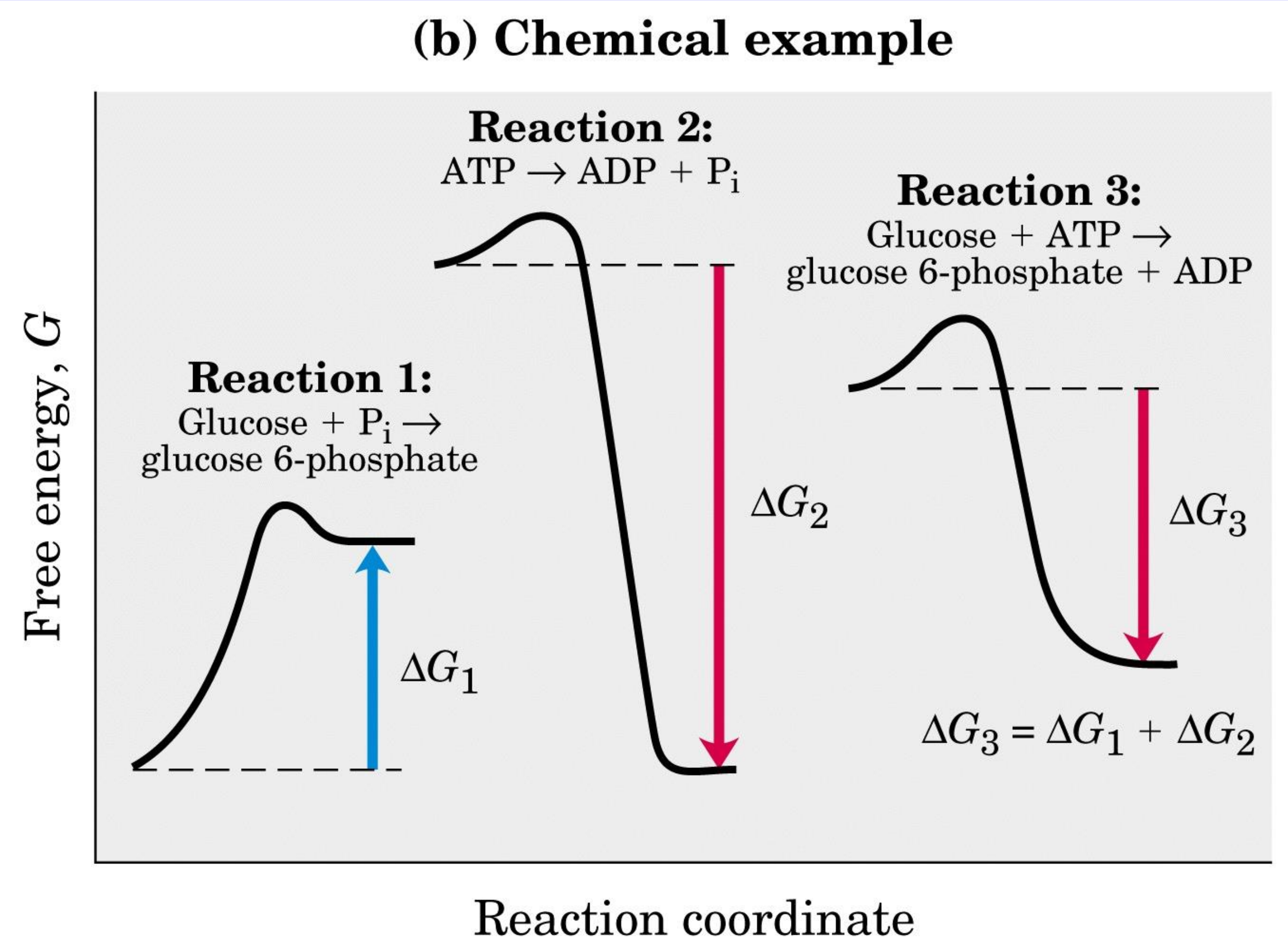
$$(2) \quad \cancel{B} \longrightarrow C \qquad \Delta G'^{\circ}_{2} = -32 \text{ kJ/mole}$$

$$\mathit{Sum:} \quad A \longrightarrow C \qquad \Delta G'^{\circ}_{1} + \Delta G'^{\circ}_{2}$$

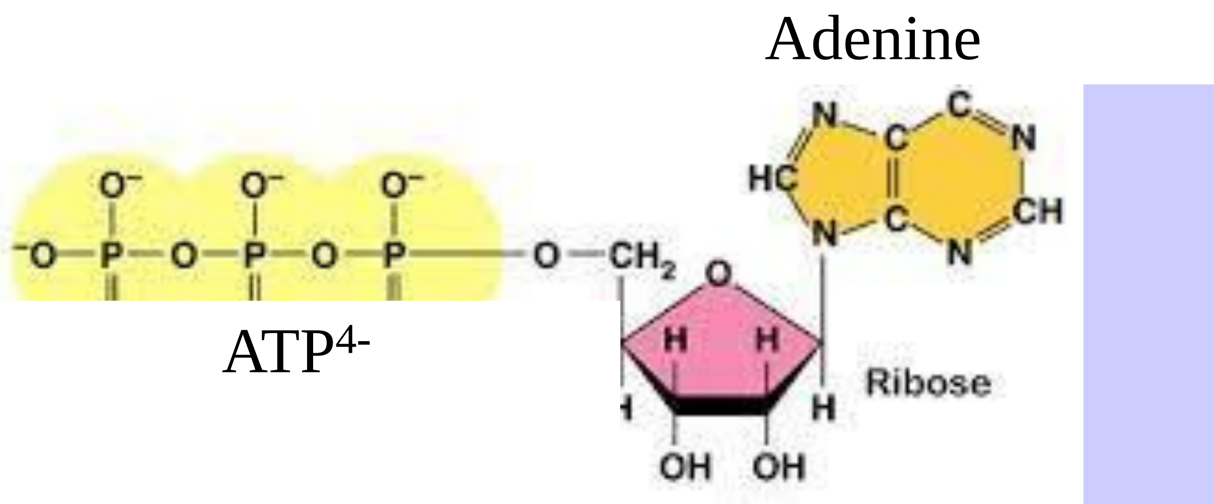
exergonic

$$= -12 \text{ kJ/mole}$$

A pratical Example



Inside the cells, many reactions with a $\Delta G > 0$ are sustained thanks to the **hydrolysis** of ATP



$$ATP^{4-} + H_2O \rightarrow ADP^{3-} + P_i^{2-} + H^+$$

$$\Delta G°' = -30.5\ KJ/mol$$

Free Energies

ATP (adenosine- 5’ triphosphate)

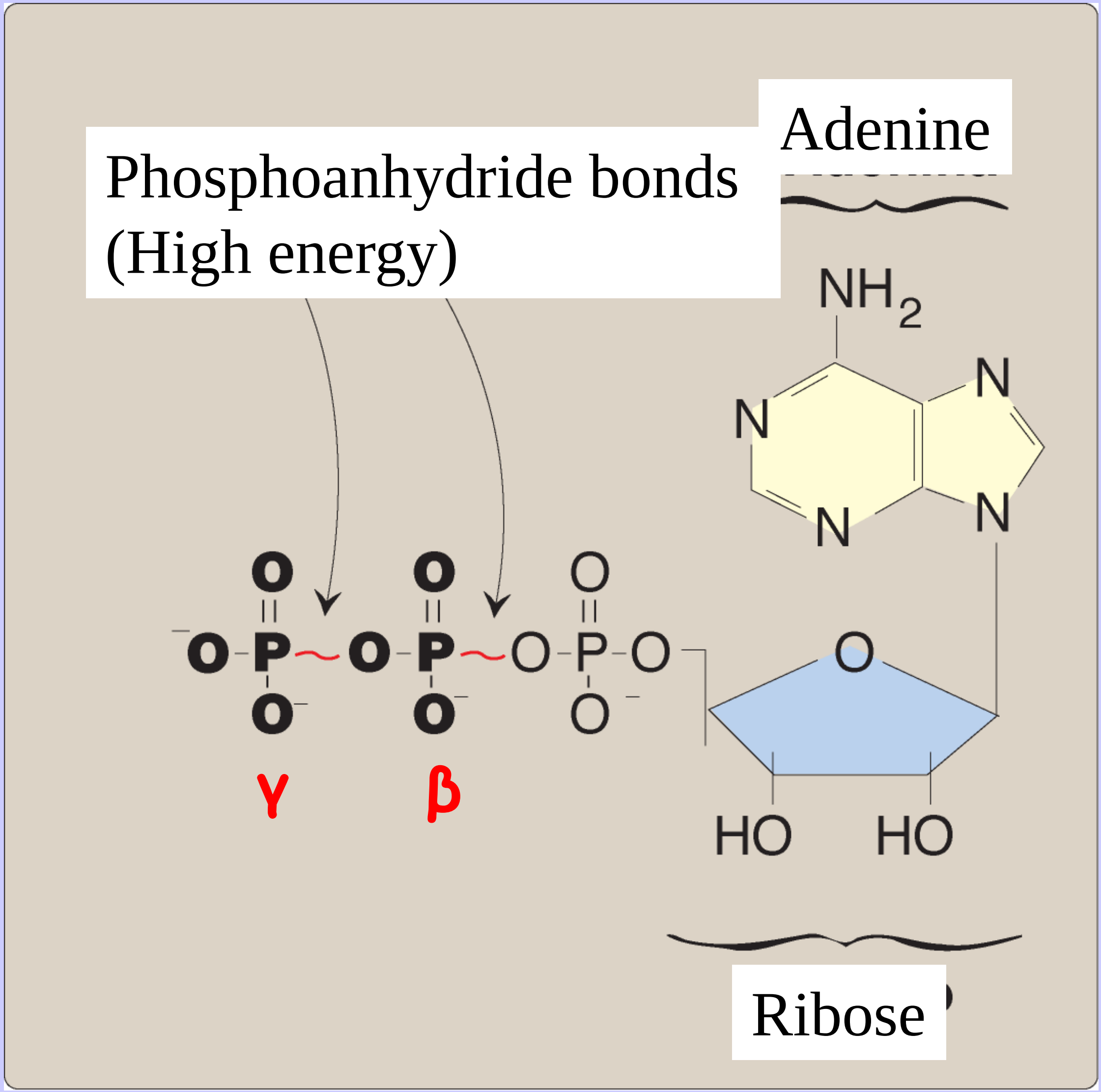
ATP: adenosine- 5' triphosphate

Many coupled reactions use hydrolysis of ATP (highly exergonic process) to make possible the metabolic pathway

Purine nucleotide in which adenine is linked with a glycosidic bond to D-ribose.

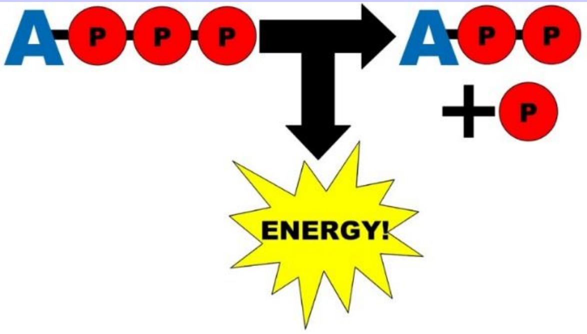
Three phosphate groups are esterified at the 5 position of the D-ribose.

Two phosphate groups (β and γ) are linked with energy-rich phosphoanhydride bonds.



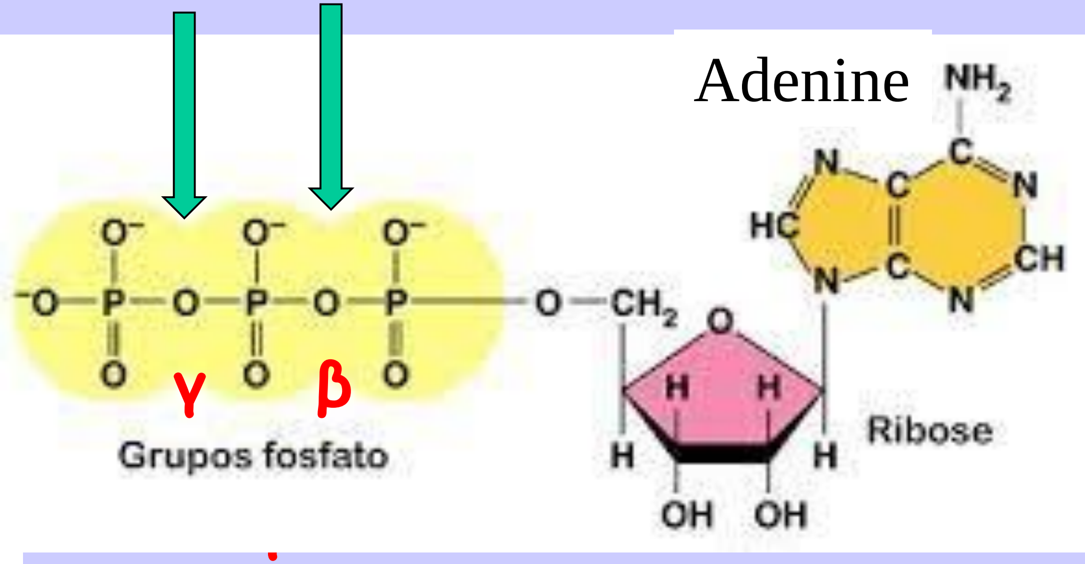
ATP: adenosine- 5' triphosphate

Many coupled reactions use hydrolysis of ATP (highly exergonic process) to make possible the metabolic pathway



Hydrolysis and transfer of terminal phosphate.

Phosphoanidridic bond (High energy)

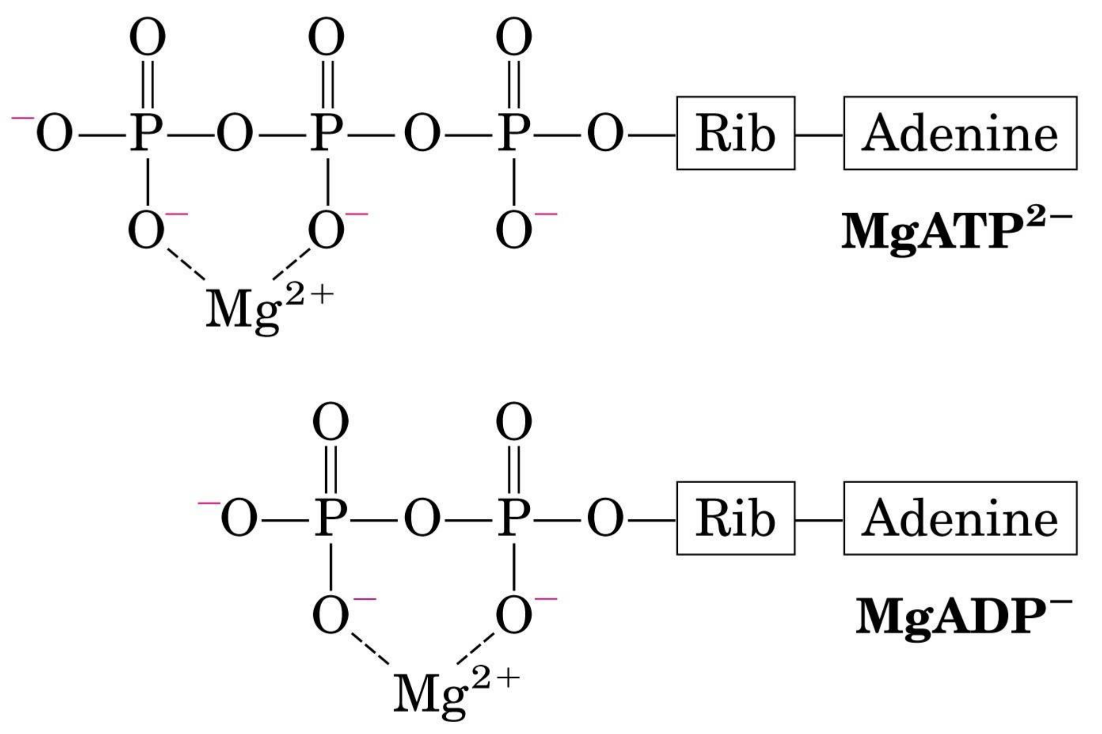


$ATP^{4-} + H_2O \rightarrow ADP^{3-} + P_i^{2-} + H^+$

The reaction is favored following the electrostatic repulsion between the negative charges of the phosphate groups

Factors that contribute to making ATP hydrolysis exergonic

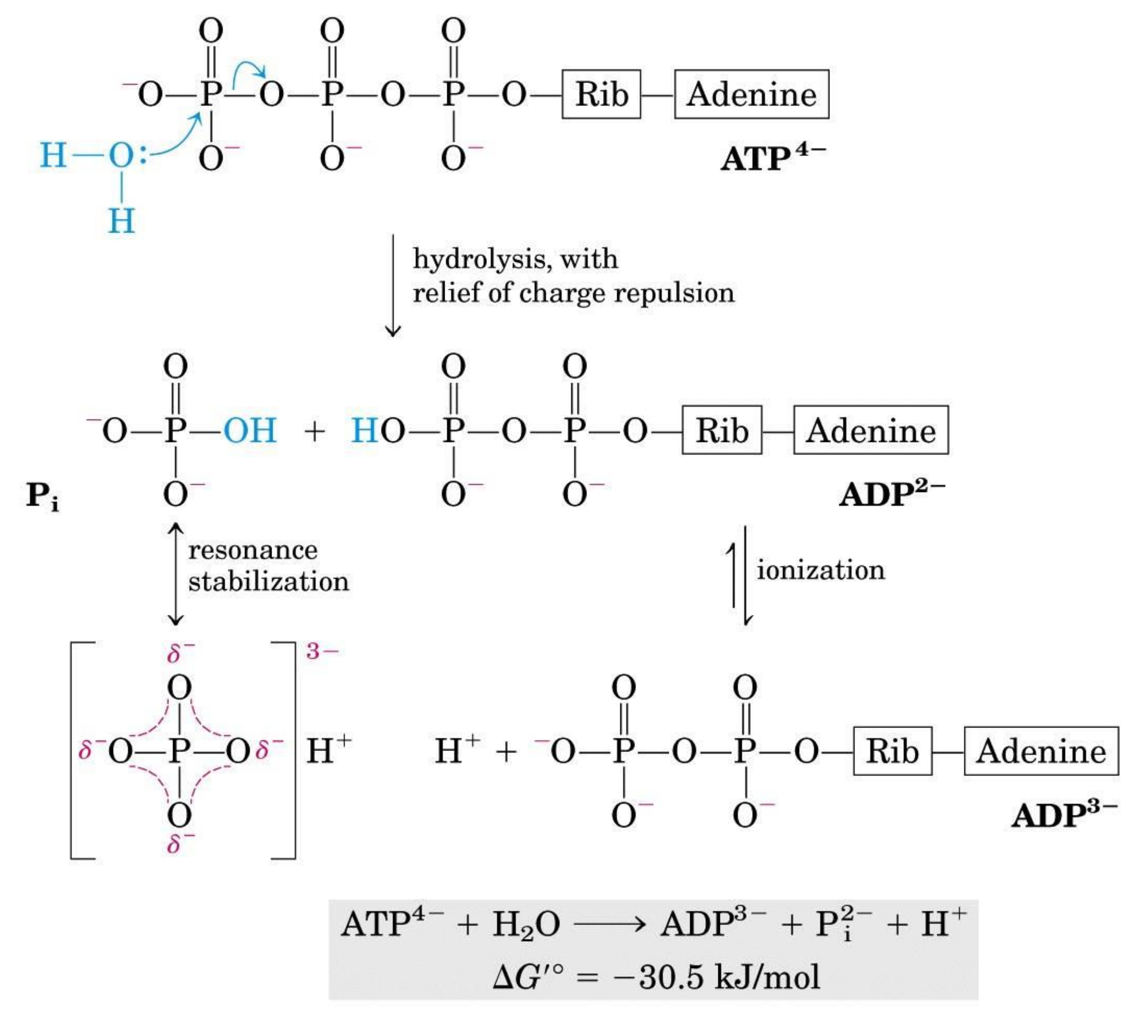
Hydrolysis products (ADP, AMP and Pi) have a greater neutralization of the negative charges of the phosphoanhydride groups by the Mg^{2+} ions (decrease in electrostatic repulsion)



ATP and ADP are chelated with bivalent cation such as magnesium in physiological condition

Formation of Mg2+ complexes partially shields the negative charges and influences the conformation of the phosphate groups in nucleotides such as ATP and ADP.

Factors that contribute to making ATP hydrolysis exergonic



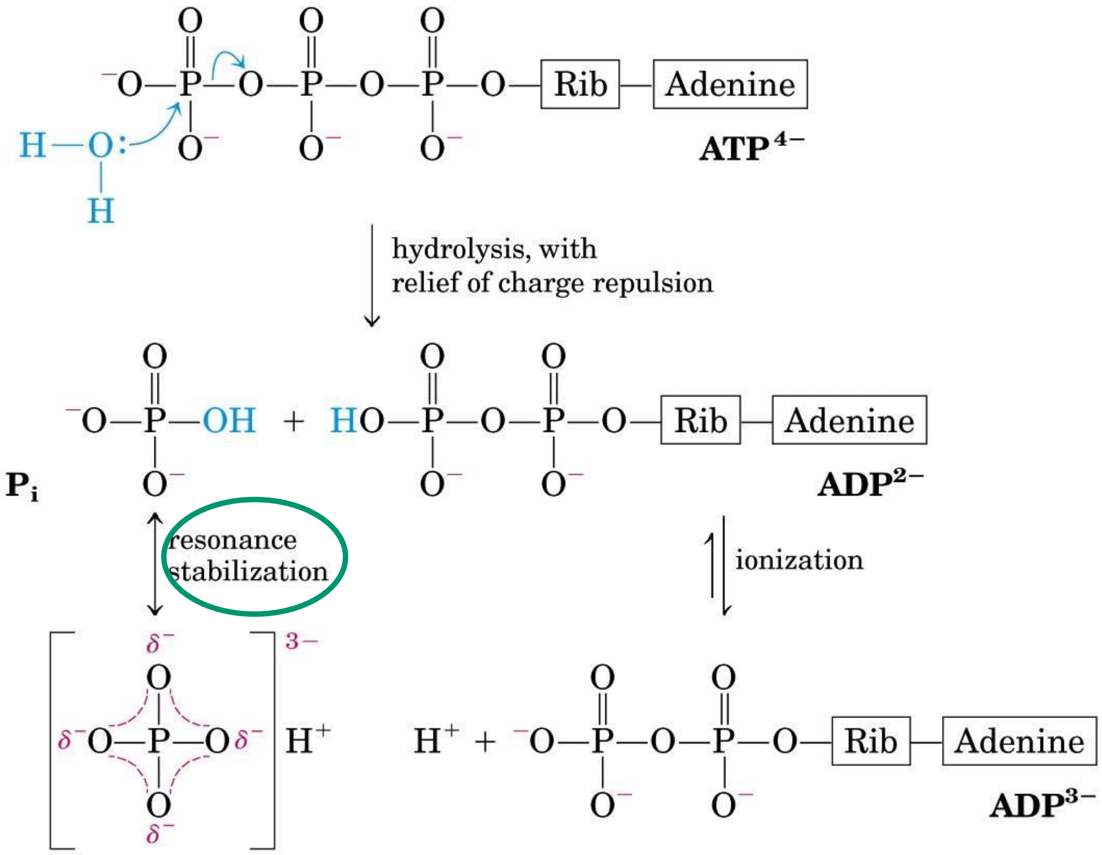
$$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + P_i^{2-} + H^+$$

$$\Delta G'^{\circ} = -30.5 \text{ kJ/mol}$$

The hydrolysis products (ADP, AMP, Pi) are better solvated than ATP itself. The solvation sphere helps shield negative charges.

Factors that contribute to making ATP hydrolysis exergonic

Resonance of organic phosphate



$$\mathrm{ATP^{4-}} + \mathrm{H_2O} \longrightarrow \mathrm{ADP^{3-}} + \mathrm{P_i^{2-}} + \mathrm{H^+}$$

$$\Delta G'^{\circ} = -30.5\ \mathrm{kJ/mol}$$

The hydrolysis products (ADP, AMP, Pi) are more stable than ATP.

Stabilization occurs by resonance: the electrons on the terminal oxygen atoms are more delocalized than on the oxygens that act as a bridge in the phosphoanhydride bonds.

EXERGONIC REACTION

$ATP + H_2O \rightarrow ADP + P_i$ $\Delta G^{\circ\prime} = -30.5$ KJ/mol

ENDERGONIC REACTION

$ADP + P_i \rightarrow ATP + H_2O$ $\Delta G^{\circ\prime} = +30.5$ KJ/mol

$\Delta G^{\circ\prime} < 0$

Spontaneus reaction
(EXERGONIC)

$\Delta G^{\circ\prime} > 0$

Not-spontaneus reaction
(ENDERGONIC)

$\Delta G^{\circ\prime} = 0$

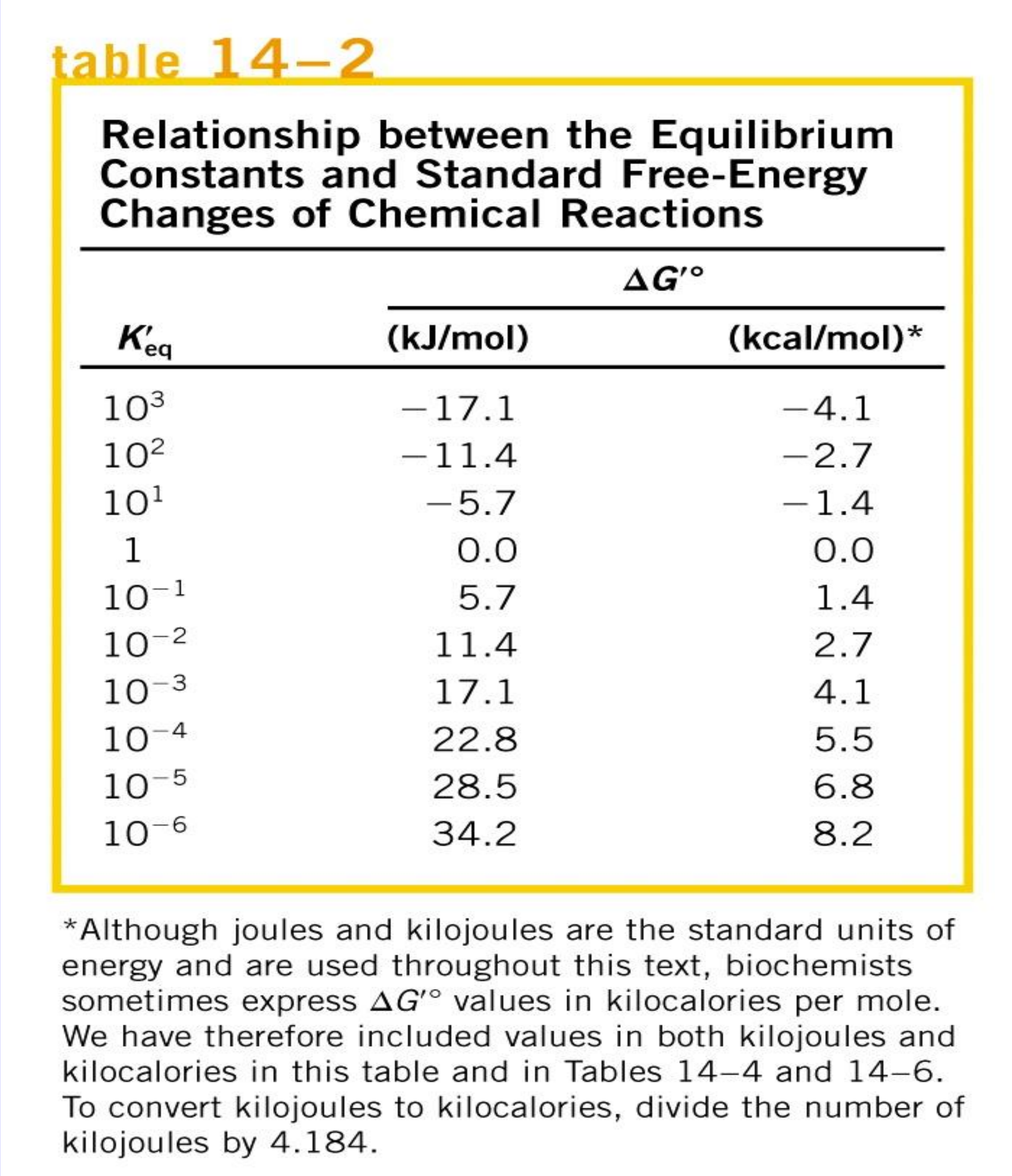


table 14–2

Relationship between the Equilibrium Constants and Standard Free-Energy Changes of Chemical Reactions

	$\Delta G'^{\circ}$	
K'_{eq}	(kJ/mol)	(kcal/mol)*
10^{3}	−17.1	−4.1
10^{2}	−11.4	−2.7
10^{1}	−5.7	−1.4
1	0.0	0.0
10^{-1}	5.7	1.4
10^{-2}	11.4	2.7
10^{-3}	17.1	4.1
10^{-4}	22.8	5.5
10^{-5}	28.5	6.8
10^{-6}	34.2	8.2

*Although joules and kilojoules are the standard units of energy and are used throughout this text, biochemists sometimes express $\Delta G'^{\circ}$ values in kilocalories per mole. We have therefore included values in both kilojoules and kilocalories in this table and in Tables 14–4 and 14–6. To convert kilojoules to kilocalories, divide the number of kilojoules by 4.184.

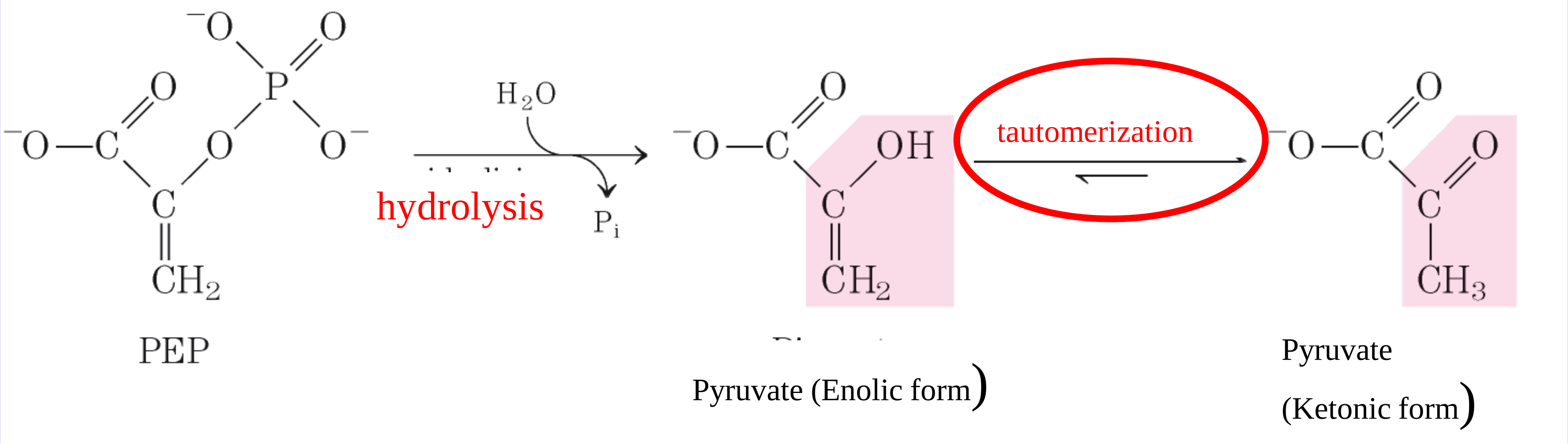
ATP is not unique intermediate which storages energy

ΔG°' (High)

Phosphorylated Compounds have Large Free Energies of Hydrolysis

Phosphoenolpyruvate (PEP) contains a phosphate ester bond that undergoes hydrolysis to yield the enol form of pyruvate, and this direct product can tautomerize to the more stable keto form.

PEP has only one form (enol) and the product (pyruvate) has two possible forms, the product is stabilized respect to the reactant.
This is the greatest contributing factor to the high standard free energy of hydrolysis of phosphoenolpyruvate: ΔG= -61.9 kJ/mol



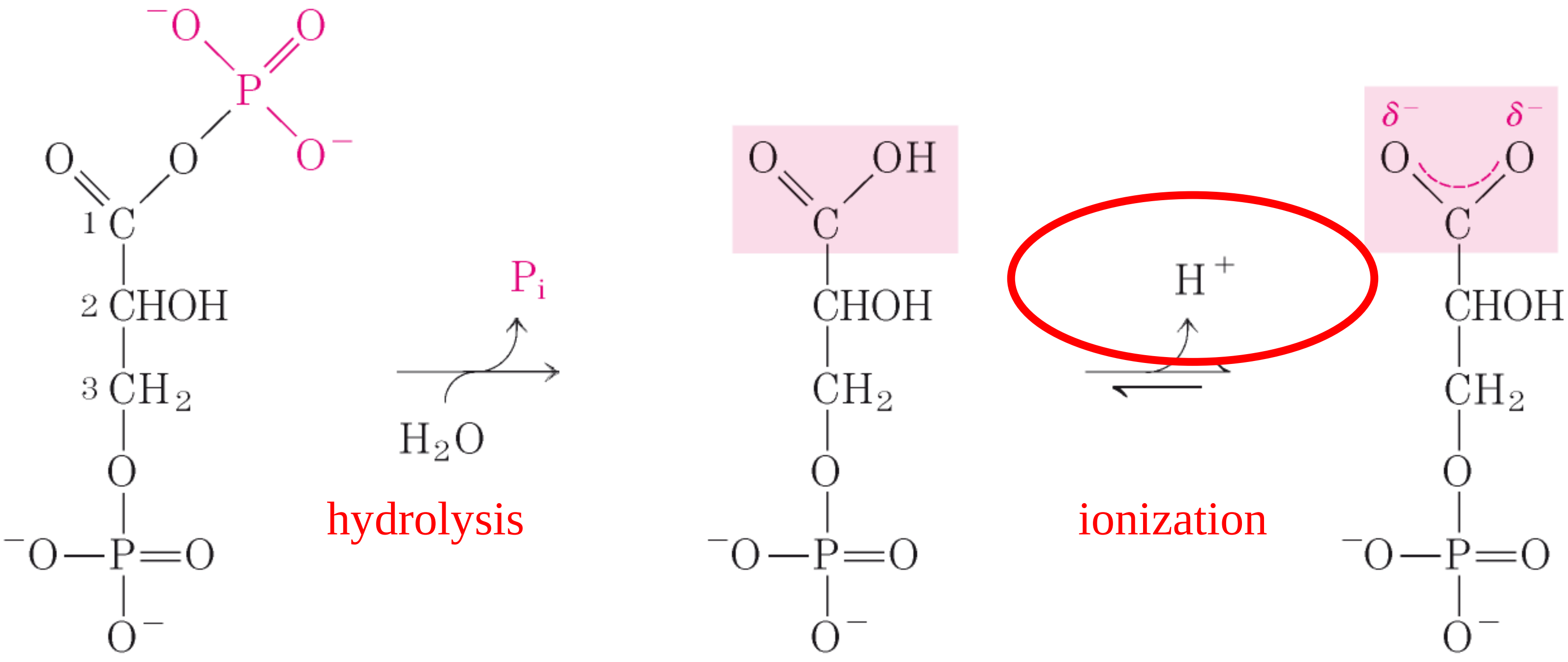
$$PEP^{3-} + H_2O \longrightarrow \text{Pyruvate}^{-} + P_i^{2-}$$
$$\Delta G'^{\circ} = -61{,}9 \text{ kJ/mole}$$

1,3 BISPHOSPHOGLYCERATE (1,3-BPG)

1,3-bisphosphoglycerate contains an anhydride bond between the C-1 carboxyl group and a phosphoric acid.

Hydrolysis of this acyl phosphate is accompanied by a large, negative, standard free-energy (ΔG= - 49.3 kJ/mol).

Anhydride bond



hydrolysis

ionization

risonance

1,3-bisphosphoglycerate,

3-phosphoglyceric acid

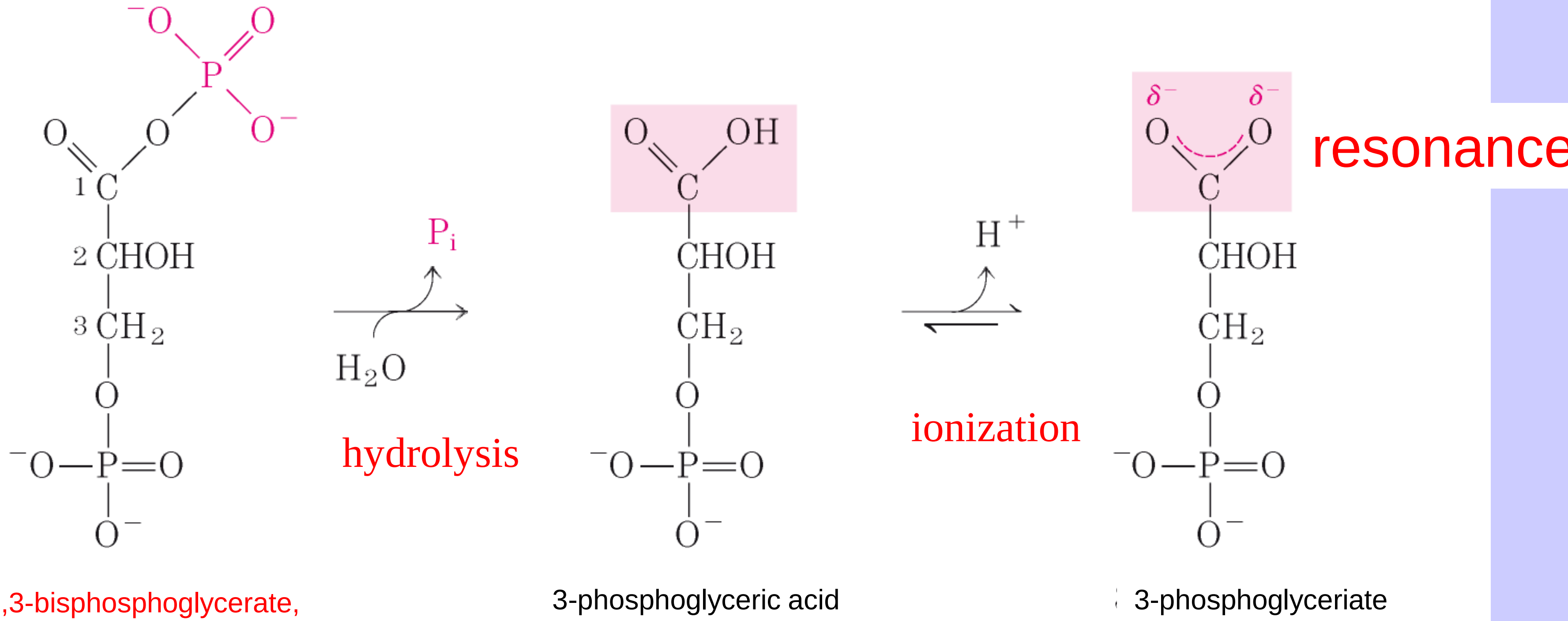
3-phosphoglyceric acid

$$\text{1,3-bisphosphoglycerate,}^{4-} + H_2O \longrightarrow \text{3-phosphoglyceric acid}^{3-} + P_i^{2-} + H^+$$

$$\Delta G'^\circ = -49{,}3 \text{ kJ/mole}$$

.Addition of H_2O is added to 1,3-bisphosphoglycerate,

The product 3-phosphoglyceric acid lose a proton to give the carboxylate ion,
3-phosphoglycerate, which is stabilization by resonance



1,3-bisphosphoglycerate,$^{4-}$ $+ H_2O \longrightarrow$ 3-phosphoglyceric acid^{3-} $+ P_i^{2-} + H^+$

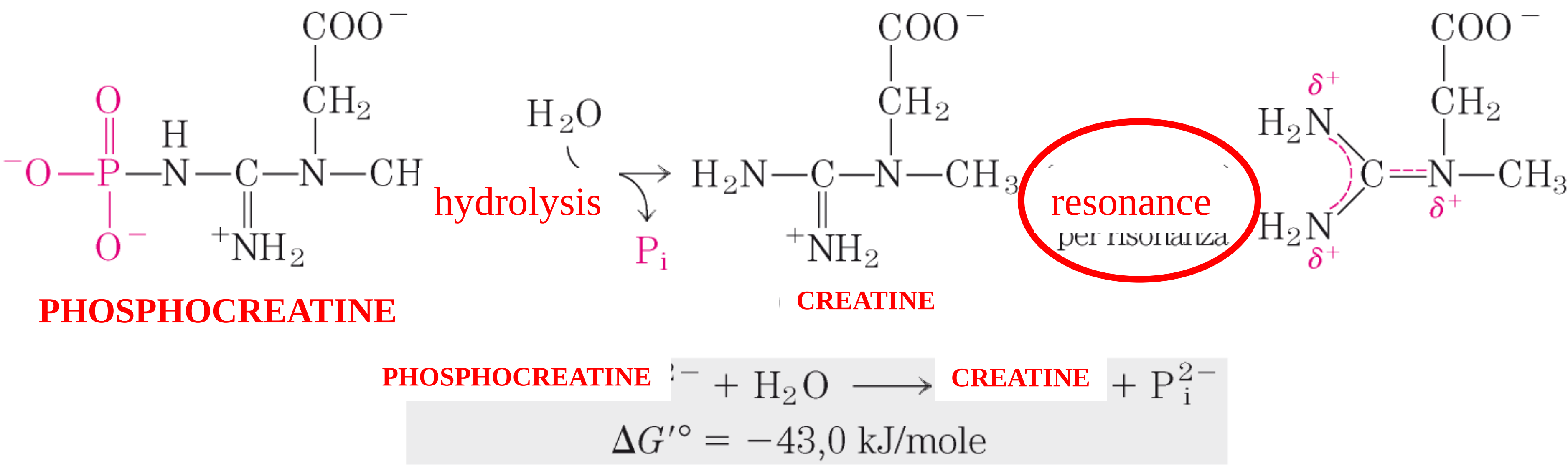
$$\Delta G'^\circ = -49{,}3 \text{ kJ/mole}$$

PHOSPHOCREATINE

Phosphocreatine contains a P-N bond.
Phosphocreatine can be hydrolized to generate free creatine and Pi.

The release of Pi and resonance stabilization of creatine favour the forward reaction.

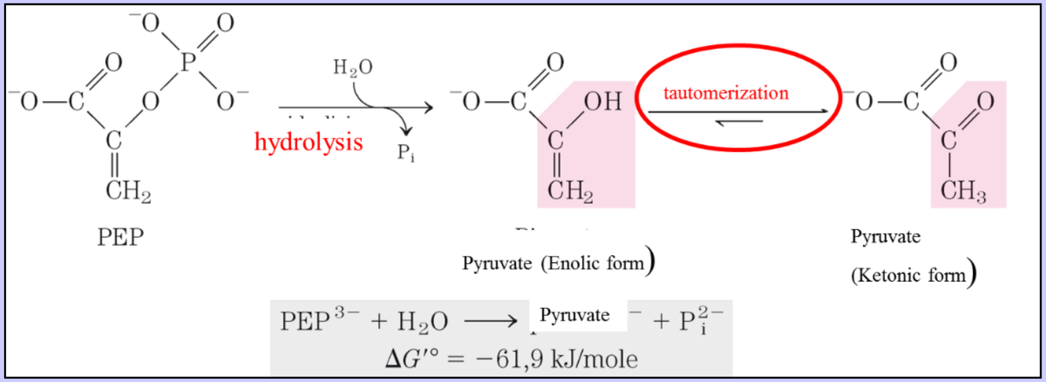
The standard free-energy change of phosphocreatine hydrolysis is again large, -43.0 kJ/mol.



$$\text{PHOSPHOCREATINE} + H_2O \longrightarrow \text{CREATINE} + P_i^{2-}$$

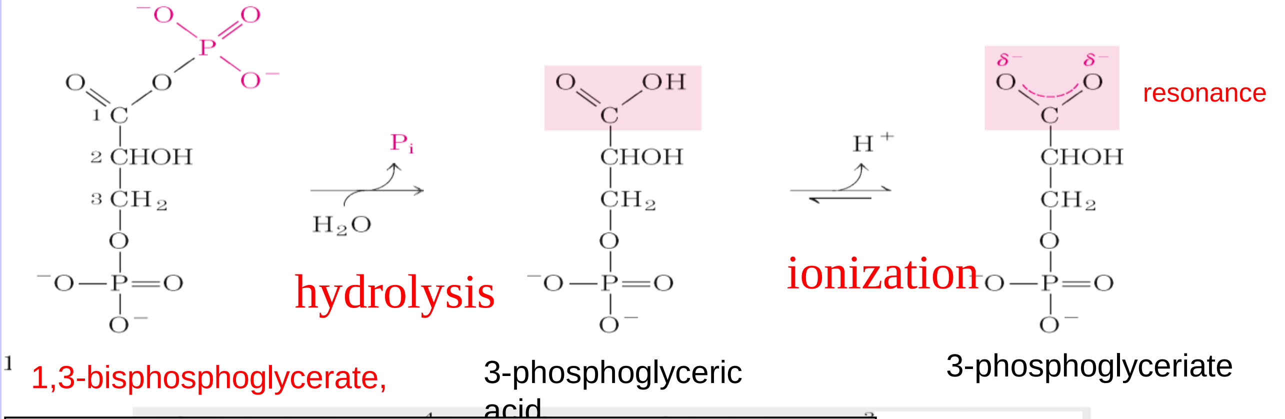
$$\Delta G'^\circ = -43{,}0 \text{ kJ/mole}$$

All these phosphate-releasing reactions, the several resonance forms available to Pi stabilize this product relative to the reactant, contributing to already negative free-energy change



$$PEP^{3-} + H_2O \longrightarrow Pyruvate^{-} + P_i^{2-}$$

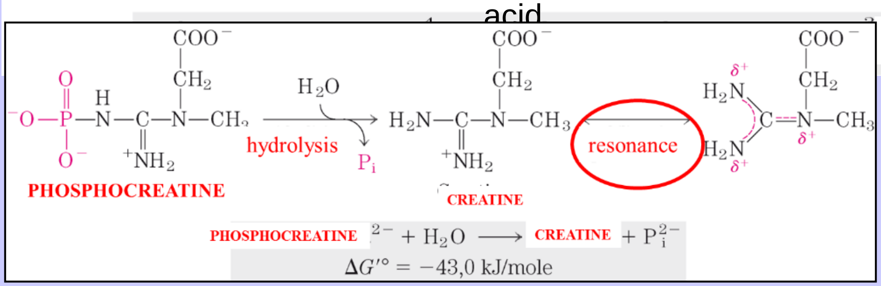
$$\Delta G'^{\circ} = -61{,}9 \text{ kJ/mole}$$



1,3-bisphosphoglycerate,

3-phosphoglyceric acid

3-phosphoglyceriate



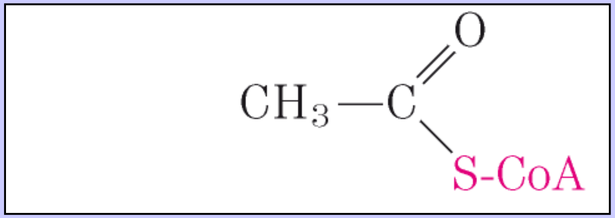
$$PHOSPHOCREATINE^{2-} + H_2O \longrightarrow CREATINE + P_i^{2-}$$

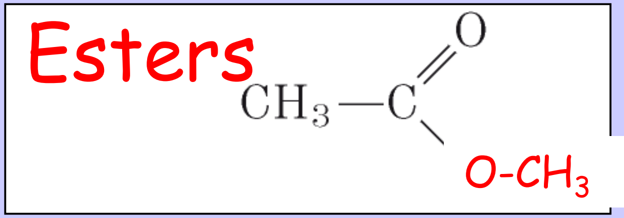
$$\Delta G'^{\circ} = -43{,}0 \text{ kJ/mole}$$

Thioesters have Large Free Energy of Hydrolysis

Acetyl-coenzyme A

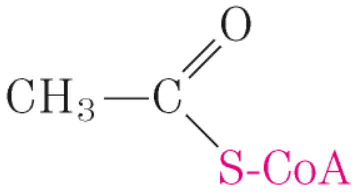
Acetyl-coenzyme A, or acetyl-CoA is one of many thioesters important in metabolism.




Esters

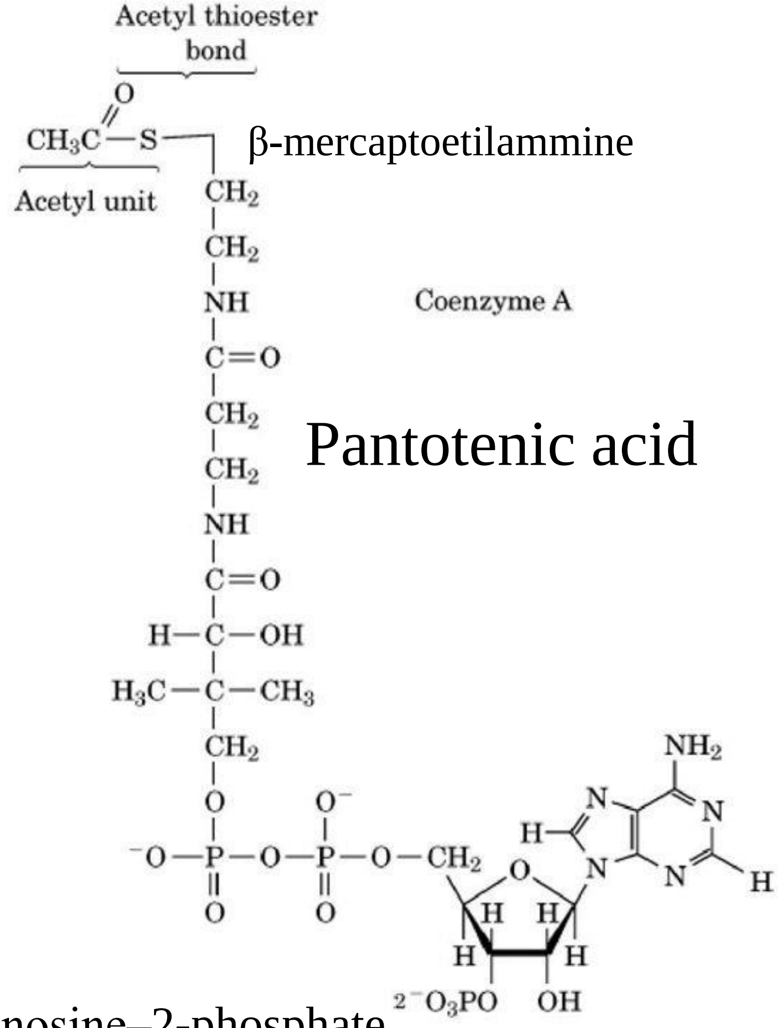
A sulfur atom replaces the usual oxygen in the ester bond,
Acetyl-CoA have large, negative, standard free energy of hydrolysis.

Thioesters have Large Free Energies of Hydrolysis



Acetil-CoA

ADP
Pantotenic acid
β-mercaptoetilammine



β-mercaptoetilammine

Pantotenic acid

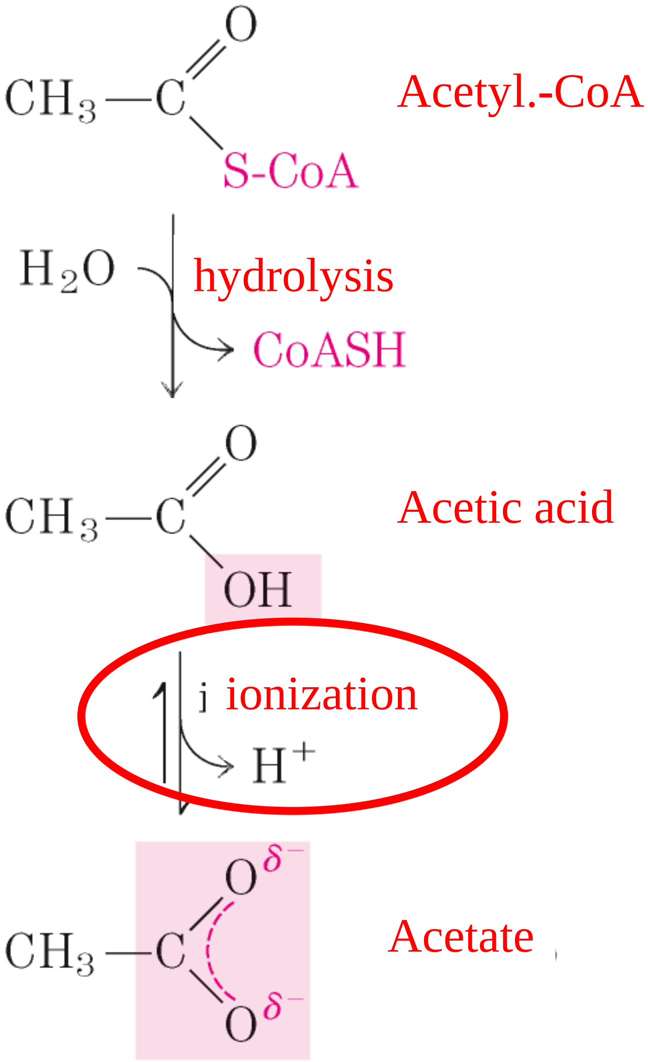
- Adenosine–2-phosphate

Pantotenic acid:
pantoic acid+ β -alanine

Thioesters have Large Free Energy of Hydrolysis

Hydrolysis of the ester generates a carboxylic acid, which can ionize and assume several resonance forms.

Its hydrolysis products *are* resonance-stabilized,



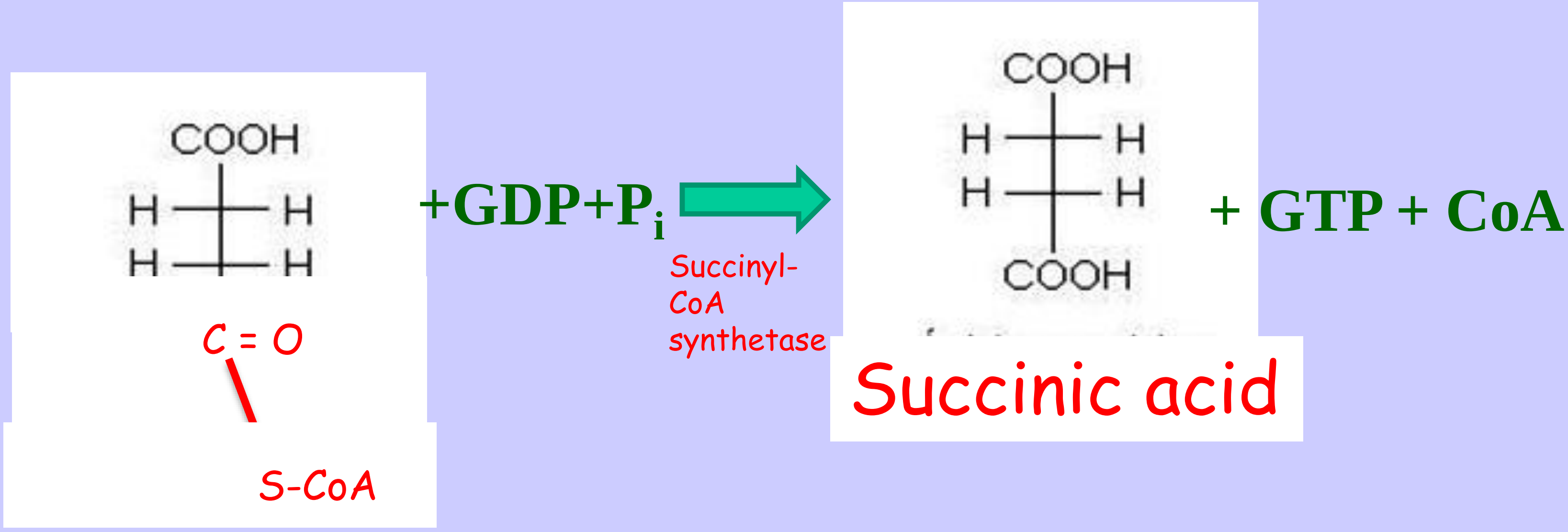
resonance

The result is a large, negative

Acetyl-CoA $+ H_2O \longrightarrow$ Acetate $+$ CoA $+ H^+$

$\Delta G'^\circ = -31{,}4$ kJ/mole

High Free Energy of Thioester is used in Krebs Circle



C = O

S-CoA

Succinil-CoA

$+GDP+P_i$

Succinyl-CoA synthetase

Succinic acid

+ GTP + CoA

Considerations

1) The tension of the bonds in the reactants, due to electrostatic repulsion, decreases following the separation of charges (ATP)

2) The products are stabilized by ionization (ATP, thioesters)

3) The products are stabilized by tautomerization (PEP)

4) The products are stabilized by resonance (Phosphocreatine)

table 14–6

Standard Free Energies of Hydrolysis of Some Phosphorylated Compounds and Acetyl-CoA (a Thioester)

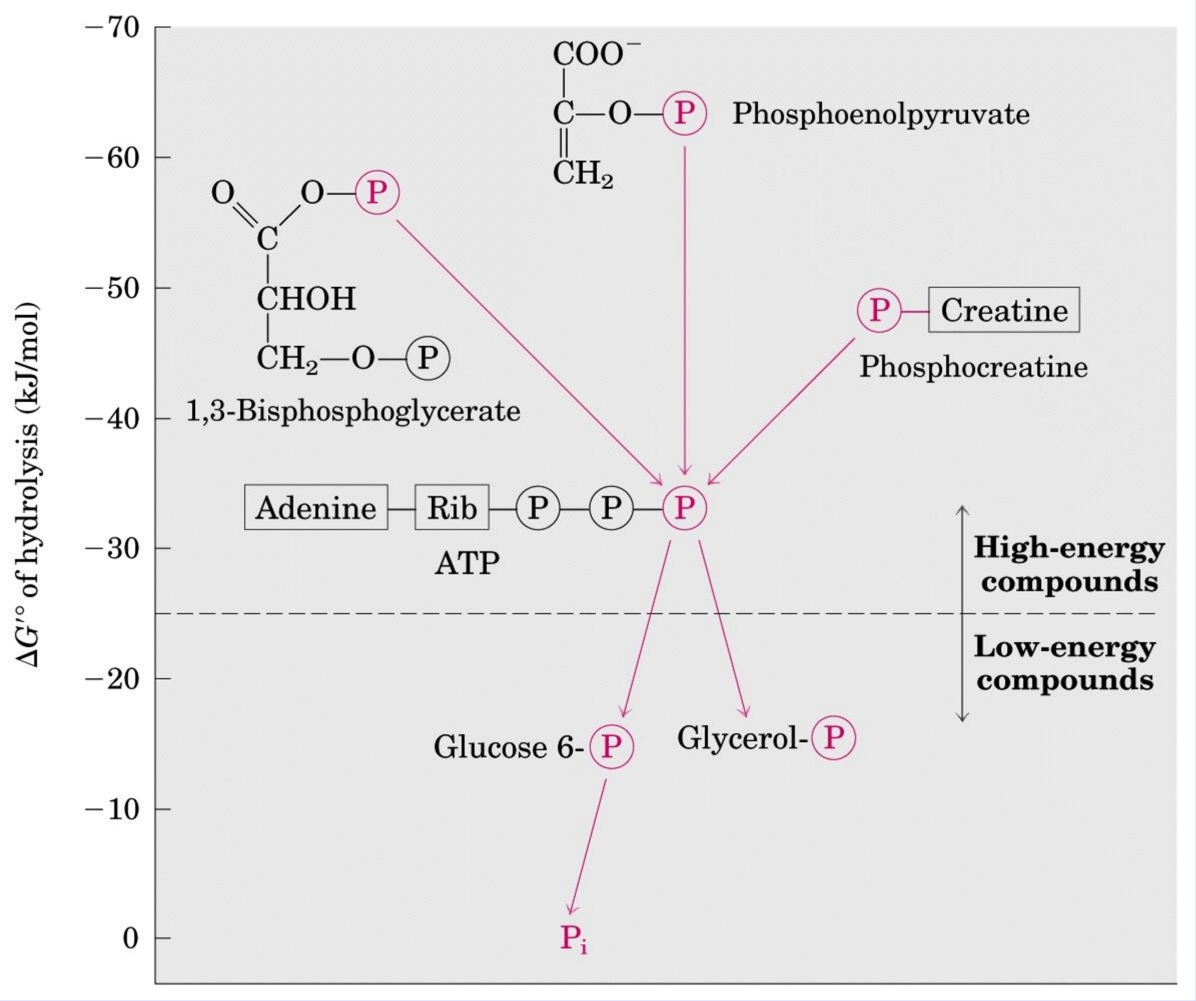
	$\Delta G'^\circ$	
	(kJ/mol)	(kcal/mol)
Phosphoenolpyruvate	−61.9	−14.8
1,3-bisphosphoglycerate (→ 3-phosphoglycerate + P_i)	−49.3	−11.8
Phosphocreatine	−43.0	−10.3
ADP (→ AMP + P_i)	−32.8	−7.8
ATP (→ ADP + P_i)	−30.5	−7.3
ATP (→ AMP + PP_i)	−45.6	−10.9
AMP (→ adenosine + P_i)	−14.2	−3.4
PP_i (→ $2P_i$)	−19	−4.0
Glucose 1-phosphate	−20.9	−5.0
Fructose 6-phosphate	−15.9	−3.8
Glucose 6-phosphate	−13.8	−3.3
Glycerol 1-phosphate	−9.2	−2.2
Acetyl-CoA	−31.4	−7.5

Source: Data mostly from Jencks, W.P. (1976) in *Handbook of Biochemistry and Molecular Biology,* 3rd edn (Fasman, G.D., ed.), *Physical and Chemical Data,* Vol. I, pp. 296–304, CRC Press, Boca Raton, FL.

Metabolites with large, negative, free energy of hydrolysis are classified in two classes:

-High energy compounds: phosphoenolpyruvate
1,3-bisphosphoglycerate
Phosphocreatine

They are good donors of the phosphoryl group.



Another way of transferring energy in addition to the use of ATP, is through the transfer of electrons.

The electron acceptor in oxidation processes is oxygen

Electrons are not directly transferred to oxygen.

Electrons are transferred by special transporters: pyridine nucleotides or flavins

The energy of an electroosmotic gradient of protons straddling the inner mitochondrial membrane is converted into chemical energy during the synthesis of ATP

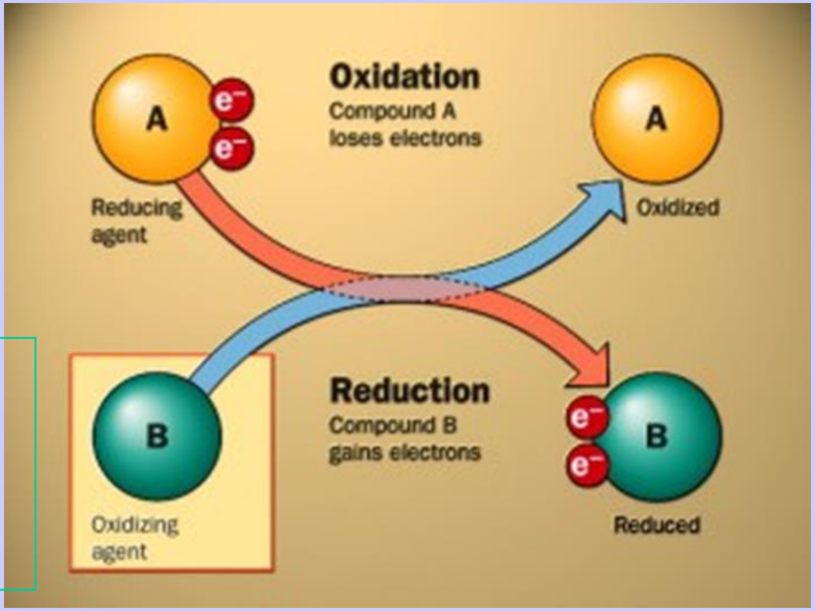
REDOX is a type of chemical reaction in which the oxidation states of atoms are changed.

Redox reactions are characterized by the actual or formal transfer of electrons

between

chemical species (reducing agent) that is oxidized (lose electrons)

another species (oxidizing agent) that is reduced (acquire electrons).



Oxidation is the loss of electrons or an increase in the oxidation state of an atom, an ion, or of certain atoms in a molecule.

Reduction is the acquire of electrons or a decrease in the oxidation state of an atom, an ion, or of certain atoms in a molecule.

There are three main ways in the cell for electron transfer:

1) Transfer of electrons in the form of hydride ion H^- formed by two electrons and a proton (: H) (i.e NAD)

2) Transfer of two hydrogen atoms (two electrons and two protons). (i.e: FAD)

(

3) Direct electron transfer from a redox reaction

$$Fe^{2+} + Cu^{2+} \rightleftharpoons Fe^{3+} + Cu^{+}$$

WHAT are REDUCING EQUIVALENTs?

- Electron: Fe^{2+}/Fe^{3+}
- Hydride (H^-) (2 electrons and 1 proton)
- Hydrogen atoms

Enzymes involved in oxidation and reduction are called oxidoreductases and are they are classified into four groups:

Oxidases, → catalyze reactions in which molecular oxygen is reduced to H_2O or H_2O_2

Dehydrogenases, → Transfer hydrogen from one substrate to another in a coupled oxidation/reduction reaction

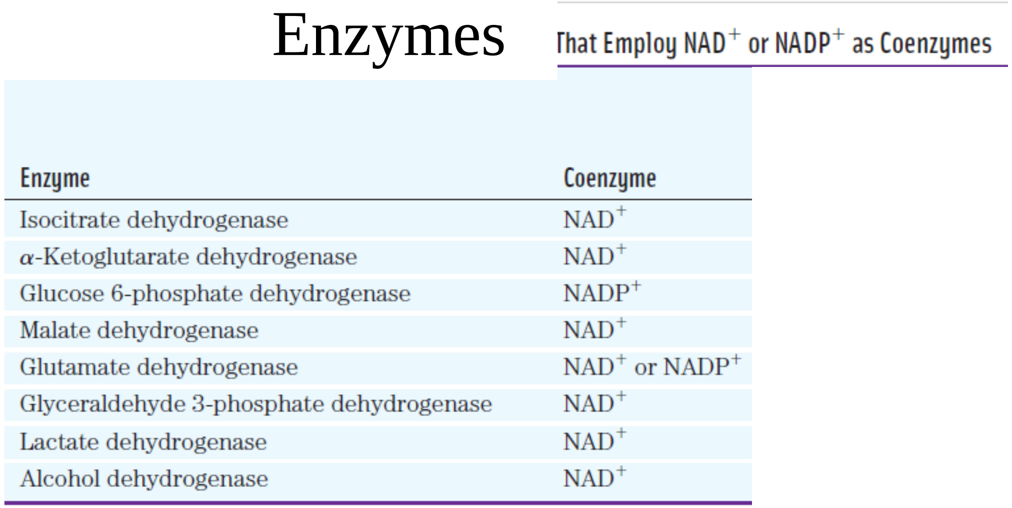
Hydroperoxidases → they have peroxides as a substrate and therefore perform protective functions
(catalase, peroxidase)

Oxygenases. → They catalyze the oxidation of a substrate through the direct incorporation of O_2
(O atom is reduced)

DEHYDROGENASES:

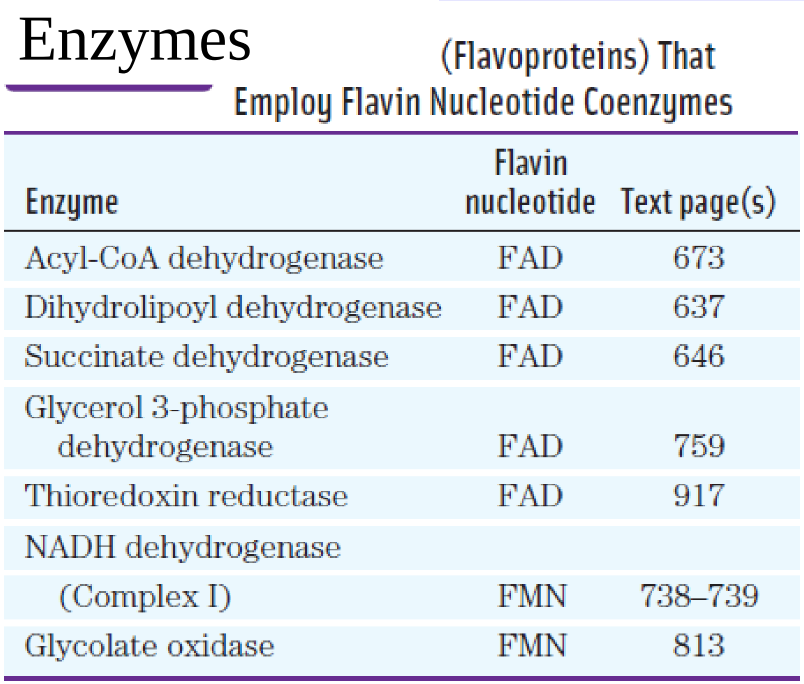
Transfer hydrogen from one substrate to another in a coupled oxidation/reduction reaction.

These dehydrogenases are specific for their substrates but often utilize common coenzymes or hydrogen carriers,



Enzymes That Employ NAD$^+$ or NADP$^+$ as Coenzymes

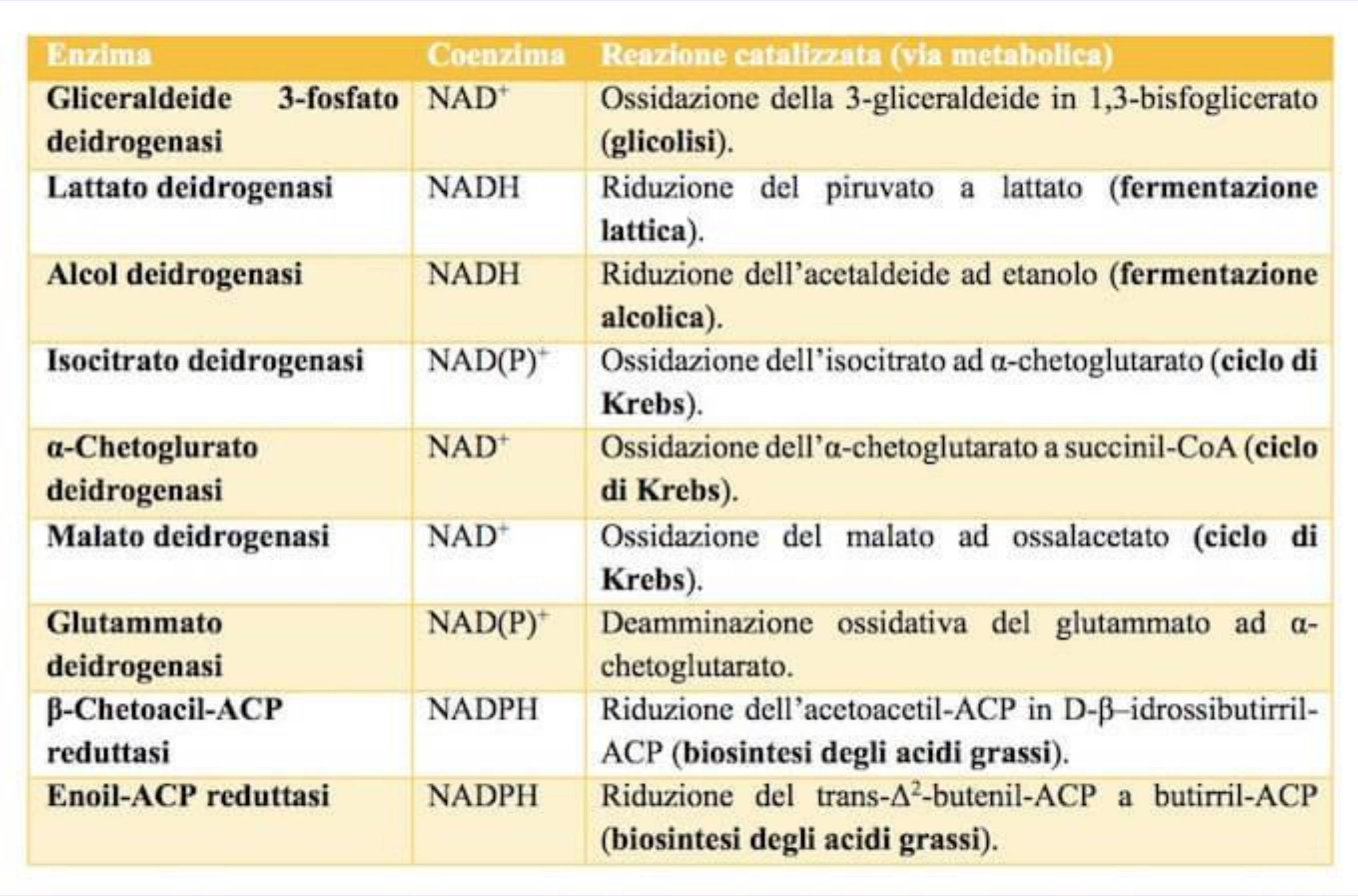
Enzyme	Coenzyme
Isocitrate dehydrogenase	NAD$^+$
α-Ketoglutarate dehydrogenase	NAD$^+$
Glucose 6-phosphate dehydrogenase	NADP$^+$
Malate dehydrogenase	NAD$^+$
Glutamate dehydrogenase	NAD$^+$ or NADP$^+$
Glyceraldehyde 3-phosphate dehydrogenase	NAD$^+$
Lactate dehydrogenase	NAD$^+$
Alcohol dehydrogenase	NAD$^+$



Enzymes (Flavoproteins) That Employ Flavin Nucleotide Coenzymes

Enzyme	Flavin nucleotide	Text page(s)
Acyl-CoA dehydrogenase	FAD	673
Dihydrolipoyl dehydrogenase	FAD	637
Succinate dehydrogenase	FAD	646
Glycerol 3-phosphate dehydrogenase	FAD	759
Thioredoxin reductase	FAD	917
NADH dehydrogenase (Complex I)	FMN	738–739
Glycolate oxidase	FMN	813

Enzyme NAD(P$^+$) dependent

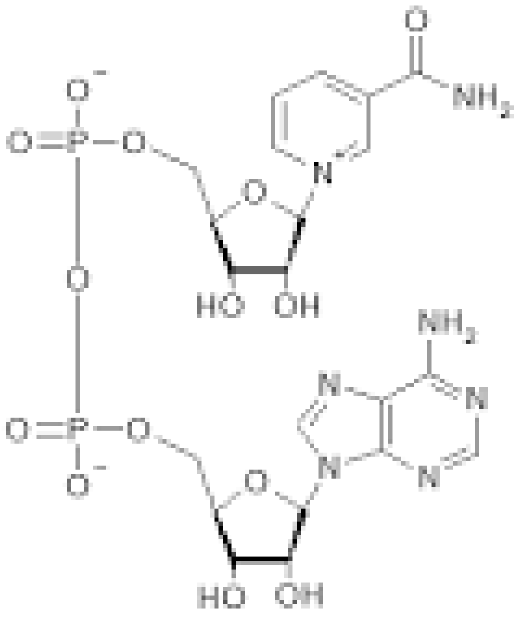


Enzima	Coenzima	Reazione catalizzata (via metabolica)
Gliceraldeide 3-fosfato deidrogenasi	NAD^+	Ossidazione della 3-gliceraldeide in 1,3-bisfoglicerato (**glicolisi**).
Lattato deidrogenasi	NADH	Riduzione del piruvato a lattato (**fermentazione lattica**).
Alcol deidrogenasi	NADH	Riduzione dell'acetaldeide ad etanolo (**fermentazione alcolica**).
Isocitrato deidrogenasi	$NAD(P)^+$	Ossidazione dell'isocitrato ad α-chetoglutarato (**ciclo di Krebs**).
α-Chetoglurato deidrogenasi	NAD^+	Ossidazione dell'α-chetoglutarato a succinil-CoA (**ciclo di Krebs**).
Malato deidrogenasi	NAD^+	Ossidazione del malato ad ossalacetato **(ciclo di Krebs**).
Glutammato deidrogenasi	$NAD(P)^+$	Deamminazione ossidativa del glutammato ad α-chetoglutarato.
β-Chetoacil-ACP reduttasi	NADPH	Riduzione dell'acetoacetil-ACP in D-β–idrossibutirril-ACP (**biosintesi degli acidi grassi**).
Enoil-ACP reduttasi	NADPH	Riduzione del trans-Δ^2-butenil-ACP a butirril-ACP (**biosintesi degli acidi grassi**).

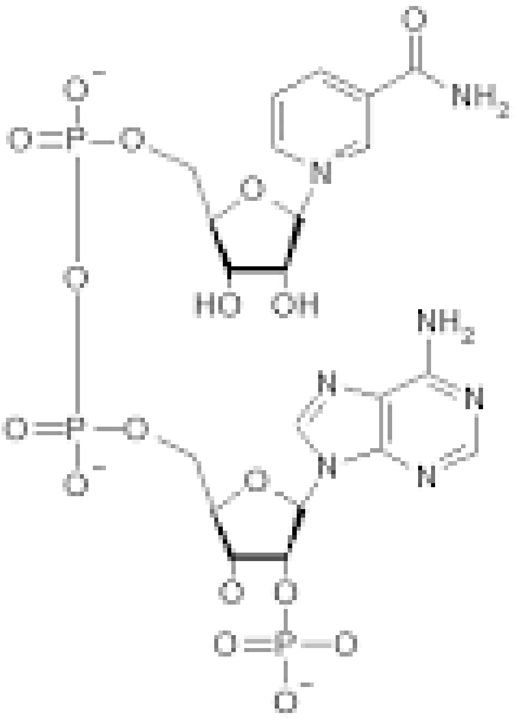
NADH and NADPH

Nicotinamide adenine dinucleotide (NAD)
Nicotinamide adenine dinucleotide phosphate (NADP)

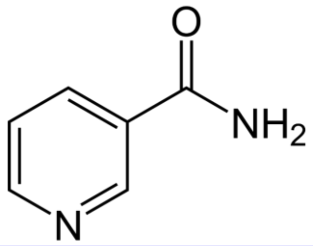
NAD$^+$ is oxidized form
NADP+ is oxidized



NAD



NADP



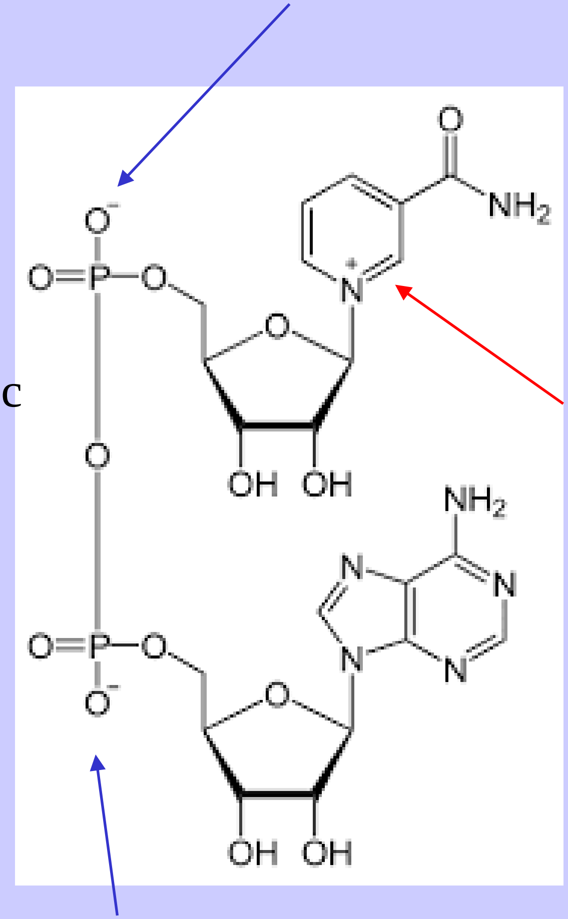
nicotinamide

synthesized from niacin

nicotinic acid
(niacin)
Vitamin PP (Pellagra Preventing)
(vitamin B3)

NAD+

The molecule is indicated with a +, but it is not positively charged as a whole, but is negative due to the presence of negative charges on the phosphate groups

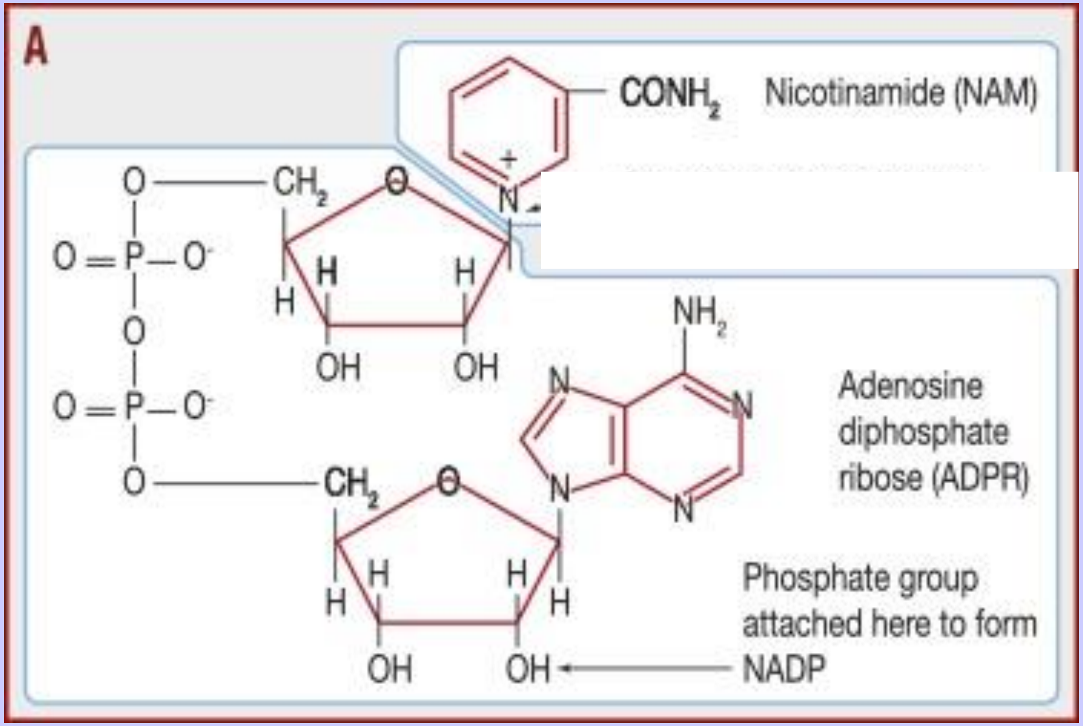


Nicotinammide
mononuleotide
(NMN)

Phoanhydridic
bond

Adenosine
monophosphate
(AMP)

NAD+

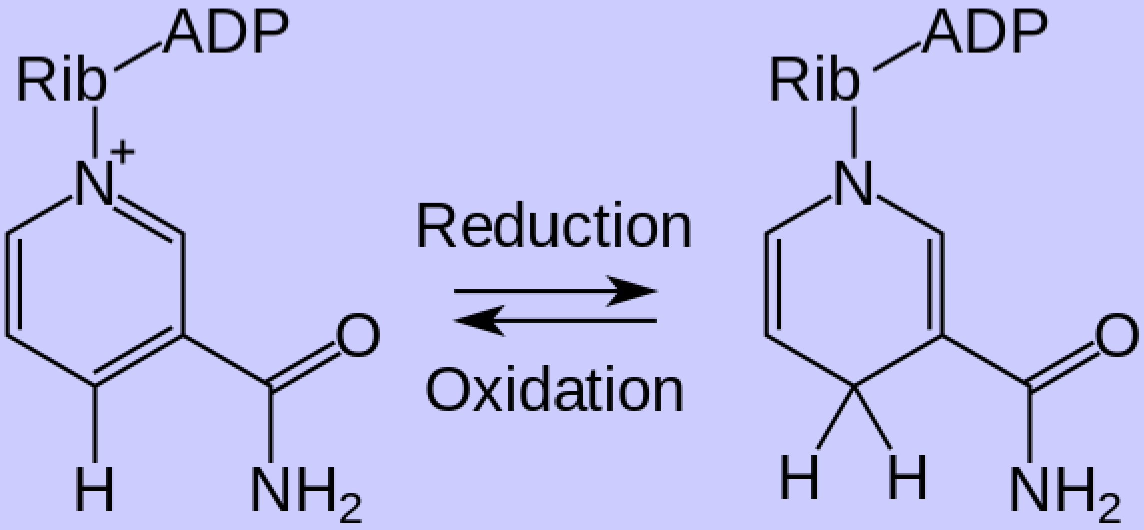


NAD $^+$ accepts one proton and two electrons

Each molecule of NAD$^+$ accepts two electrons and one proton.
(hydride ion H :-)

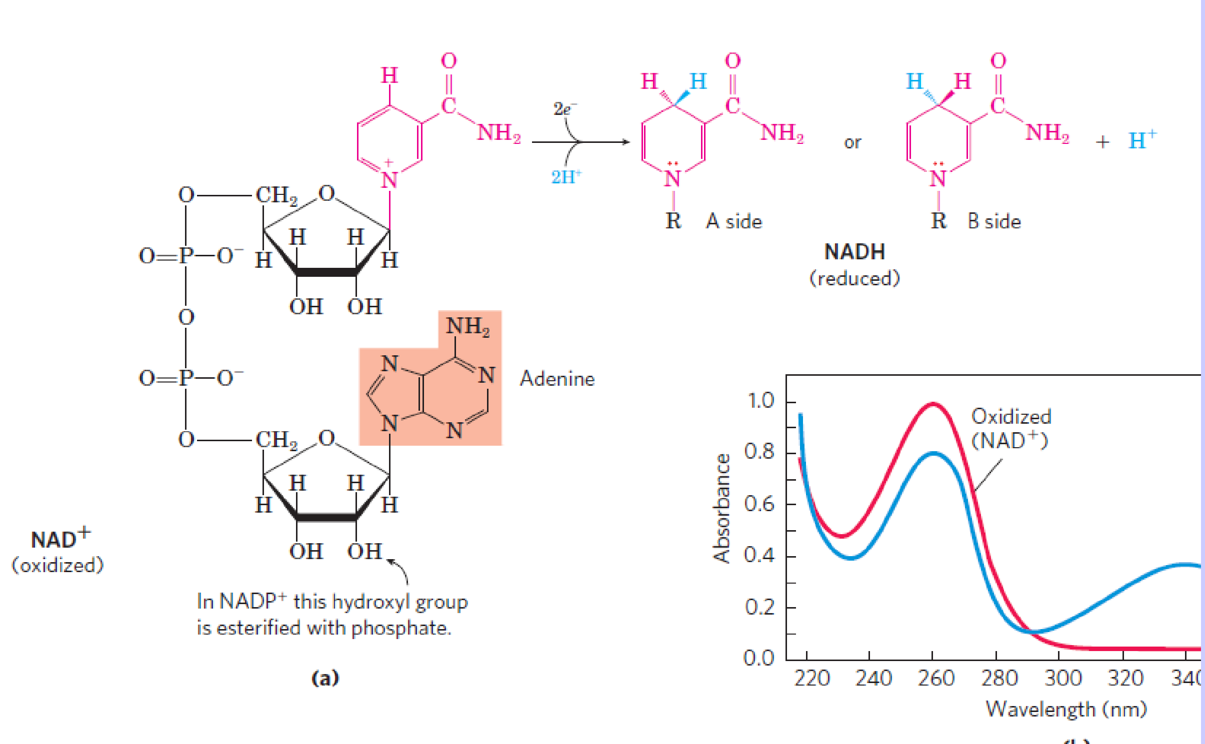
$$NAD^+ + 2H = NADH + H^+$$

The other proton removed from the substrate.



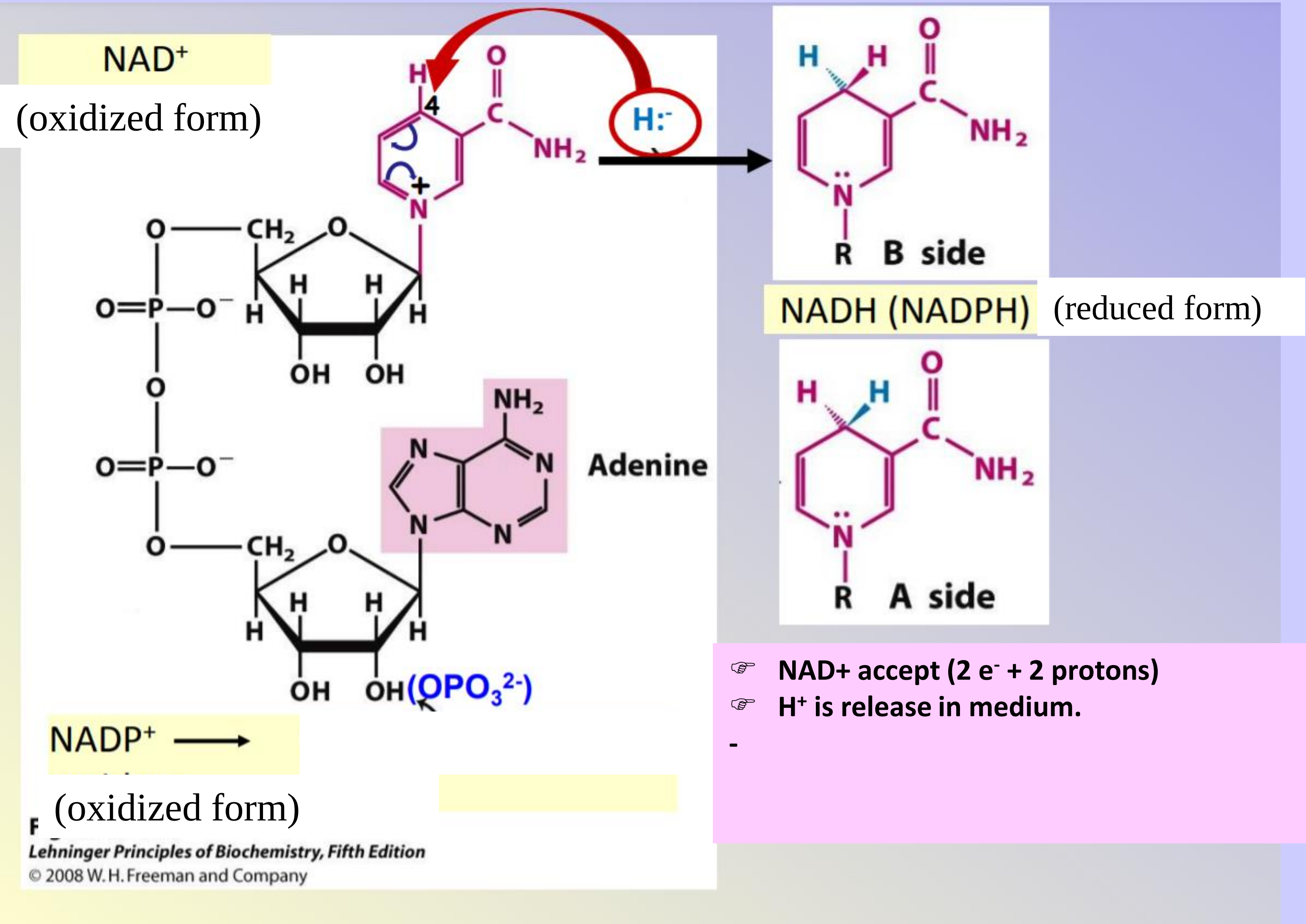
NAD+
(oxidated form)

NADH
(reducing form)



In NADP⁺ this hydroxyl group is esterified with phosphate.

Reduces accepting $H:^-$ hydride ion



Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company

- **NAD+ accept (2 e^- + 2 protons)**
- **H^+ is release in medium.**

-

$$NAD^+ + 2H = NADH + H^+$$

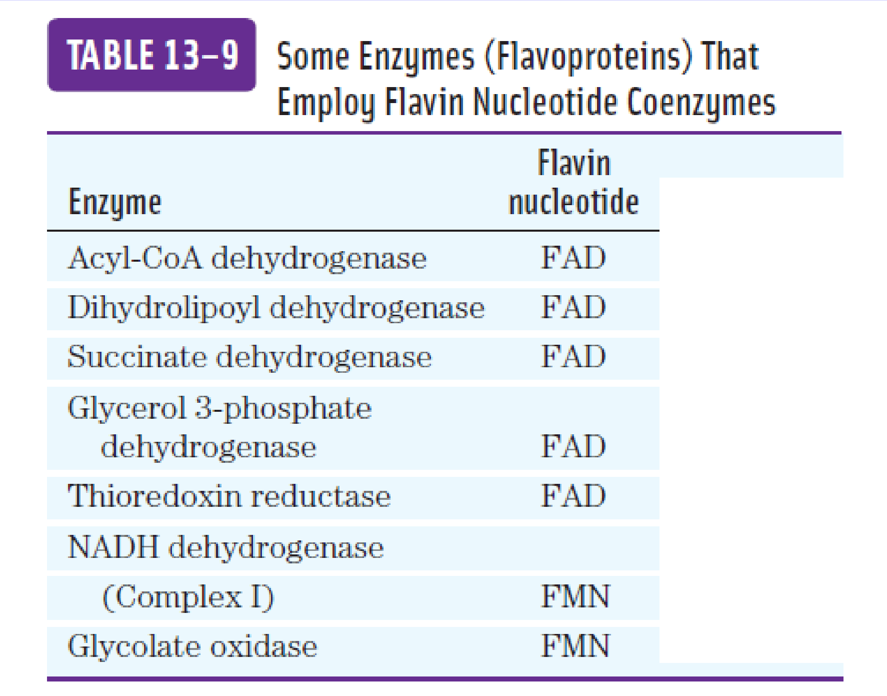


TABLE 13–9 Some Enzymes (Flavoproteins) That Employ Flavin Nucleotide Coenzymes

Enzyme	Flavin nucleotide
Acyl-CoA dehydrogenase	FAD
Dihydrolipoyl dehydrogenase	FAD
Succinate dehydrogenase	FAD
Glycerol 3-phosphate dehydrogenase	FAD
Thioredoxin reductase	FAD
NADH dehydrogenase (Complex I)	FMN
Glycolate oxidase	FMN

The flavin nucleotide in most flavoproteins is bound rather tightly to the protein, and in some enzymes, such as succinate dehydrogenase, it is bound covalently.

FAD,

flavin-adenin-dinucleotide

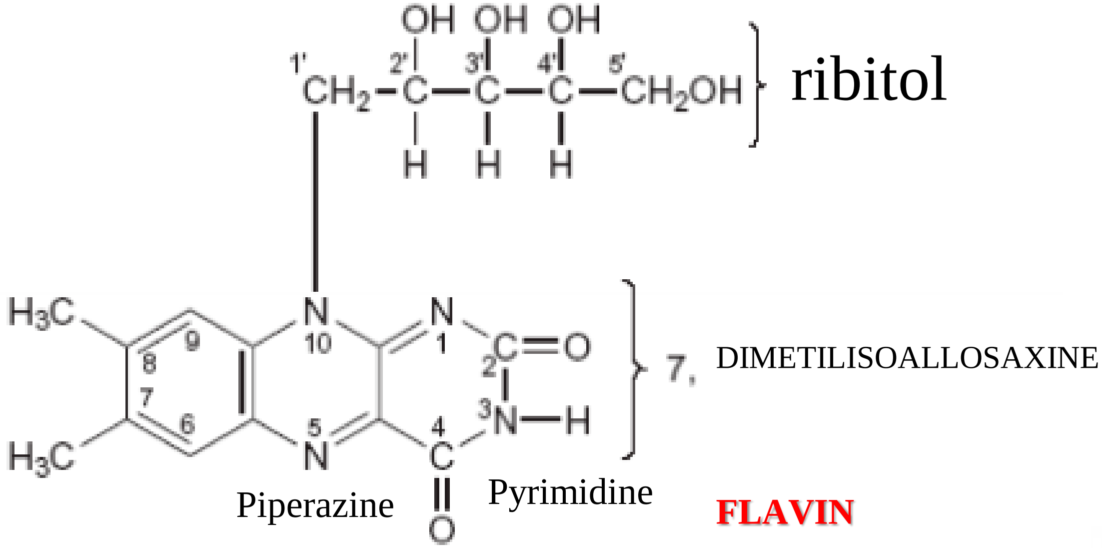
FMN

flavin-mononucleotide

FAD, **flavin-adenin-dinucleotide**

FMN **flavin-mononucleotide**

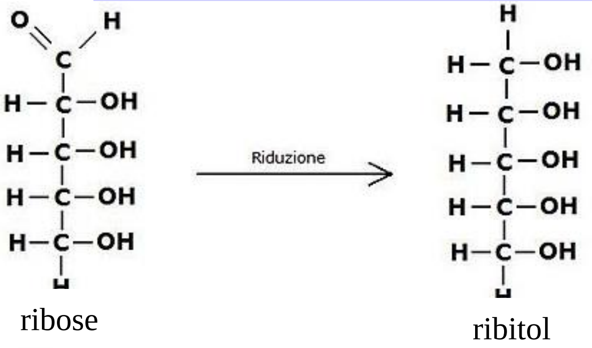
Derivative from Vitamin B2 (riboflavin)



RIBOFLAVIN

Riboflavin

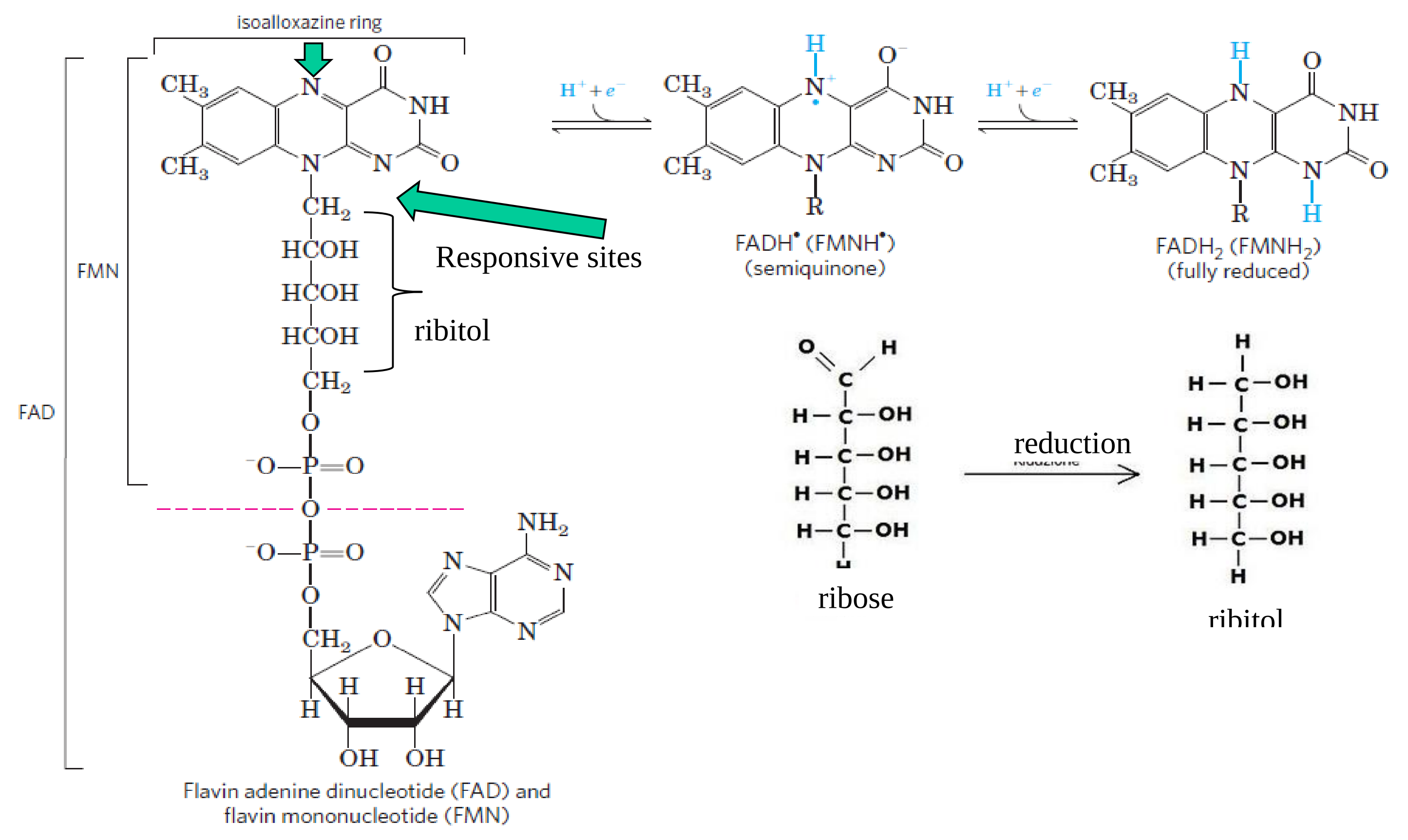
It consists of five carbon atoms of the ribitol molecule bonded to the N10 atom of isallosaxine



ribose

ribitol

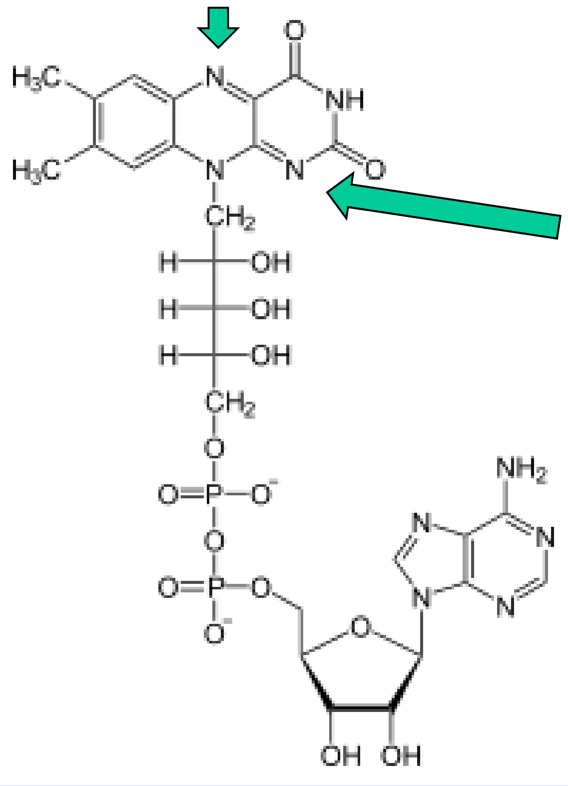
The Flavin nucleotides accept two hydrogen atoms (two electrons and two protons), both of which appear in the flavin ring system.
When FAD or FMN accepts only one hydrogen atom, the semiquinone, a stable freeradical, forms.



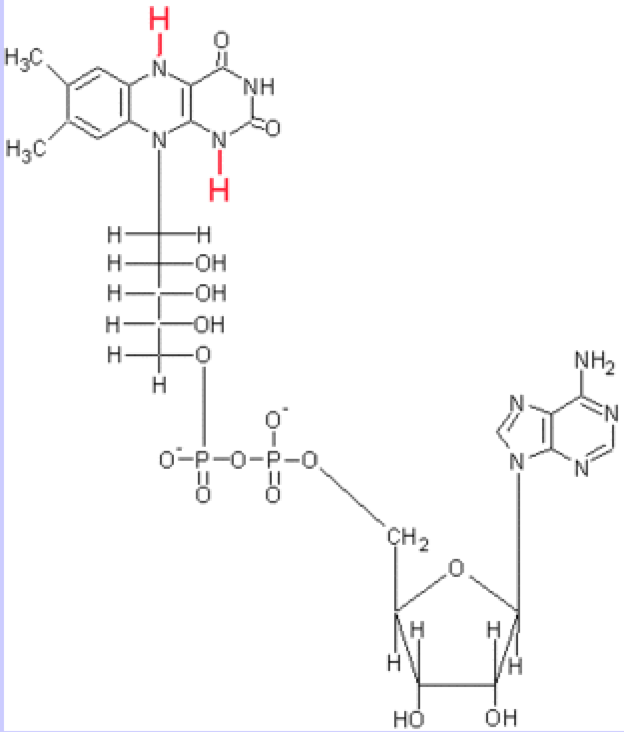
Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

Flavin-adenin-dinucleotide (FAD)

$FAD+ + 2H > FADH_2$



FAD

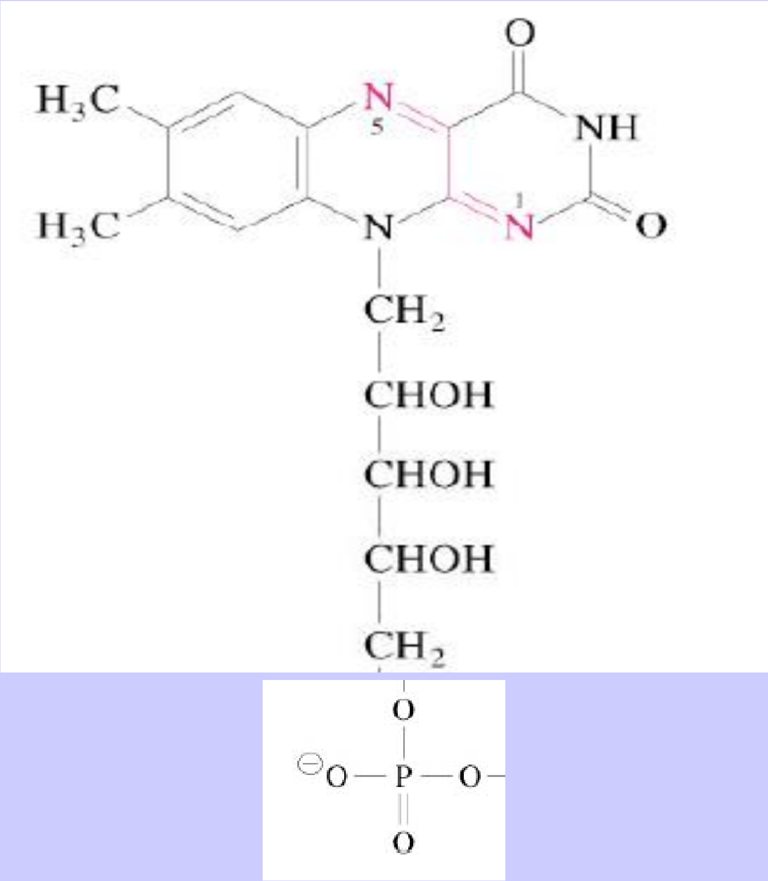


$FADH_2$

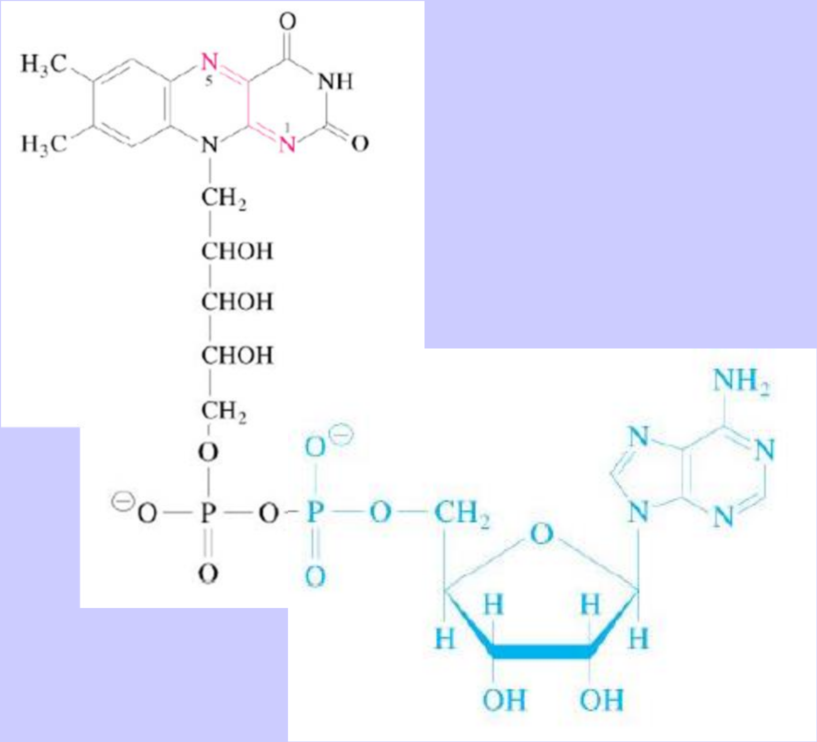
FAD, in its fully oxidized form, or quinone form, accepts two electrons and two protons to become $FADH_2$ (hydroquinone form).

FAD and FMN are a redox-active coenzyme associated with various proteins, which is involved with several enzymatic reactions in metabolism. A flavoprotein is a protein that contains a flavin group, which may be in the form of FAD or flavin mononucleotide (FMN).

Flavin-adenin mononucleotide (FMN)

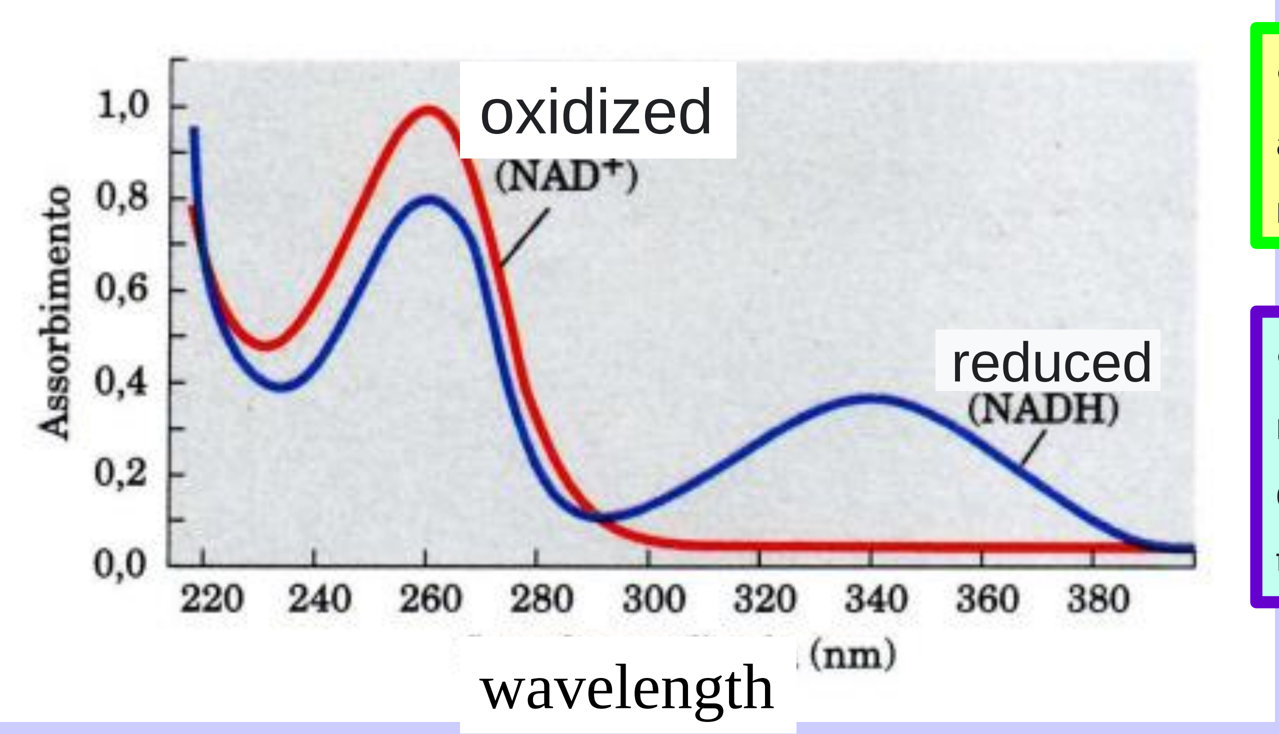


Flavin-adenin-dinucleotide (FAD)



Enzymes that contain NAD⁺/NADH + H⁺ can be followed by spectophotometric tecniques

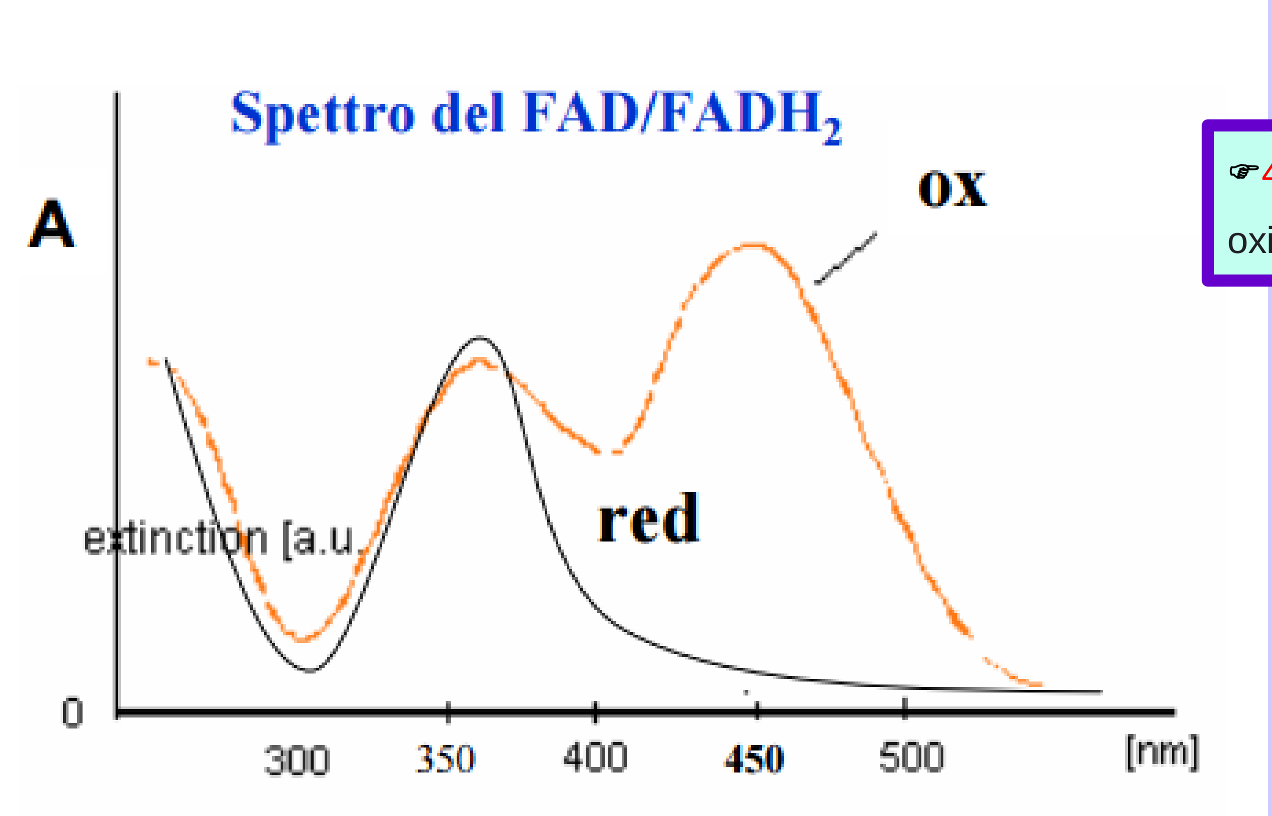
They have absorbance at 340 nm in the reduced form, but not in the oxidized forms



☞270 nm is characteristic of the adenosine moiety and is not modified

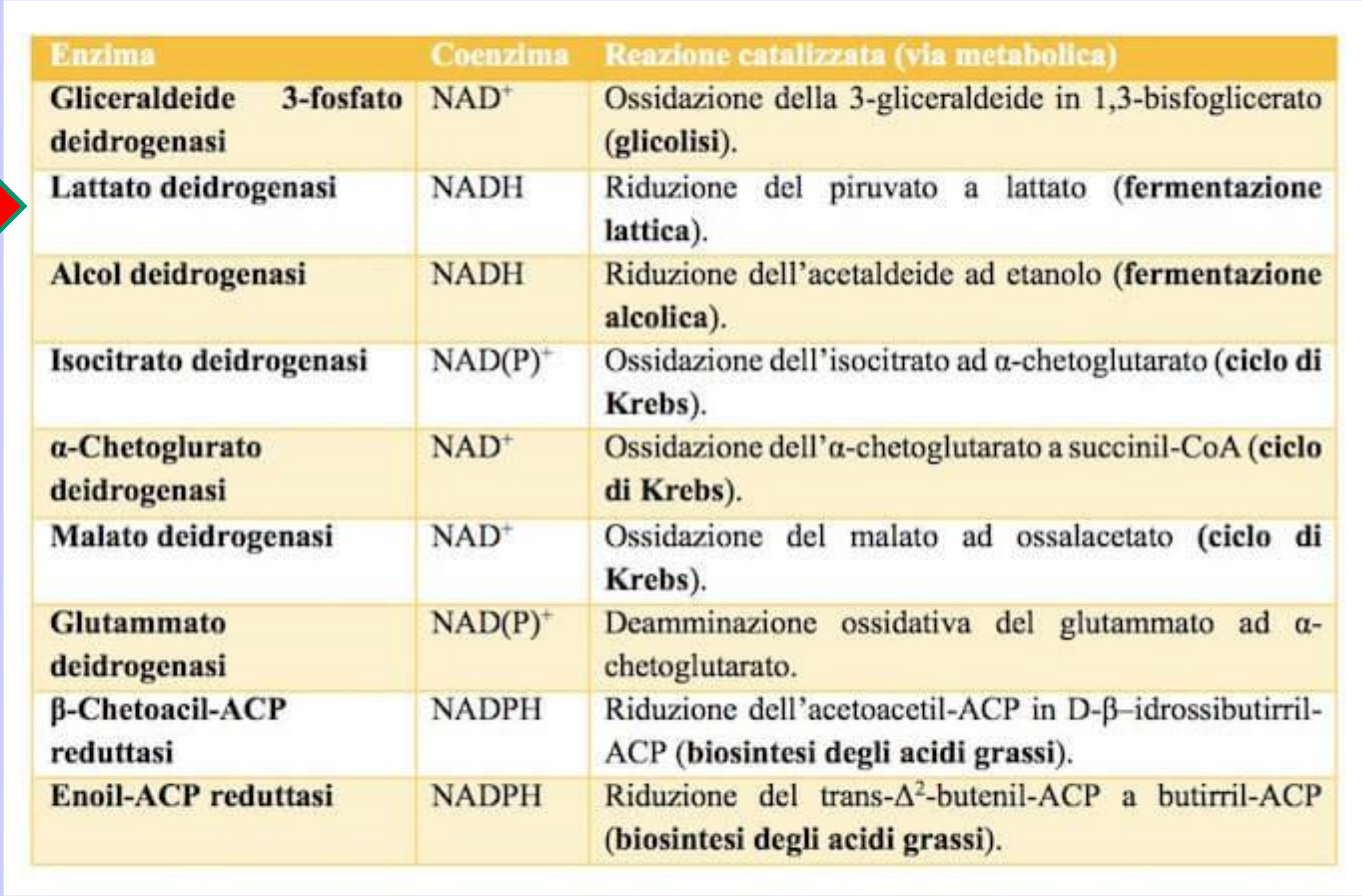
☞340 nm is characteristic in the reduced forms and depends on the different position of the electrons in the NADH pyridine ring

Enzymes that contain FAD/FADH can be followed by spectophotometric tecniques



☞450 nm is characteristic in the oxidized forms

Enzyme NAD(P^+) dependent



Enzima	Coenzima	Reazione catalizzata (via metabolica)
Gliceraldeide 3-fosfato deidrogenasi	NAD^+	Ossidazione della 3-gliceraldeide in 1,3-bisfoglicerato (**glicolisi**).
Lattato deidrogenasi	NADH	Riduzione del piruvato a lattato (**fermentazione lattica**).
Alcol deidrogenasi	NADH	Riduzione dell'acetaldeide ad etanolo (**fermentazione alcolica**).
Isocitrato deidrogenasi	$NAD(P)^+$	Ossidazione dell'isocitrato ad α-chetoglutarato (**ciclo di Krebs**).
α-Chetoglurato deidrogenasi	NAD^+	Ossidazione dell'α-chetoglutarato a succinil-CoA (**ciclo di Krebs**).
Malato deidrogenasi	NAD^+	Ossidazione del malato ad ossalacetato (**ciclo di Krebs**).
Glutammato deidrogenasi	$NAD(P)^+$	Deamminazione ossidativa del glutammato ad α-chetoglutarato.
β-Chetoacil-ACP reduttasi	NADPH	Riduzione dell'acetoacetil-ACP in D-β–idrossibutirril-ACP (**biosintesi degli acidi grassi**).
Enoil-ACP reduttasi	NADPH	Riduzione del trans-Δ^2-butenil-ACP a butirril-ACP (**biosintesi degli acidi grassi**).