ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

INTRODUCTION TO METABOLISM.
ATP SYNTHESIS AND UTILIZATION.

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METABOLISM

Metabolism is the ensemble of the enzyme-catalysed reactions that take place in the body to provide energy and building materials for biosynthetic reactions.

Metabolic reactions take place within cells, and hence they are compartmentalised across cellular and subcellular membranes: this implies knowledge of **membrane structure**, transport mechanisms, and the **electrical potentials** established across them. In addition, metabolic reactions are strongly regulated and take place according to the needs of the body; regulation implies mechanisms occurring at the **enzyme level** and at the **genetic level** and is largely dependent on **specific signals** originating in the same or other cells (hormones, neurotransmitters): this implies knowledge of the mechanisms by which cellular receptors decode these signals.

METABOLISM

- Most diseases result from changes in metabolic reactions and/or the mechanisms that control them (e.g. hormones), triggered by genetic or exogenous alterations.
- Besides metabolic diseases (e.g. diabetes), we list cardiovascular diseases (atherosclerosis), degenerative diseases (Alzheimer’s disease, linked to impaired cerebral glucose metabolism), psychiatric diseases (bipolar disorder linked to mitochondrial dysfunction), cancer (colorectal cancer strongly linked to hyperinsulinemia), etc., having a metabolic aetiology and/or pathogenesis.

INTRODUCTION TO METABOLISM

- *Metabolic Pathways*
- *Catabolism and Anabolism*
- *General Bioenergetics*
- *Coupled Reactions*
- *Metabolic Regulation*

CATABOLISM AND ANABOLISM

Catabolism is oxidative and releases energy:

Palmitate → 8 Acetate

$CH_3(CH_2)_{14}COOH \rightarrow 8\ CH_3COOH$

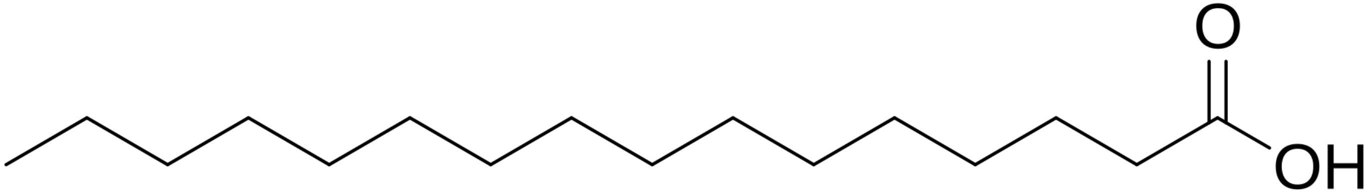
(16 C → 16 C; 32 H → 32 H; 2 O → 16 O)

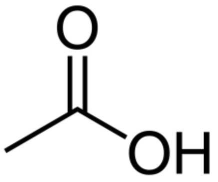
It requires 14 oxidative steps and generates 28 ATP

Symmetrically, **anabolism** is reductive and requires energy:

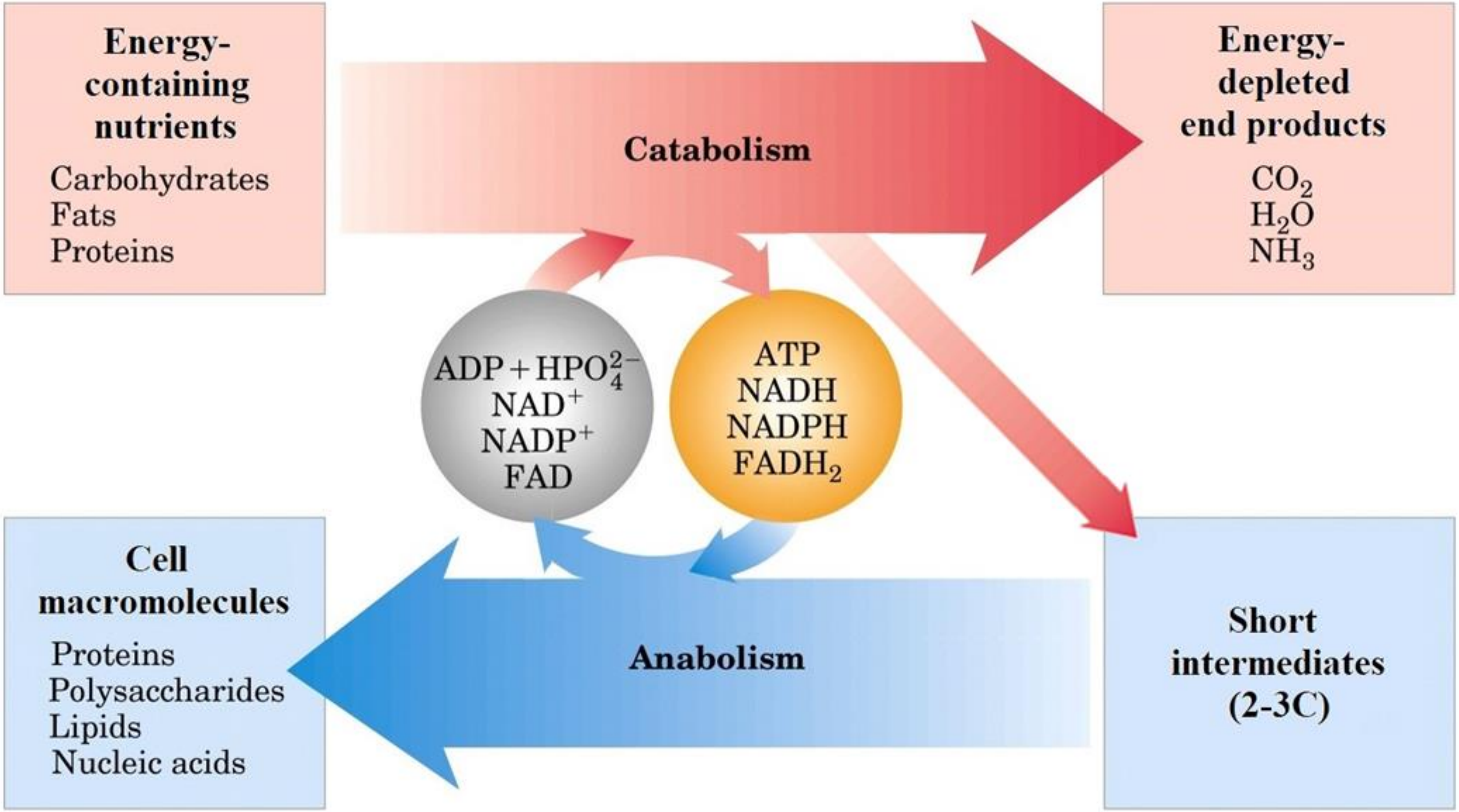
8 Acetate → palmitate

It requires 7 ATP and 14 reductions.





CATABOLISM AND ANABOLISM

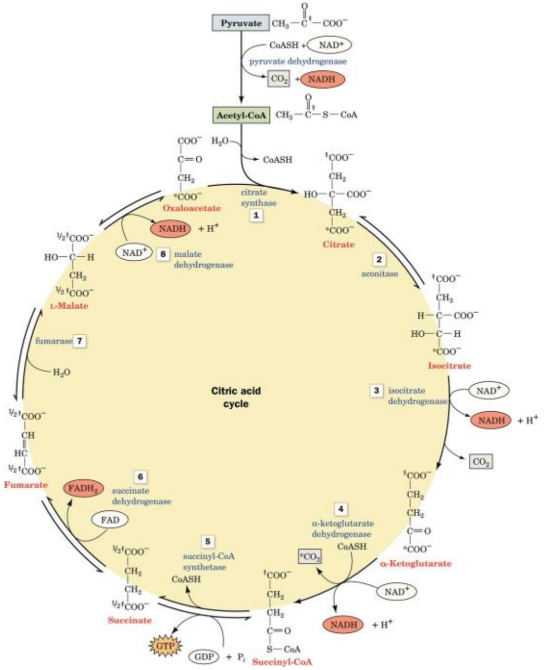


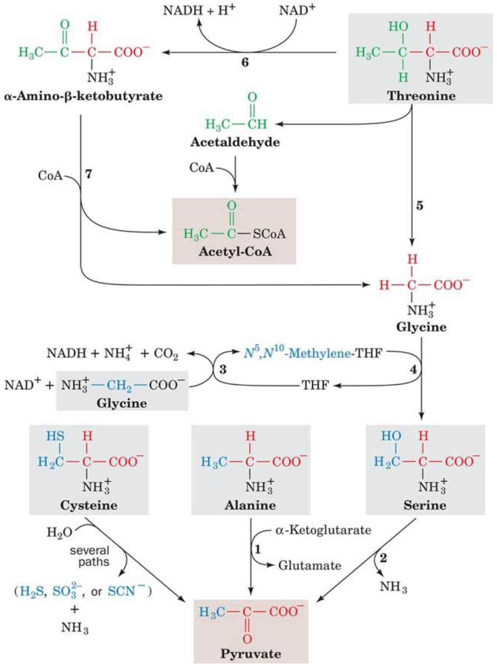
METABOLIC PATHWAYS

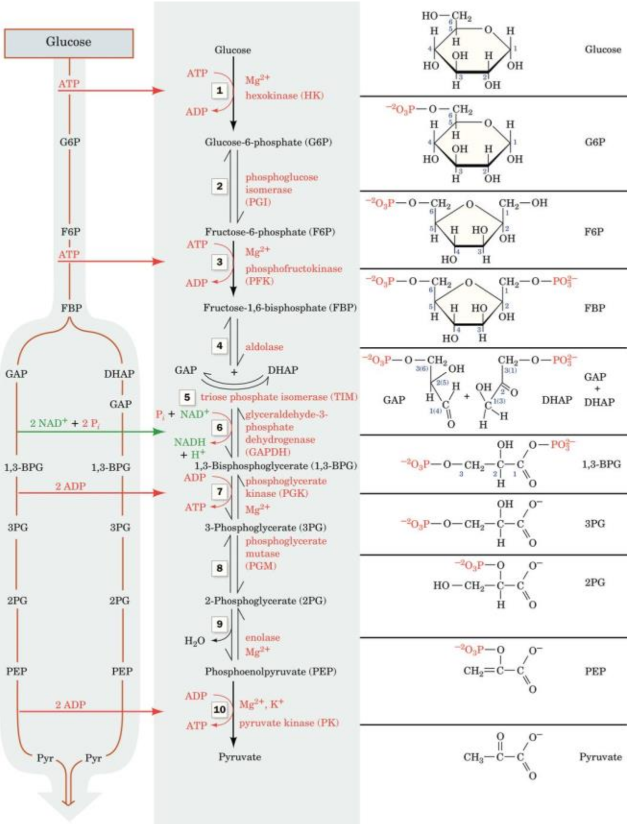
- **Linear** (e.g. *glycolysis*)
- **Branched**

    ***Converging** (e.g. to pyruvate)*

    ***Diverging** (e.g. from isopentenyl PP)*
- **Cyclic** (e.g. *TCA cycle, ornithine cycle*)







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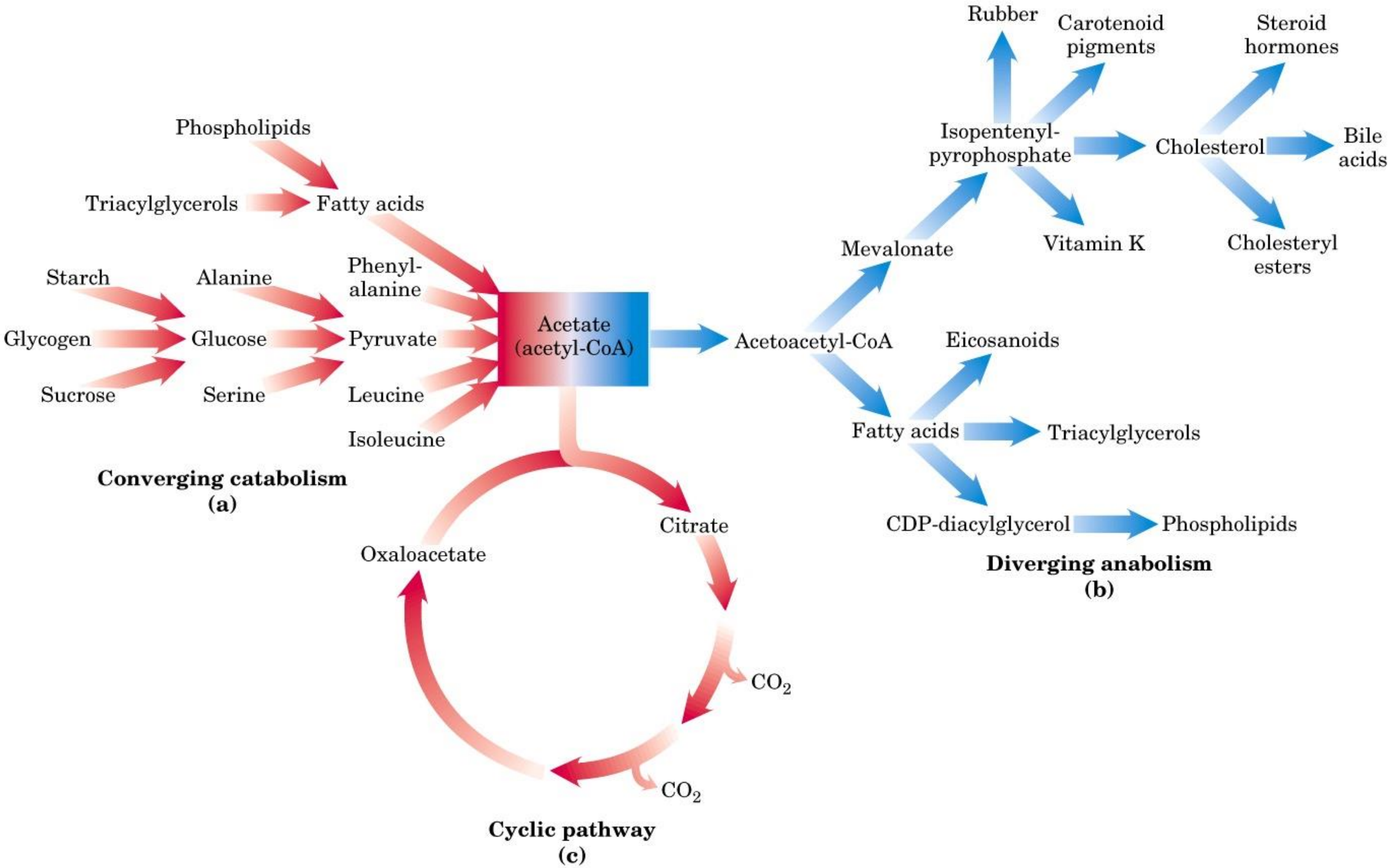
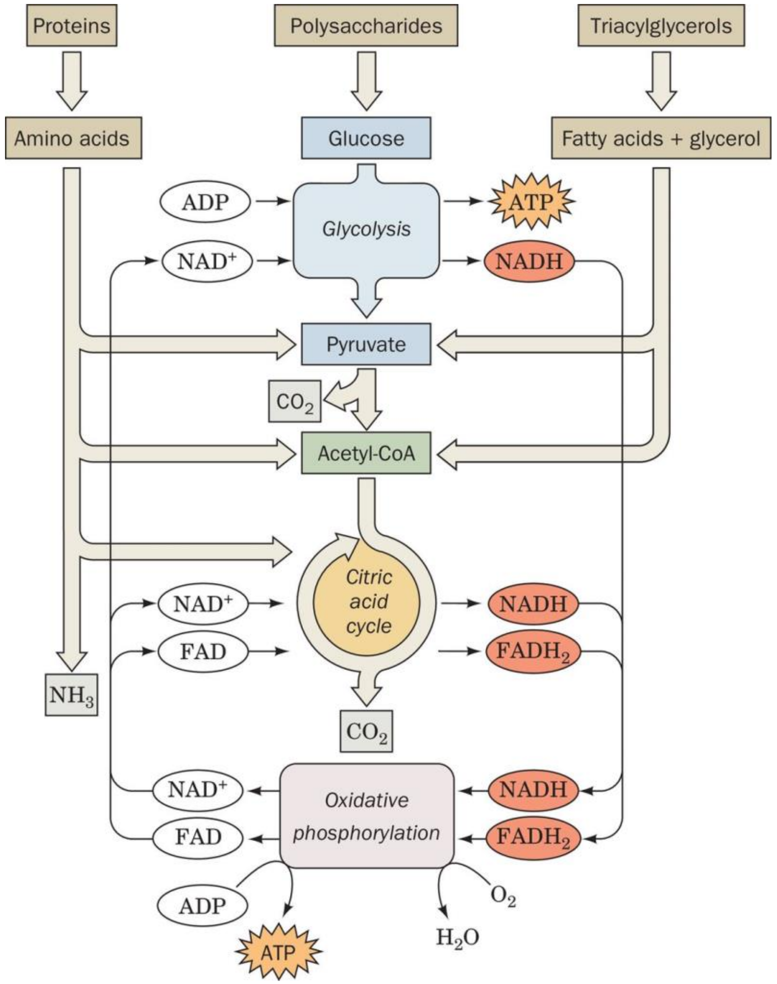
METABOLIC PATHWAYS



OVERVIEW OF CATABOLIC PROCESSES



Stage 1

Stage 2

Stage 3

Anabolic processes travel opposite pathways: stage 2 requires ATP and reducing power, stage 1 requires ATP.

MAJOR CONCEPTS IN METABOLISM

- *Metabolic flux*
- *Steady state*
- *Direction (thermodynamics)*
- *Rate (kinetics)*
- *Quantitative transformation*
- *Cell compartmentalization*
- *Metabolic control*

STEADY STATE

In a metabolic pathway:

$$A \xrightarrow{v_1} B \xrightarrow{v_2} C \xrightarrow{v_3} D \xrightarrow{v_4} E$$

The concentration of each intermediate is constant because the rate of its formation from the preceding intermediate is equal to the rate (**flux**) by which it is transformed into the subsequent intermediate: [C] remains constant because $v_2 = v_3$ **(homeostasis)**.

THERMODYNAMICS AND KINETICS

Metabolic pathways are driven by **thermodynamics:**

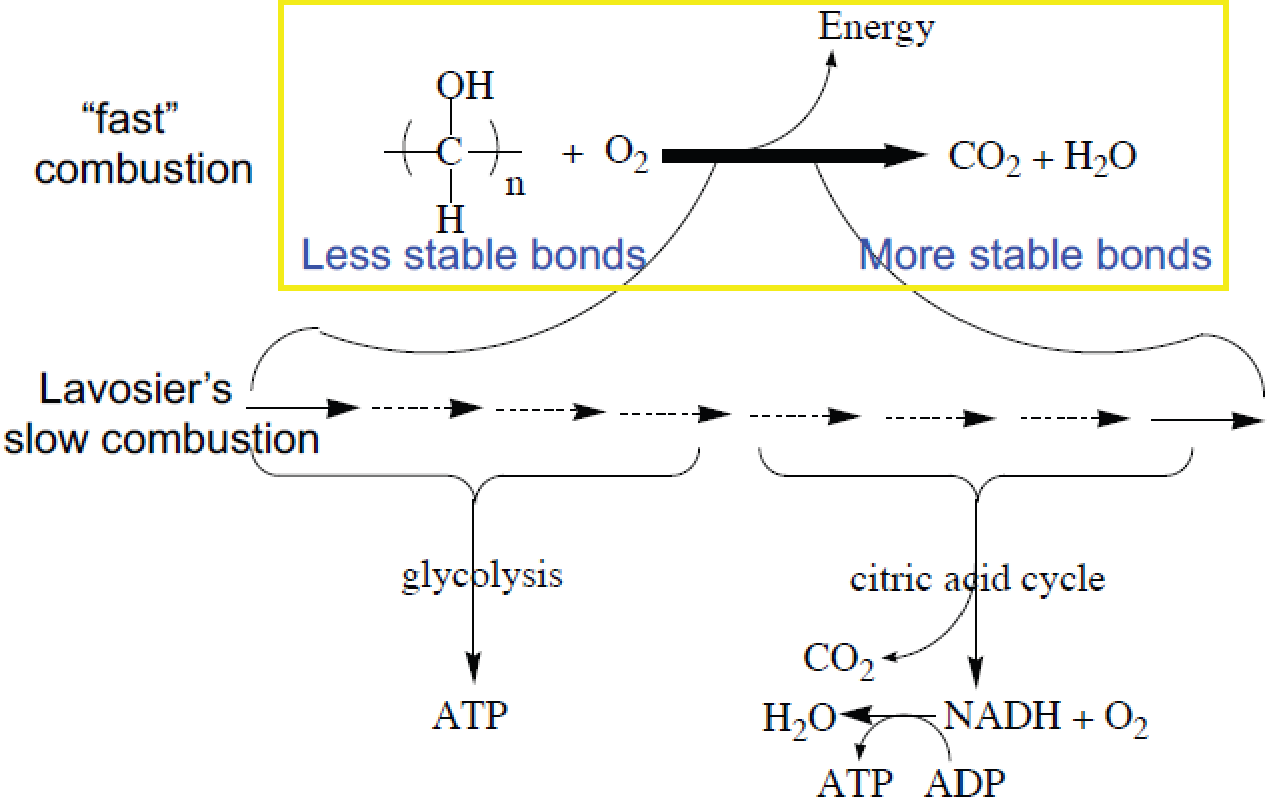
- The direction of flow is towards equilibrium;
- At steady state, the equilibrium is never reached, and the pathway proceeds at a constant rate.

Quantitative aspects are ruled by **kinetics**:

- Metabolism is the flow of matter: the amount of matter transformed in unit time is the rate of the metabolic pathway;
- The rate is established by the action and control of enzyme catalysts. Only proteins have the versatility to fine-tune chemical reactions.

FLOW OF ENERGY DURING METABOLISM

Cells obtain most of their energy by oxidation reactions,
but not by direct reaction with oxygen



“fast” combustion

Energy

$(\mathrm{CHOH})_n + O_2 \rightarrow CO_2 + H_2O$

Less stable bonds

More stable bonds

Lavosier’s slow combustion

glycolysis

ATP

citric acid cycle

CO_2

$H_2O \leftarrow NADH + O_2$

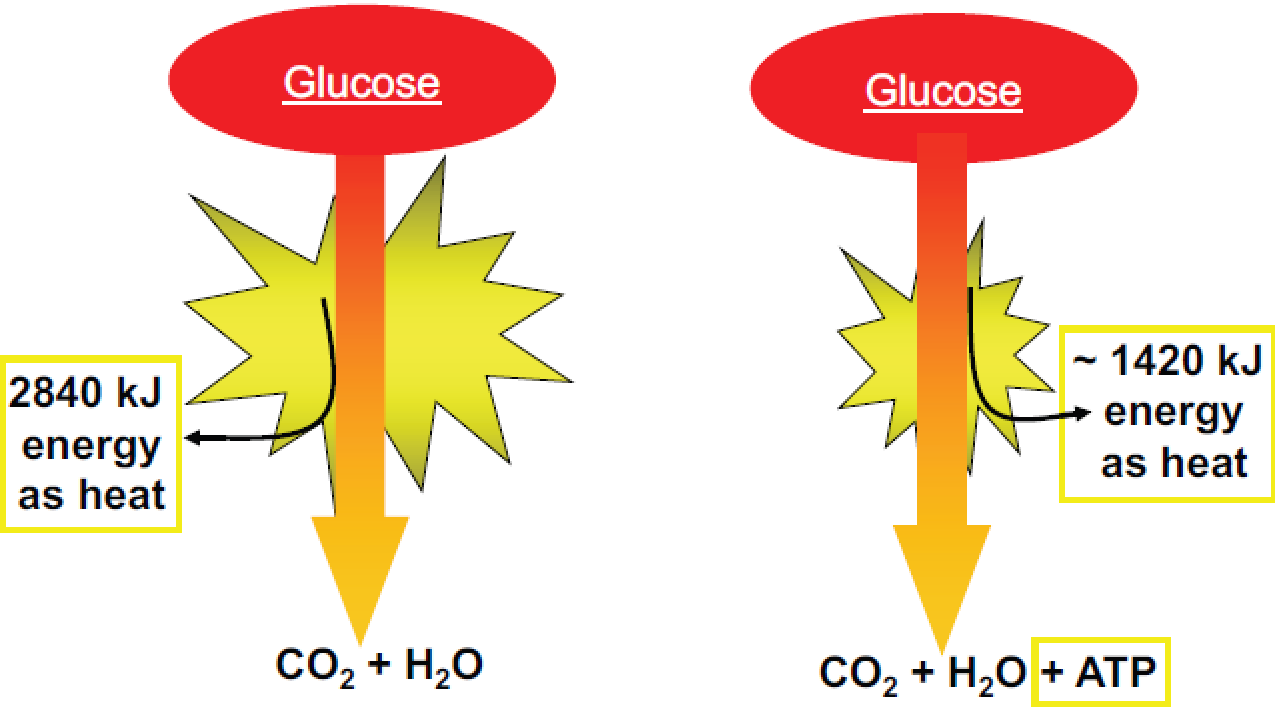
ATP ADP

FLOW OF ENERGY DURING METABOLISM

In cells, some energy of oxidations "saved" as ATP

Combustion of glucose in a bomb calorimeter

Conversion of glucose to CO_2 + ATP



Glucose

2840 kJ energy as heat

$CO_2 + H_2O$

Glucose

~ 1420 kJ energy as heat

$CO_2 + H_2O$ + ATP

FLOW OF ENERGY DURING METABOLISM

- NET reactions "go" towards equilibrium

$A + B \Longleftrightarrow C + D$

Reactants Products

$$\boxed{K'_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b}}$$

Reactants & Products defined by left/right position; not NET direction of reaction.

- Conditions can be on "either side" of equilibrium

For example, if equilibrium = 1.8 M A & B and 0.2 M C & D

Starting with "standard" conditions:
1.0 M A, B, C, D; NET $A + B \leftarrow C + D$

Starting on the "other side" of equilibrium:
1.9 M A & B; 0.1 M C & D; NET $A + B \rightarrow C + D$

FLOW OF ENERGY DURING METABOLISM

Thermodynamics: how much energy released as a reaction proceeds towards equilibrium.

$\boxed{\Delta G\text{: Gibbs free energy}}$

- Units of energy per mole (e.g., kJ/mol).
- Value of ΔG is a function of *how far from equilibrium*.

Negative ΔG (exergonic)
If rxn occurs, will proceed reactants → products.

Positive ΔG (endergonic)
If rxn occurs, will proceed products → reactants.

$\Delta G = 0$; reaction at equilibrium

FLOW OF ENERGY DURING METABOLISM

For reaction R → P

$\Delta G = \Delta G^\circ + RT \ln [P]/[R]$

$\Delta G = \Delta G^\circ + 6 \log [P]/[R]$

At Equilibrium: $\Delta G = 0$

$0 = \Delta G^\circ + 6 \log [P]/[R]$

$\Delta G^\circ = -6 \log[P]/[R]$

$\boldsymbol{\Delta G^\circ = -6 \log K_{eq}}$

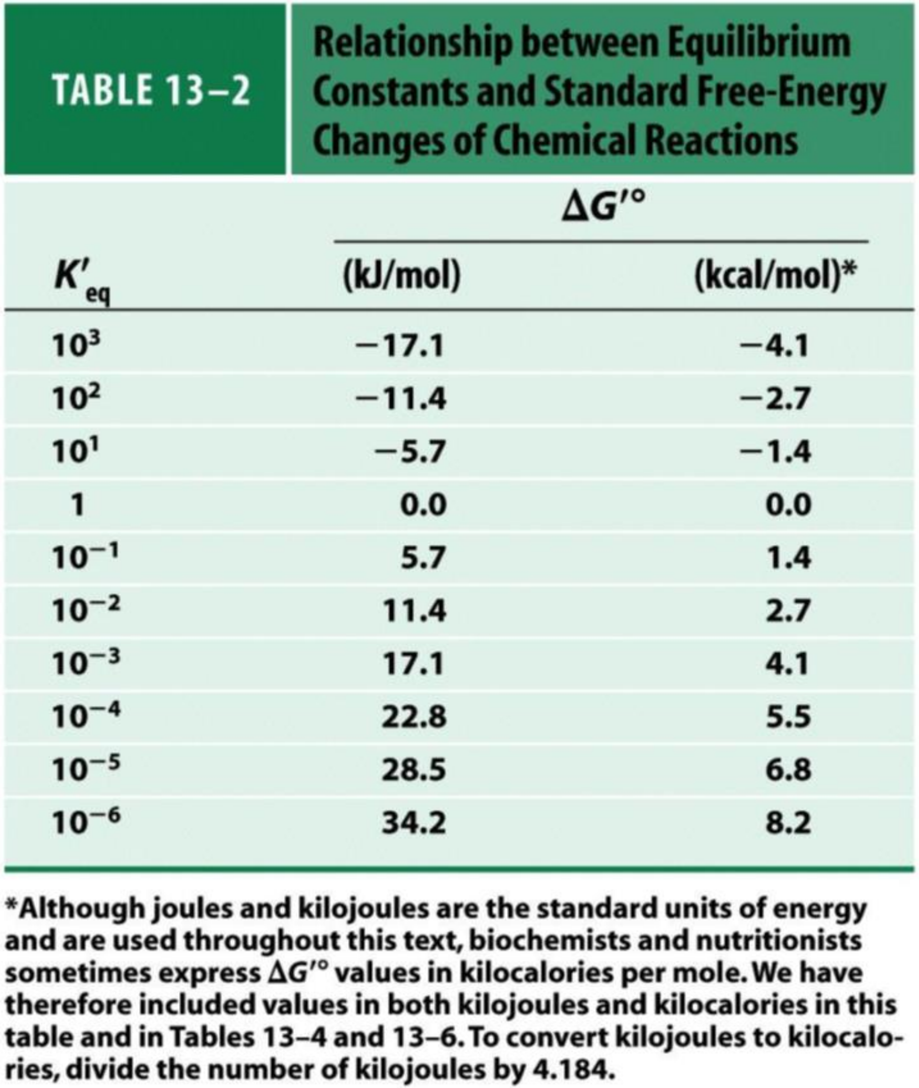


TABLE 13–2 Relationship between Equilibrium Constants and Standard Free-Energy Changes of Chemical Reactions

K'_{eq}	$\Delta G'^\circ$ (kJ/mol)	$\Delta G'^\circ$ (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 and nutritionists 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 13–4 and 13–6. To convert kilojoules to kilocalories, divide the number of kilojoules by 4.184.

FLOW OF ENERGY DURING METABOLISM

Solve these problems

1. In the reaction ATP → ADP + P_i the standard free energy change ΔG° = - 30 kJ/mol

What is the actual ΔG at the following metabolite concentrations?

[ATP] 10 mM (10^{-2} M); [ADP] = 1 mM (10^{-3} M); [P_i] = 1 mM (10^{-3} M)

The actual reaction is: ATP + H_2O → ADP + P_i

But the reaction takes place in 55.6 M H_2O, [H_2O] is constant and can be incorporated in the free energy values.

FLOW OF ENERGY DURING METABOLISM

$ATP \rightarrow ADP + P_i$ $\Delta G° = -30$ kJ/mol

[ATP] 10 mM

[ADP] = 1 mM

$[P_i] = 1$ mM

$$\mathbf{\Delta G = \Delta G° + 6 \log [P]/[R]}$$

$\Delta G = -30 + 6 \log (10^{-3} \times 10^{-3}/10^{-2})$

$\Delta G = -30 + 6 \times (-4)$

$\Delta G = -54$ kJ/mol

FLOW OF ENERGY DURING METABOLISM

2. In the reaction

Fructose 1,6-bisphosphate (FBP) → Glyceraldehyde-3-P (GAP) + dihydroxyacetone-P (DOP)

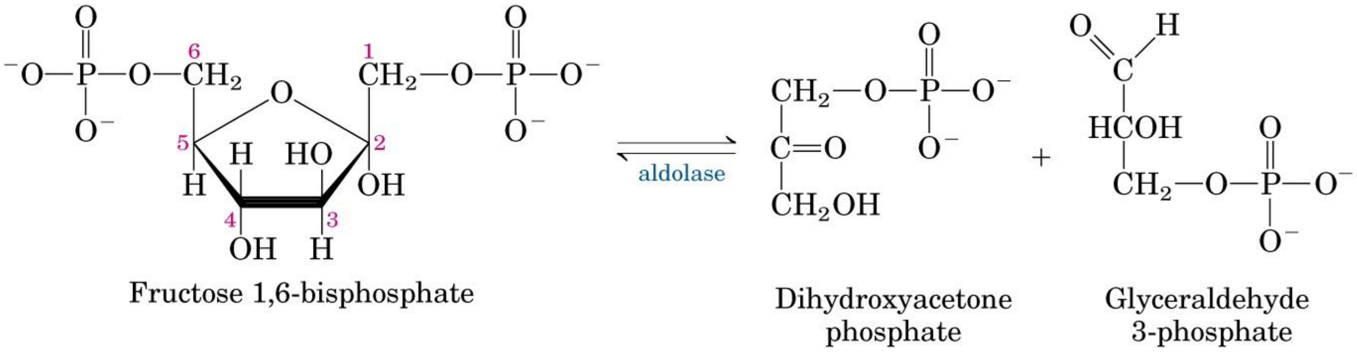
$\Delta G° = + 24$ kJ/mol.

What is the actual ΔG at the following metabolites concentration?

[FBP] = 10 mM

[GAP] = 1 mM

[DOP] = 1 mM



FLOW OF ENERGY DURING METABOLISM

(FBP) → (GAP) + (DOP) $\Delta G° = +24$ kJ/mol.

[FBP] = 10 mM

[GAP] = 1 mM

[DOP] = 1 mM

$$\boldsymbol{\Delta G = \Delta G° + 6 \log [P]/[R]}$$

$\Delta G = +24 + 6 \log (10^{-3} \times 10^{-3}/10^{-2})$

$\Delta G = +24 + 6 \log (10^{-3} \times 10^{-3}/10^{-2})$

$\Delta G = +24 + 6 \times (-4)$

$\Delta G = 0$

REDOX REACTIONS

$Fe^{2+} \leftrightarrow Fe^{3+} + e^-$

Oxidations are often **dehydrogenations**:

$AH_2 \leftrightarrow A + 2H^+ + 2\,e^-$

An oxidation is accompanied by a reduction:

$2H^+ + 2\,e^- + B \leftrightarrow BH_2$

Redox couple: Ox/Red

Fe^{3+}/Fe^{2+} A/AH_2 B/BH_2

Redox reaction:

$AH_2 + B \leftrightarrow A + BH_2$

REDOX REACTIONS

The **redox potential** is the tendency to receive electrons.

Nernst Equation

$$E = E'^{\circ} + \frac{R\,T}{n\,F} \ln \frac{[e^{-}\ acceptor]}{[e^{-}\ donor]}$$

$$E = E'^{\circ} + 0.06/n \log [ox]/[red\ ]$$

For the redox reaction: $AH_2 + B \leftrightarrow A + BH_2$

$K_{eq} = [A]\,[BH_2]/[AH_2]\,[B]\ (Eq)$ $\Delta E'^{\circ} = E'^{\circ}_B - E'^{\circ}_A = RT/nF \ln K_{eq}$

Since

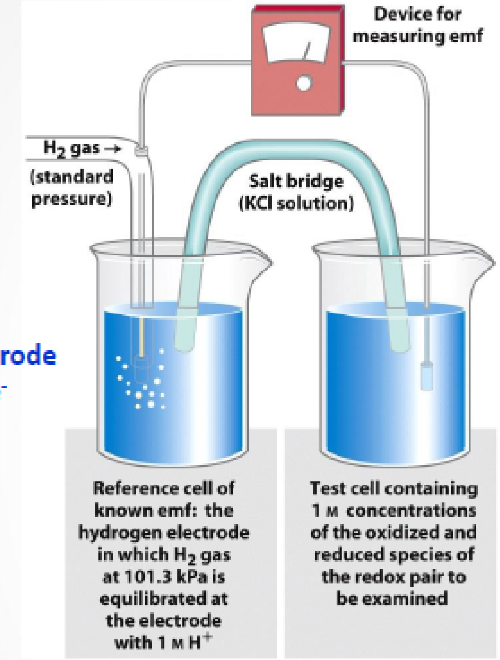
$\Delta G'^{\circ} = -\,RT \ln K_{eq} = -\,nF\,\Delta E'^{\circ}$

In general (non-standard conditions):

$$\boldsymbol{\Delta G = -nF\,\Delta E}$$

REDOX REACTIONS

Measurement of the reduction potential *ΔE* ° and ΔG° of a redox reactions (in standard condition)



Reference electrode

$H_2 \rightarrow 2H^+ + 2e^-$

$NADH + H^+ + ½\,O_2 \longrightarrow NAD^+ + H_2O$

Take e^-

$½\,O_2 + 2\,H^+ + 2\,e^- \rightarrow H_2O$ $E° = +0.816$

Yield e^-

$NAD^+ + 2\,H^+ + 2\,e^- \rightarrow NADH + H^+$ $E° = -0.320$

$\Delta E° = E^+ + E^- = 0.816 - (-)\,0.320 = 1.136$ volt

Where:

n = the number of transferred electron

F = Faraday constant that is 96.5 kJ/volt

$$\Delta G° = -\,n\,F\,\Delta E°$$

$$-\,\Delta G° = n\,F\,\Delta E° = 2 \cdot F \cdot 1.136 = 220\text{ KJ/mole)}$$

$NADH + H^+ + ½\,O_2 \longrightarrow NAD^+ + H_2O$

COUPLED REACTIONS

An unfavourable reaction ($\Delta G > 0$) can be driven to occur spontaneously by coupling with a favourable reaction ($\Delta G < 0$) if the sum of ΔG values is negative (ΔG values are additive).

$$A \leftrightarrow B \leftrightarrow C \qquad \Delta G_1,\ \Delta G_2$$

$$A \leftrightarrow C \qquad \Delta G_T = \Delta G_1 + \Delta G_2$$

Coupling takes place through a common intermediate:

$$A + X \rightarrow AX \qquad \Delta G_1 > 0$$

$$BX \rightarrow B + X \qquad \Delta G_2 < 0$$

$$A + BX \rightarrow AX + B \qquad \Delta G_1 + \Delta G_2 < 0$$

COUPLED REACTIONS

Amino acids → proteins $\Delta G > 0$ (endergonic) (1)

$ATP + H_20 \rightarrow AMP + 2P_i$ $\Delta G < 0$ (exergonic) (2)

(1) + (2): Amino acids + nATP → proteins + nAMP

Overall Δ G is negative.

$Glucose + P_i \rightarrow glucose\text{-}6\text{-}phosphate + H_2O$

$\Delta G'^\circ = 13.8$ kJ/mol (1)

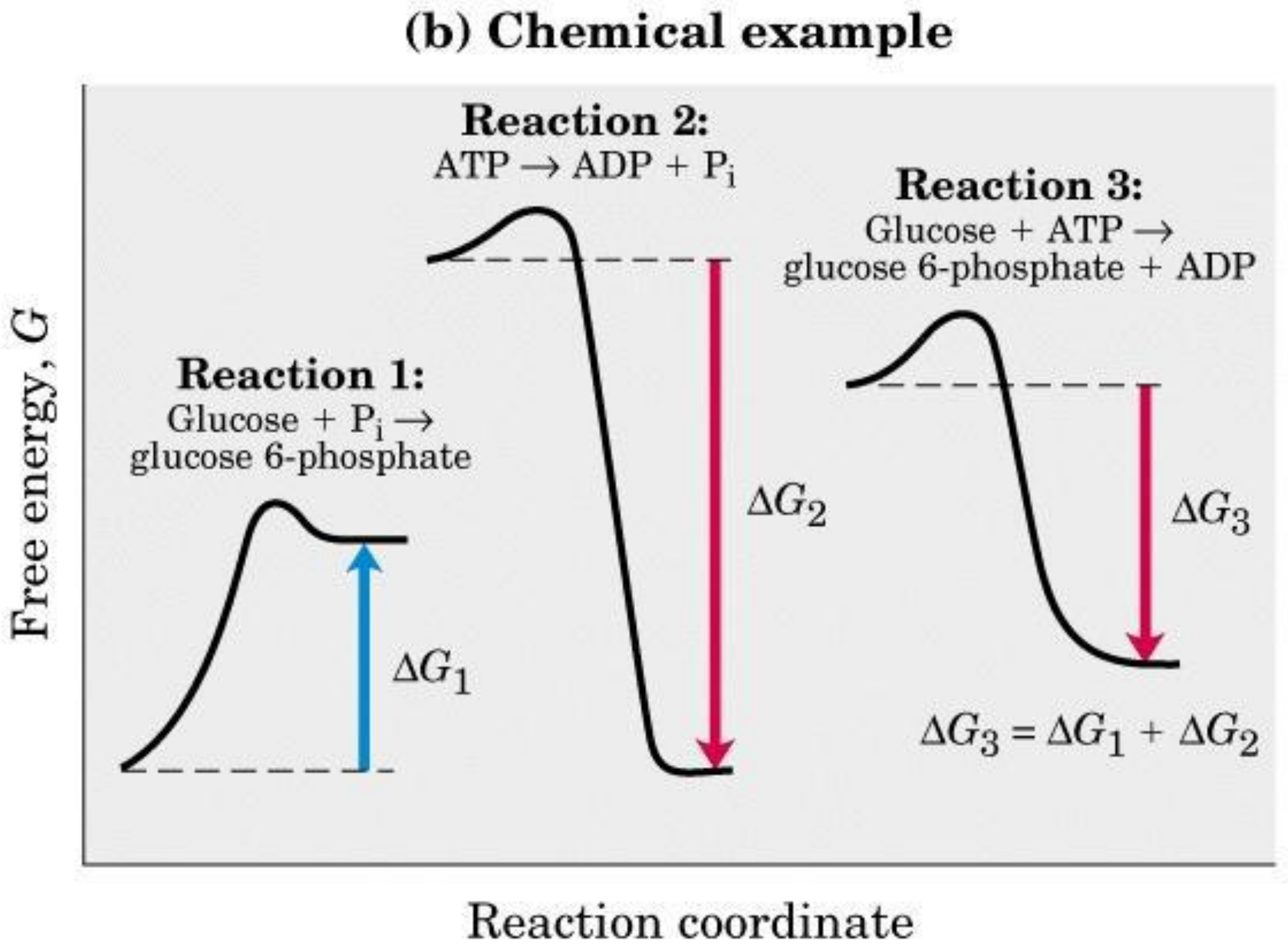
(The reverse reaction, hydrolysis of G-6-P will be spontaneous.)

$ATP + H_20 \rightarrow ADP + P_i$ $\Delta G'^\circ = -30.5$ kJ/mol (2)

(The $\Delta G'^\circ$ values of sequential reaction are additive.)

(1) + (2) = -16.7 kJ/mol

COUPLED REACTIONS



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COUPLED REACTIONS

$$\text{glutamate} + NH_3 \rightarrow \text{glutamine} + H_2O$$

$$\Delta G°' = + 3.4 \text{ kcal/mol}$$

Glutamate + NH_3 in order to happen we should increase the reagents to non-physiological concentrations.

The reaction exploits splitting of ATP to ADP and P_i ($\Delta G°' = - 7.3$ kcal/mol)

$$\text{glutamate} + ATP + NH_3 \rightarrow \text{glutamine} + ADP + P_i$$

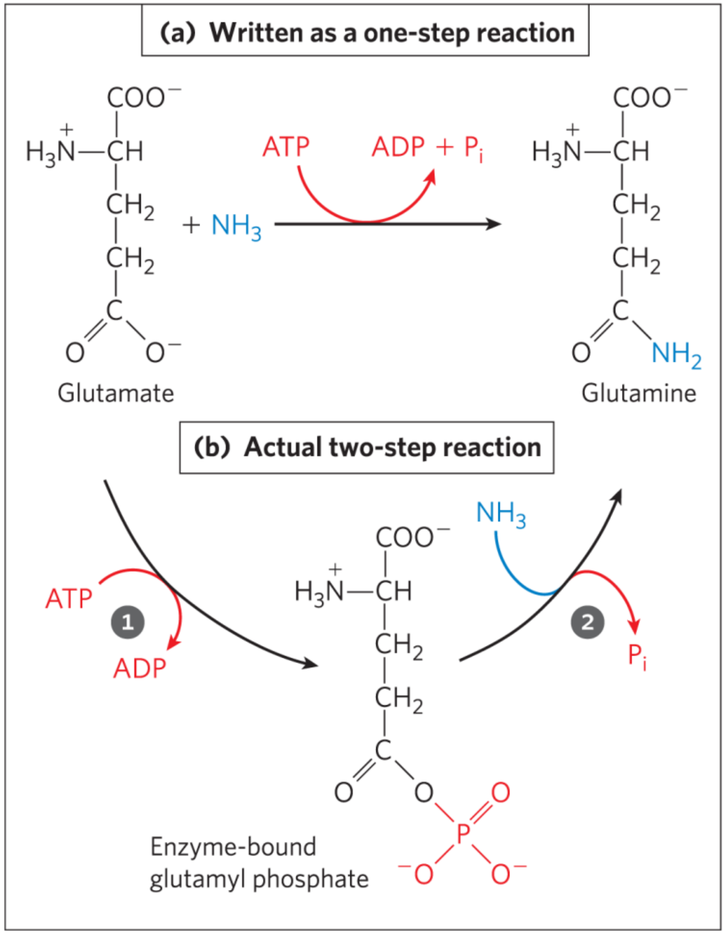
$$\Delta G°' = - 7.3 + 3.4 = - 3.9 \text{ kcal/mol}$$

$$\text{glutamate} + ATP \rightarrow \text{glutamyl-P} + ADP$$

$$\text{glutamyl-P} + NH_3 \rightarrow \text{glutamine} + Pi$$

COUPLED REACTIONS

glutamate + NH_3 → glutamine + H_2O



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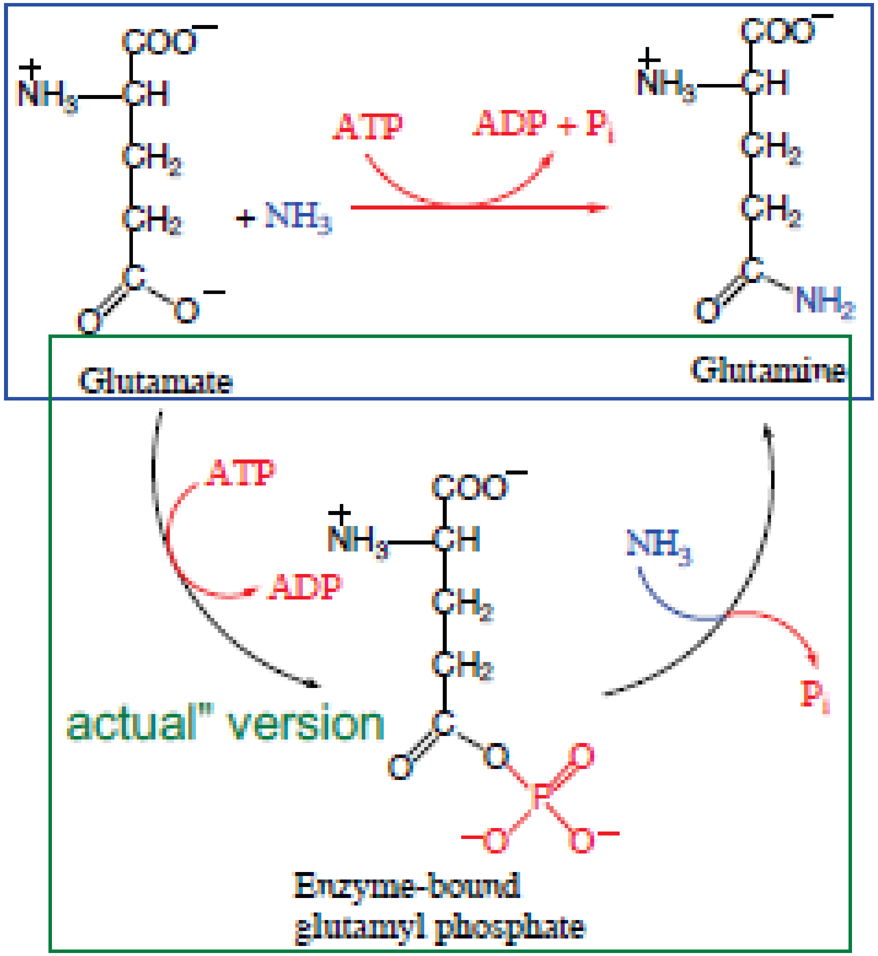
COUPLED REACTIONS

ATP Usually Provides Energy by Group Transfers, Not by Direct Hydrolysis

"shorthand" version

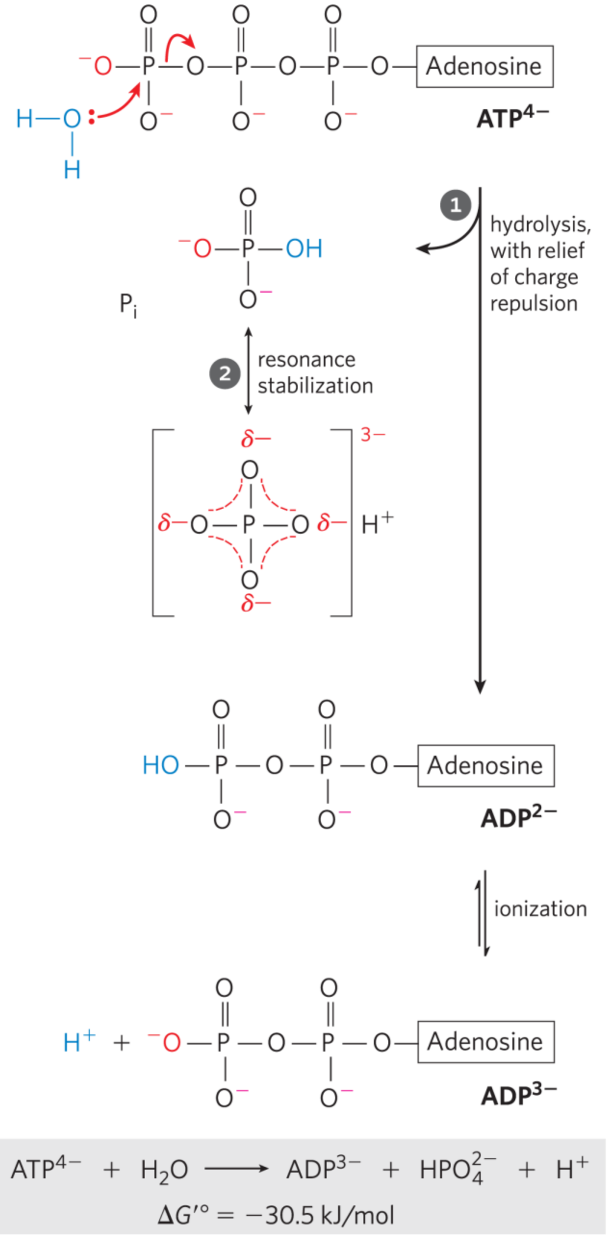


Group transfer "energizes" recipient.

"Coupling" to ATP breakdown renders a Amine formation favorable reactions.

like Figure 13-8

COUPLED REACTIONS



$ATP^{4-} + H_2O \longrightarrow ADP^{3-} + HPO_4^{2-} + H^+$

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

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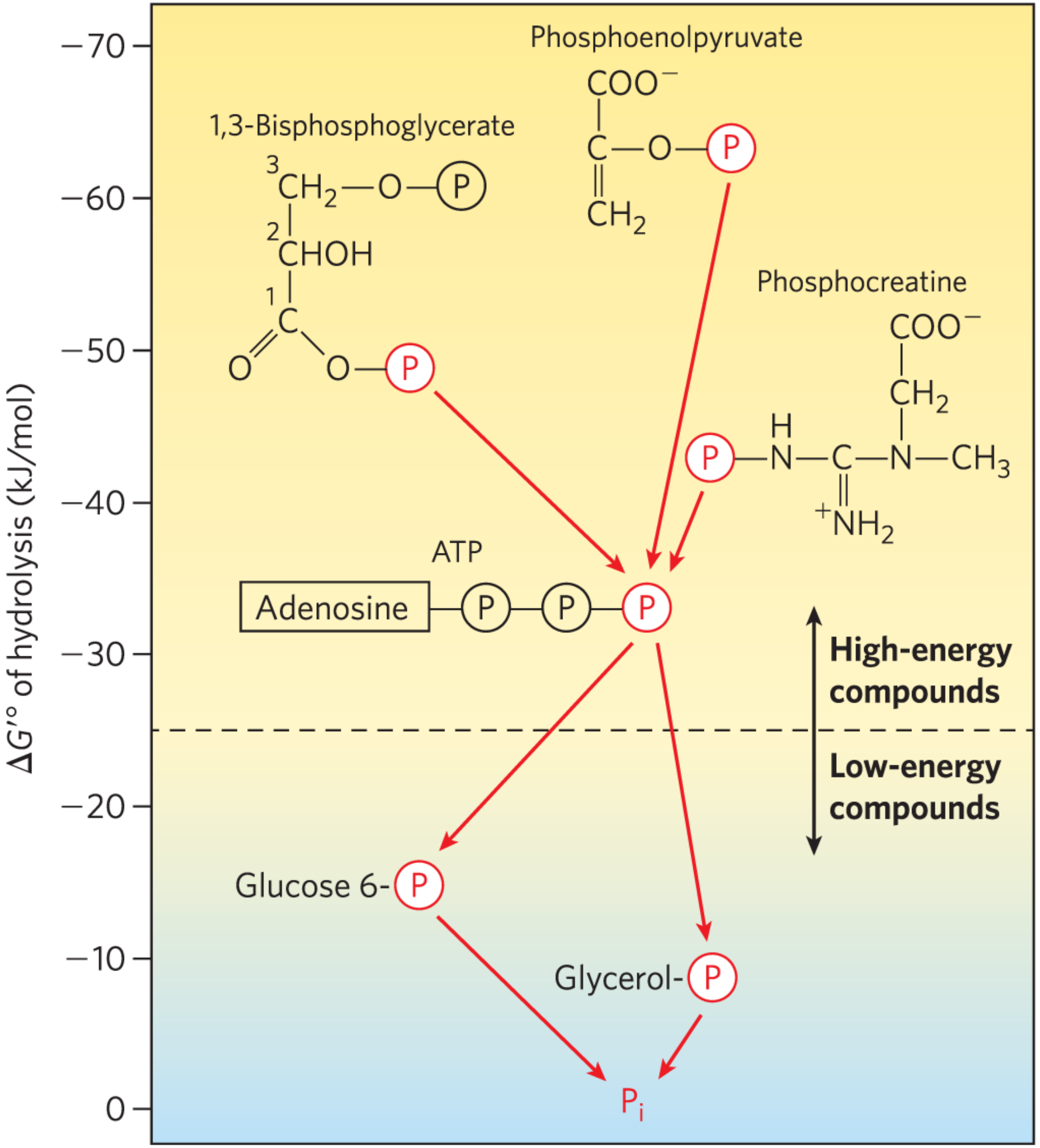
Chemical basis for the large free-energy change associated with ATP hydrolysis.

1) Charge separation.

2) P_i is stabilized by formation of a resonance hybrid

3) (not shown) greater degree of solvation of the products Pi and ADP relative to ATP.

MECHANISMS OF ATP UTILIZATION



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MECHANISMS OF ATP UTILIZATION

Mechanisms of ATP synthesis phosphorylation (of ADP):

$$\mathbf{ADP + P_i + energy \rightarrow ATP}$$

- Substrate-level phosphorylation

$AH_2 + B \rightarrow A + BH_2$ $\Delta G < 0$

$ADP + P_i \rightarrow ATP$ $\Delta G > 0$

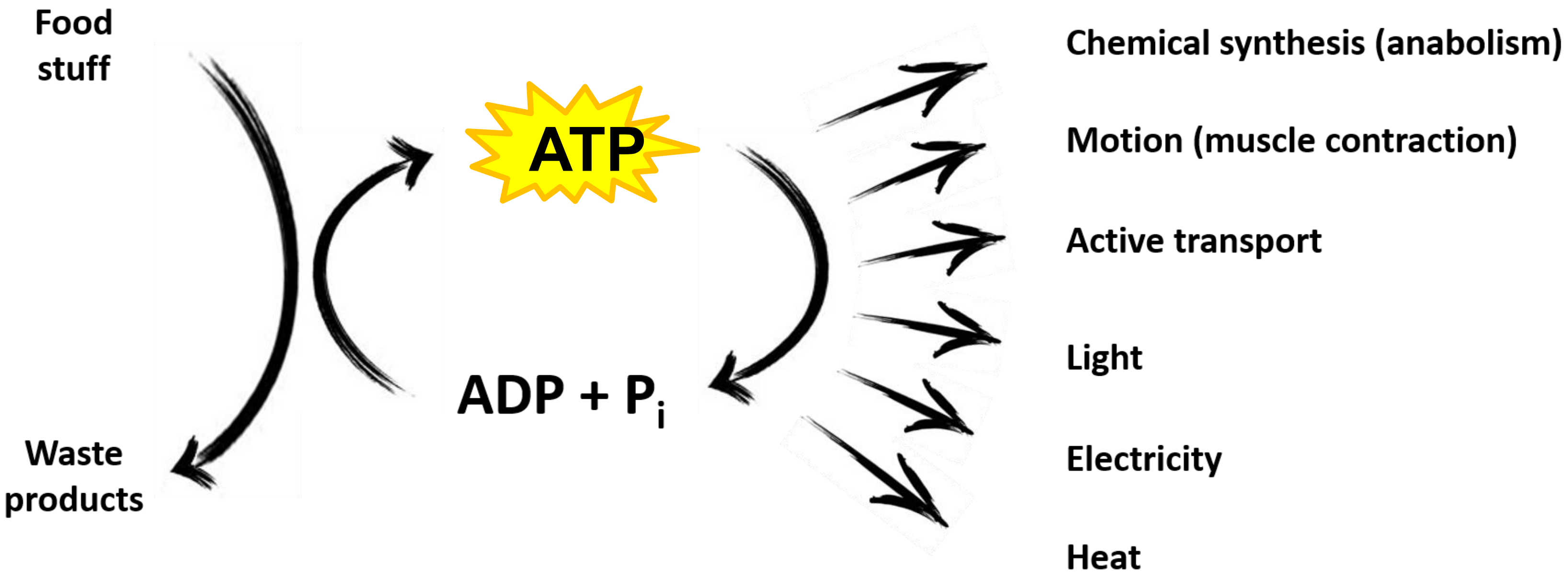
Mechanism:

$AH_2 + B + P_i \rightarrow A{\sim}P + BH_2$

$A{\sim}P + ADP \rightarrow A + ATP$

- Oxidative phosphorylation (at the level of electron transfer chain).

MECHANISMS OF ATP UTILIZATION



Food stuff

Waste products

ATP

ADP + P_i

Chemical synthesis (anabolism)

Motion (muscle contraction)

Active transport

Light

Electricity

Heat

MECHANISMS OF ATP UTILIZATION

- **Orthophosphoric cleavage**

$$X + ATP \rightarrow X\text{-}P + ADP$$

$$\text{Glucose} + ATP \rightarrow \text{glucose-6-P} + ADP$$

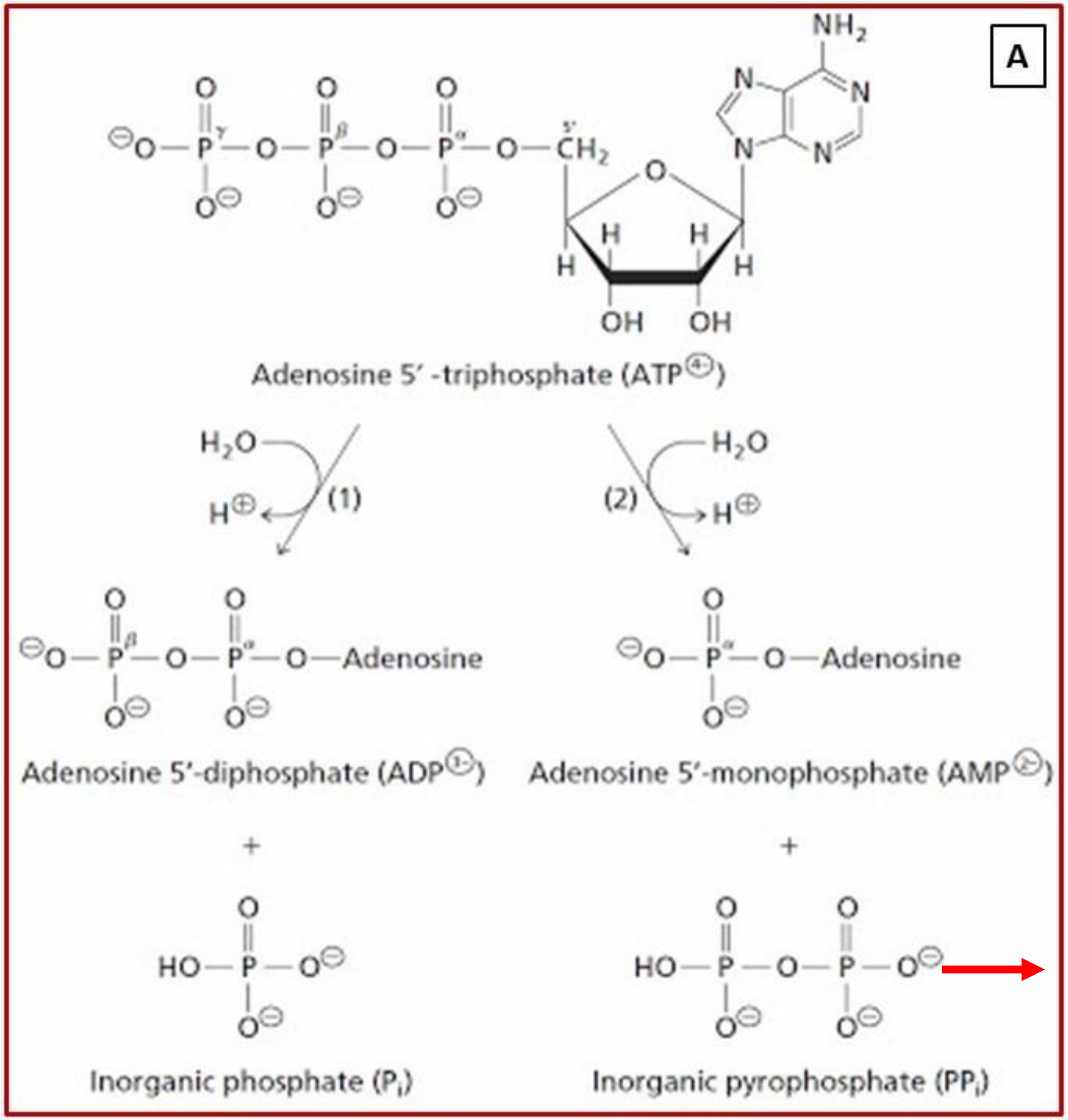
- **Pyrophosphoric cleavage**

$$X + ATP \rightarrow X\text{-}AMP + PP_i$$

$$\text{Aminoacid} + ATP \rightarrow \text{aminoacyl-AMP} + PP_i$$

The reaction is shifted to the right by PP_i *hydrolysis:* $PP_i \rightarrow 2\ P_i$

MECHANISMS OF ATP UTILIZATION



Enzymatic (pyrophosphatase) cleavage to 2 PO_4^{3-} groups

Orthophosphoric cleavage Pyrophosphoric cleavage

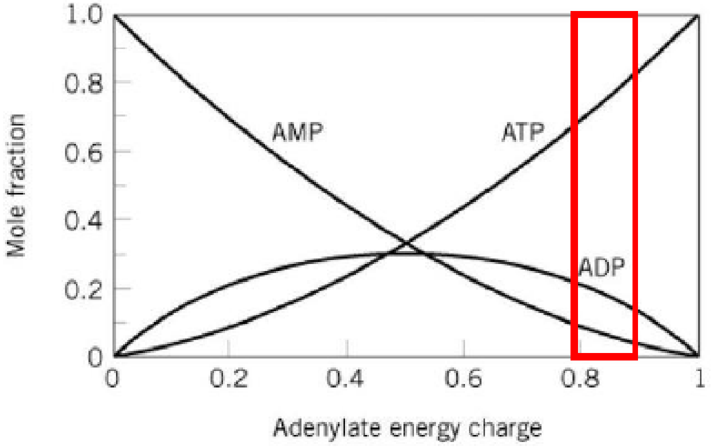
MECHANISMS OF ATP UTILIZATION

The energy charge of the adenylate pool:metabolic regulatory parameter

Atkinson and Walton,1967

$$\text{Energy charge} = \frac{[\text{ATP}] + \frac{1}{2}[\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]}$$

A system containing only ATP is fully charged, with an adenylate charge value of 1.0, and one containing only AMP is fully discharged, with an adenylate charge of 0. The charge value would be 0.5 if only ADP were present.



The energy charge of most cells range from 0.8 to 0.95

MECHANISMS OF ATP UTILIZATION

Examples of energy charge:

ATP = 10 mM, ADP = 10 mM, AMP = 10 mM

E.C. = (10 + 5)/(10 + 10 + 10) = 0.5

ATP = 0, ADP = 10 mM, AMP = 0

E.C. = 5/10 = 0.5

ATP = 10 mM, ADP and AMP 0

E.C. = 10 / 10 = 1

METABOLISM AND CELLULAR STRUCTURE

Metabolism is integrated into the cell and is tightly linked to the cell’s structure and **compartmentation**, which ensure metabolite concentration, separation from the environment, and selective permeability.

Proteins are cellular machines that act by specifically interacting with other biomolecules. Such interactions are inherent in the protein's three-dimensional structure, which is determined by the genetically encoded amino acid sequence.

ROLE OF ENZYMES

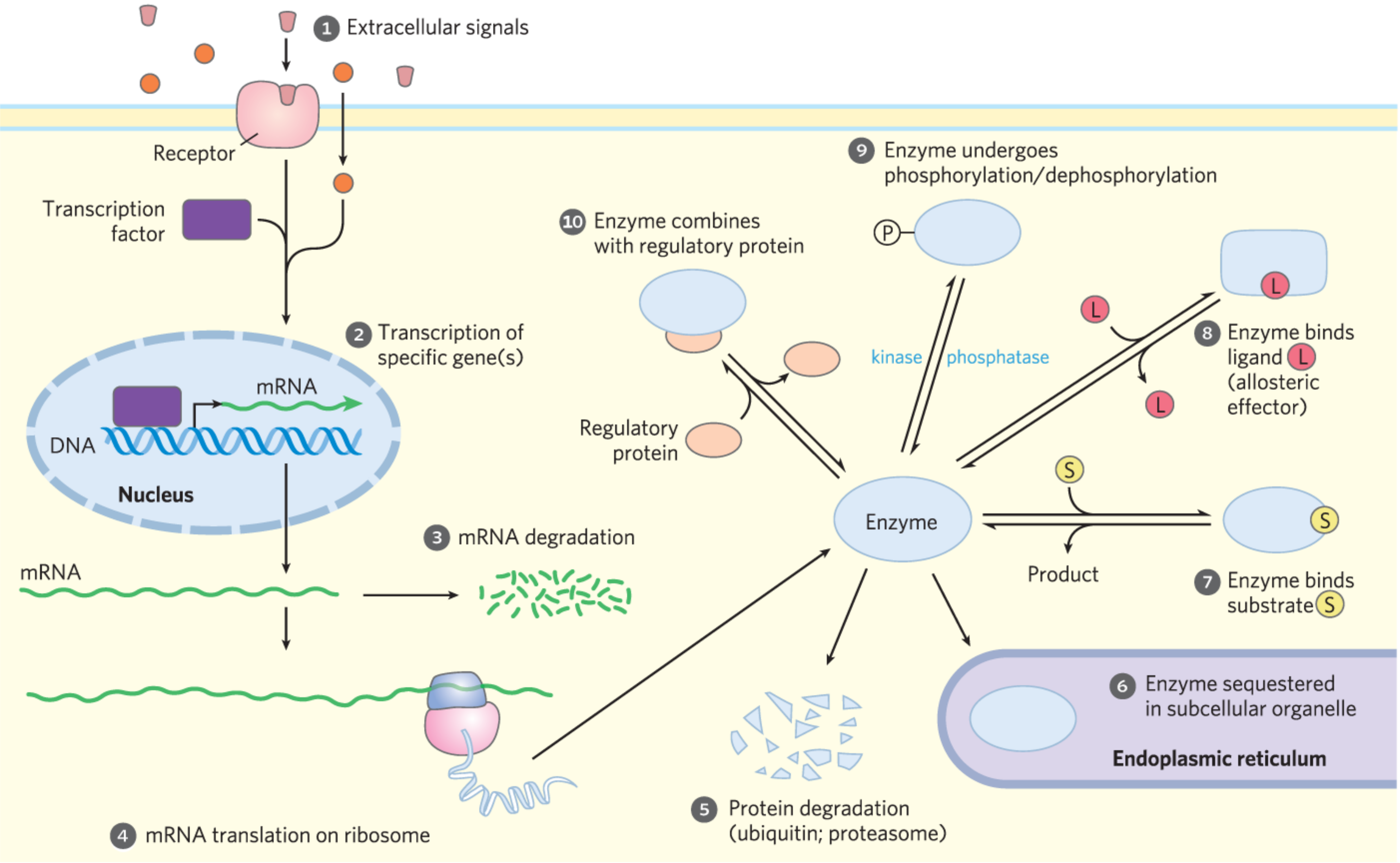
Metabolism is regulated at the enzyme level:

- Compartmentalization
- Thermodynamic and kinetic control
- Allosteric regulation
- Covalent regulation (proteolytic activation, enzyme degradation, reversible phosphorylation, etc.)
- Genetic regulation

metabolic regulation = processes that serve to maintain homeostasis at the molecular level

metabolic control = processes that lead to a change in the output of a metabolic pathway over time

ROLE OF ENZYMES



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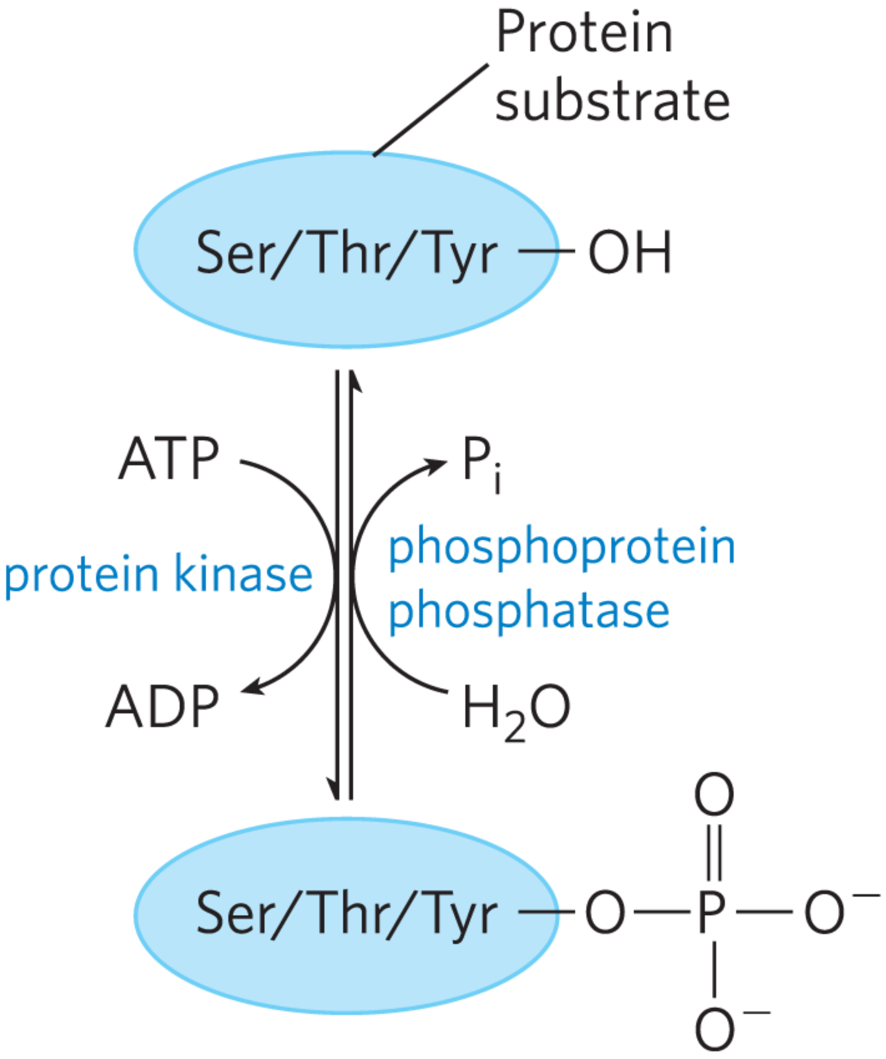
ROLE OF ENZYMES



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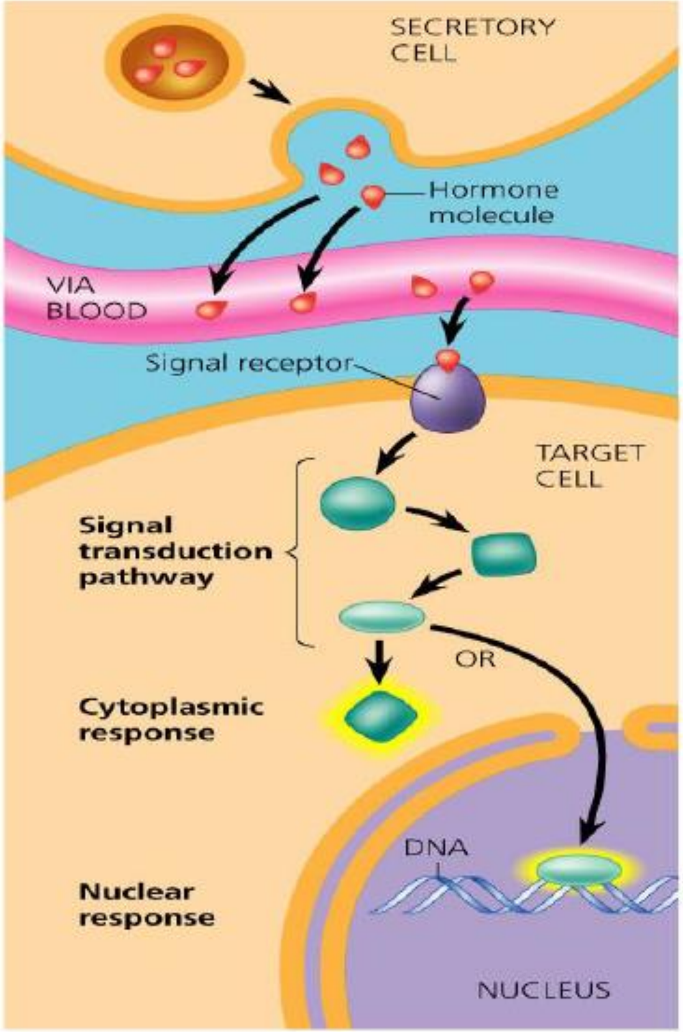
ROLE OF ENZYMES



(a) Receptor in plasma membrane

Hydrophilic hormones bind to a receptor on the cell membrane which causes several reactions known as a signal transduction pathway. This can affect the properties of enzymes/proteins (for instance phosphorylation) or it may affect gene expression.

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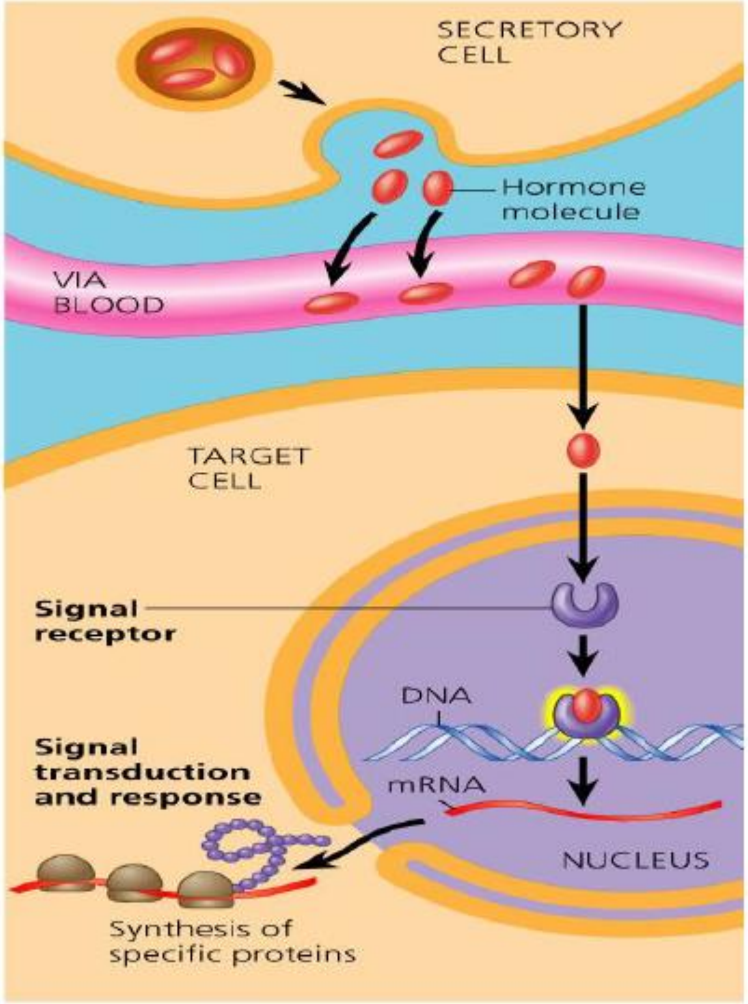
ROLE OF ENZYMES



(b) Receptor in cell nucleus

Hydrophobic messengers pass into cell (and sometimes the nucleus) where they bind with transcription factors which affect gene expression.

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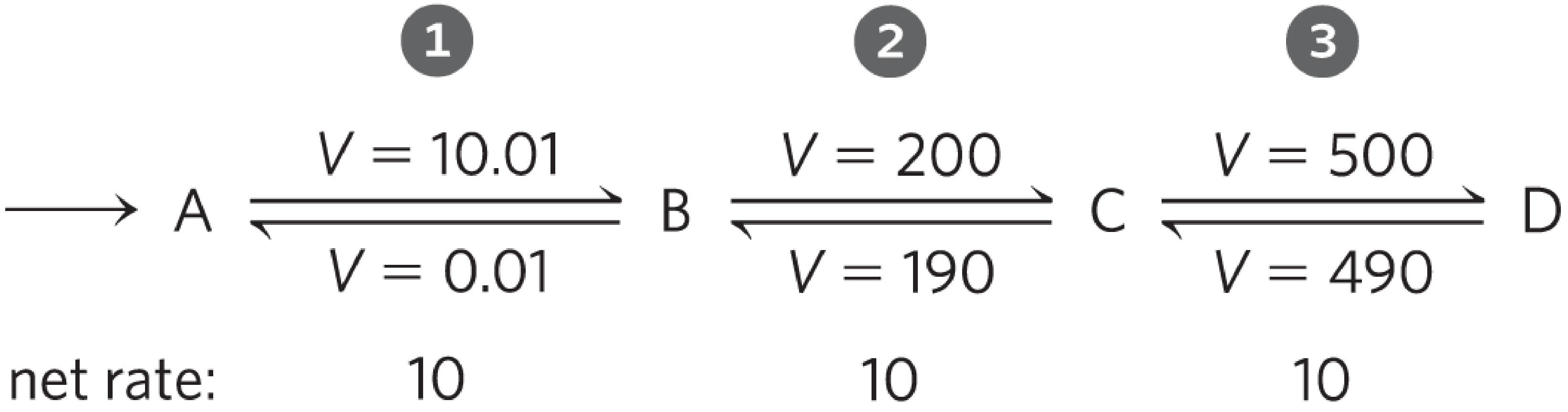
ROLE OF ENZYMES

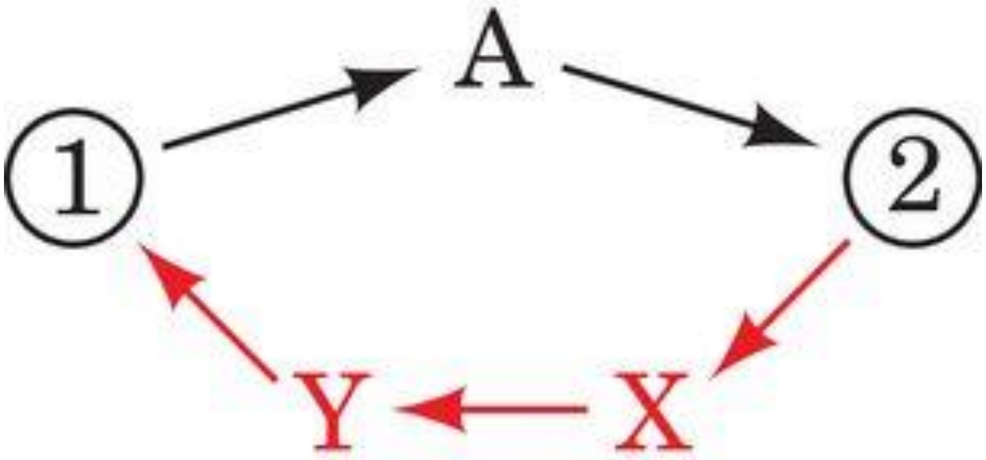
An irreversible step is obtained at the level of a rate-limiting enzyme. A high negative ΔG° helps but is not required.



Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company

Kinetic control is exerted at **irreversible** steps.

ROLE OF ENZYMES



Separation of catabolism and anabolism

Feedback inhibition

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