生物化学（下）授课安排

2018.02.26

课程名称: 生物化学（下） **学分** 3

教师姓名: 李勤喜、徐庆妍、赵同金、陈航姿、钟传奇

邮箱: liqinxi@xmu.edu.cn; xuqingyan@xmu.edu.cn;
zhaotj@xmu.edu.cn; chenhz@xmu.edu.cn;
zhongcq@xmu.edu.cn

电话: 13850006392；2181722；18650175031;
15959391425; 18649632837

助教： 张妍
地址：黄朝阳楼D424；E-mail: 361151210@qq.com
Tel：18850338306

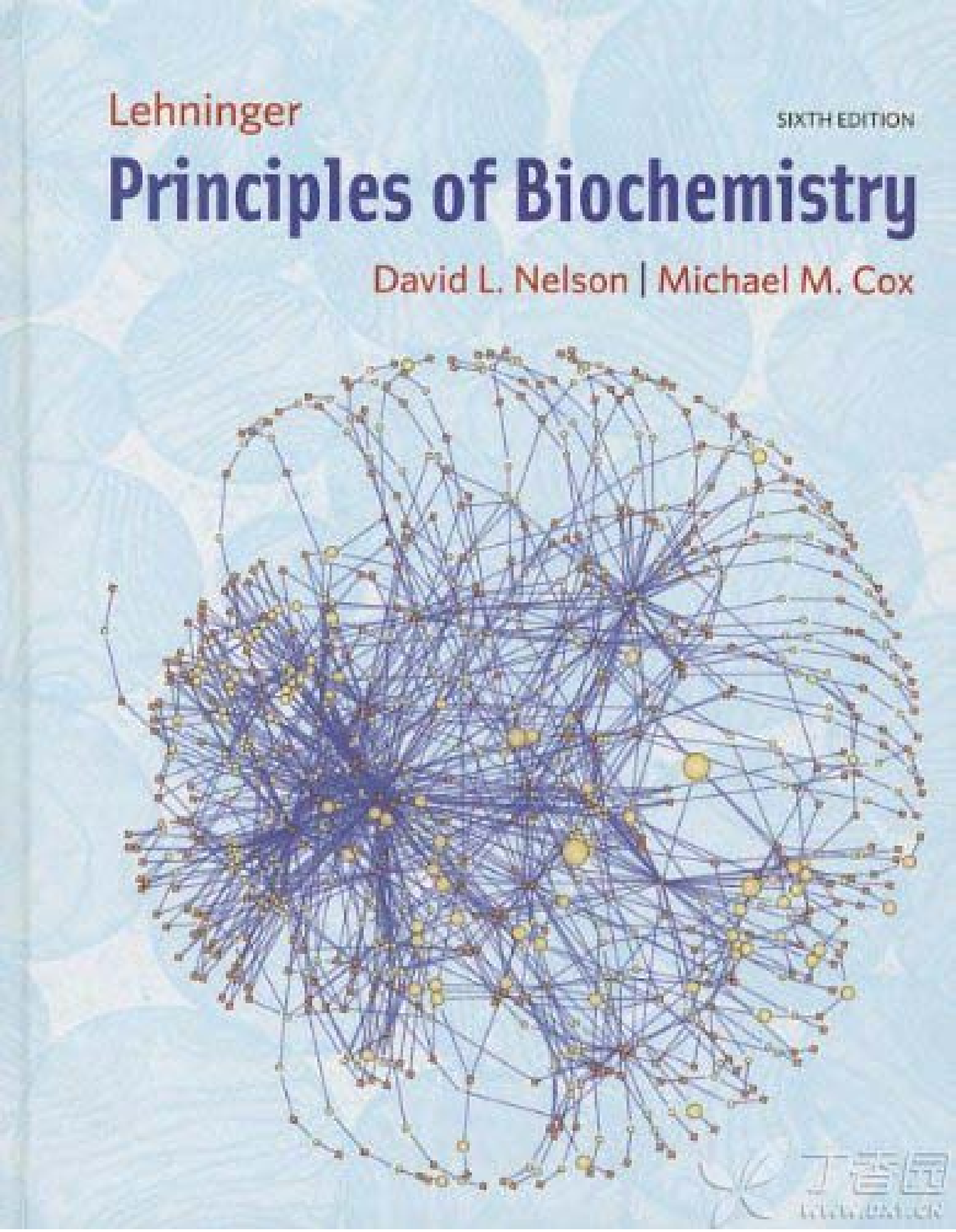
李勤喜（第1-4周）

研究领域：肿瘤代谢 Tumor Metabolism

地址：黄朝阳楼D422

E-mail：liqinxi@xmu.edu.cn

Tel：13850006392



提供PDF格式的
电子版教科书

周数(按校历)	各章节教学内容纲要	课时	主讲人	备注
1	Metabolism-An overview; Chapter 13, Bioenergetics and Biochemical Reaction types	3	李勤喜	第1周
2	Chapter 14, Glycolysis, Gluconeogenesis, Pentose phosphate pathway	3	李勤喜	第2周
3	Chapter 14, Glycolysis, Gluconeogenesis, Pentose phosphate pathway	3	李勤喜	第3周
4	Chapter15, Principles of metabolic regulation, Glycogen synthesis and breakdown	3	李勤喜	第4周
5	Chapter15, Principles of metabolic regulation, Glycogen synthesis and breakdown	3	陈航姿	第5周
6	Chapter 16, The Tricarboxylic Acid Cycle I	3	陈航姿	第6周
7	Chapter 16, The Tricarboxylic Acid Cycle II Chapter 17, Fatty Acid Catabolism	3	陈航姿	第7周
8	Chapter 18, Amino acid oxidation and the production of urea	3	陈航姿	第8周
9	Chapter 18, Amino acid oxidation and the production of urea Chapter 19, Electron Transport and Oxidative Phosphorylation	3	钟传奇	第9周
10	Chapter 19, Electron Transport and Oxidative Phosphorylation	3	钟传奇	第10周
11	Chapter 22, Biosynthesis of amino acids, nucleotides and related molecules	3	钟传奇	第11周
12	Chapter 22, Biosynthesis of amino acids, nucleotides and related molecules	3	徐庆妍	第12周
13	Chapter 21, Lipid biosynthesis	3	徐庆妍	第13周
14	Chapter 21, Lipid biosynthesis	3	徐庆妍	第14周
15	Chapter23, Hormone Regulation and Integration of Mammalian Metabolism	3	赵同金	第15周
16-17	复习、期末考试			期末考试

目的与任务：生物化学（下）主要讲授糖类、脂类、蛋白质及核酸在体内的代谢转化过程（包括合成代谢和分解代谢两大部分），通过学习不仅要让学生熟练掌握相关的基础知识，而且要很好地理解和感悟生物体内相互联系、相互制约和相互变化的反应是生命存在的重要特征，从而为进一步深入学习生命科学其它专业打下坚实的基础

教学大纲、教学进度表、课件及复习题均已上传到课程网站学生可自行下载：http://course.xmu.edu.cn
也可以直接在课堂copy

Text book: Lehninger Principles of Biochemistry, Sixth edition, PDF

考核方式： 考试+考查

成绩由期中成绩(占50%)和期末成绩（占50%）组成。

期中和期末成绩均由平时考查成绩（包括课堂考勤、课堂测验、课后作业，共占50%）和考试成绩（占50%）构成。

PART II
Bioenergetics and Metabolism

p501

All is flux, nothing stays still.

Nothing endures but changes.

You could not step twice into the same river.

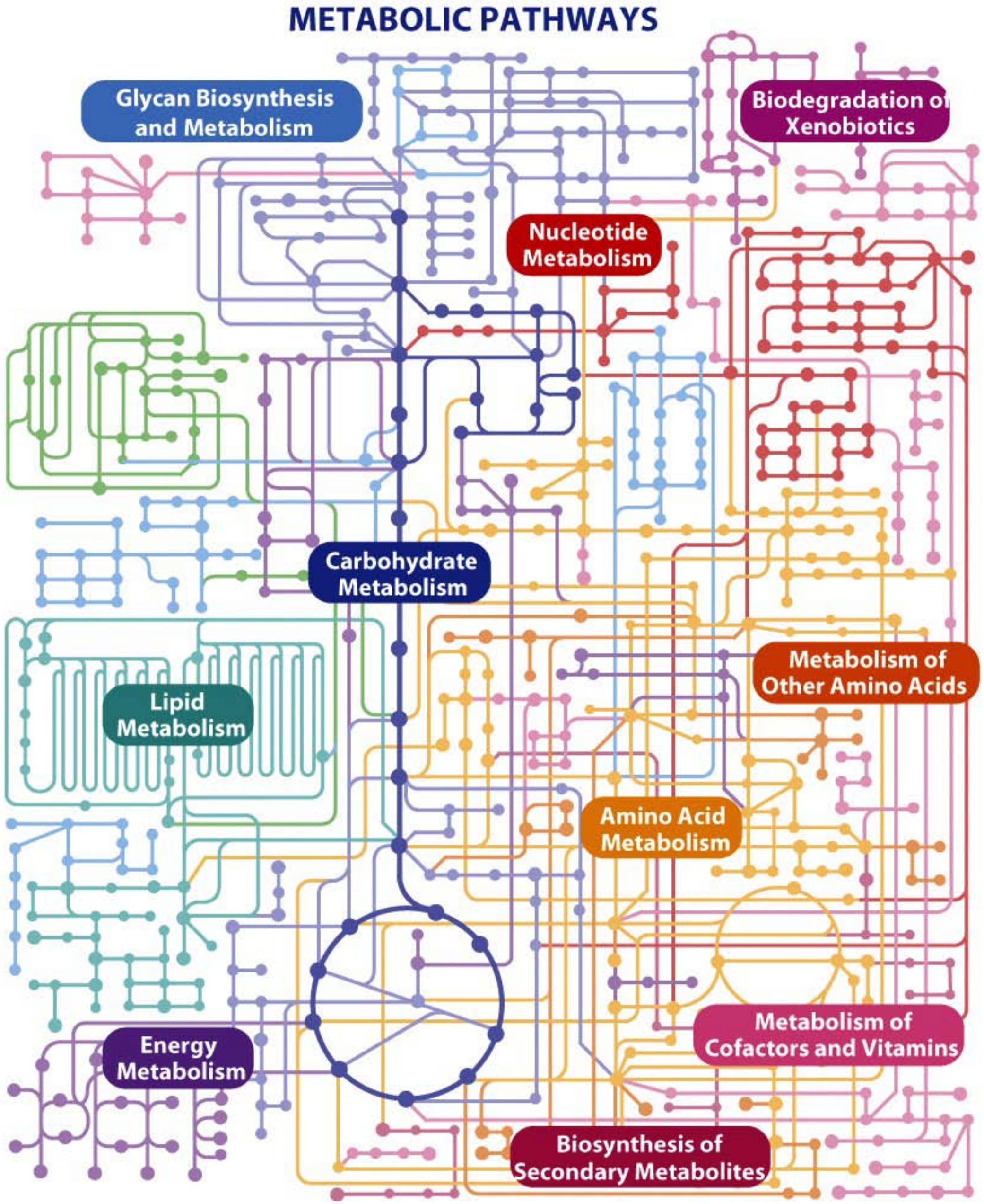
Heraclitus (535 BC-475 BC), Greek philosopher, known for his doctrine of change being central to the universe

Metabolism, the sum of all the chemical transformations taking place in a cell or organism, occurs through a series of enzyme-catalyzed reactions that constitute metabolic pathways.

The sum of catabolism and anabolism

Importance:

(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; (4) synthesize and degrade biomolecules required for specialized cellular functions, such as membrane lipids, intracellular messengers and pigments. (p501)



www.genome.ad.jp/kegg/pathway/map/map01100.html

Characters :

- Multiple pathways
- Every pathway proceeds in a stepwise fashion
  Intermediates
- Crosstalk between pathways
- Well regulated
- Major pathways are highly conserved. Glycolysis

Metabolic Diversity

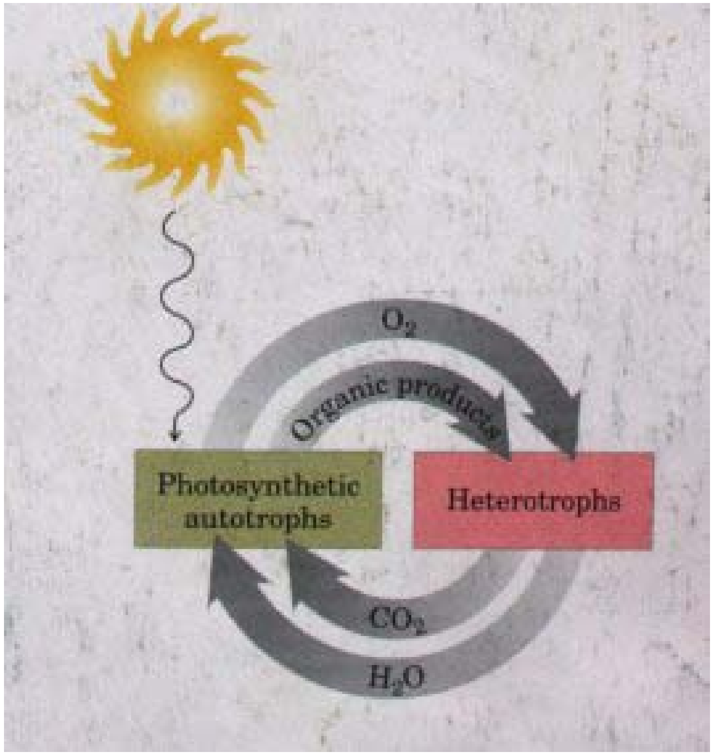
Classification based on carbon requirement

Autotrophs-----Heterotrophs

Classification based on energy sources

Phototrophs-----Chemotrophs

Five Kingdoms: Monera, Fungi, Protozoa, Plants and Animals



About **4×10^{11}** metric **tons** of carbon are turned over in the biosphere annually

The Flow of Energy in the Biosphere and the Carbon and Oxygen Cycles are Intimately Related

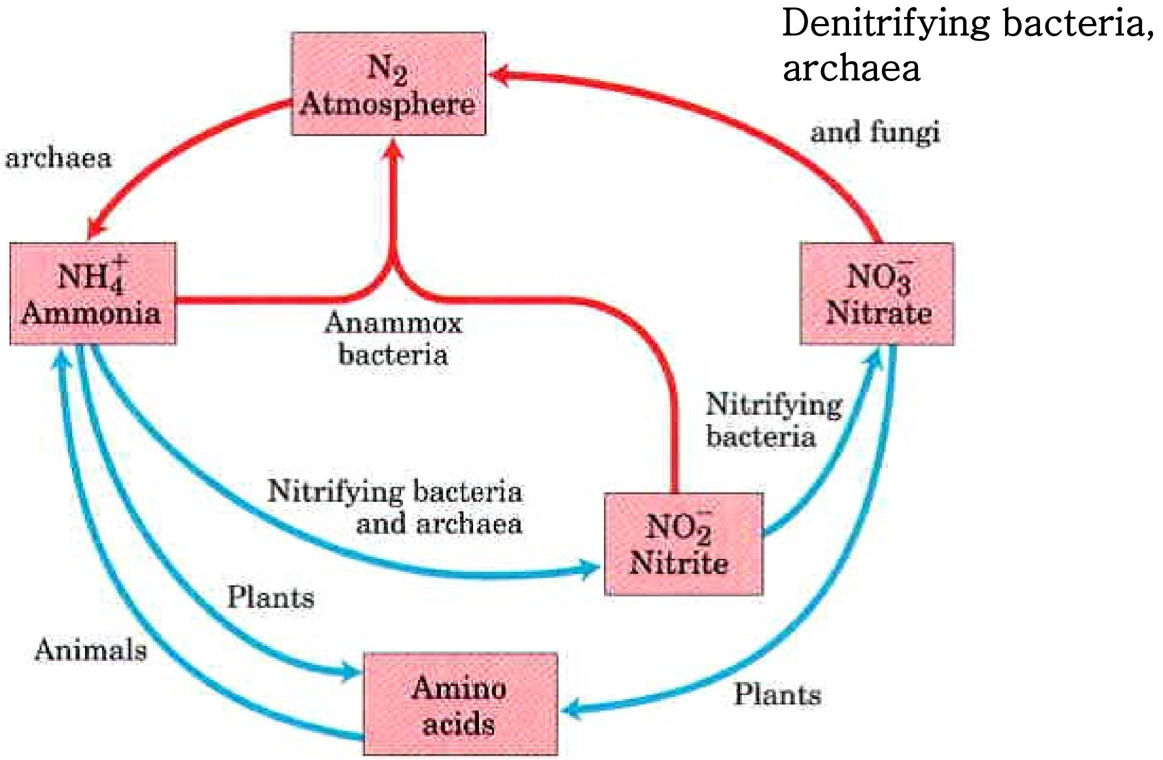
The Role of O_2 in Metabolism

Aerobes------anaerobes

Obligate aerobes------obligate anaerobes

（*Clostridium botulinum*）

Facultative aerobes



Cycling of nitrogen in the biosphere

Gaseous nitrogen(N_2) makes up 80% of the earth's atmosphere

Catabolism:

1. catabolism involves the oxidative degradation of complex nutrient molecules (carbohydrates, lipids and proteins).

2. catabolism leads to the formation of simpler molecules such as lactic acid, ethanol, CO_2, urea or ammonia, that is to say, the pathways of catabolism converge to a few end products.

3. usually exergonic, and chemical energy released is captured in the form of ATP

The three stage of catabolism.

Building block

Common degradation product

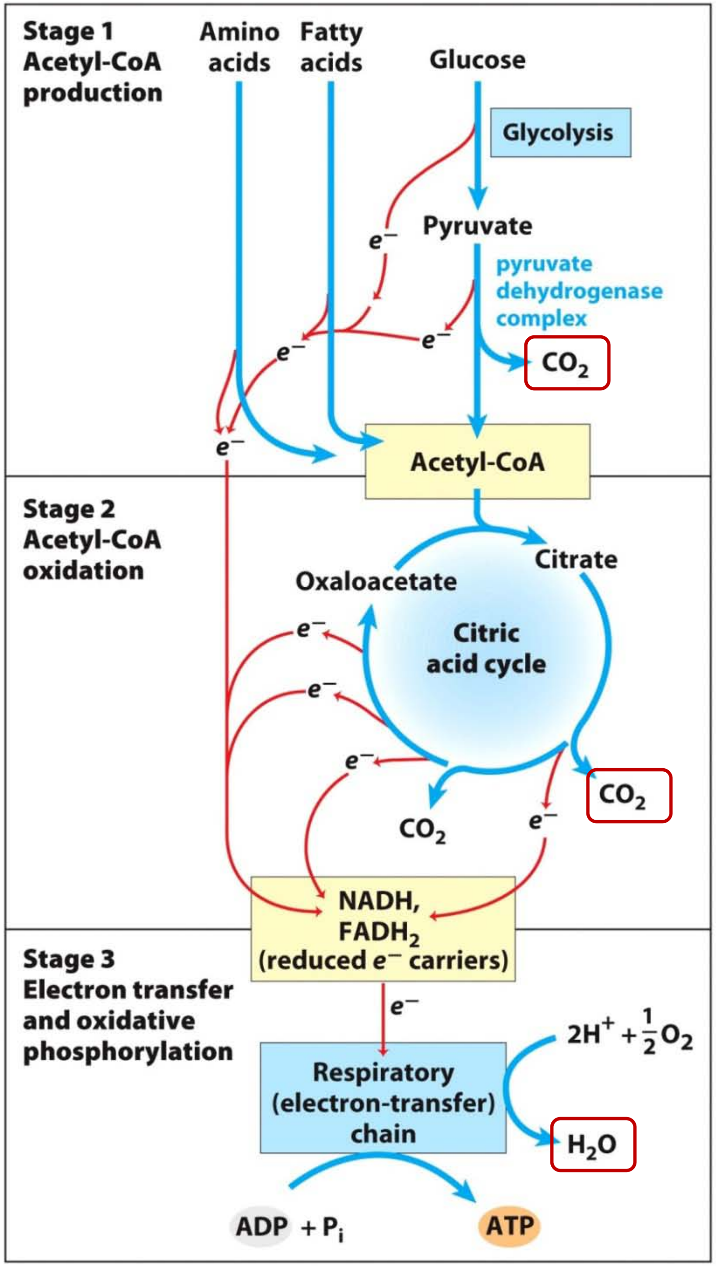
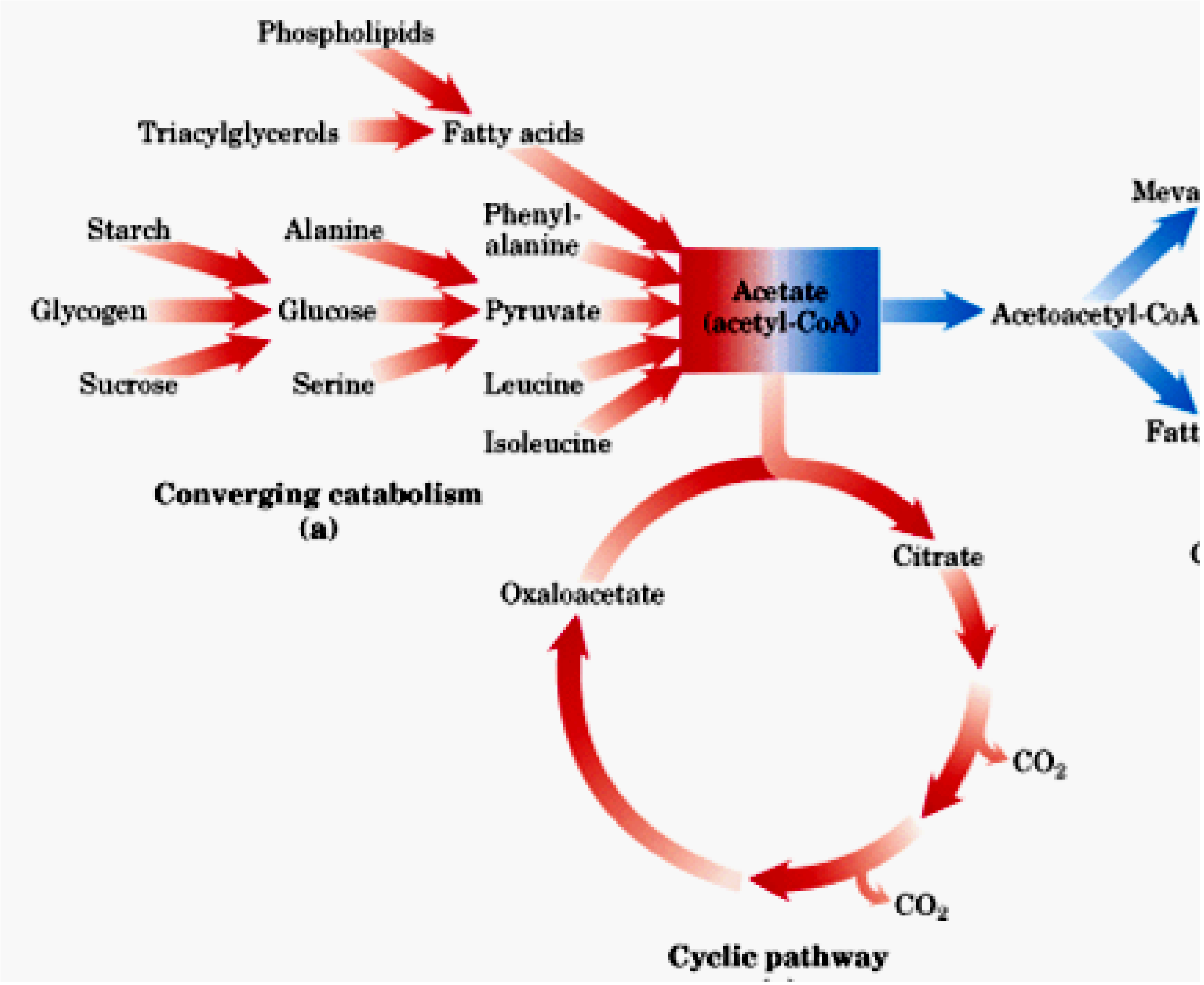


Figure 16-1

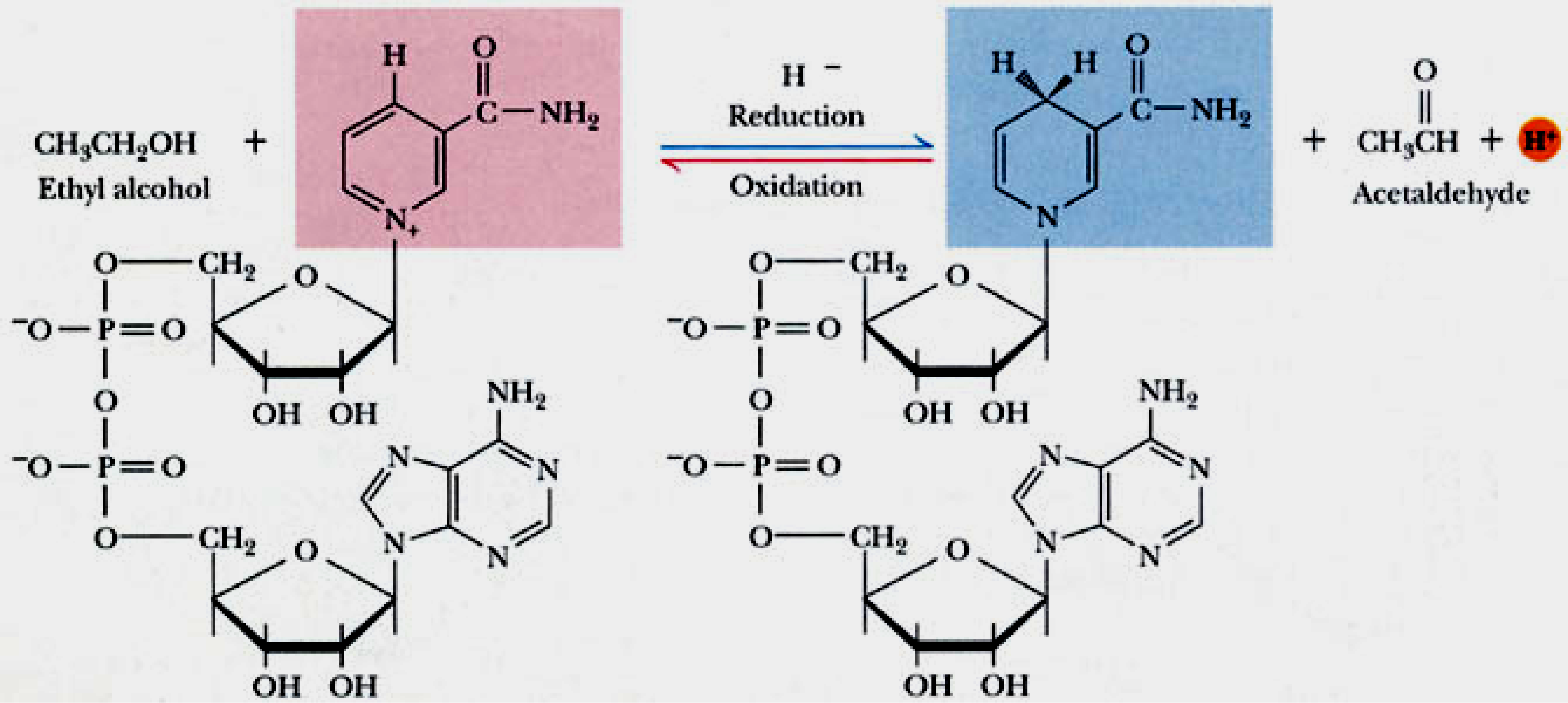
Simple, small end product

NH_3, urea



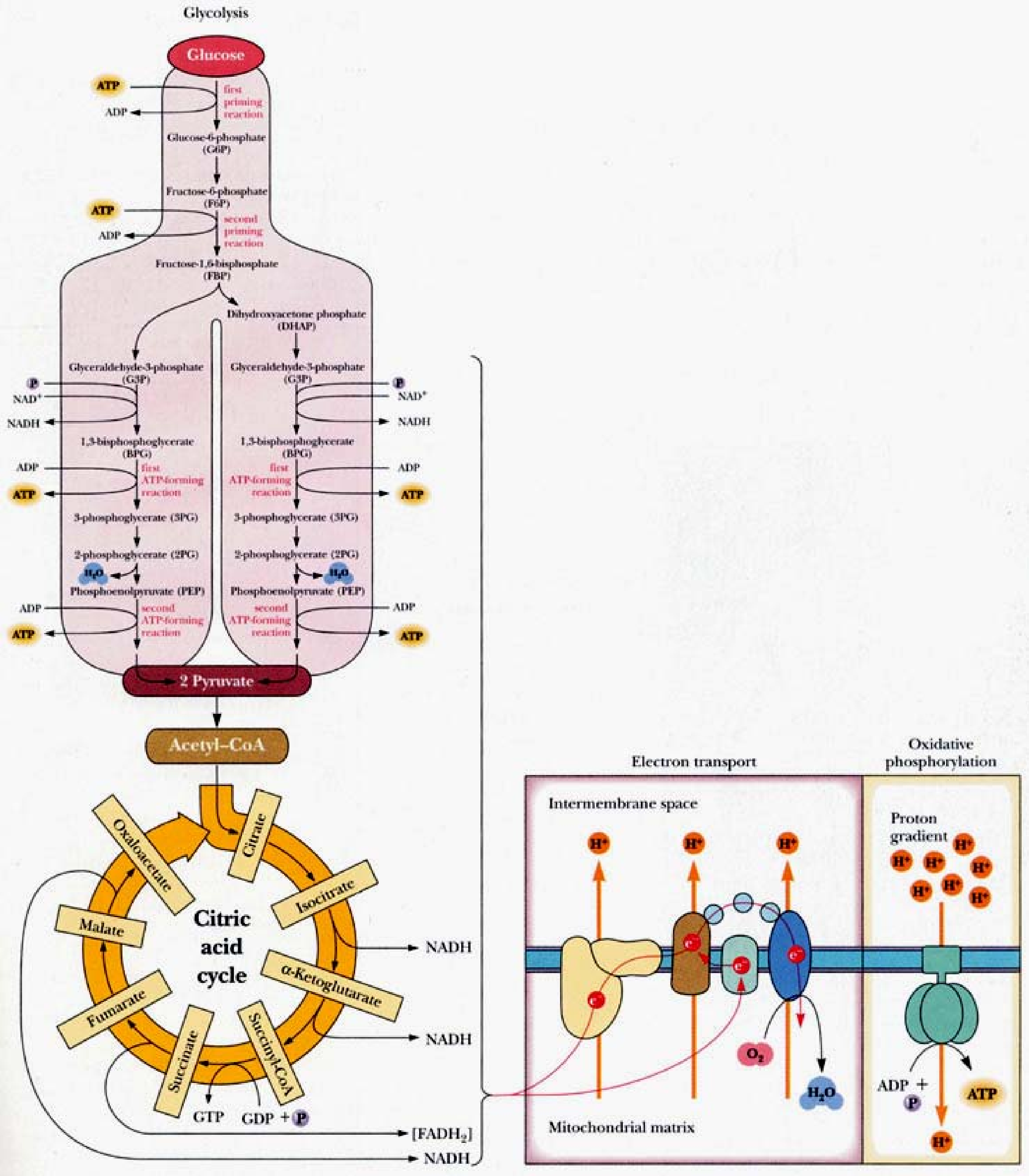
Catabolism is converging

4. Part of the chemical energy are conserved by the coenzymes NAD^+ and $NADP^+$ as the form of NADH and NADPH. Oxidation of **NADH back to NAD^+ serves to generate ATP**

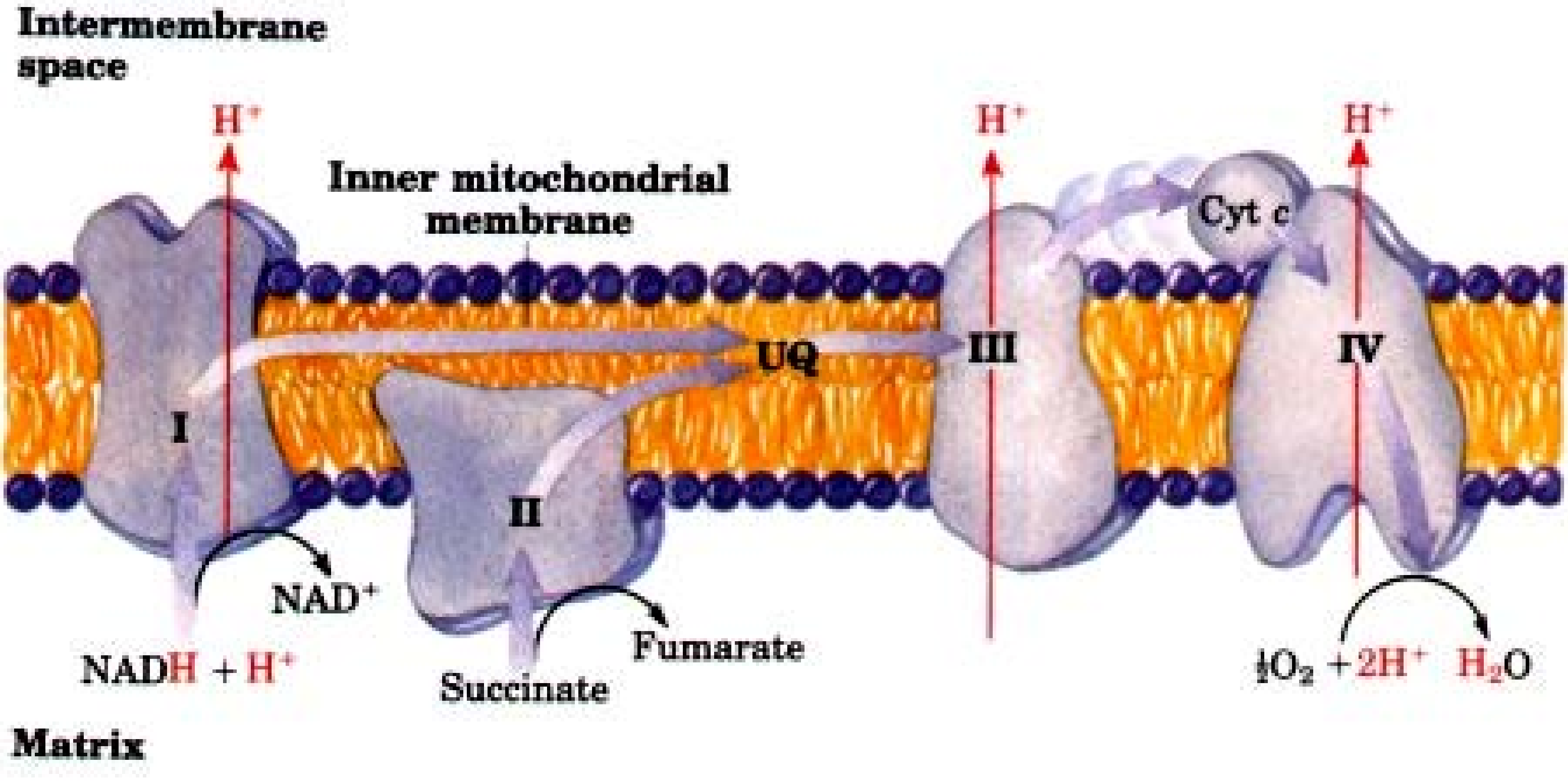


↑
In NADP$^+$ this hydroxyl group is esterified with phosphate

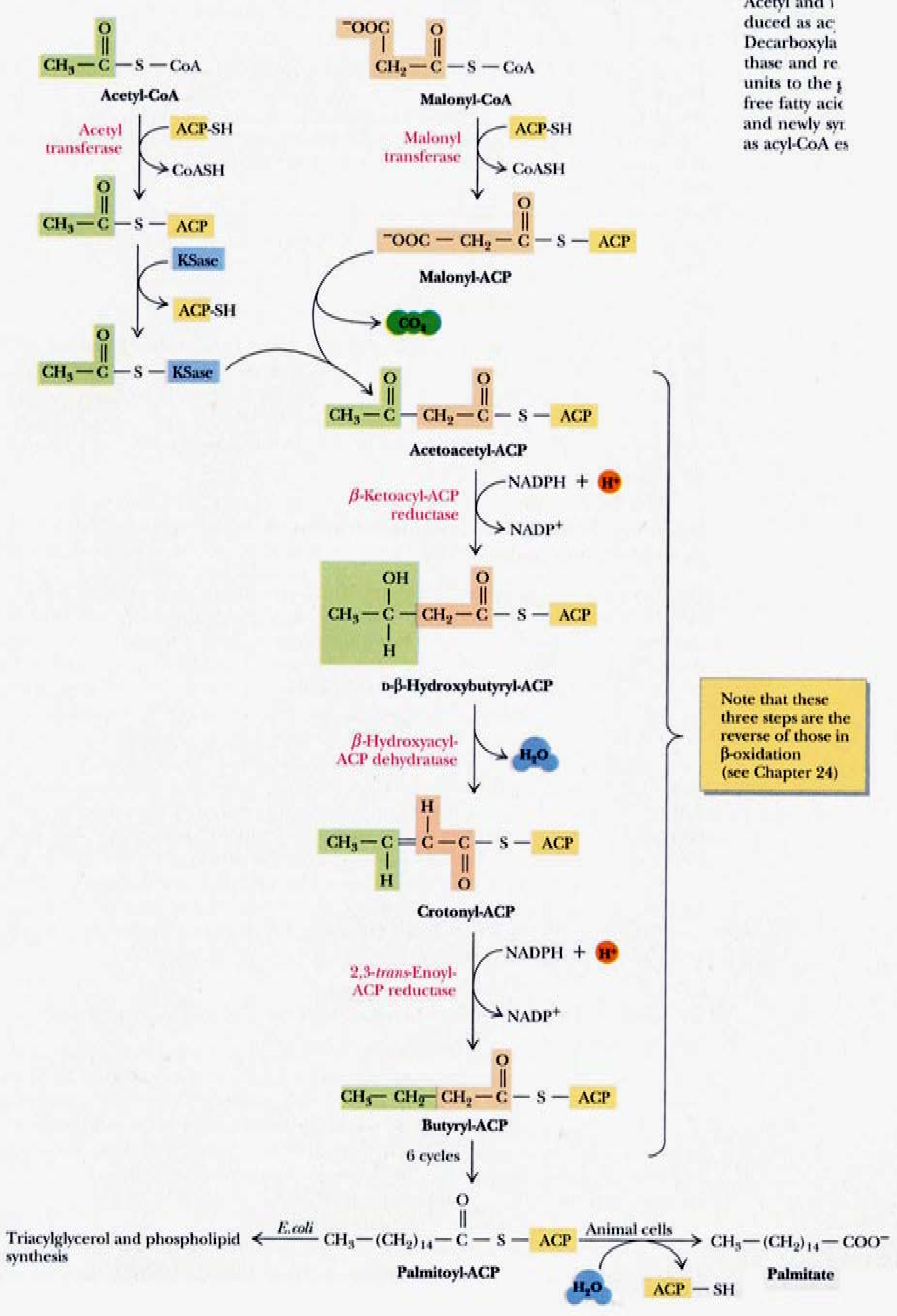
$$AH_2 + NAD^+ \rightarrow A + NADH + H^+$$



NAD$^+$ collects electrons released in catabolism.



NADH is oxidized back to NAD$^+$, and the electrons are transferred along the electron transport chain to oxygen, followed by the synthesis of ATPs



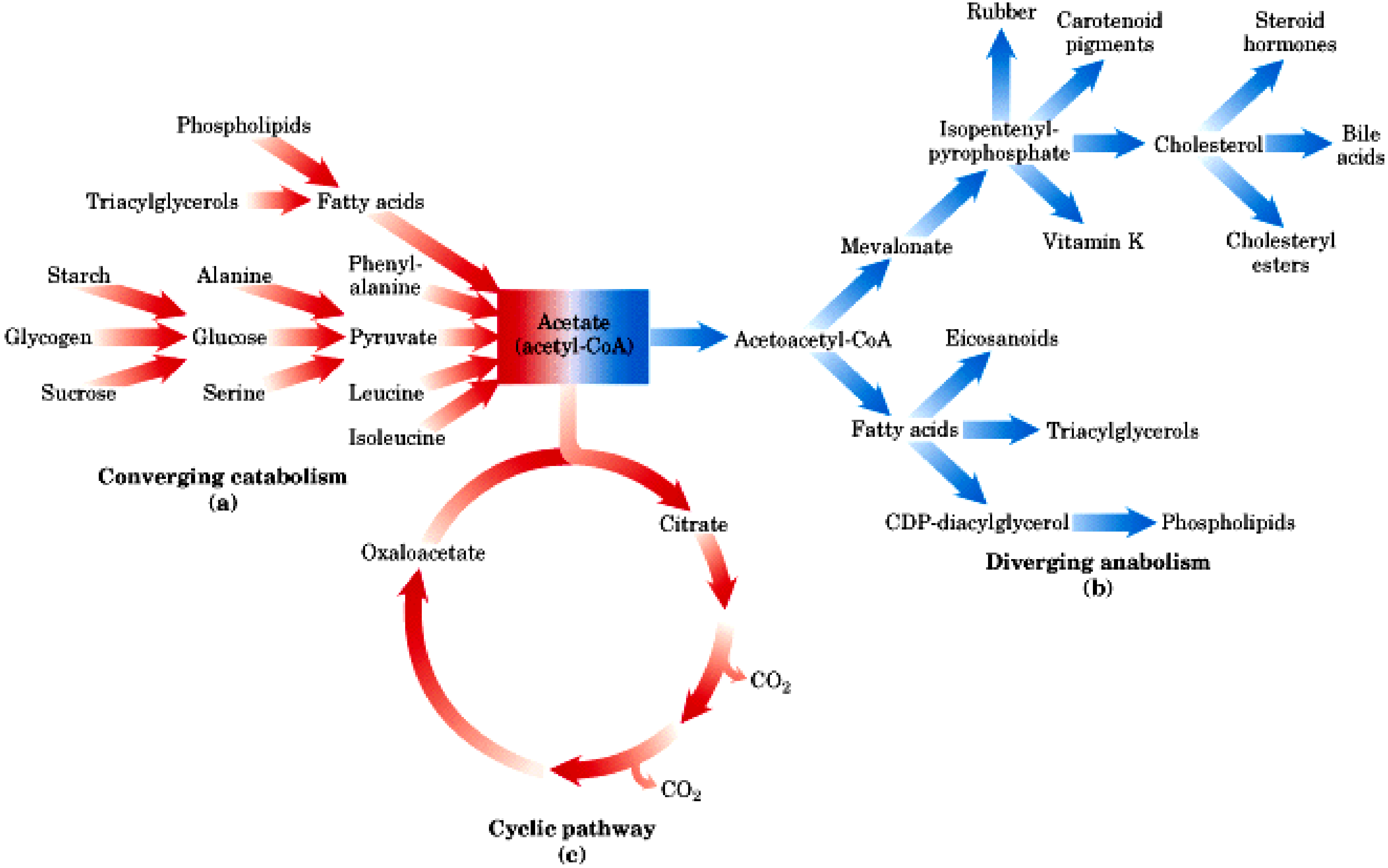
Acetyl and
duced as ac
Decarboxyla
thase and re
units to the
free fatty acic
and newly syr
as acyl-CoA es

Note that these three steps are the reverse of those in β-oxidation (see Chapter 24)

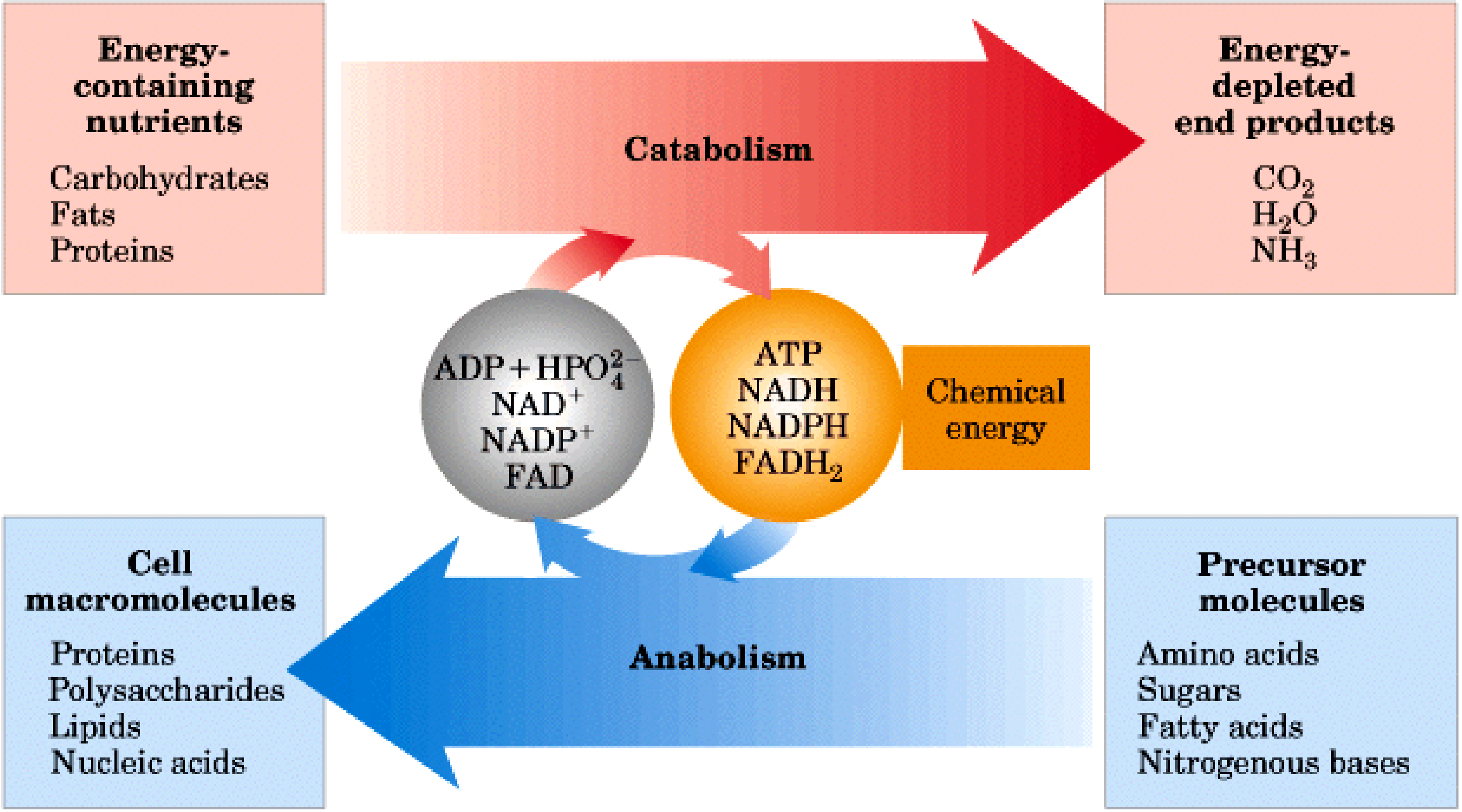
NADPH provides the reducing power for anabolic process.

Anabolism:

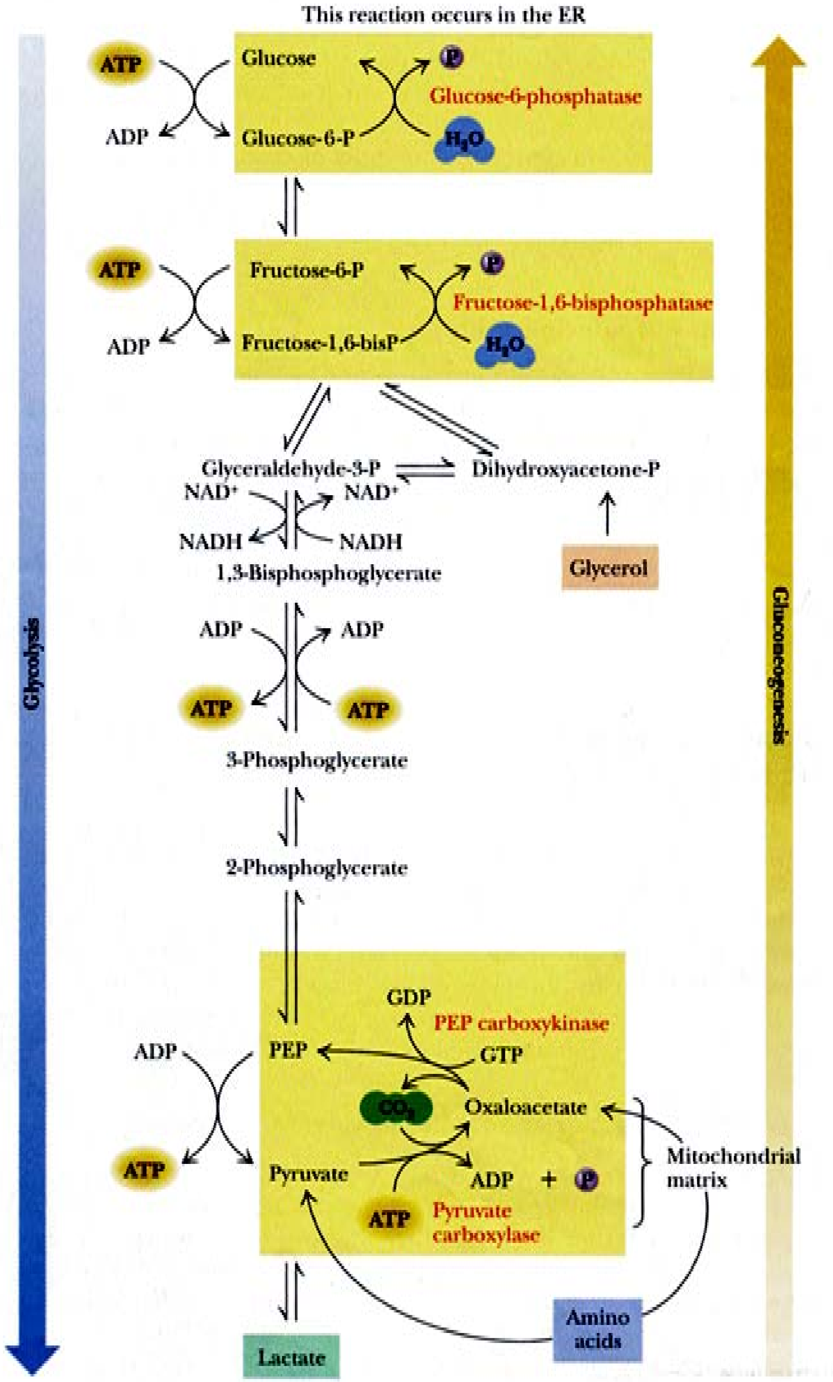
1. Anabolism is a reductive and synthetic process in which simple precursors are assembled to varied and complex biomolecules.
2. A limited set of building blocks are synthesized into a variety of biomolecules. The processes of anabolism are Diverging
3. Anabolism is endergonic , ATP generated by catabolism provides the energy.
4. NADPH is a donor of high-energy electrons for reductive reaction of anabolism



Anabolism is diverging.



Anabolism and catabolism are interrelated both in energy and intermediates, and many metabolites can serve in both catabolism and anabolism. (perpetual motion machines ?)

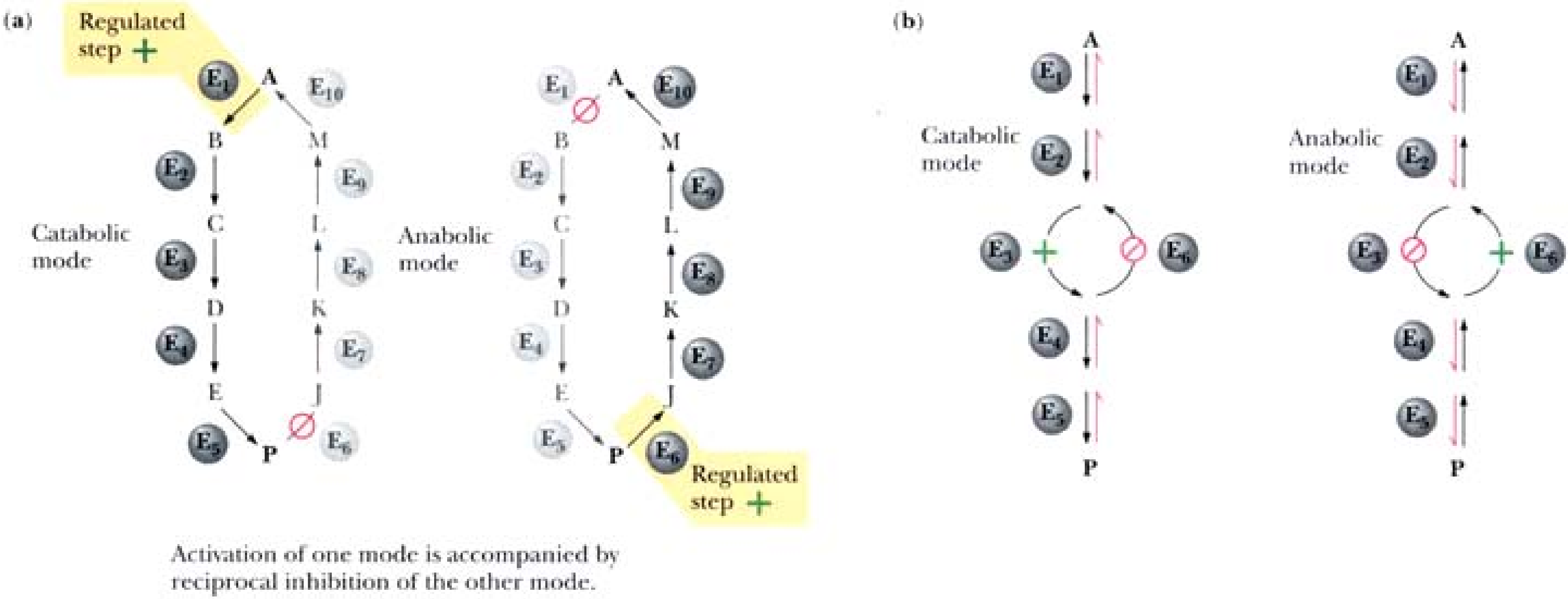


Some intermetabolites are amphibolic.

Glycolysis and gluconeogenesis

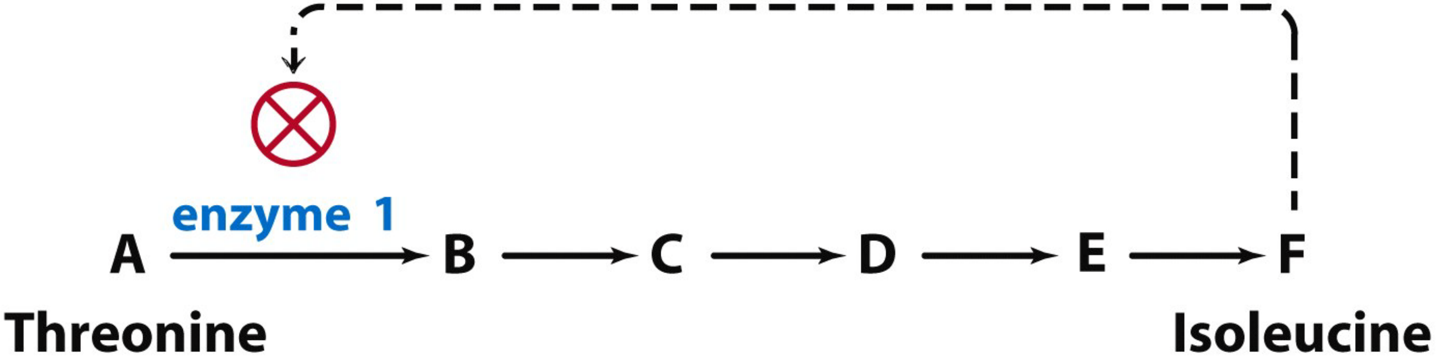
Some of the intermediates may be common to steps in both pathways, but anabolism and catabolism are not mutually exclusive:

1. There must be at least one enzymatic reaction is different between catabolism and anabolism;
2. Different pathways serving in opposite metabolic direction must be independently regulated.



Activation of one mode is accompanied by reciprocal inhibition of the other mode.

Parallel pathways of catabolism and anabolism must differ in at least one metabolic step .



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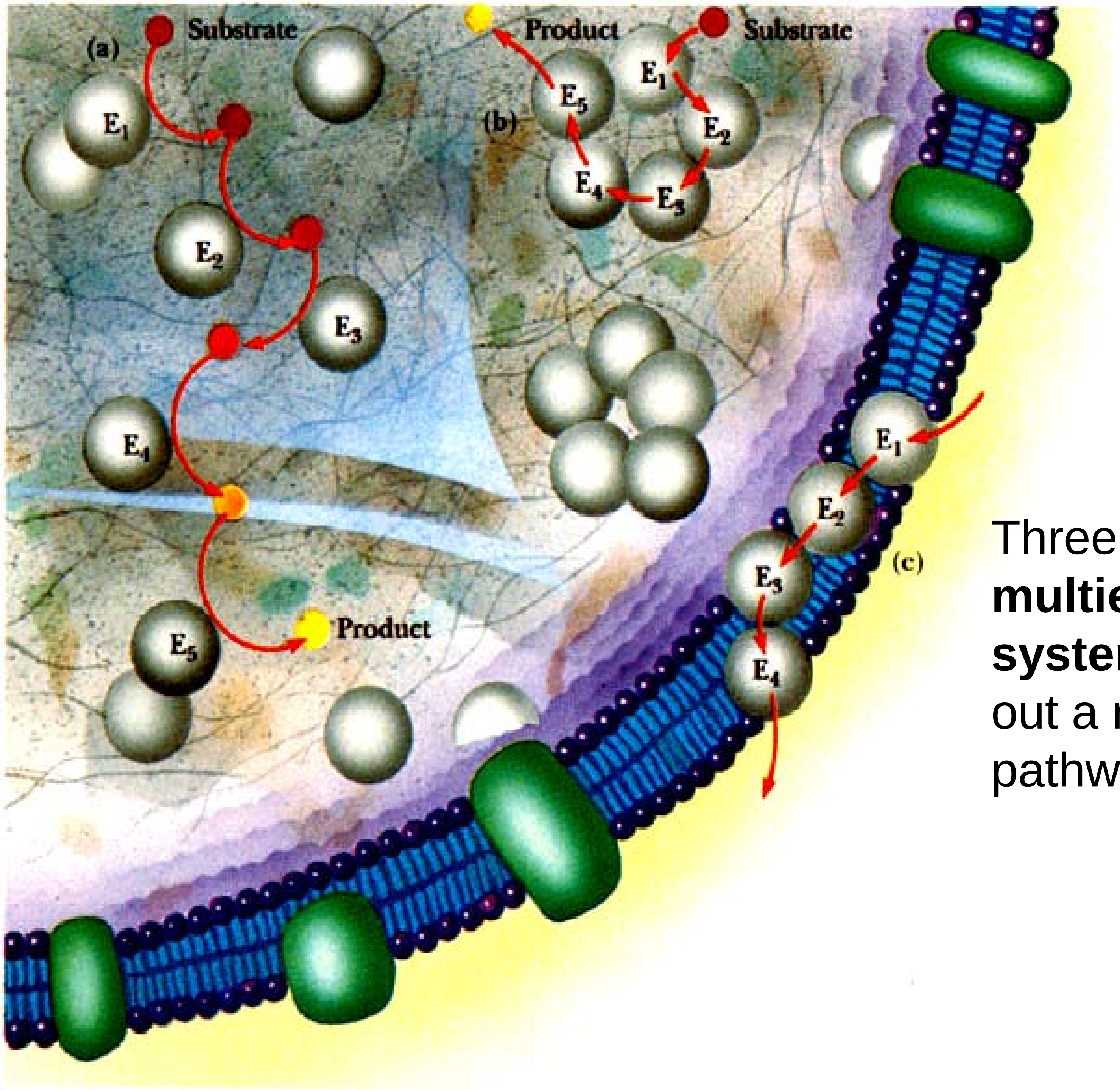


Three types of **multienzyme systems** carrying out a metabolic pathway.

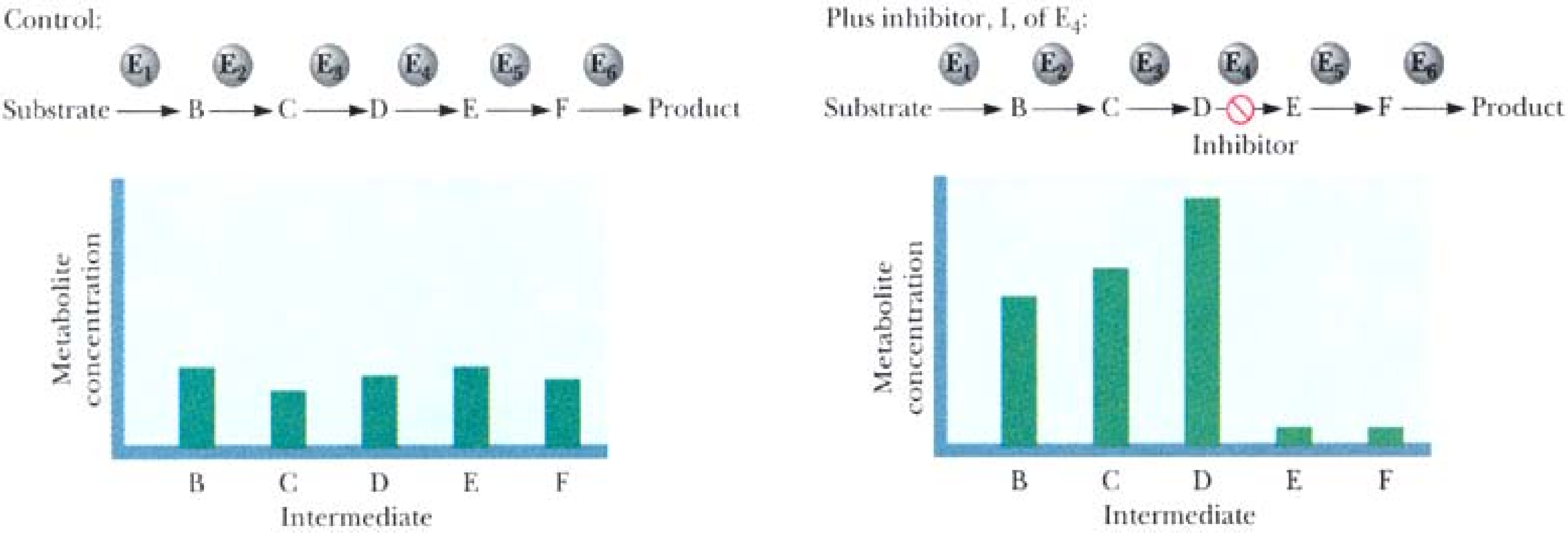
Metabolic pathways are compartmentalized within cells

A prokaryotic cell shows some segregation of metabolism.

A eukaryotic cell is extensively compartmentalized by an endomembrane system: the endoplasmic reticulum, the Golgi complex, mitochondria, lysosomes, vacuoles, microbodies.

Experimental Methods to Reveal Metabolic Pathways

1. The use of metabolic inhibitors

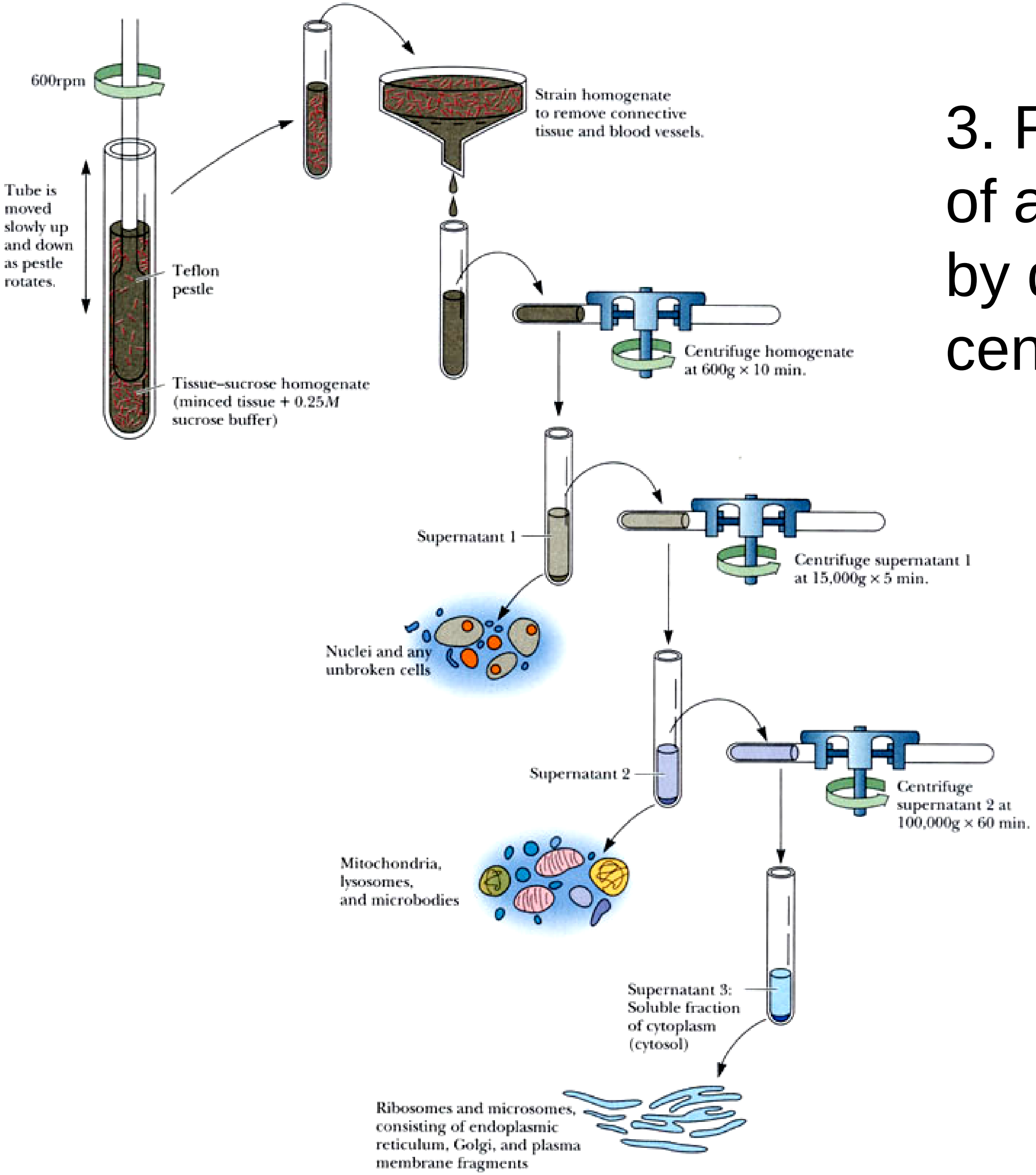


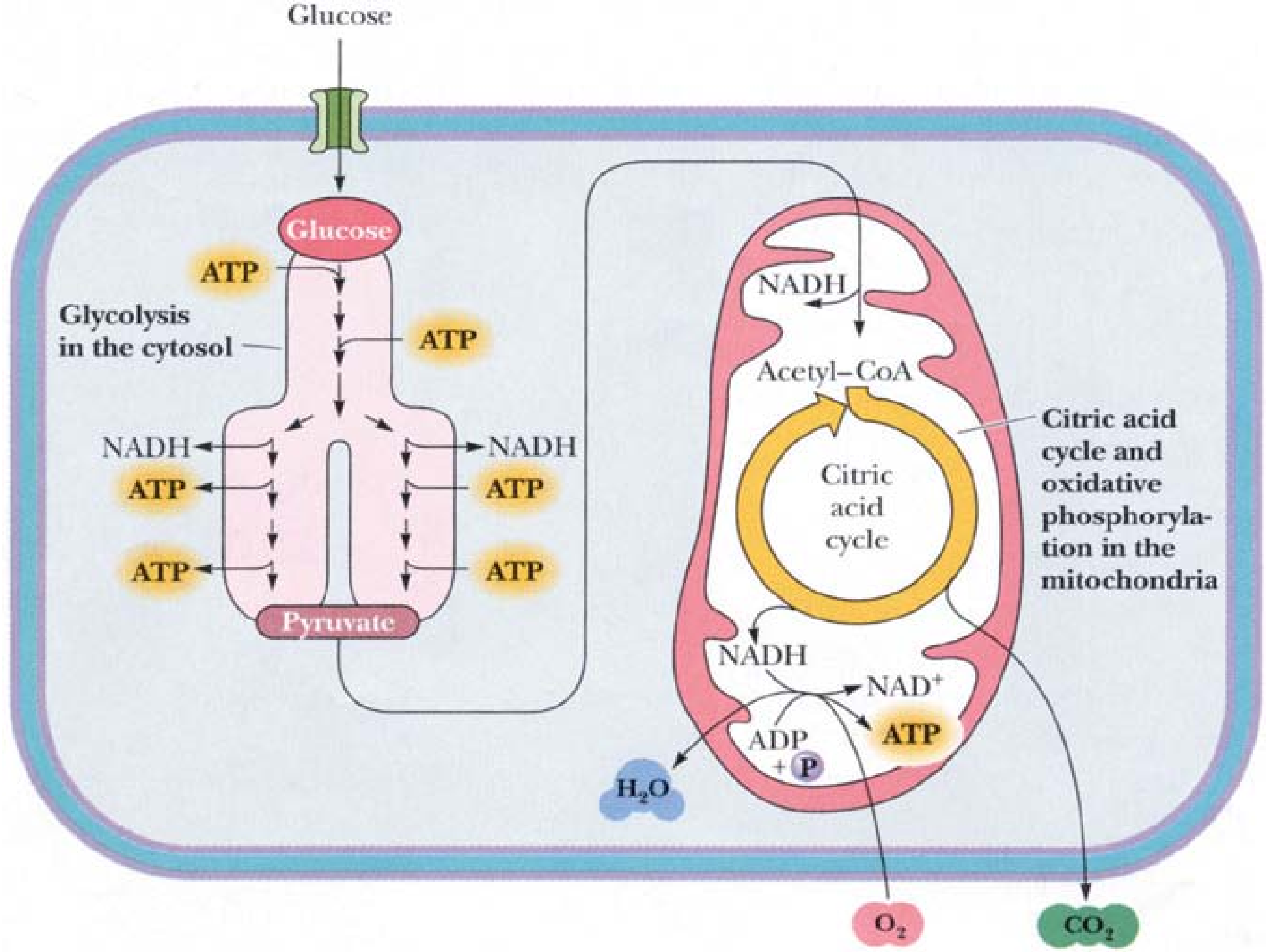
The use of inhibitors to reveal the sequences in a metabolic pathway.

2. Mutations create specific metabolic blocks

Mutation in a gene encoding an enzyme often results in an inability to synthesize the enzyme in an active form, which leads to a block in the metabolic pathway and accumulation of the enzyme’s substrate.

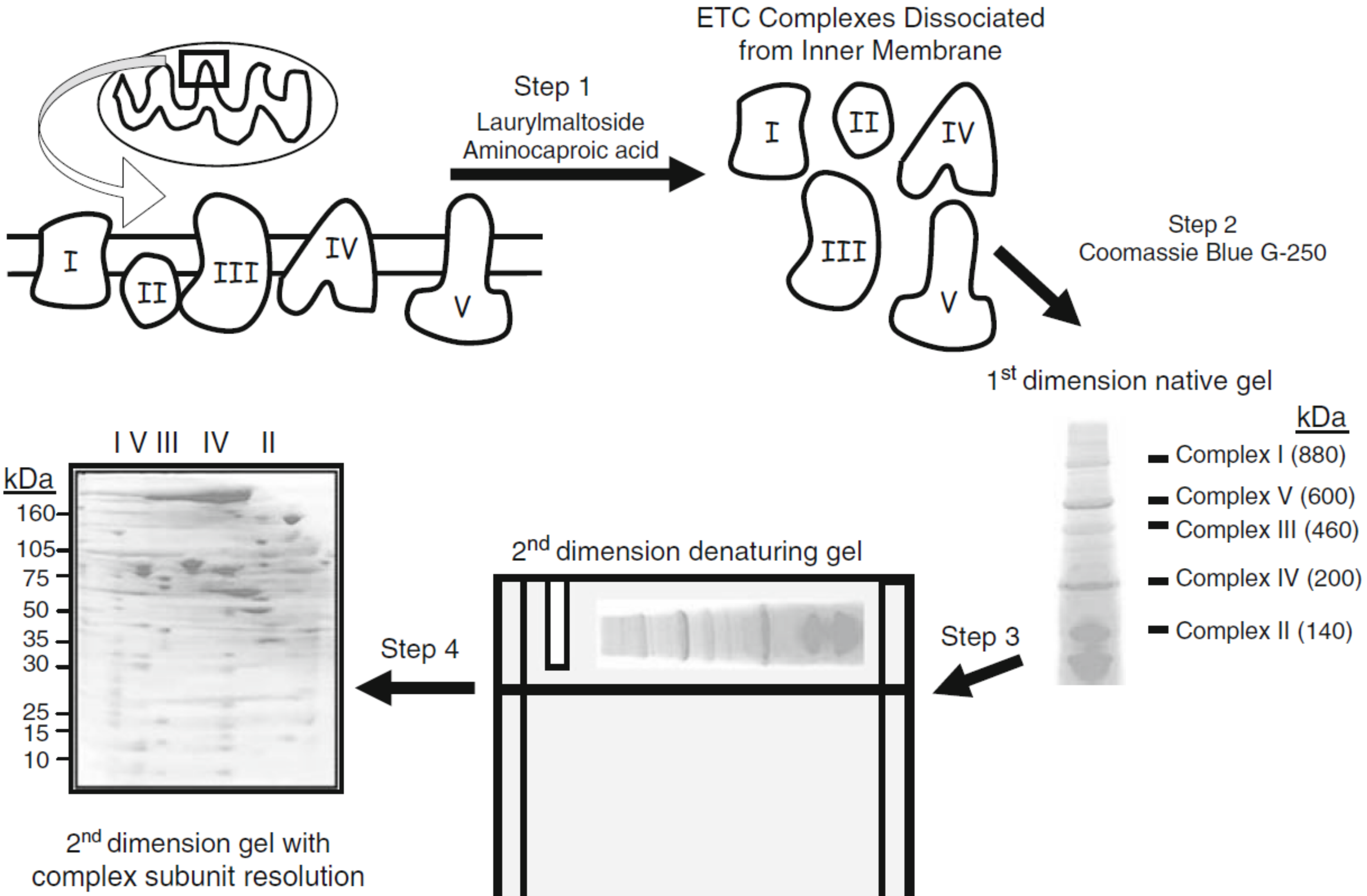
3. Fractionation of a cell extract by differential centrifugation.





Localization

4. BN-PAGE proteomics



Step 1
Laurylmaltoside
Aminocaproic acid

ETC Complexes Dissociated from Inner Membrane

Step 2
Coomassie Blue G-250

1^{st} dimension native gel

Step 3

2^{nd} dimension denaturing gel

Step 4

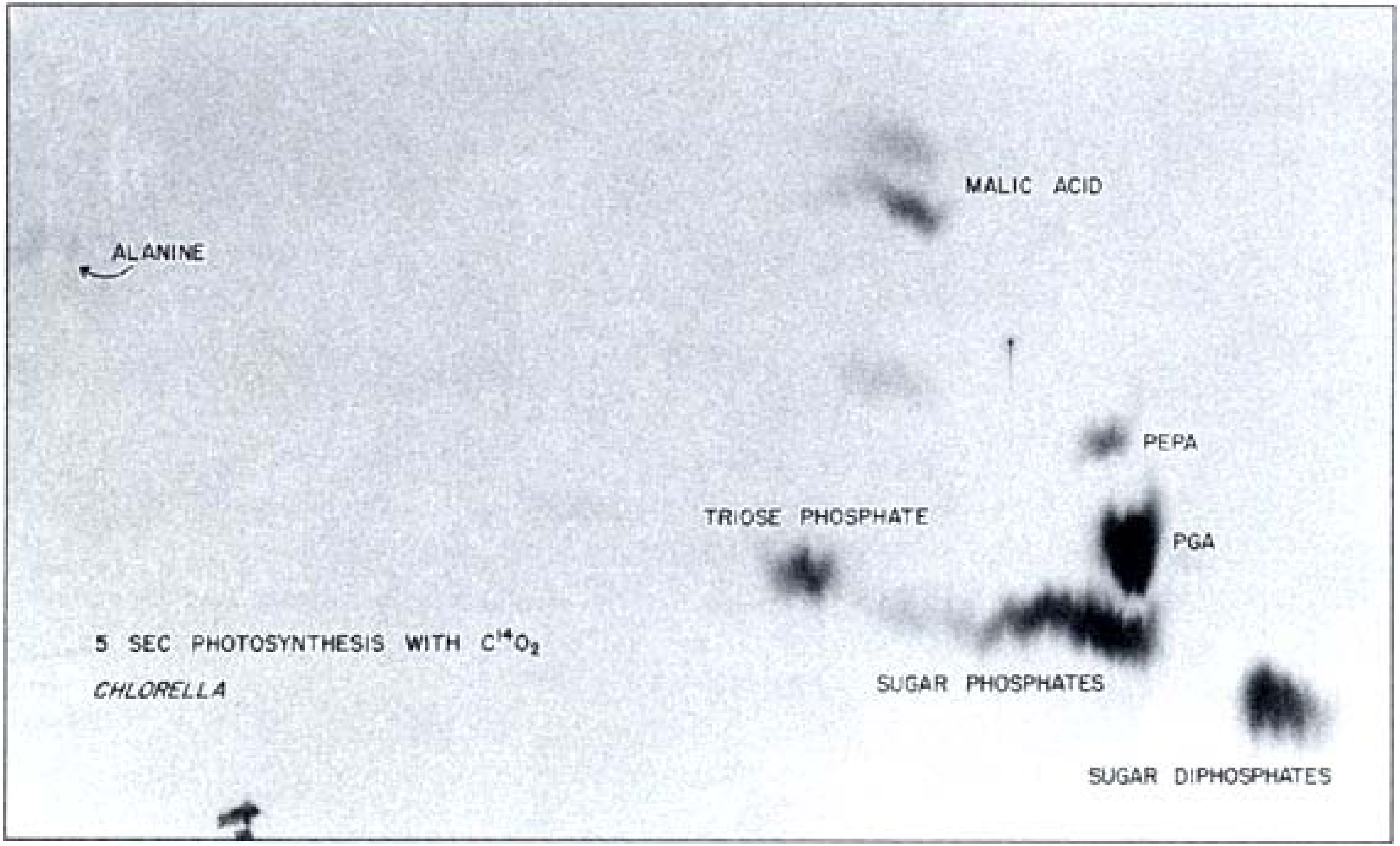
2^{nd} dimension gel with complex subunit resolution

5. Isotopic tracer as metabolic probes

Radioactive isotopes : ^{14}C, ^{32}P, ^{35}S, ^{131}I

stable “heavy” isotopes : ^{13}C, ^{15}N,^{18}O,

^{14}C-UBT(urea breath test) is performed to detect helicobacter *pylori*



Experiments using a radioactive isotope as a metabolic tracer

Cells of chlorella were exposed to ^{14}C labelled CO_2 for 5 sec

$$C^{18}O_2 + 2H_2O \longrightarrow (CH_2{}^{18}O) + O_2 + H_2{}^{18}O$$

$$C^{16}O_2 + 2H_2{}^{18}O \longrightarrow (CH_2{}^{16}O) + {}^{18}O_2 + H_2{}^{16}O$$

^{18}O was used as a tracer of the fate of the oxygen atoms in water and carbon dioxide.

CHAPTER 13
Bioenergetics and Biochemical Reaction Types

p505-542

13.1 Bioenergetics and Thermodynamics

The first law of thermodynamics

In any physical or chemical change, the total amount of energy in the universe remains constant, although the form of the energy may change.

The second law of thermodynamics

The total entropy of the universe is continually increasing.

The universe always tends toward increasing disorder

Heat and Work.

Lavoisier

Antoine Lavoisier
1743–1794

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French nobleman and chemist who is widely considered to be the **"Father of Modern Chemistry"**

1. Lavoisier's great accomplishments in chemistry largely stem from the fact that he **changed the science from a qualitative to a quantitative.**
2. Discovered the **role oxygen plays in combustion**. He recognized and named oxygen (1778) and hydrogen (1783) and opposed the phlogiston theory.
3. Lavoisier helped to **construct the metric system**, wrote the first extensive list of elements, and helped to reform chemical nomenclature.
4. He predicted the existence of **silicon** (1787) and was also the first to **establish that sulfur was an element** (1777) rather than a compound.
5. He discovered that, **although matter may change its form or shape, its mass always remains the same**.

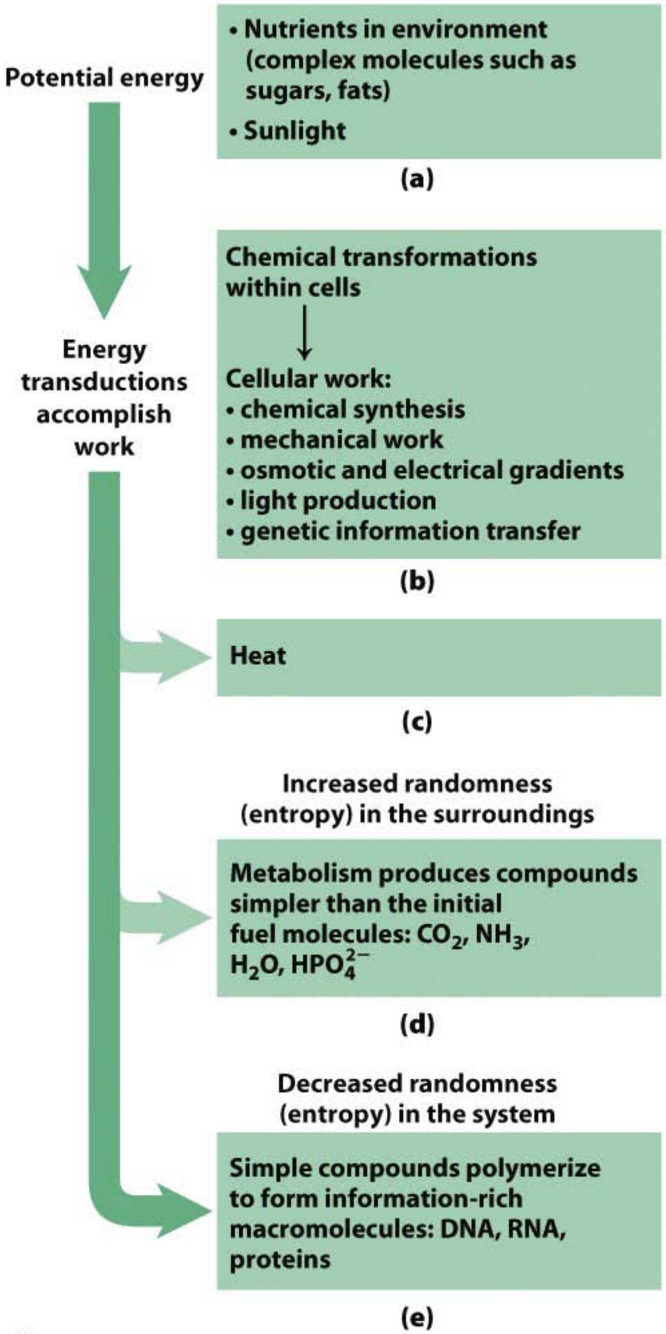
……in general, respiration is nothing but a slow combustion of carbon and hydrogen, which is entirely similar to that which occurs in a lighted lamp or candle, and that, from this point of view, animals that respire are true combustible bodies that burn and consume themselves……the torch of life lights itself at the moment the infant breathes for the first time, and it does not extinguish itself except at death.*

*From a memoir by Armand Seguin and Antoine Lavoisier, dated 1789, quoted in Lavoisier, A (1862) Oeuvres de Lavoisier, Imprimerie Imperiale, Paris

Living organisms exist in a dynamic steady state, never at equilibrium with their surroundings

Organism transform energy and matters from their surroundings

Isolated system
Closed system
Open system



Potential energy

- Nutrients in environment (complex molecules such as sugars, fats)
- Sunlight

(a)

Energy transductions accomplish work

Chemical transformations within cells

↓

Cellular work:
- chemical synthesis
- mechanical work
- osmotic and electrical gradients
- light production
- genetic information transfer

(b)

Heat

(c)

Increased randomness (entropy) in the surroundings

Metabolism produces compounds simpler than the initial fuel molecules: CO_2, NH_3, H_2O, HPO_4^{2-}

(d)

Decreased randomness (entropy) in the system

Simple compounds polymerize to form information-rich macromolecules: DNA, RNA, proteins

(e)

Figure 1-24
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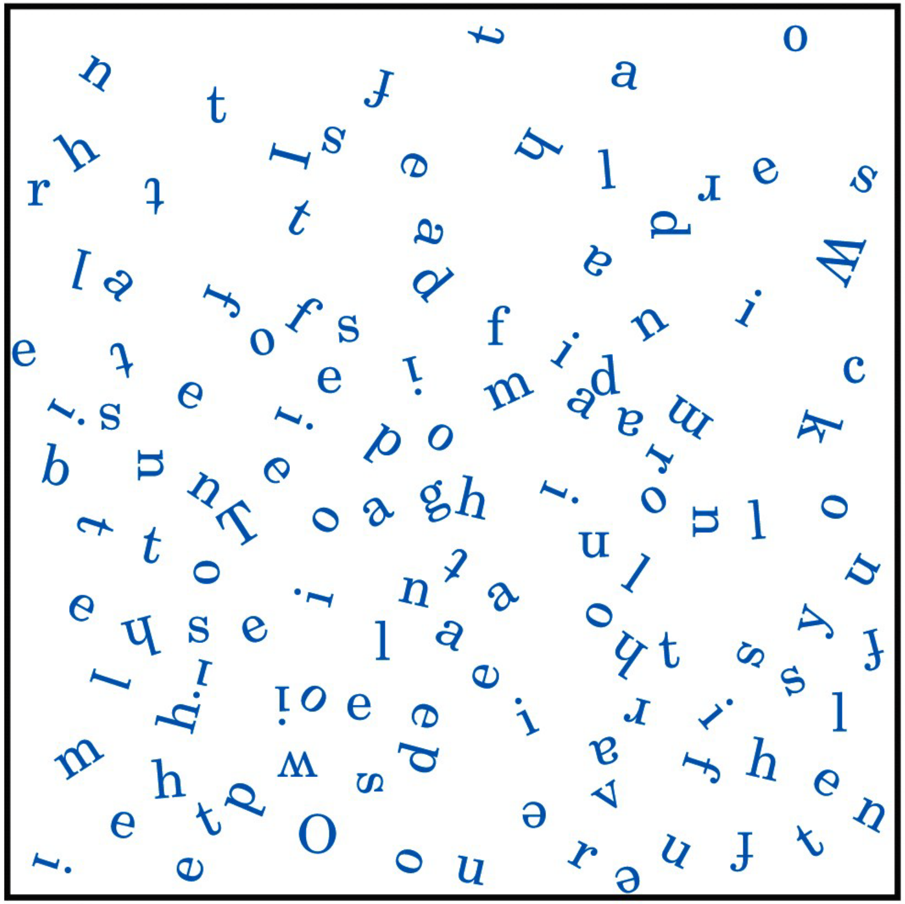
Some energy transformations in living organisms



Box 1-3b
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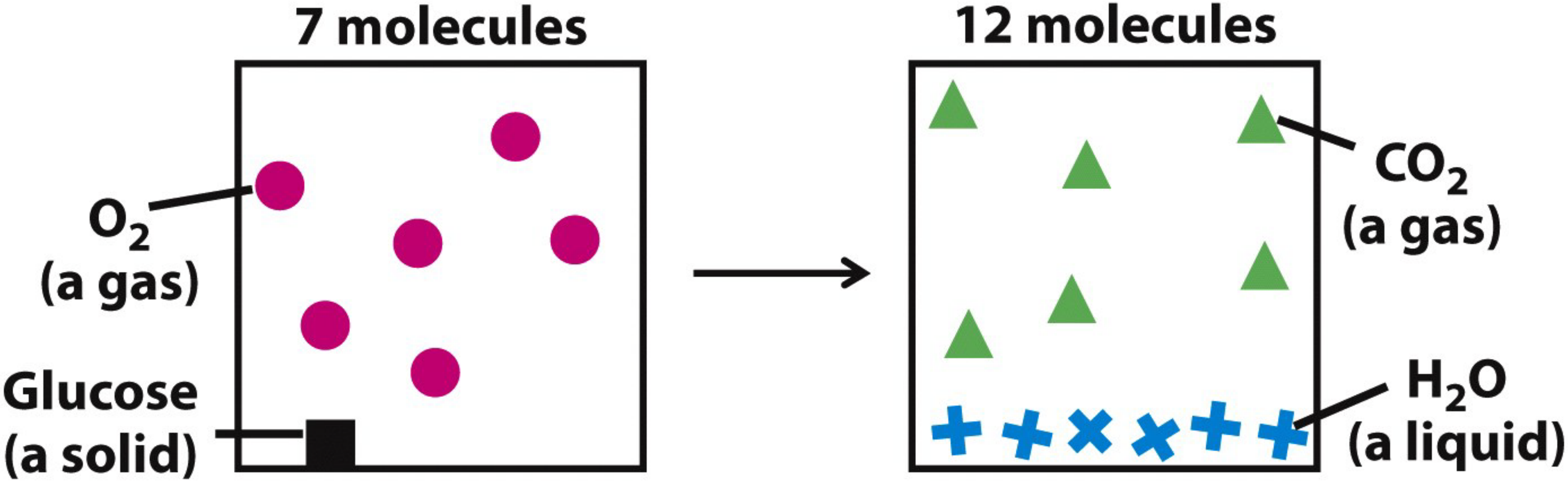
Information is a form of energy

There is a tide in the affairs of men, Which, taken at the flood , leads on to fortune;
Omitted, all the voyage of their life Is bound in sallows and in miseries



Box 1-3a
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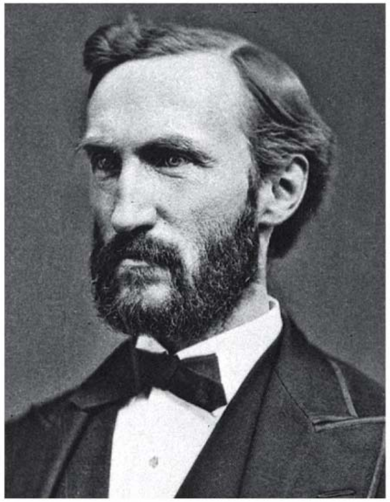
$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

Gibbs free energy, ***G***, expresses the amount of an energy capable of doing work during a reaction at constant temperature and pressure. $\triangle G$ J/mole

Enthalpy, *H*, is the heat content of the reacting system. $\triangle H$ J/mole

Entropy, *S*, is a quantitative expression for the randomness or disorder in a system
$\triangle S$ J/mole•K



J. Williard Gibbs, 1839–1903

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$$A \rightleftharpoons B$$

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

A process tends to occur spontaneously only if it is negative

Creating and maintaining order requires work and energy

$$A \rightleftharpoons B$$

process proceeds spontaneously to a final state of lower free energy $\Delta G = G_B - G_A$

$\Delta G = 0$, process is at equilibrium

$\Delta G < 0$, process proceeds spontaneously forward

$\Delta G > 0$, process proceeds spontaneously in the reverse direction

The **standard-state free energy change** ΔG^o

Standard-state: 298 K, l atm (1.013 x 10^5 pa), l mol/L

Standard transformed free energy change $\Delta G'^o$

pH=7, $[H_2O]$=55.5mM

$$A + B \rightleftharpoons C + D$$

$$\boldsymbol{\Delta G'^o} = - RT\ln K'_{eq}$$

R is the gas constant, defined as R = 8.314 J/mol· K

TABLE 13–1 Some Physical Constants and Units Used in Thermodynamics

Boltzmann constant, k = 1.381×10^{-23} J/K

Avogadro's number, N = 6.022×10^{23} mol^{-1}

Faraday constant, $\mathcal{F}$ = 96,480 J/V · mol

Gas constant, R = 8.315 J/mol · K
(= 1.987 cal/mol · K)

Units of ΔG and ΔH are J/mol (or cal/mol)
Units of ΔS are J/mol · K (or cal/mol · K)
1 cal = 4.184 J

Units of absolute temperature, T, are Kelvin, K
25 °C = 298 K
At 25 °C, RT = 2.478 kJ/mol
(= 0.592 kcal/mol)

Table 13-1
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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.

Table 13-2

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TABLE 13–3 Relationships among K'_{eq}, $\Delta G'^{\circ}$, and the Direction of Chemical Reactions

When K'_{eq} is . . .	$\Delta G'^{\circ}$ is . . .	Starting with all components at 1 M, the reaction . . .
>1.0	negative	proceeds forward
1.0	zero	is at equilibrium
<1.0	positive	proceeds in reverse

Table 13-3
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TABLE 13–4 Standard Free-Energy Changes of Some Chemical Reactions

Reaction type	$\Delta G'^\circ$ (kJ/mol)	$\Delta G'^\circ$ (kcal/mol)
Hydrolysis reactions		
Acid anhydrides		
Acetic anhydride + $H_2O \longrightarrow$ 2 acetate	−91.1	−21.8
ATP + $H_2O \longrightarrow$ ADP + P_i	−30.5	−7.3
ATP + $H_2O \longrightarrow$ AMP + PP_i	−45.6	−10.9
$PP_i + H_2O \longrightarrow 2P_i$	−19.2	−4.6
UDP-glucose + $H_2O \longrightarrow$ UMP + glucose 1-phosphate	−43.0	−10.3
Esters		
Ethyl acetate + $H_2O \longrightarrow$ ethanol + acetate	−19.6	−4.7
Glucose 6-phosphate + $H_2O \longrightarrow$ glucose + P_i	−13.8	−3.3
Amides and peptides		
Glutamine + $H_2O \longrightarrow$ glutamate + NH_4^+	−14.2	−3.4
Glycylglycine + $H_2O \longrightarrow$ 2 glycine	−9.2	−2.2
Glycosides		
Maltose + $H_2O \longrightarrow$ 2 glucose	−15.5	−3.7
Lactose + $H_2O \longrightarrow$ glucose + galactose	−15.9	−3.8
Rearrangements		
Glucose 1-phosphate $\longrightarrow$ glucose 6-phosphate	−7.3	−1.7
Fructose 6-phosphate $\longrightarrow$ glucose 6-phosphate	−1.7	−0.4
Elimination of water		
Malate $\longrightarrow$ fumarate + H_2O	3.1	0.8
Oxidations with molecular oxygen		
Glucose + $6O_2 \longrightarrow 6CO_2 + 6H_2O$	−2,840	−686
Palmitate + $23O_2 \longrightarrow 16CO_2 + 16H_2O$	−9,770	−2,338

Table 13-4
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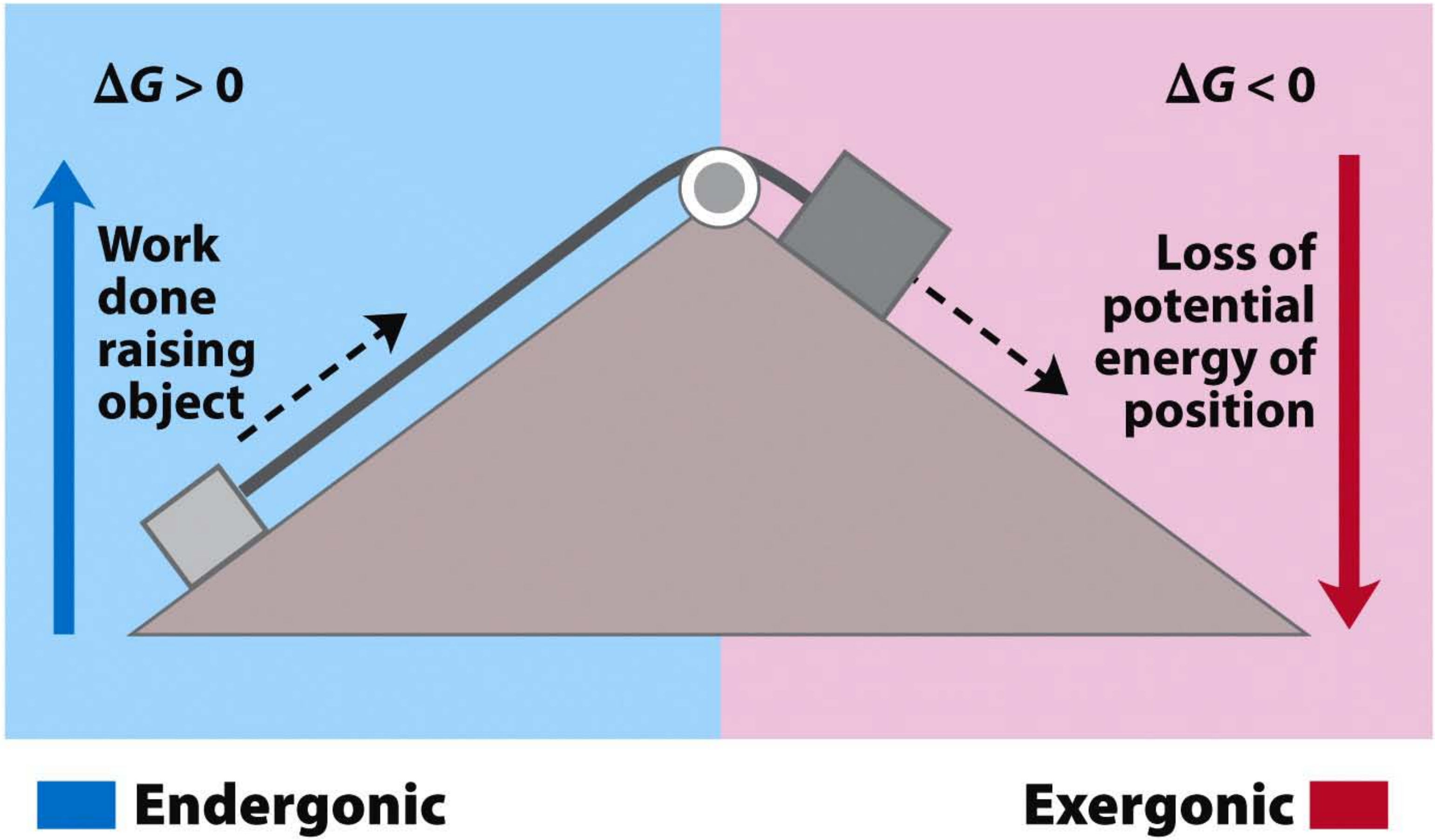
Actual free-energy changes depend on reactant and product concentrations

$$\Delta G = \Delta G'^{o} + RT \ln([C]^{c}[D]^{d} / [A]^{a}[B]^{b})$$
$$= \Delta G'^{o} + RT \ln Q$$

Q is called mass-action ratio



Mechanical example

Figure 1-26a
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Energy coupling in mechanical and chemical processes

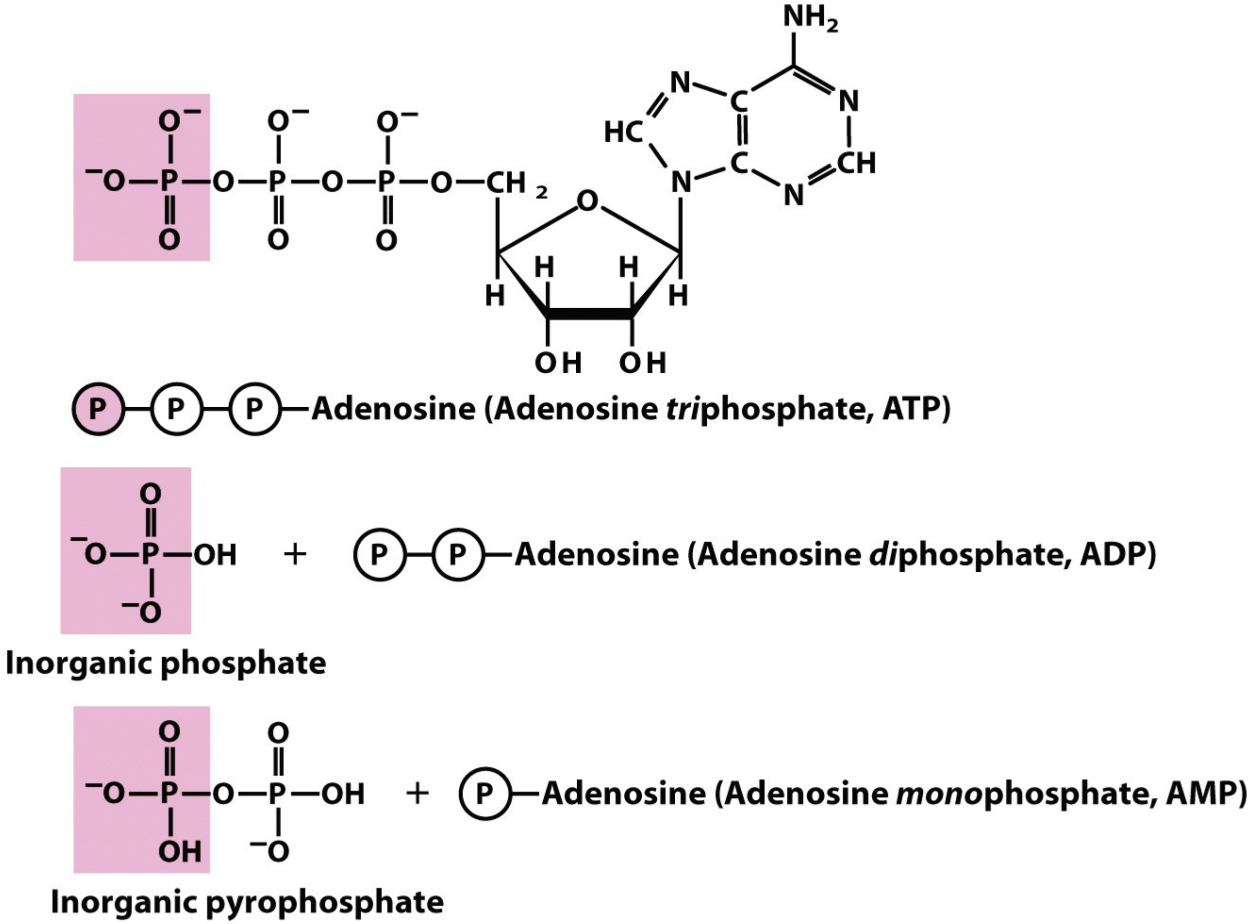

Figure 1-25
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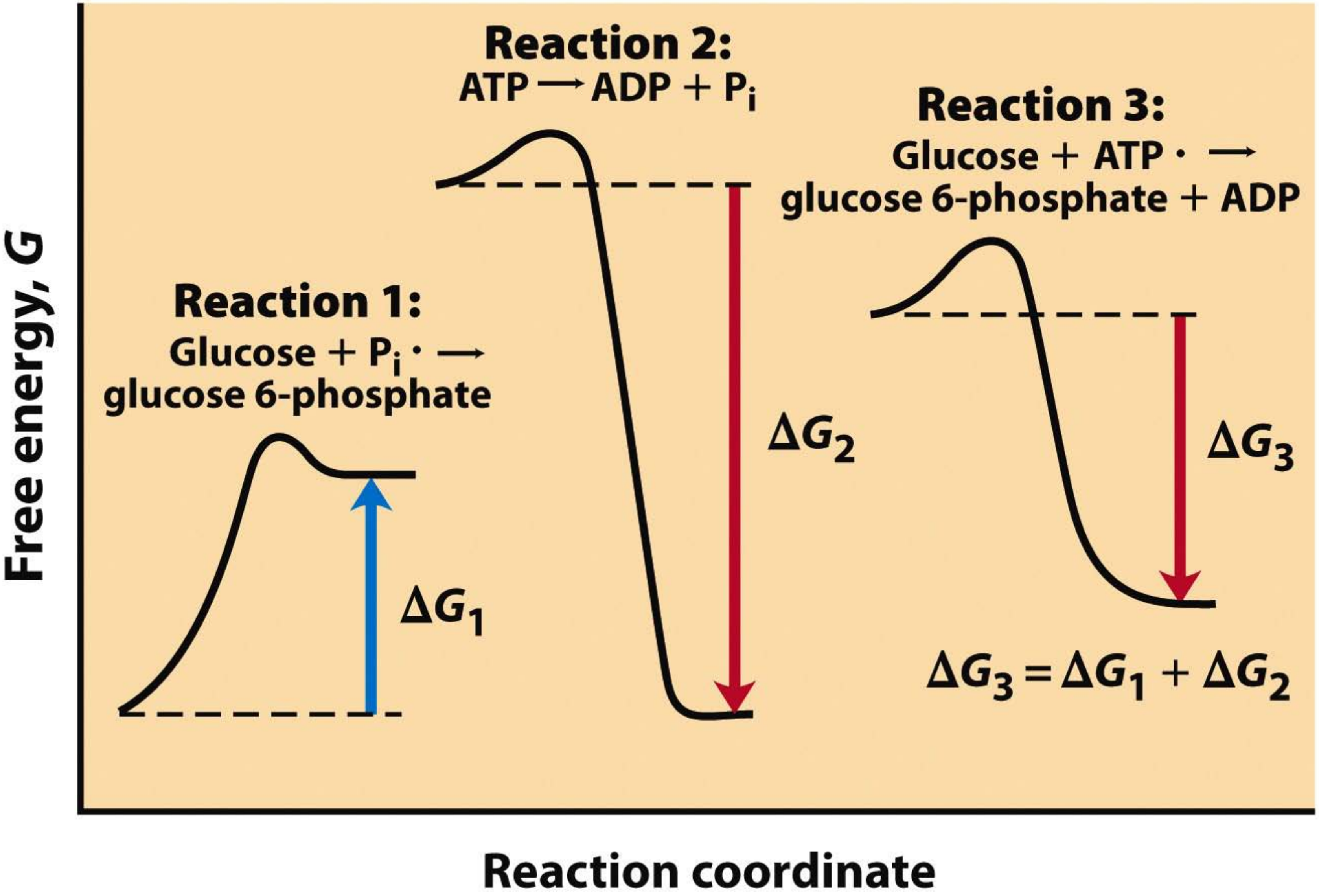
The majority of energy-consuming reactions are coupled to hydrolysis of adenosine triphosphate (ATP)

Chemical example



Reaction 1:
Glucose + P_i · ⟶
glucose 6-phosphate

Reaction 2:
$ATP \longrightarrow ADP + P_i$

Reaction 3:
Glucose + ATP · ⟶
glucose 6-phosphate + ADP

$\Delta G_3 = \Delta G_1 + \Delta G_2$

Figure 1-26b
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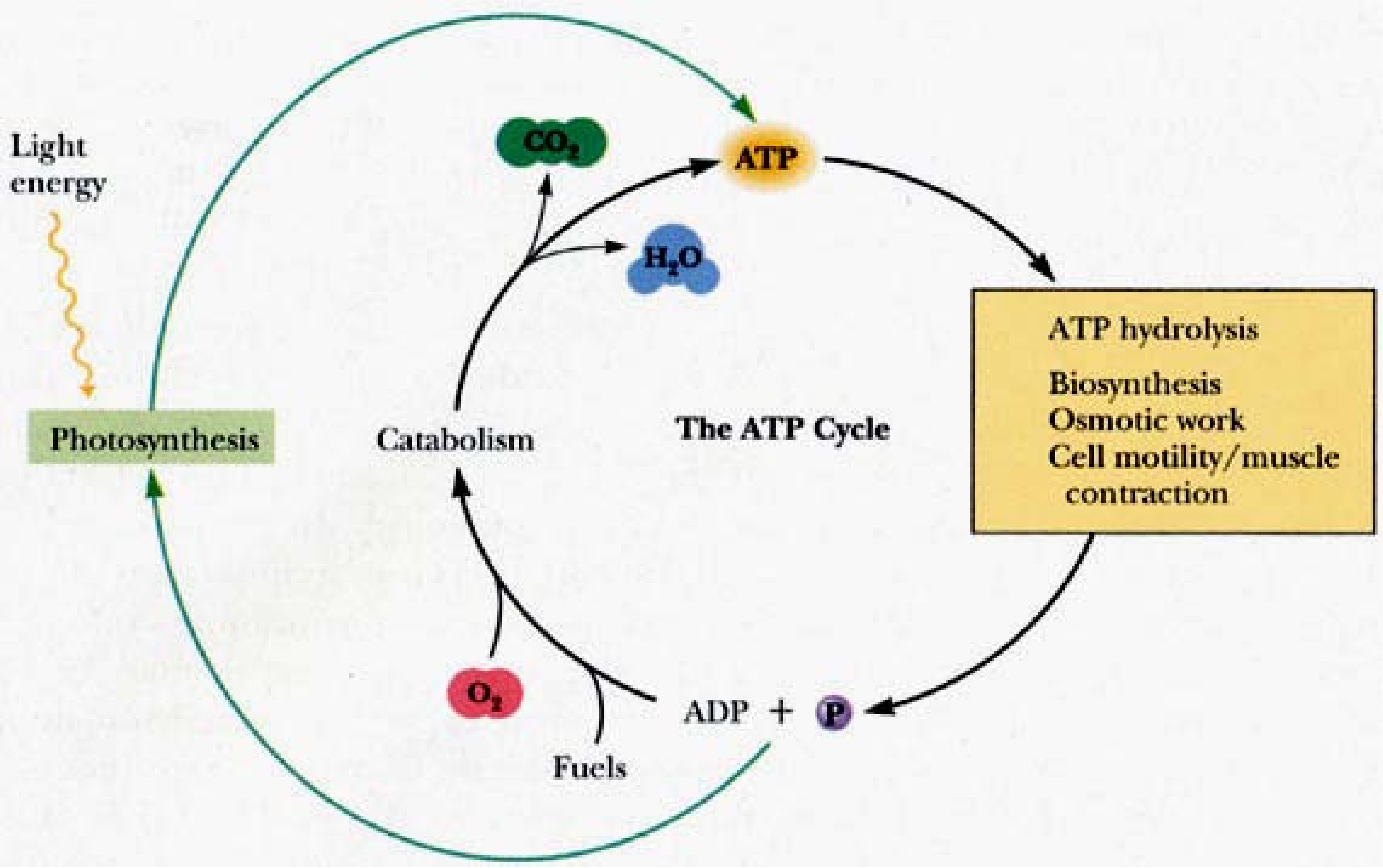

The ATP circle in the cell.

Standard free-energy change are additive

$$\begin{array}{lll}
(1) & \mathrm{A} \longrightarrow \mathrm{B} & \Delta G_1'^{\circ} \\
(2) & \mathrm{B} \longrightarrow \mathrm{C} & \Delta G_2'^{\circ} \\
\hline
\textit{Sum:} & \mathrm{A} \longrightarrow \mathrm{C} & \Delta G_1'^{\circ} + \Delta G_2'^{\circ}
\end{array}$$

$$\text{Glucose} + \mathrm{P_i} \longrightarrow \text{glucose 6-phosphate} + \mathrm{H_2O}$$
$$\Delta G'^{\circ} = 13.8\ \text{kJ/mol}$$

$$\text{ATP} + \mathrm{H_2O} \longrightarrow \text{ADP} + \mathrm{P_i} \qquad \Delta G'^{\circ} = -30.5\ \text{kJ/mol}$$

$$\begin{array}{lll}
(1) & \text{Glucose} + \mathrm{P_i} \longrightarrow \text{glucose 6-phosphate} + \mathrm{H_2O} \\
(2) & \text{ATP} + \mathrm{H_2O} \longrightarrow \text{ADP} + \mathrm{P_i} \\
\hline
\textit{Sum:} & \text{ATP} + \text{glucose} \longrightarrow \text{ADP} + \text{glucose 6-phosphate}
\end{array}$$

$$\Delta G'^{\circ} = 13.8\ \text{kJ/mol} + (-30.5\ \text{kJ/mol}) = -16.7\ \text{kJ/mol}$$

13.2 Chemical Logic and Common Biochemical Reactions

p495

The reactions in living cells are divided into five general categories:

(1) reactions that make or break carbon-carbon bonds;

(2) Internal rearrangements, isomerizations, and eliminations;

(3) free-radical reactions;

(4) group transfers;

(5) oxidation-reductions.

Two basic chemical principles:

First, a covalent bond consists of a shared pair of electrons, and the bond can be broken in two general types.

In **homolytic cleavage**, each atom leaves the bond as a **radical**, carrying one unpaired electron.

In **heterolytic cleavage**, one atom retains both bonding electrons.

The second, biochemical reactions involve interactions between nucleophiles and electrophiles.

Nucleophiles: functional groups rich in and capable of donating electrons.
Electrophiles: electron-deficient functional groups that seek electrons.

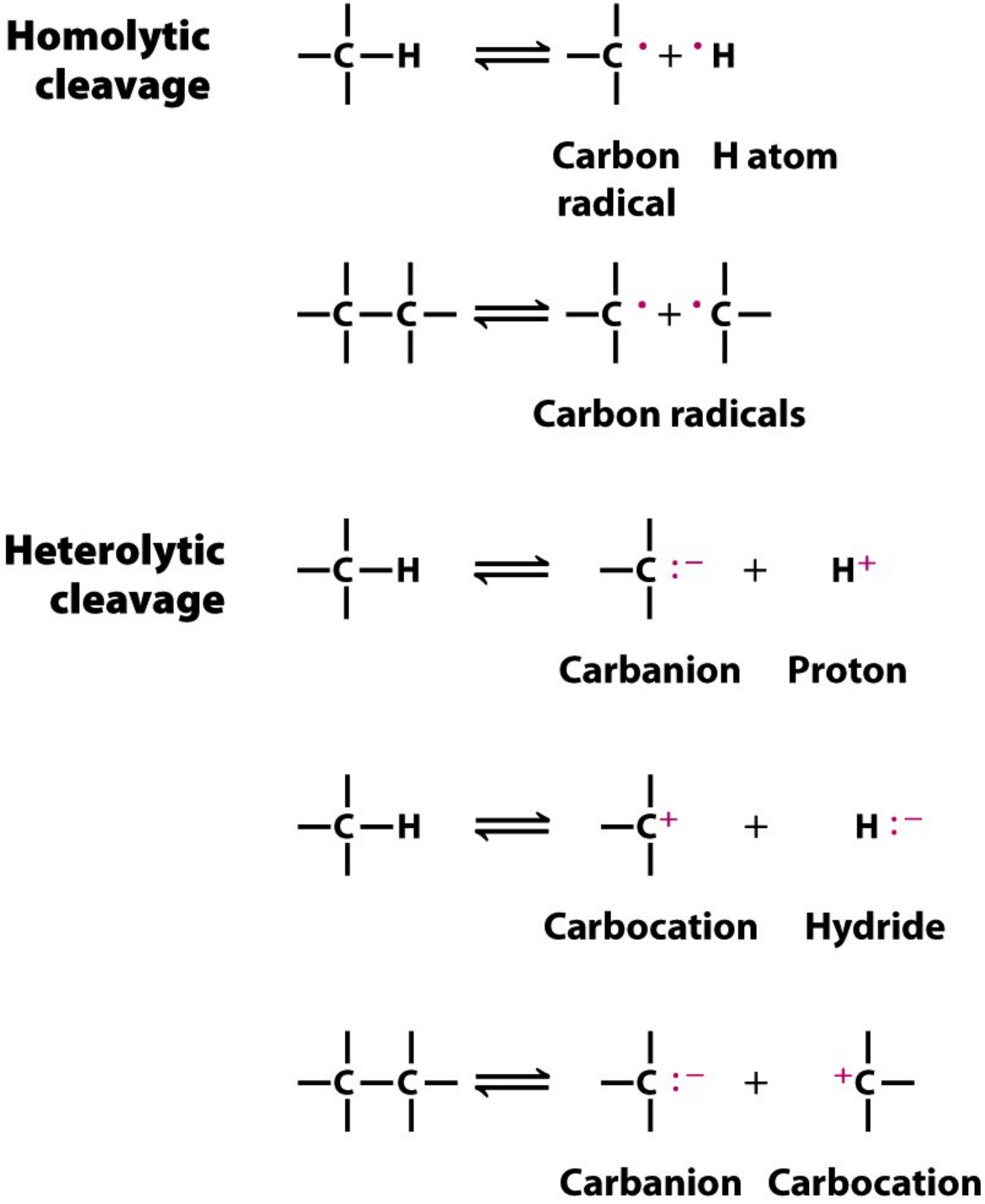

Figure 13-1
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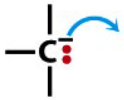
Two mechanisms for cleavage of a C—C or C—H bond

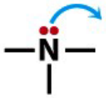
Nucleophiles

Negatively charged oxygen (as in an unprotonated hydroxyl group or an ionized carboxylic acid)

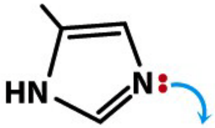
Negatively charged sulfhydryl



Carbanion



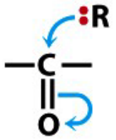
Uncharged amine group



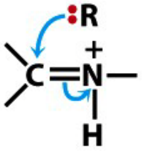
Imidazole

Hydroxide ion

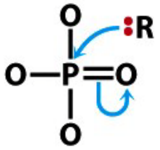
Electrophiles



Carbon atom of a carbonyl group (the more electronegative oxygen of the carbonyl group pulls electrons away from the carbon)



Pronated imine group (activated for nucleophilic attack at the carbon by protonation of the imine)



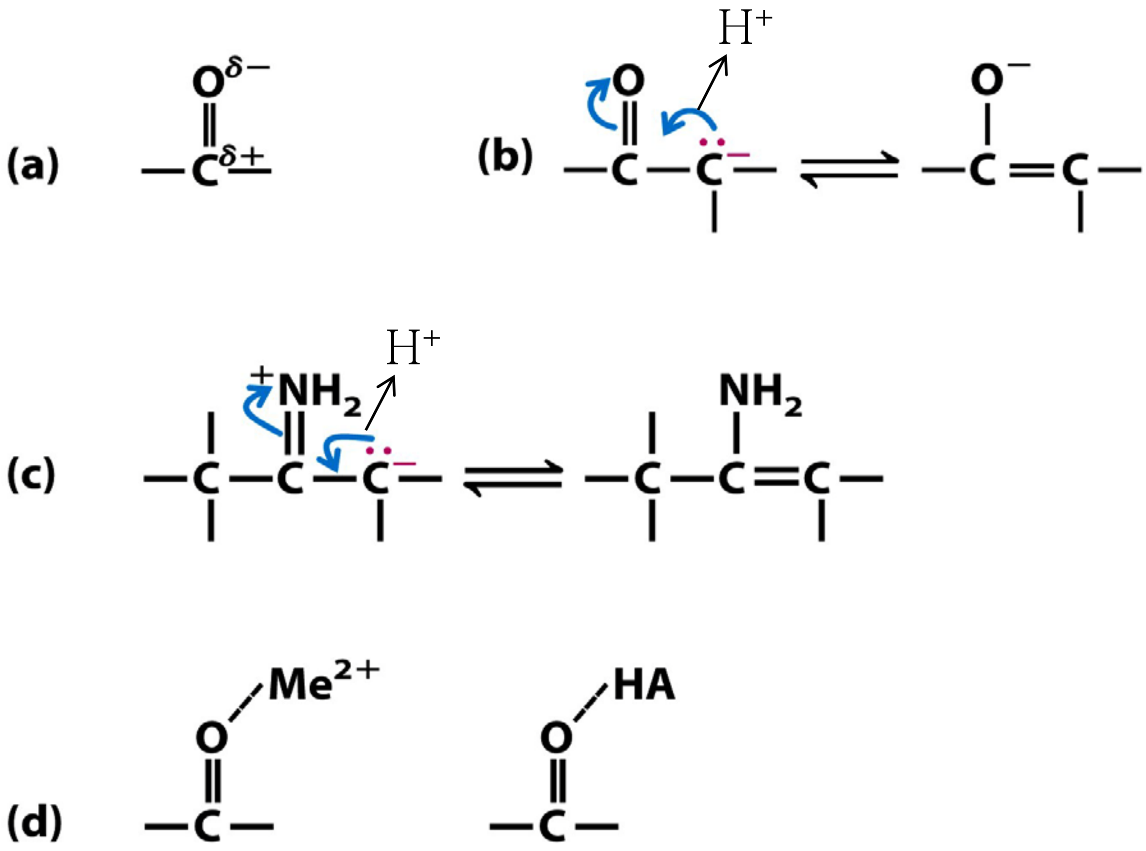
Phosphorus of a phosphate group

Proton

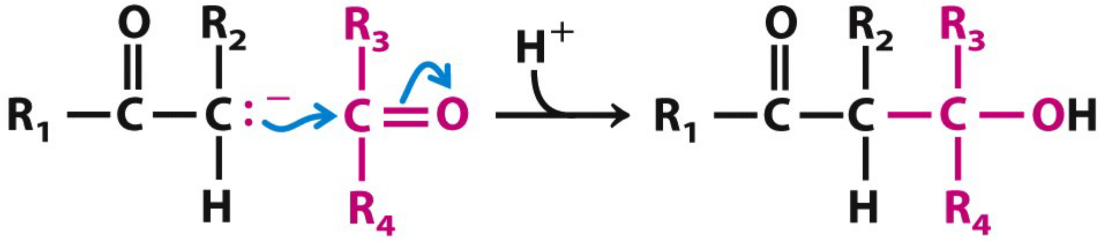
Common nucleophiles and electrophiles in biochemical reactions

(1) reactions that make or break carbon-carbon bonds

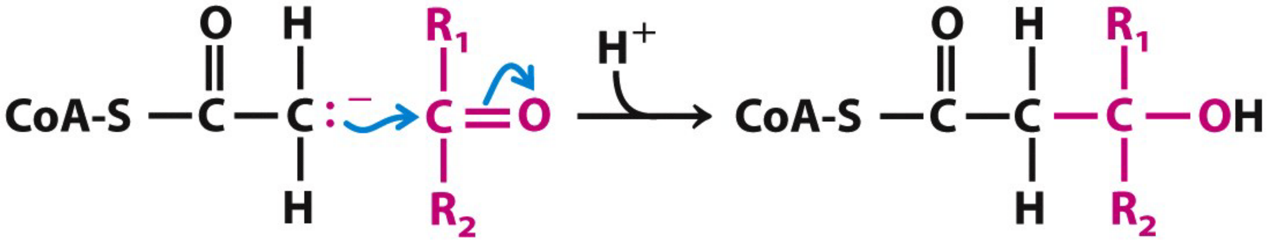
Carbonyl group plays crucial roles in the reactions that make or break C-C bonds



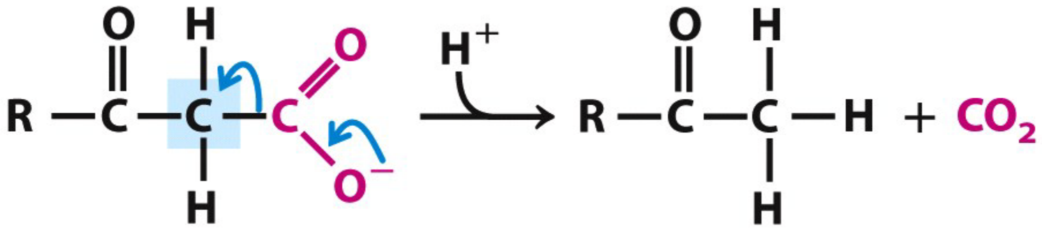
Chemical properties of carbonyl groups



Aldol condensation “aldol” (aldehyde + alcohol)



Claisen ester condensation



Decarboxylation of a β-keto acid

Figure 13-4

Some common reactions that form and break C–C bonds with the ajacent carbonyl group

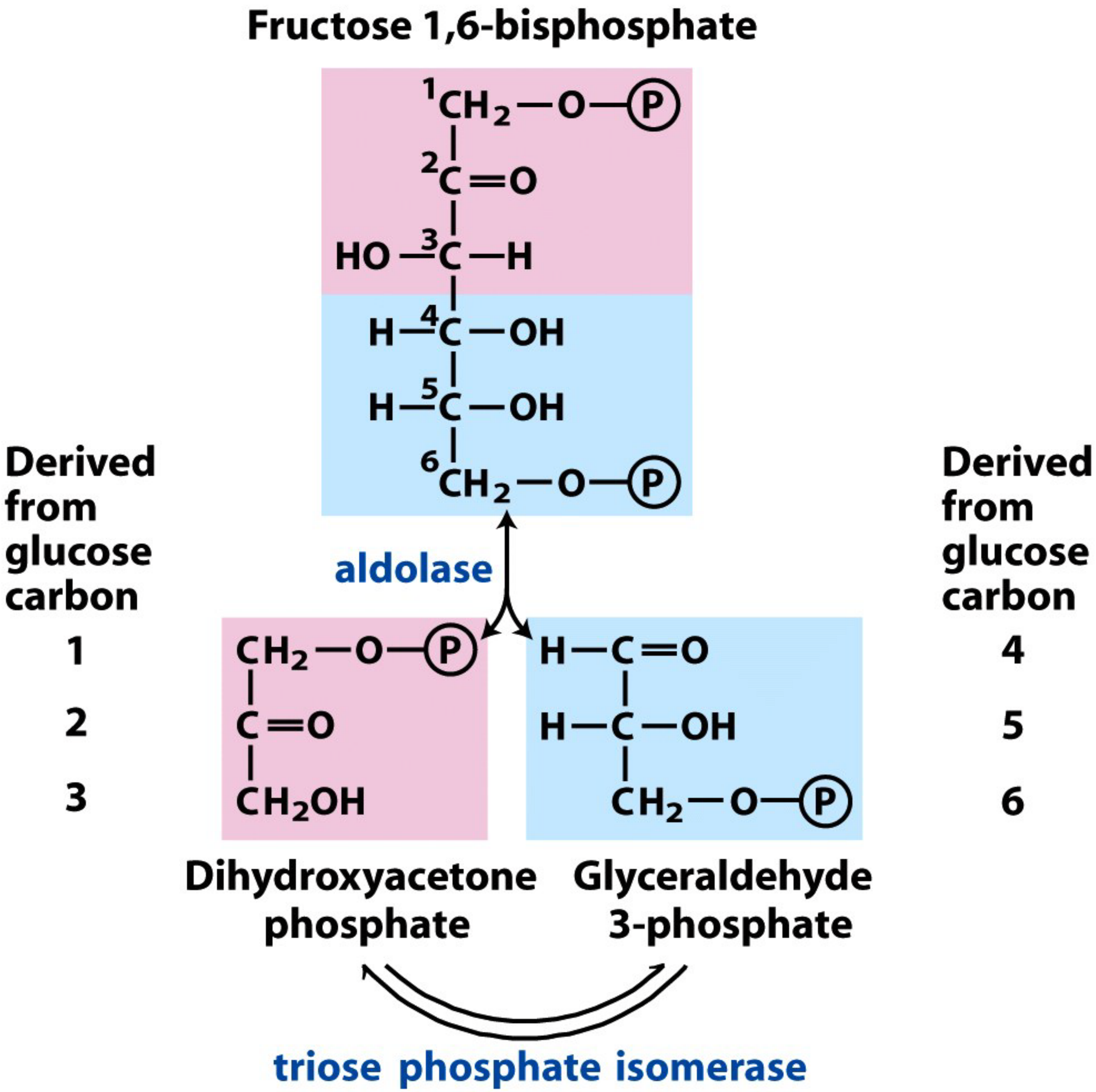


Figure 14-6a

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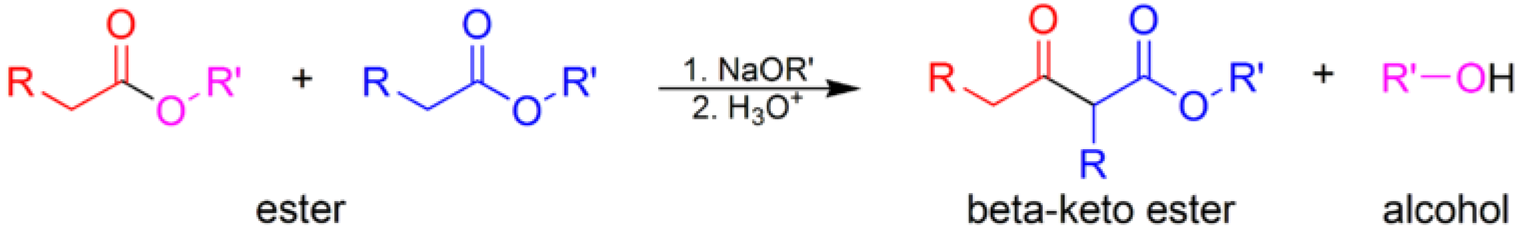
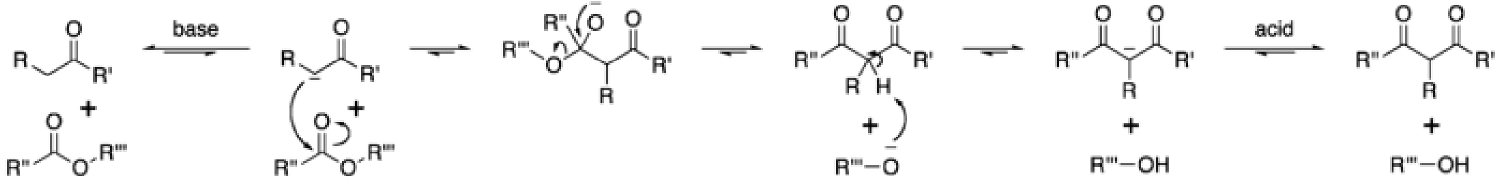
The **Claisen condensation** is a carbon–carbon bond forming reaction that occurs between two esters or one ester and another carbonyl compound in the presence of a strong base, resulting in a β–keto ester or a β–diketone.It is named after Rainer Ludwig Claisen, who first published his work on the reaction in 1887





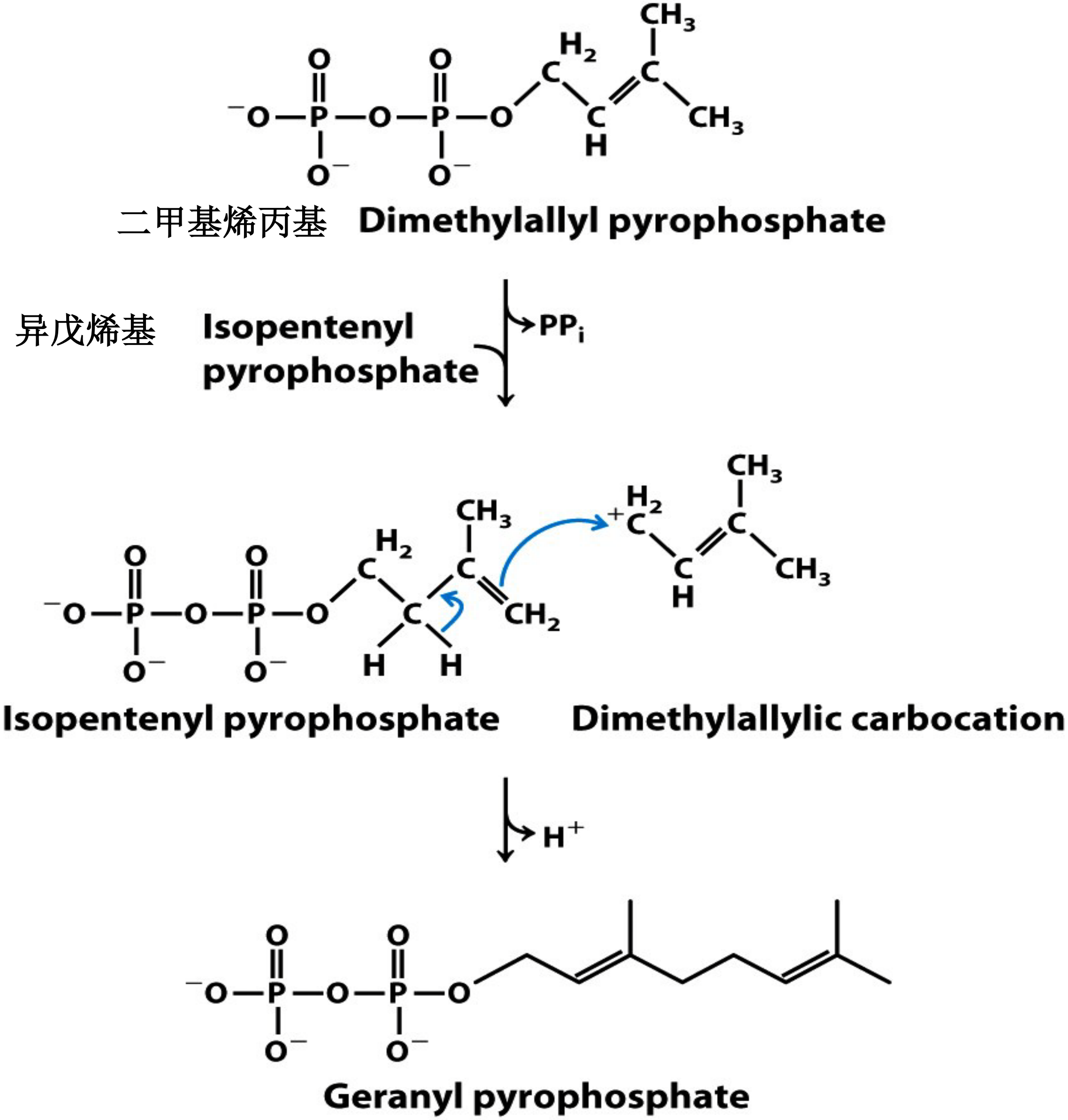
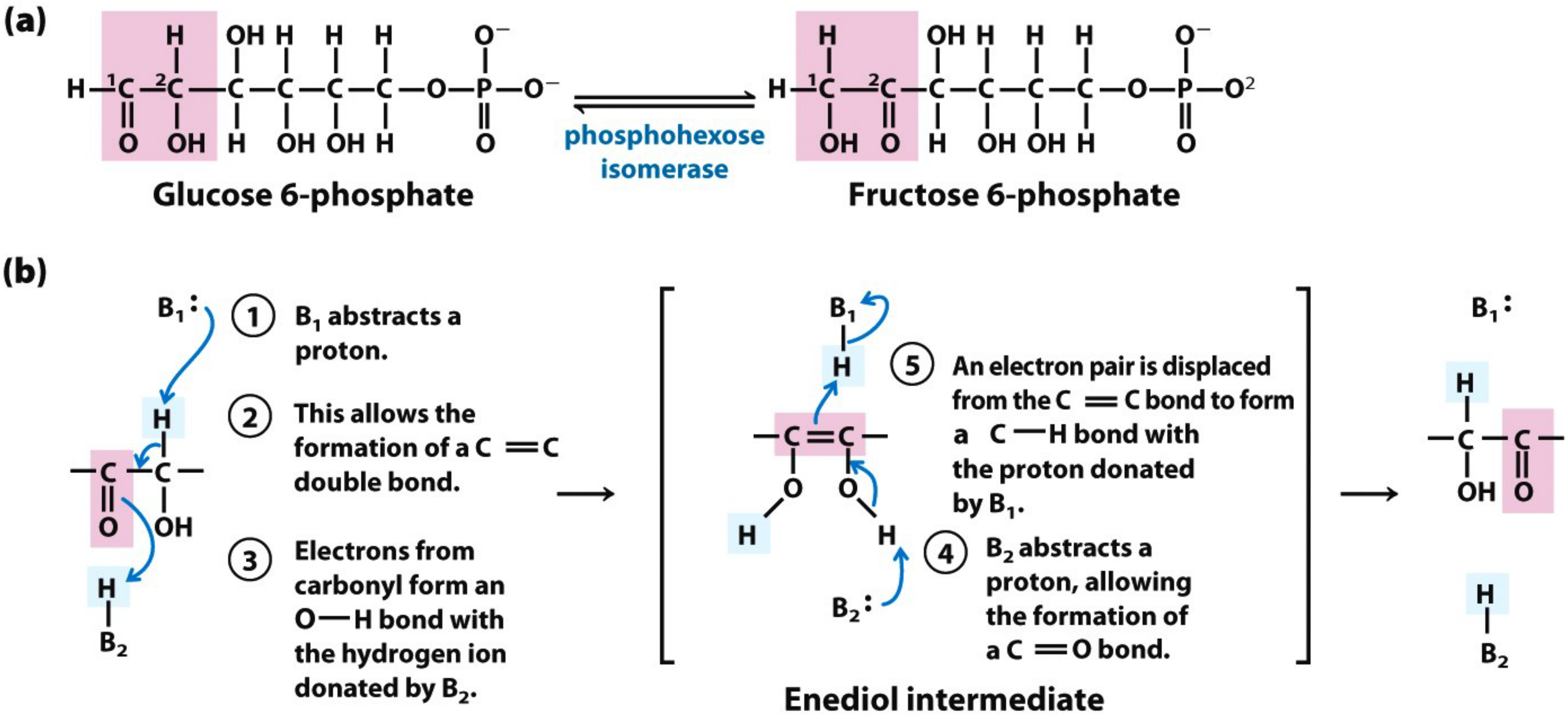


Figure 13-5

Carbocations in carbon-carbon bond formation

(2) Internal rearrangements, isomerizations, and eliminations



① B_1 abstracts a proton.

② This allows the formation of a C ═C double bond.

③ Electrons from carbonyl form an O—H bond with the hydrogen ion donated by B_2.

④ B_2 abstracts a proton, allowing the formation of a C ═O bond.

⑤ An electron pair is displaced from the C ═C bond to form a C—H bond with the proton donated by B_1.

Figure 13-6
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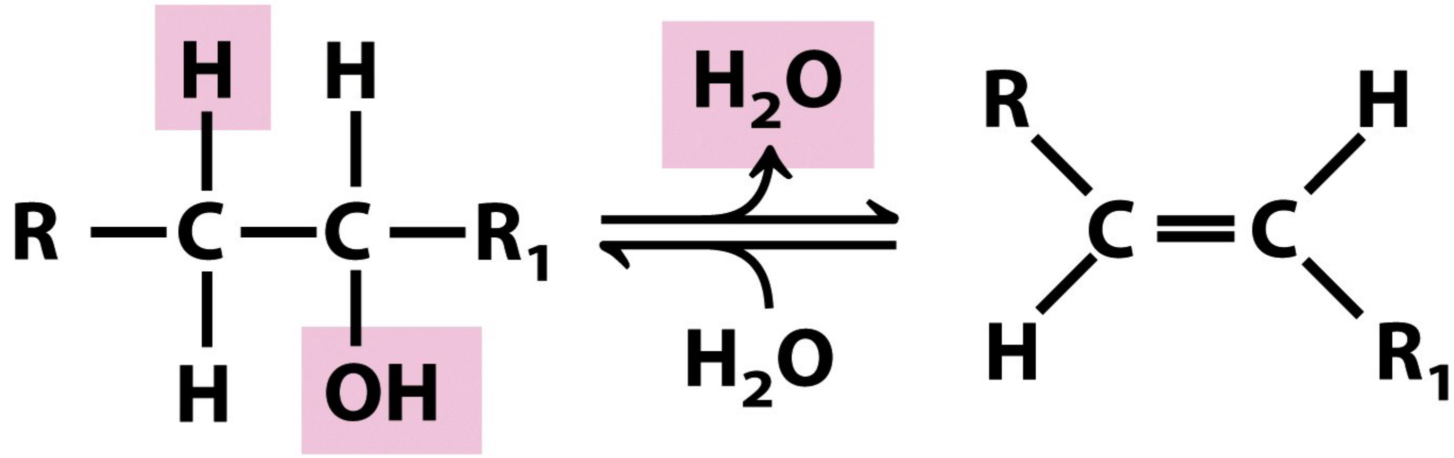
Isomerization and elimination reactions



Unnumbered 13 p498
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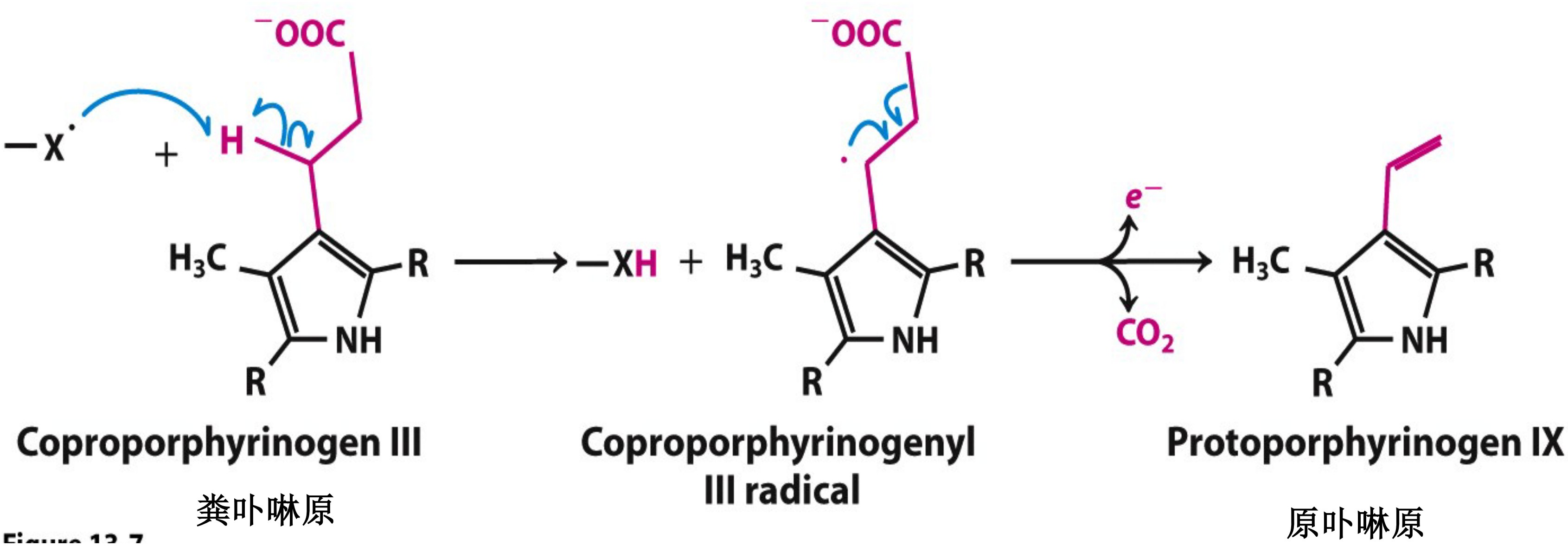
Elimination reaction in a C-C bond

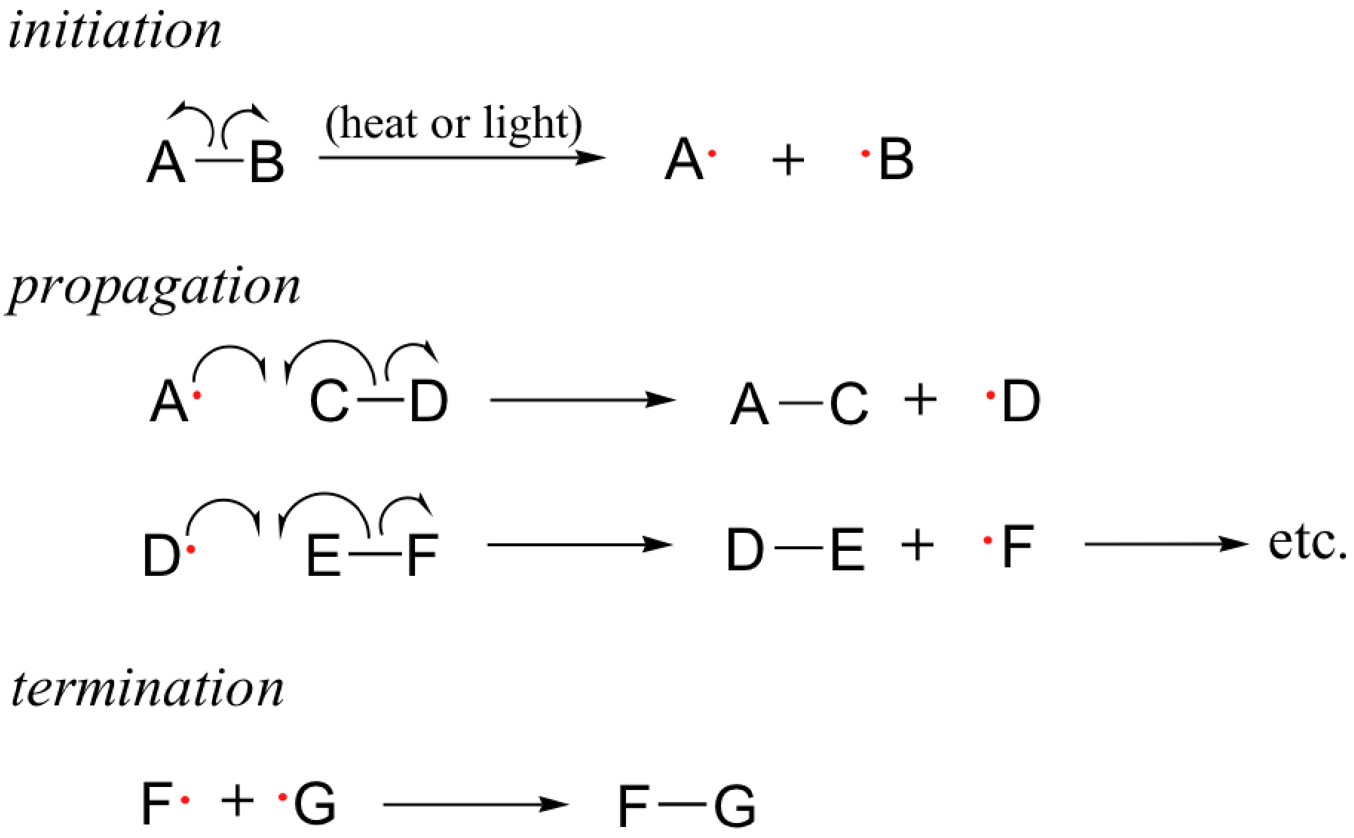
(3) Free-radical reactions



粪卟啉原

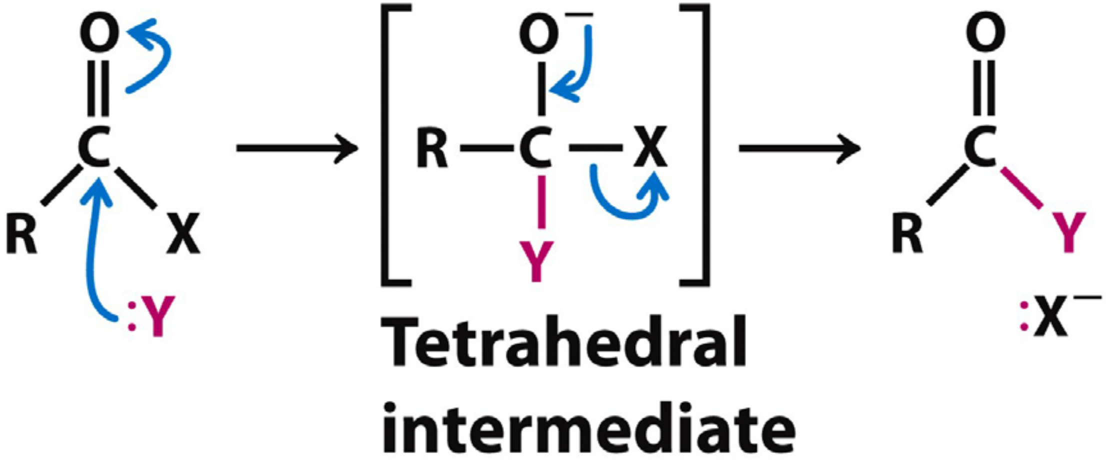
原卟啉原

A free radical-initiated decarboxylation reaction



Radical chain reactions

(4) Group transfer reactions



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Acyl group transfer generally involves the addition of a nucleophile to the carbonyl carbon of an acyl group to form a tetrahedral intermediate

Phosphoryl group transfers

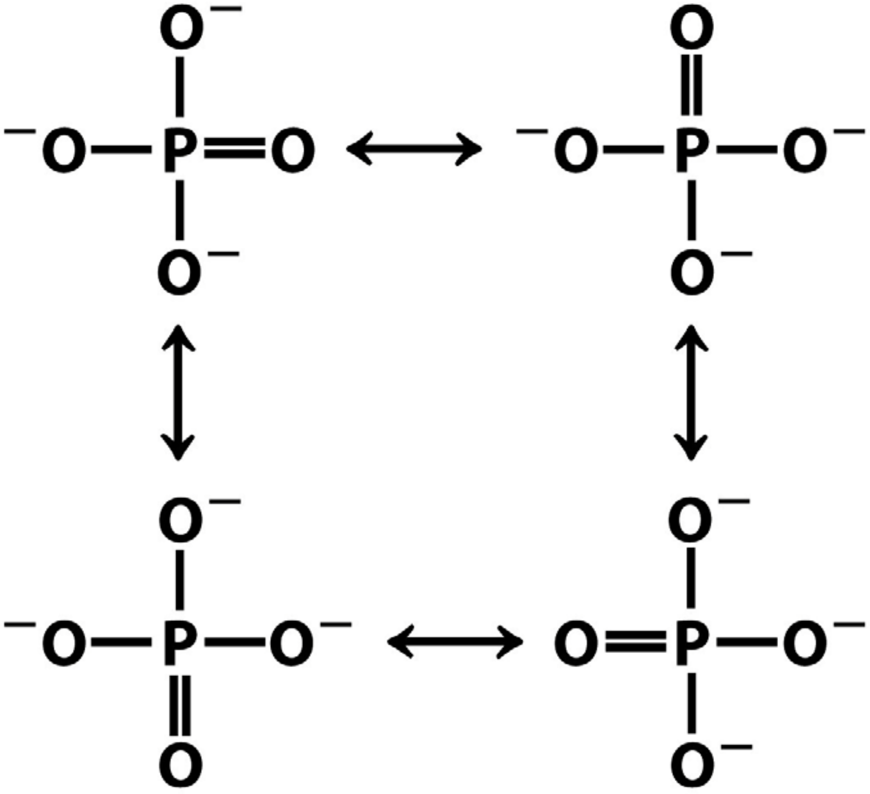

Figure 13-8a
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Four different resonance structures of inorganic phosphate

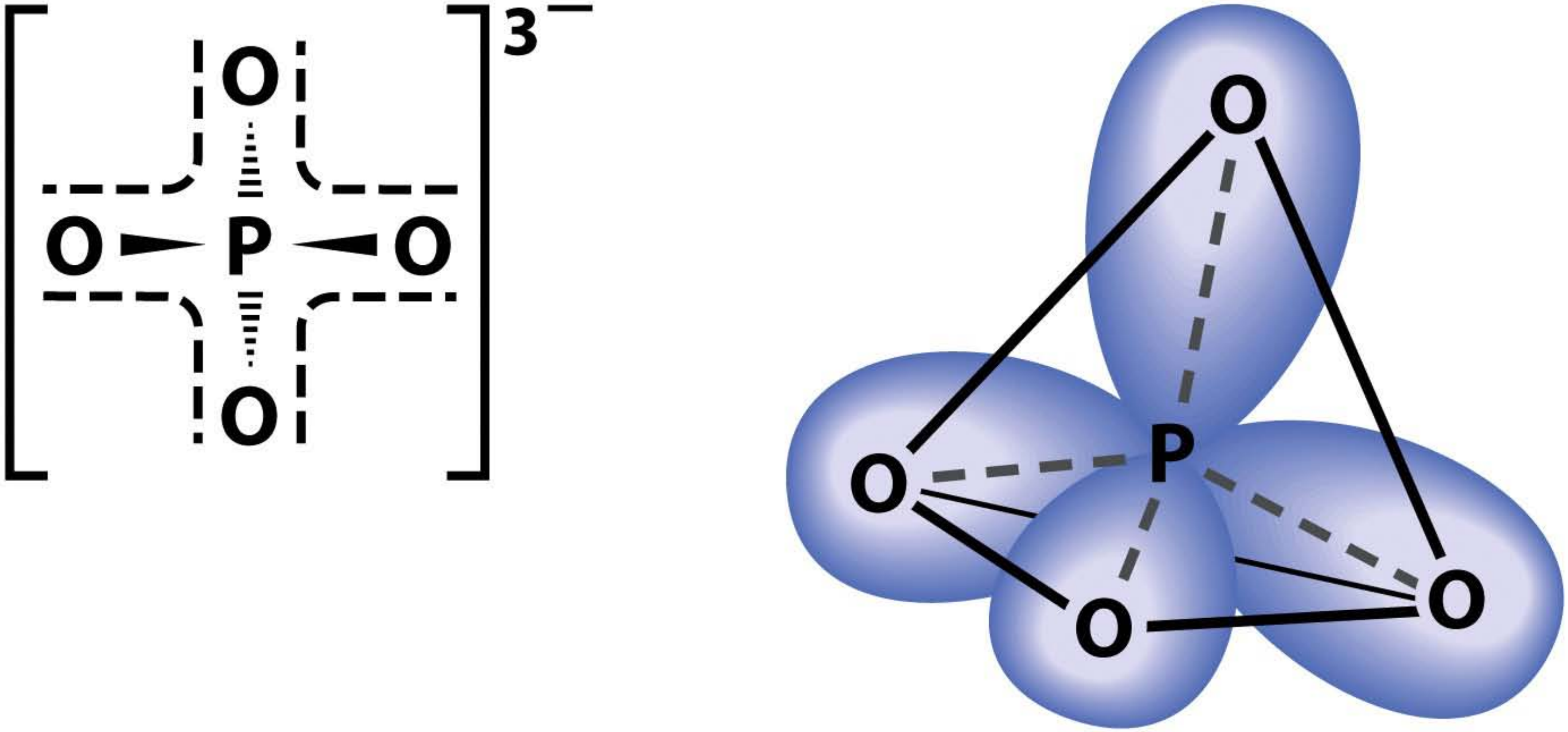

Figure 13-8b
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The resonance structure of phosphate is a tetrahedron

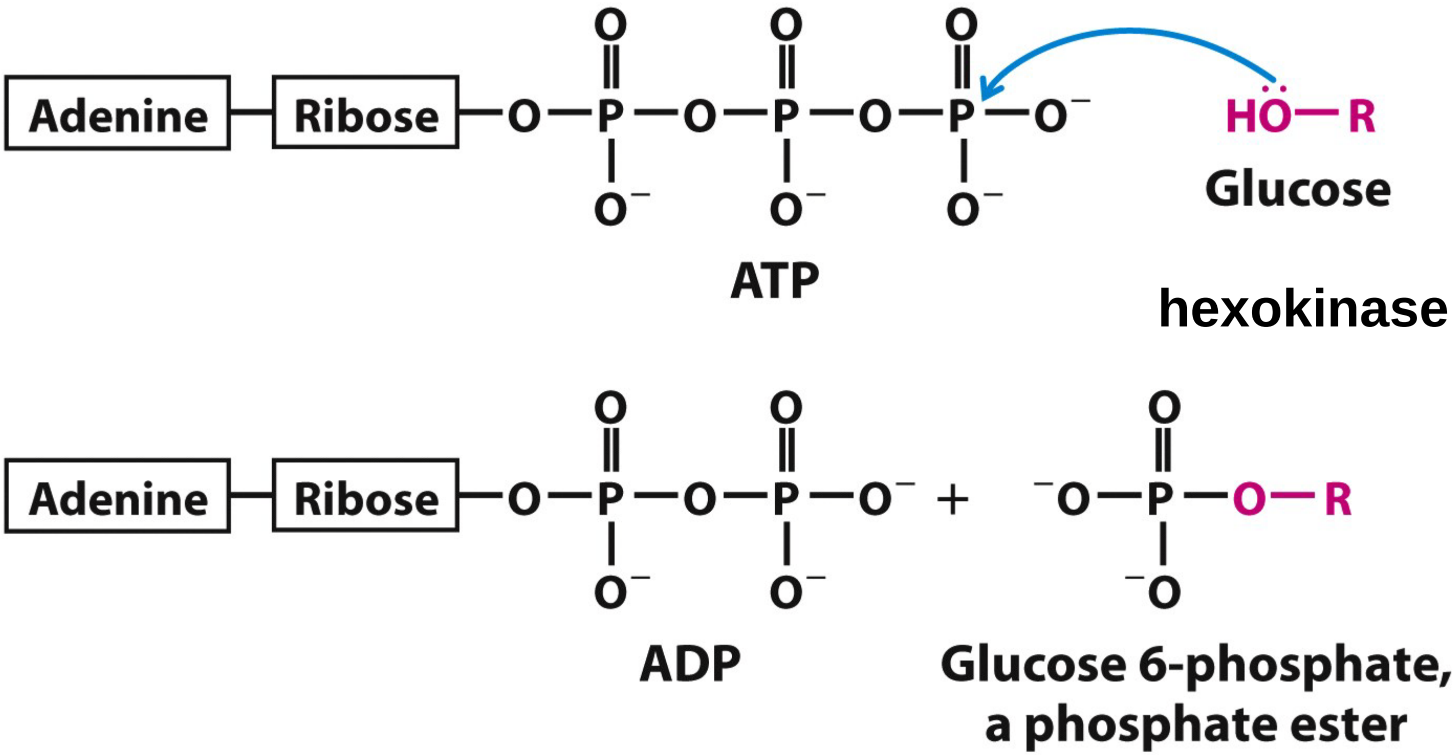
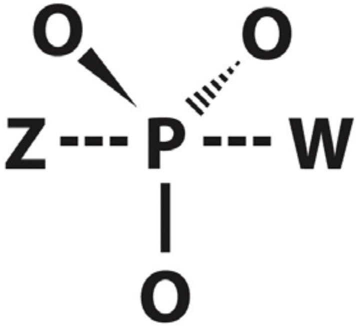


Figure 13-8c



Z = R — OH

W = ADP

(4) Oxidation-reduction reactions

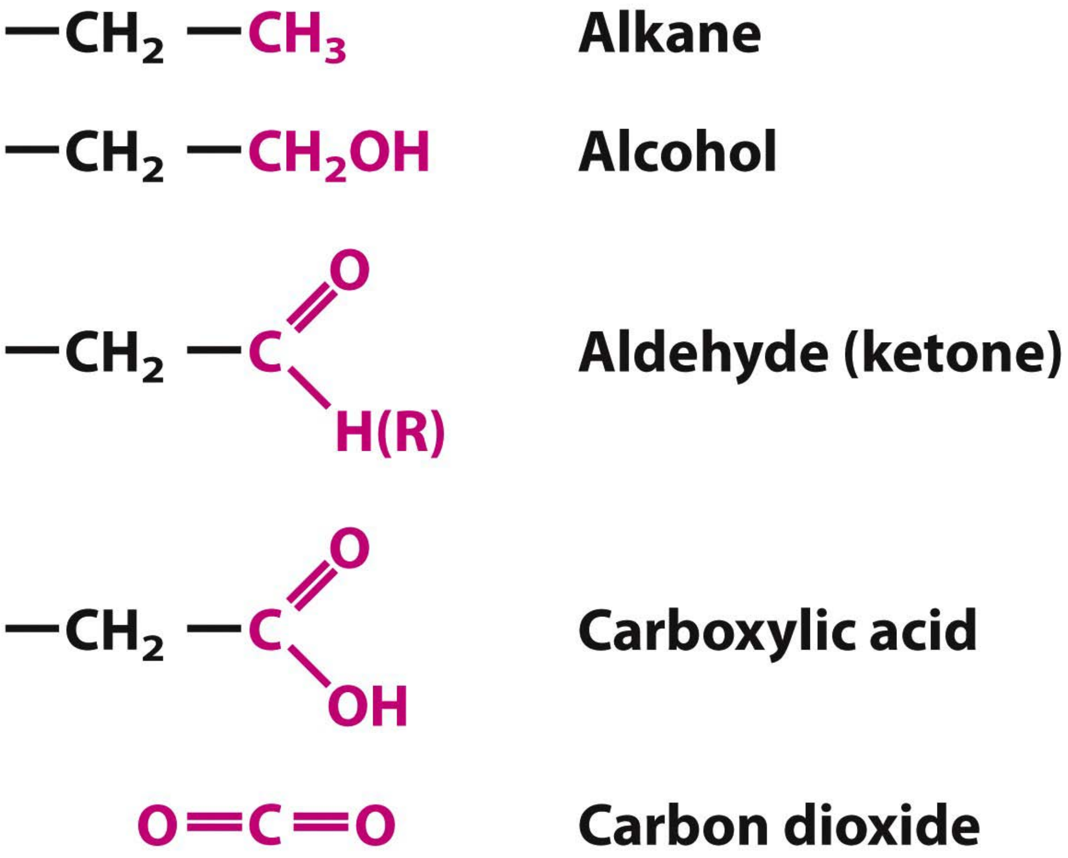
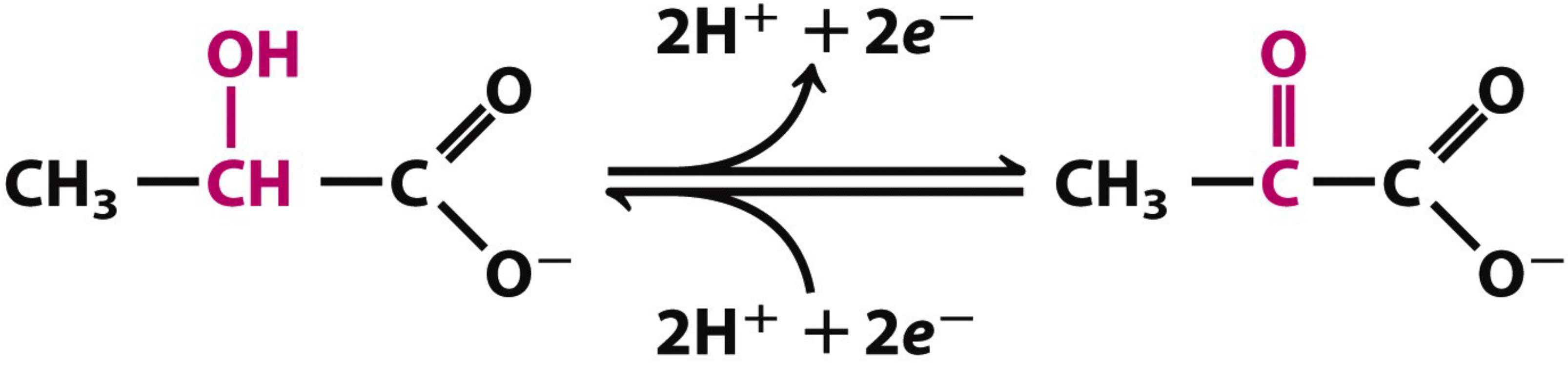


Figure 13-9

The oxidation states of carbon in biomolecules



Lactate **lactate dehydrogenase** **Pyruvate**

Figure 13-10
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13.3 Phosphoryl Group Transfers and ATP

Why is ATP suitable for acting as the energy currency in cells?

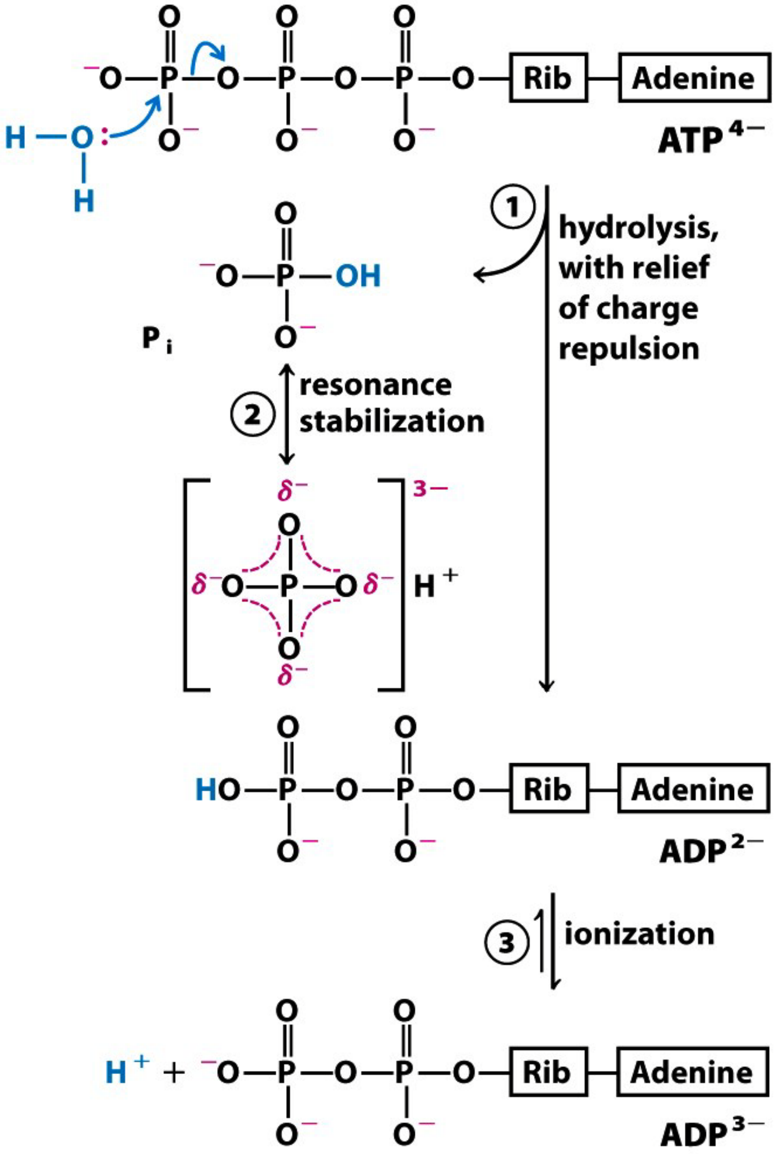

Figure 13-11

The free-energy change for ATP hydrolysis is large and negative

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

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

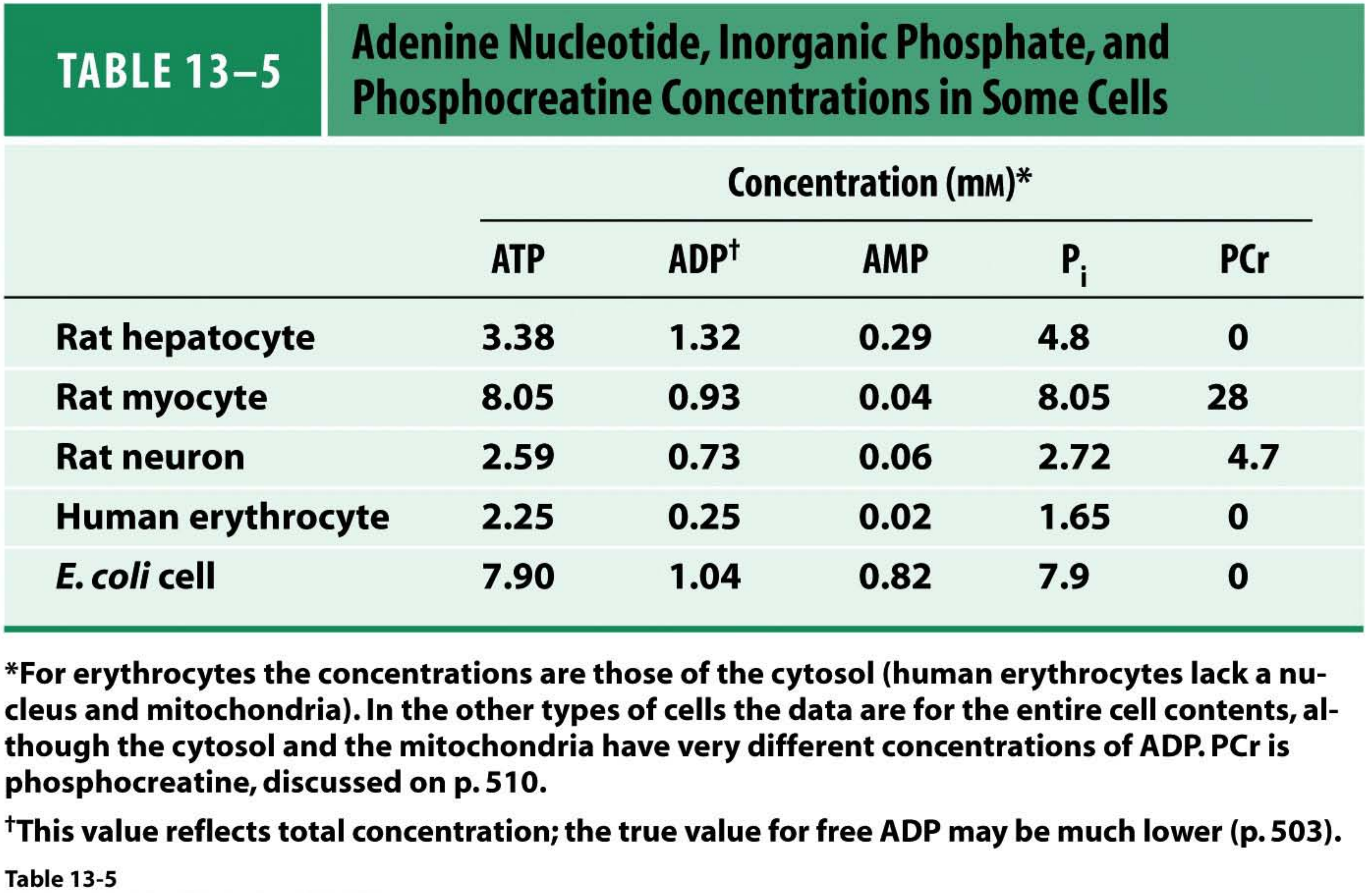

TABLE 13–5 **Adenine Nucleotide, Inorganic Phosphate, and Phosphocreatine Concentrations in Some Cells**

	Concentration (mM)*				
	ATP	ADP†	AMP	P_i	PCr
Rat hepatocyte	3.38	1.32	0.29	4.8	0
Rat myocyte	8.05	0.93	0.04	8.05	28
Rat neuron	2.59	0.73	0.06	2.72	4.7
Human erythrocyte	2.25	0.25	0.02	1.65	0
E. coli cell	7.90	1.04	0.82	7.9	0

*For erythrocytes the concentrations are those of the cytosol (human erythrocytes lack a nucleus and mitochondria). In the other types of cells the data are for the entire cell contents, although the cytosol and the mitochondria have very different concentrations of ADP. PCr is phosphocreatine, discussed on p. 510.

†This value reflects total concentration; the true value for free ADP may be much lower (p. 503).

Table 13-5

The actual free energy change of hydrolysis of ATP under intracellular conditions is often called its **phosphorylation potential, ΔG_p**

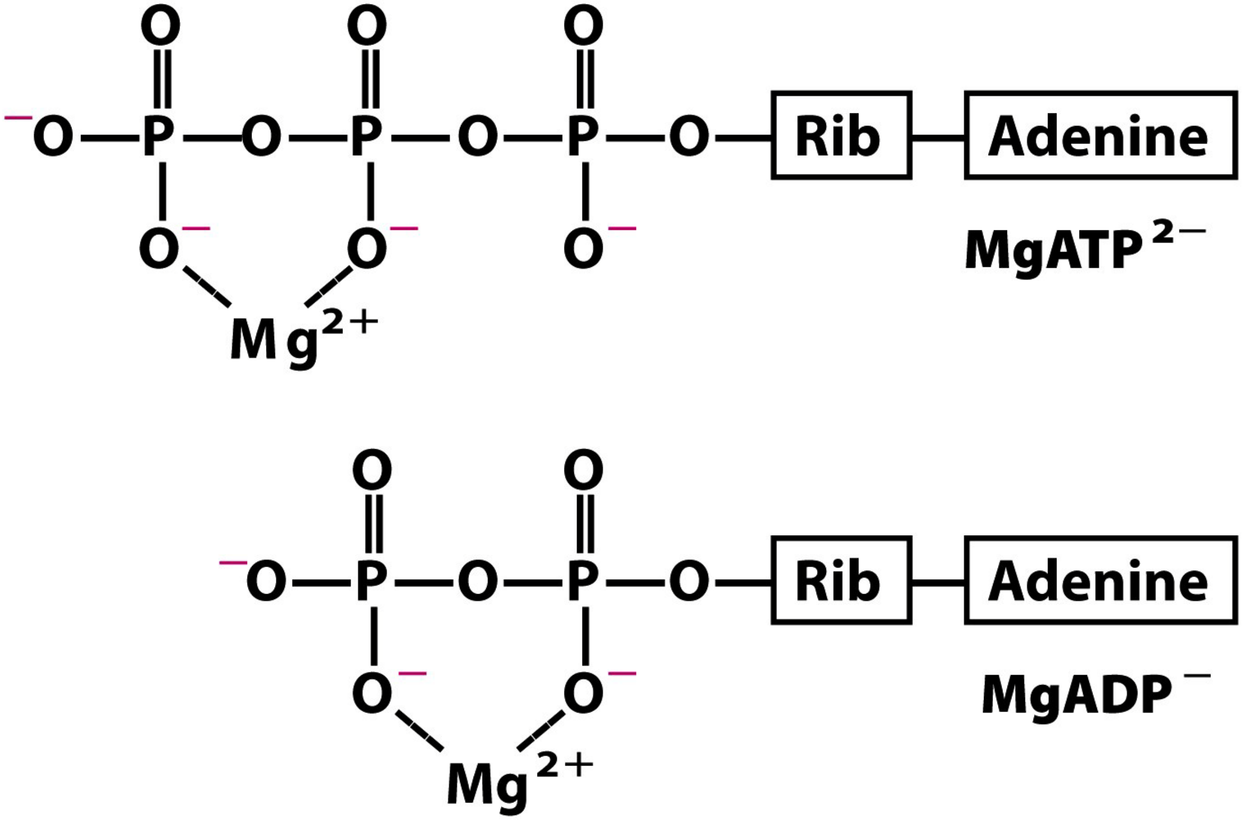

Figure 13-12
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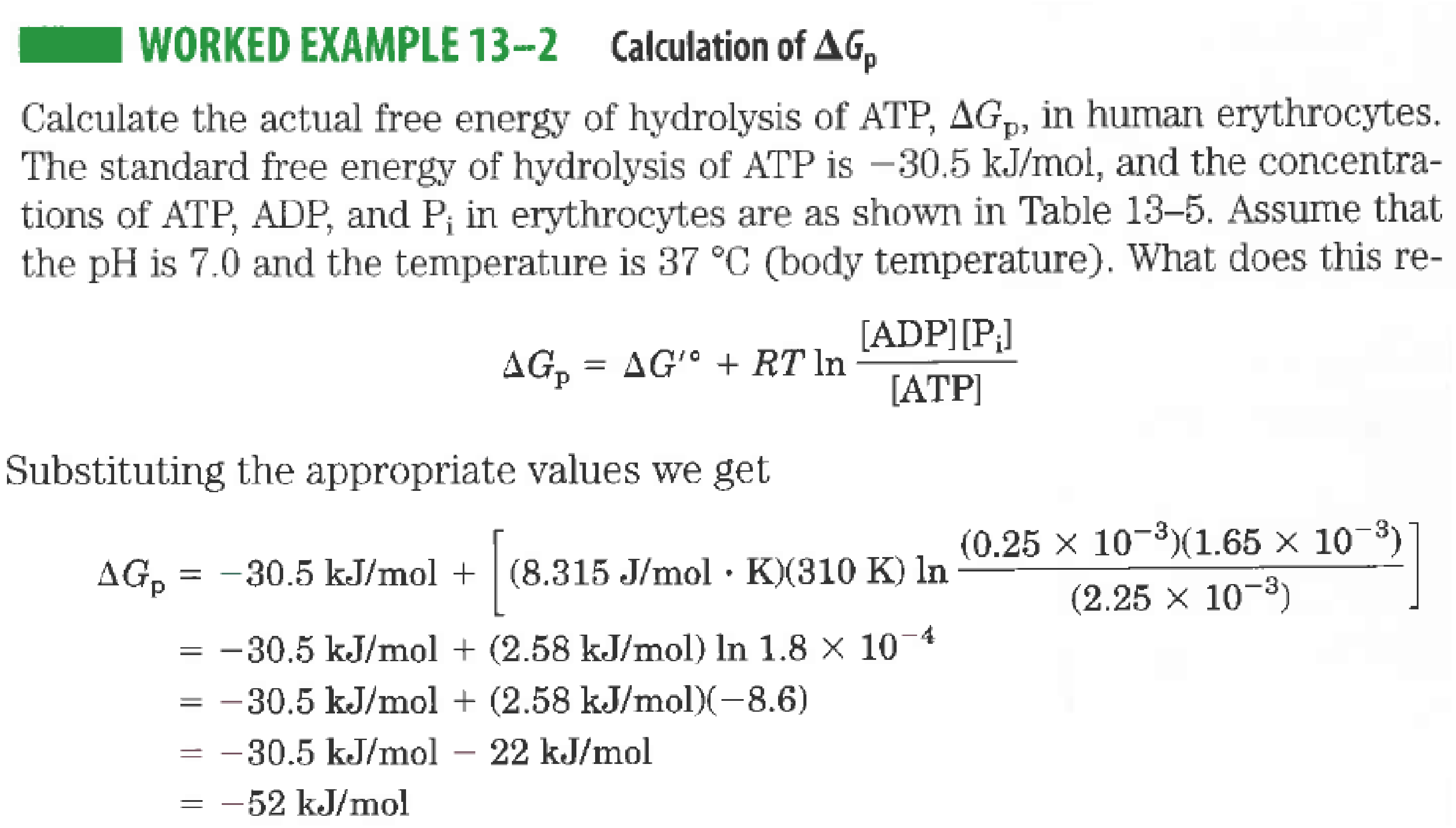


WORKED EXAMPLE 13–2 Calculation of ΔG_p

Calculate the actual free energy of hydrolysis of ATP, ΔG_p, in human erythrocytes. The standard free energy of hydrolysis of ATP is −30.5 kJ/mol, and the concentrations of ATP, ADP, and P_i in erythrocytes are as shown in Table 13–5. Assume that the pH is 7.0 and the temperature is 37 °C (body temperature). What does this re-

$$\Delta G_p = \Delta G'^\circ + RT \ln \frac{[\mathrm{ADP}][\mathrm{P_i}]}{[\mathrm{ATP}]}$$

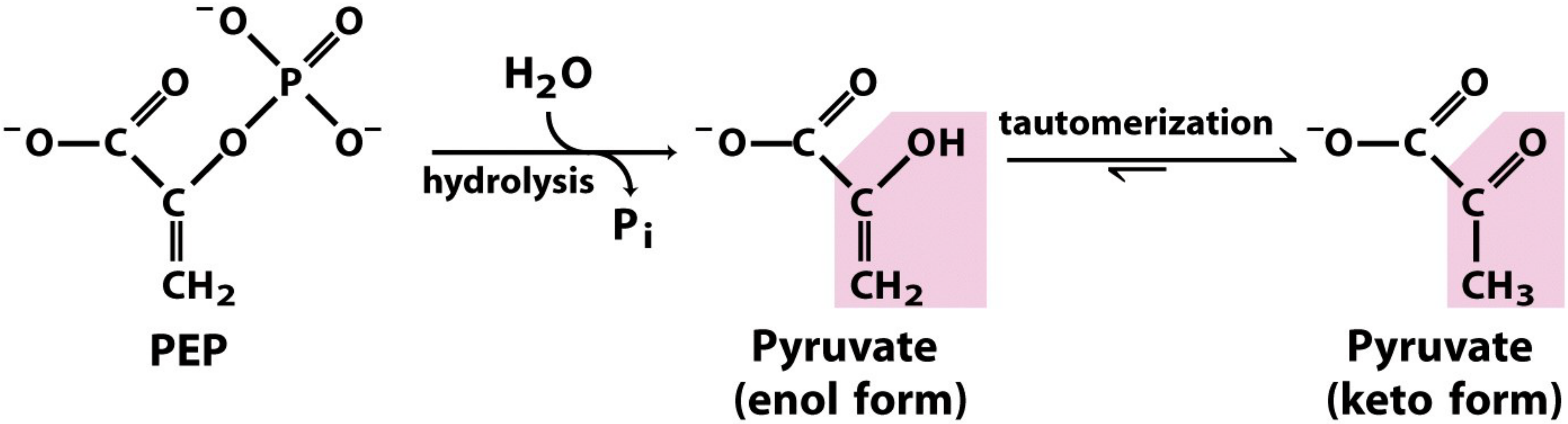
Substituting the appropriate values we get

$$\begin{aligned}
\Delta G_p &= -30.5\ \text{kJ/mol} + \left[(8.315\ \text{J/mol}\cdot\text{K})(310\ \text{K}) \ln \frac{(0.25 \times 10^{-3})(1.65 \times 10^{-3})}{(2.25 \times 10^{-3})}\right] \\
&= -30.5\ \text{kJ/mol} + (2.58\ \text{kJ/mol}) \ln 1.8 \times 10^{-4} \\
&= -30.5\ \text{kJ/mol} + (2.58\ \text{kJ/mol})(-8.6) \\
&= -30.5\ \text{kJ/mol} - 22\ \text{kJ/mol} \\
&= -52\ \text{kJ/mol}
\end{aligned}$$

Actually, in vivo, the energy released by ATP hydrolysis is greater than the standard free-energy change, $\Delta G'^{o}$.

In the Course of **evolution**, there has been a very strong **selective pressure** for regulatory mechanisms that hold celIular ATP concentrations far above the equilibrium concentrations for the hydrolysis reaction.

Other phosphorylated compounds and thioesters also have large free energies of hydrolysis



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

$$\Delta G'^{\circ} = -61.9\ \mathrm{kJ/mol}$$

Figure 13-13
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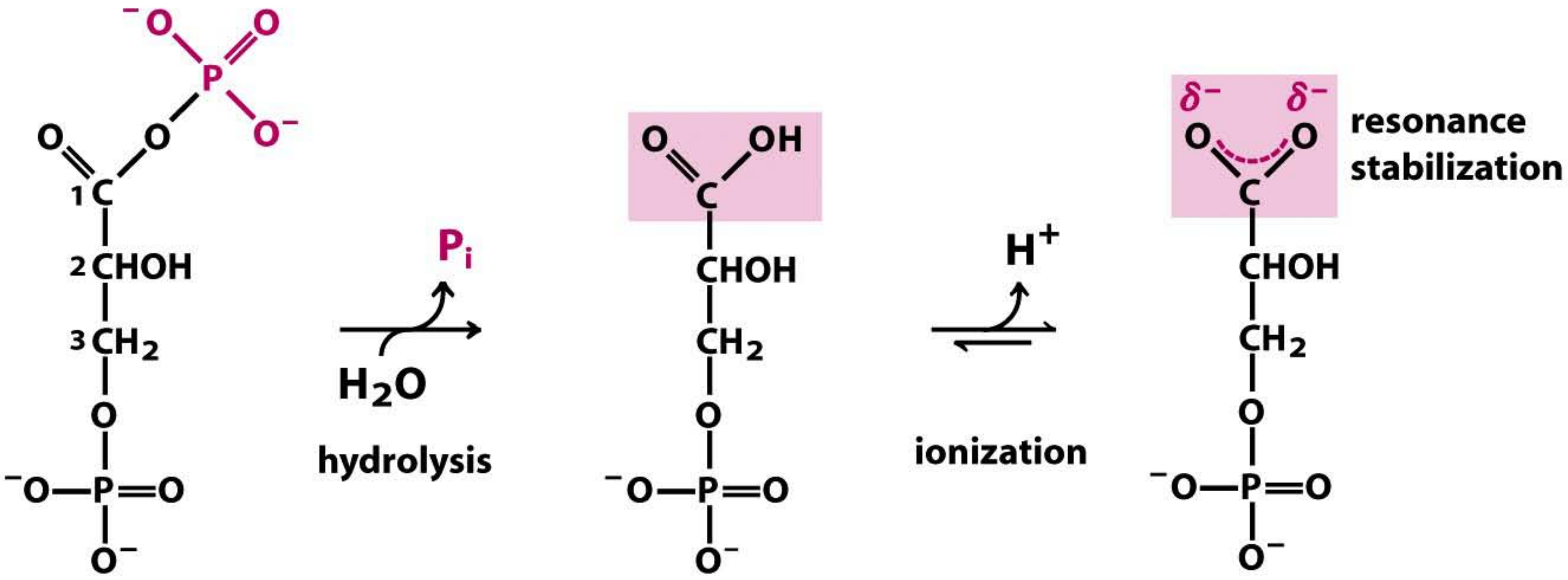
Hydrolysis of phosphoenolpyruvate (PEP)

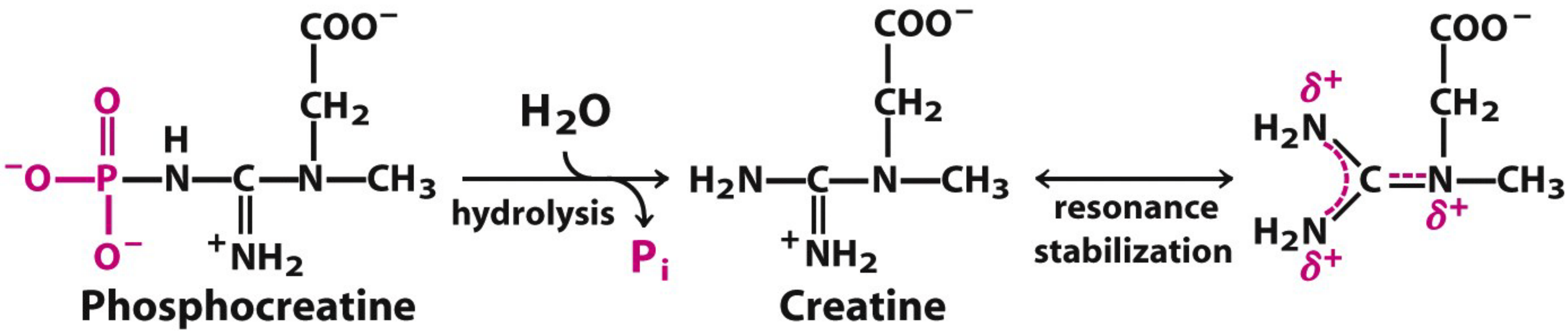


1,3-Bisphosphoglycerate   3-Phosphoglyceric acid   3-Phosphoglycerate

$$\text{1,3-Bisphosphoglycerate}^{4-} + H_2O \longrightarrow \text{3-phosphoglycerate}^{3-} + P_i^{2-} + H^+$$

$$\Delta G'^{\circ} = -49.3 \text{ kJ/mol}$$

Figure 13-14



$$\text{Phosphocreatine}^{2-} + H_2O \longrightarrow \text{creatine} + P_i^{2-}$$

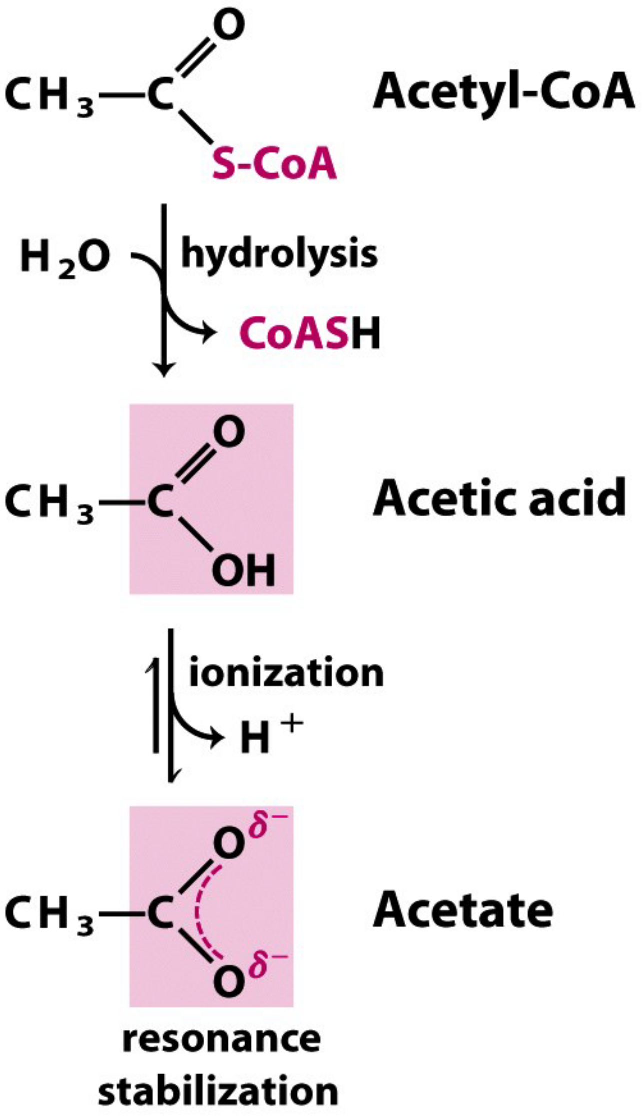
$$\Delta G'^{\circ} = -43.0 \text{ kJ/mol}$$

Figure 13-15

TABLE 13–6 Standard Free Energies of Hydrolysis of Some Phosphorylated Compounds and Acetyl-CoA (a Thioester)

	$\Delta G'^\circ$ (kJ/mol)	$\Delta G'^\circ$ (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 (→ $2P_i$)	−19.2	−4.0
Glucose 3-phosphate	−20.9	−5.0
Fructose 6-phosphate	−15.9	−3.8
Glucose 6-phosphate	−13.8	−3.3
Glycerol 3-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. 1, pp. 296–304, CRC Press, Boca Raton, FL. The value for the free energy of hydrolysis of PP_i is from Frey, P.A. & Arabshahi, A. (1995) Standard free-energy change for the hydrolysis of the α-β-phosphoanhydride bridge in ATP. *Biochemistry* **34**, 11,307–11,310.



$$\text{Acetyl-CoA} + H_2O \longrightarrow \text{acetate}^- + \text{CoA} + H^+$$
$$\Delta G'^\circ = -31.4 \text{ kJ/mol}$$

Figure 13-16
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Thioesters having large, negative, standard free energies of hydrolysis

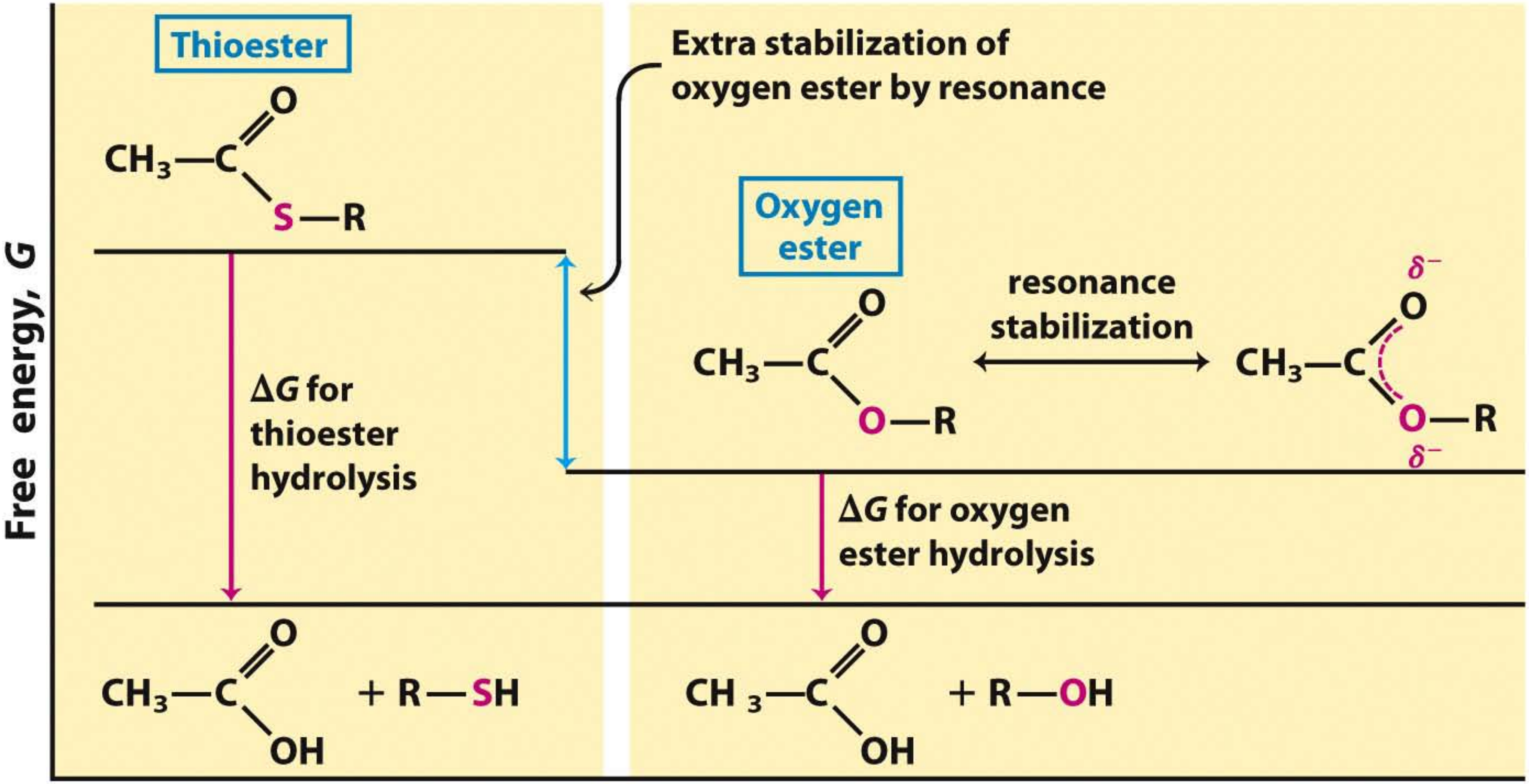

Figure 13-17

Free energy of hydrolysis for thioesters is greater than for comparable oxygen esters

To summarize, for hydrolysis reactions with large, negative, standard free-energy changes, the products are more stable than the reactants for one or more of the following reasons: (1) the bond strain in reactants due to electrostatic repulsion is relieved by charge separation, as for ATP; (2) the products are stabilized by ionization, as for ATP, acyl phosphates and thioesters; (3) the products are stabilized by isomerization (tautomerization), as for PEP; and./or (4) the products are stabilized by resonance, as for creatine released from phosphocreatine, carboxylate ion released from acyl phosphates and thioesters, and phosphate (P) released from anhydride or ester linkages.

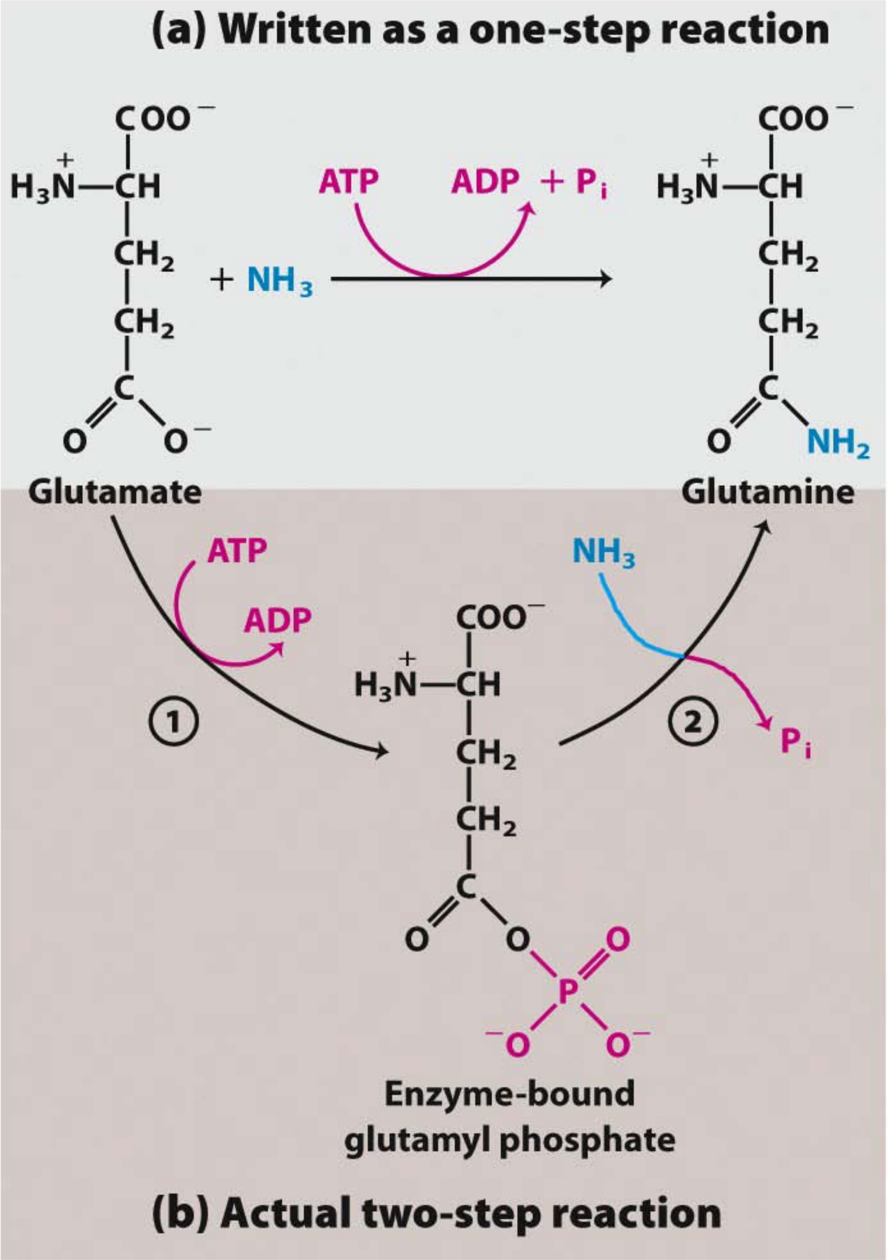

Figure 13-18

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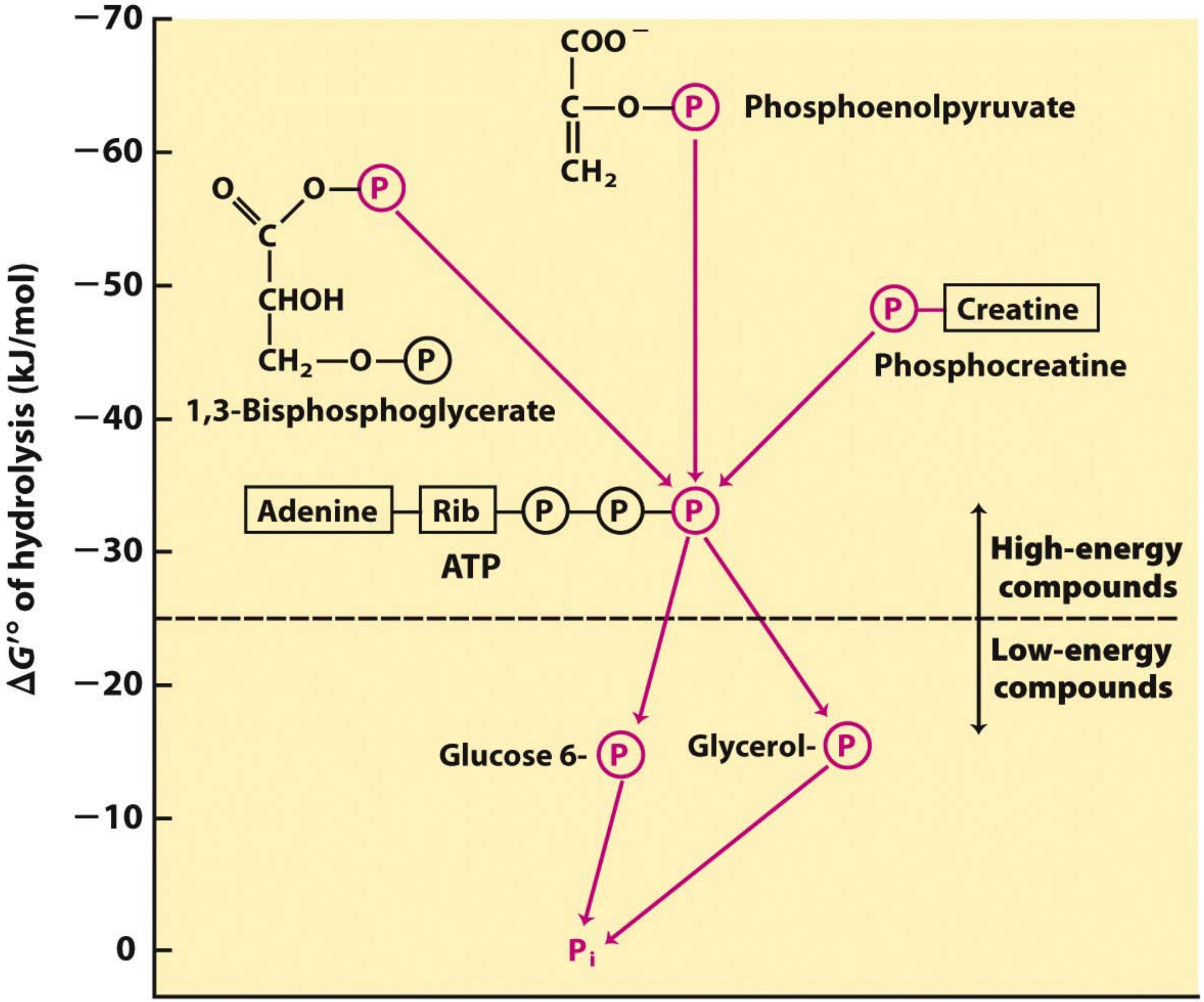
ATP provides energy by group transfers, not by simple hydrolysis



25 kJ/mol

Figure 13-19

Ranking of biological phosphate compounds by standard free energies of hydrolysis

ATP is kinetically stable in aqueous solution, because huge activation energies (200 to 400 kJ/mol) is required for uncatalyzed cleavage of its phosphoanhydride bonds

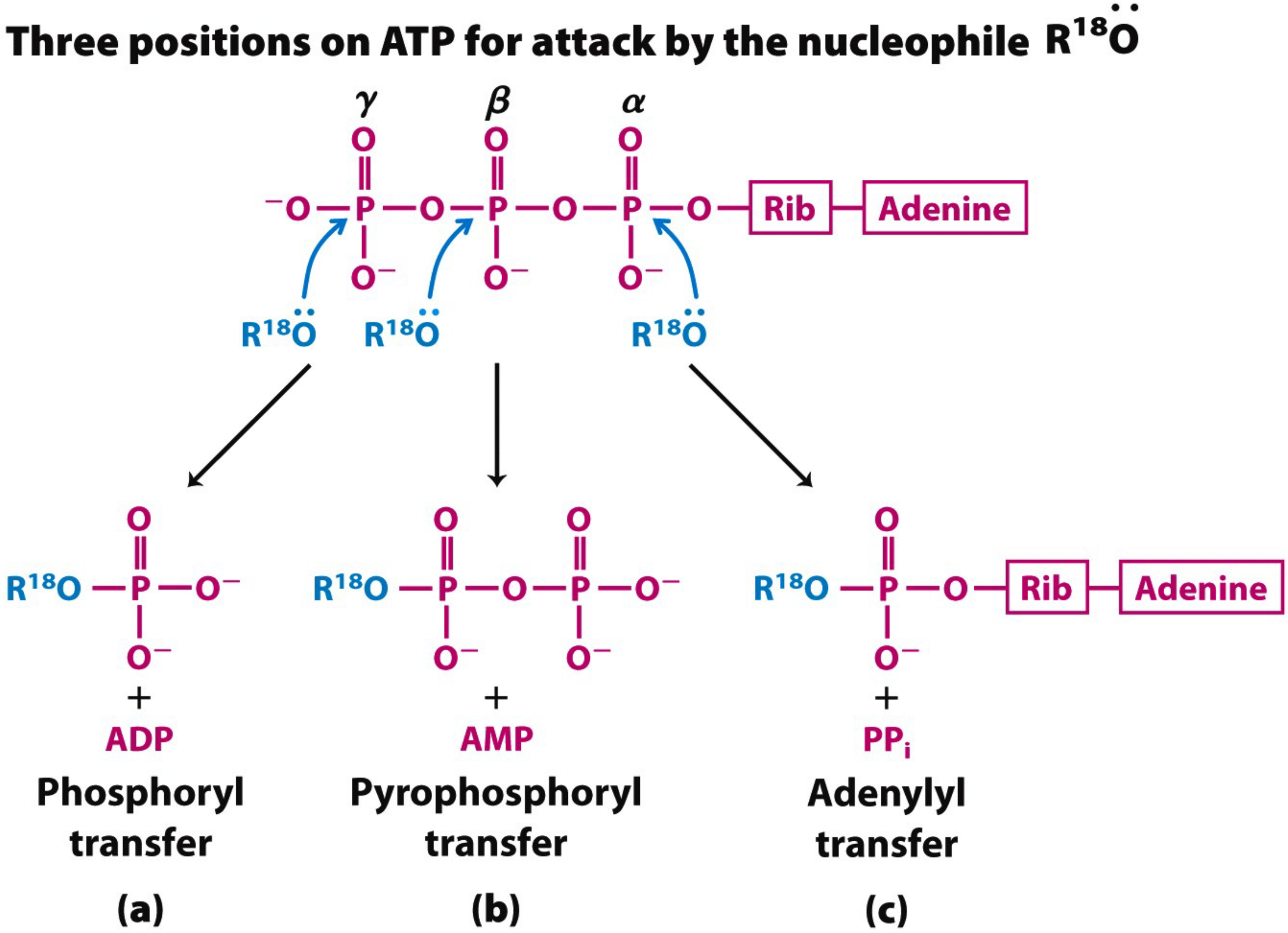
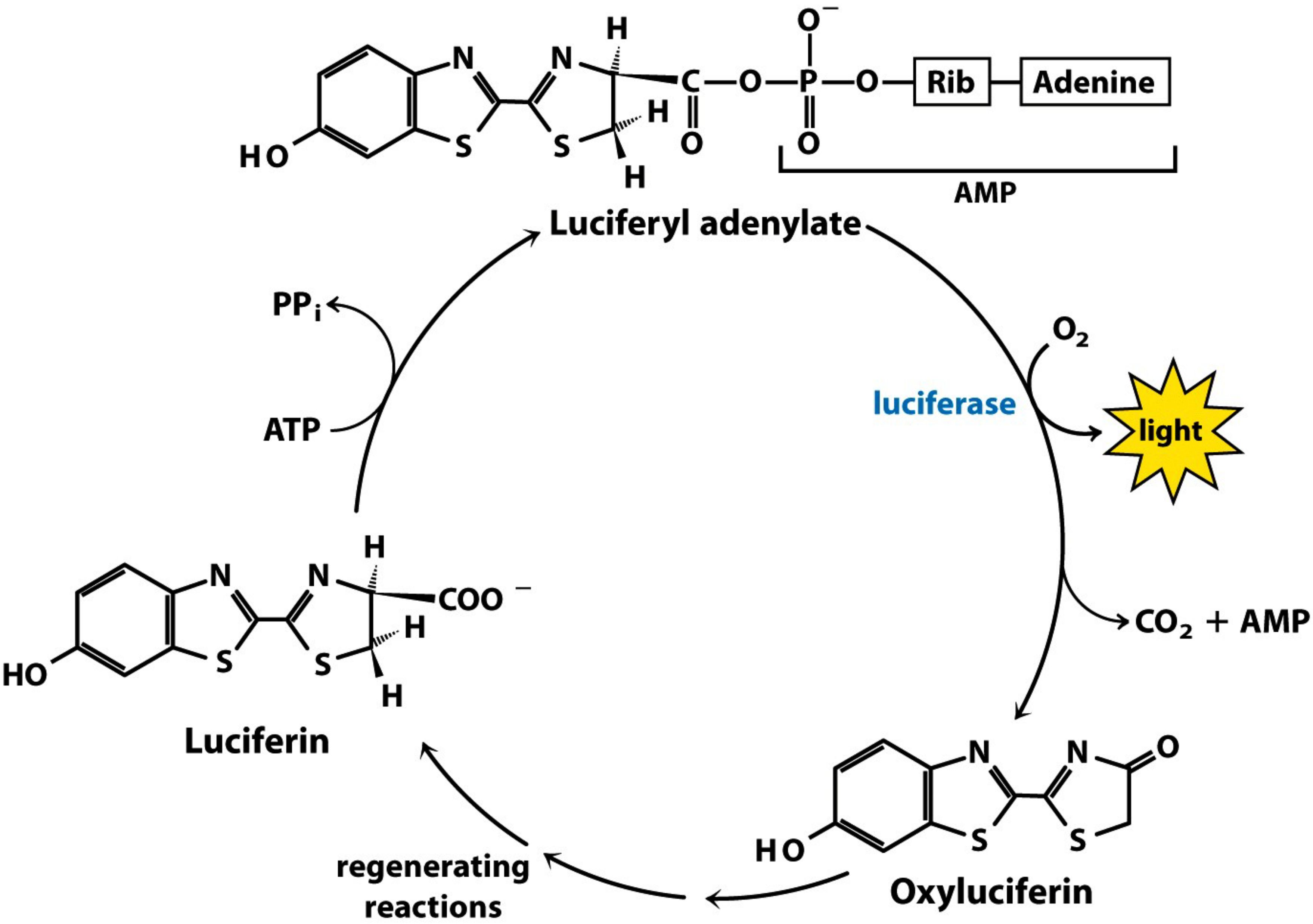

Three positions on ATP for attack by the nucleophile $R^{18}\ddot{O}$

Figure 13-20

Hydrolysis of the α−β phosphoanhydride bond releases considerably more energy (−46 kJ/mol) than hydrolysis of the β−γ bond (−31 kJ/mol)

Box 13-1 figure 1a

Lehninger Principles of Biochemistry, Fifth Edition



Box 13-1 figure 1b
Lehninger Principles of Biochemistry, Fifth Edition

Hydrolysis of ATP provides energy for the flashes of firefly

Assembly of Informational macromolecules requires energy

ATP energizes active transport and muscle contraction

Nucleoside diphosphate kinase, found in all cells, catalyzes the reaction

$$\text{ATP} + \text{NDP (or dNDP)} \xrightleftharpoons{\text{Mg}^{2+}} \text{ADP} + \text{NTP (or dNTP)} \qquad \Delta G'^{\circ} \approx 0$$

Adenylate kinase

$$2\text{ADP} \xrightleftharpoons{\text{Mg}^{2+}} \text{ATP} + \text{AMP} \qquad \Delta G'^{\circ} \approx 0$$

creatine kinase

$$\text{ADP} + \text{PCr} \xrightleftharpoons{\text{Mg}^{2+}} \text{ATP} + \text{Cr} \qquad \Delta G'^{\circ} = -12.5\,\text{kJ/mol}$$

Transphosphorylations between nucleotides occurs in all cell types

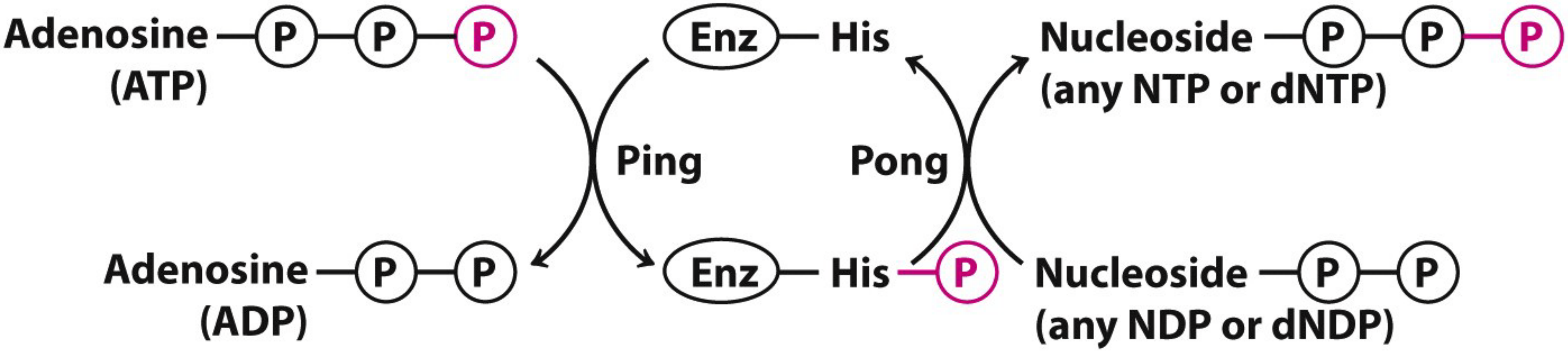
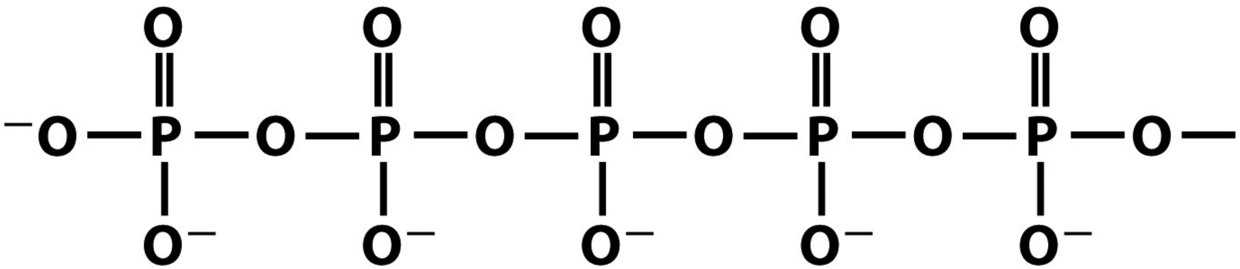


Figure 13-21

Ping-Pong mechanism of nucleoside diphosphate kinase



Inorganic polyphosphate (polyP)

PPK1 polyphosphate kinase

$$\text{ATP} + \text{polyP}_n \xrightleftharpoons{\text{Mg}^{2+}} \text{ADP} + \text{polyP}_{n+1}$$

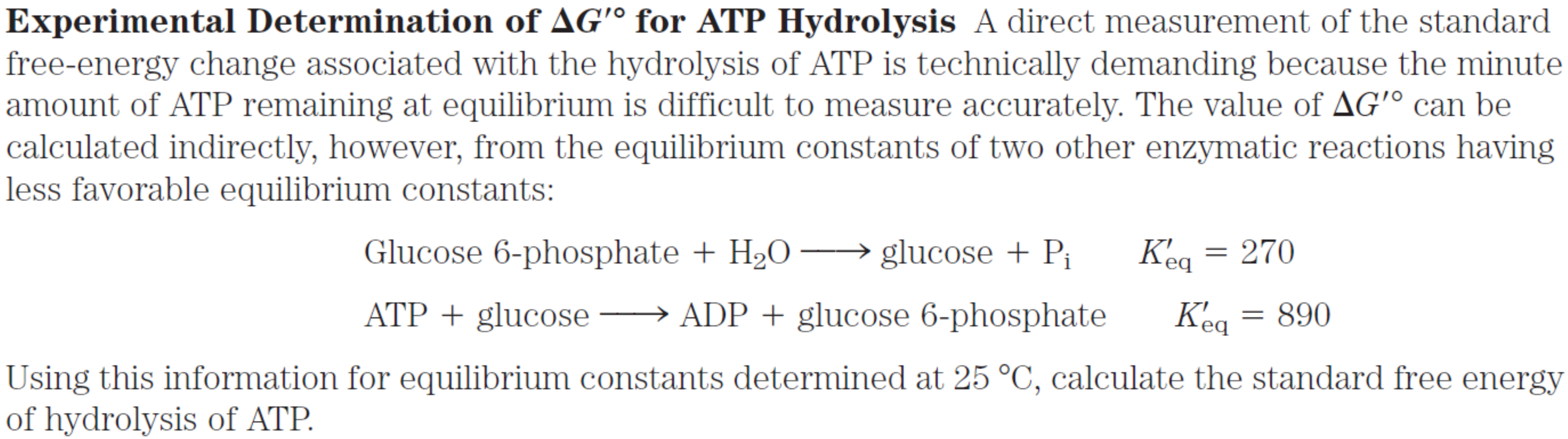
$$\Delta G'^{\circ} = -20\,\text{kJ/mol}$$

PPK2

$$\text{GDP} + \text{polyP}_{n+1} \xrightleftharpoons{\text{Mn}^{2+}} \text{GTP} + \text{polyP}_n$$

polyP is to serve as a phosphagen, a reservoir of phosphoryl groups that can be used to generate ATP, as creatine phosphate is used in muscle.

Homework



Experimental Determination of $\Delta G'^\circ$ for ATP Hydrolysis A direct measurement of the standard free-energy change associated with the hydrolysis of ATP is technically demanding because the minute amount of ATP remaining at equilibrium is difficult to measure accurately. The value of $\Delta G'^\circ$ can be calculated indirectly, however, from the equilibrium constants of two other enzymatic reactions having less favorable equilibrium constants:

$$\text{Glucose 6-phosphate} + \text{H}_2\text{O} \longrightarrow \text{glucose} + \text{P}_\text{i} \qquad K'_\text{eq} = 270$$

$$\text{ATP} + \text{glucose} \longrightarrow \text{ADP} + \text{glucose 6-phosphate} \qquad K'_\text{eq} = 890$$

Using this information for equilibrium constants determined at 25 °C, calculate the standard free energy of hydrolysis of ATP.