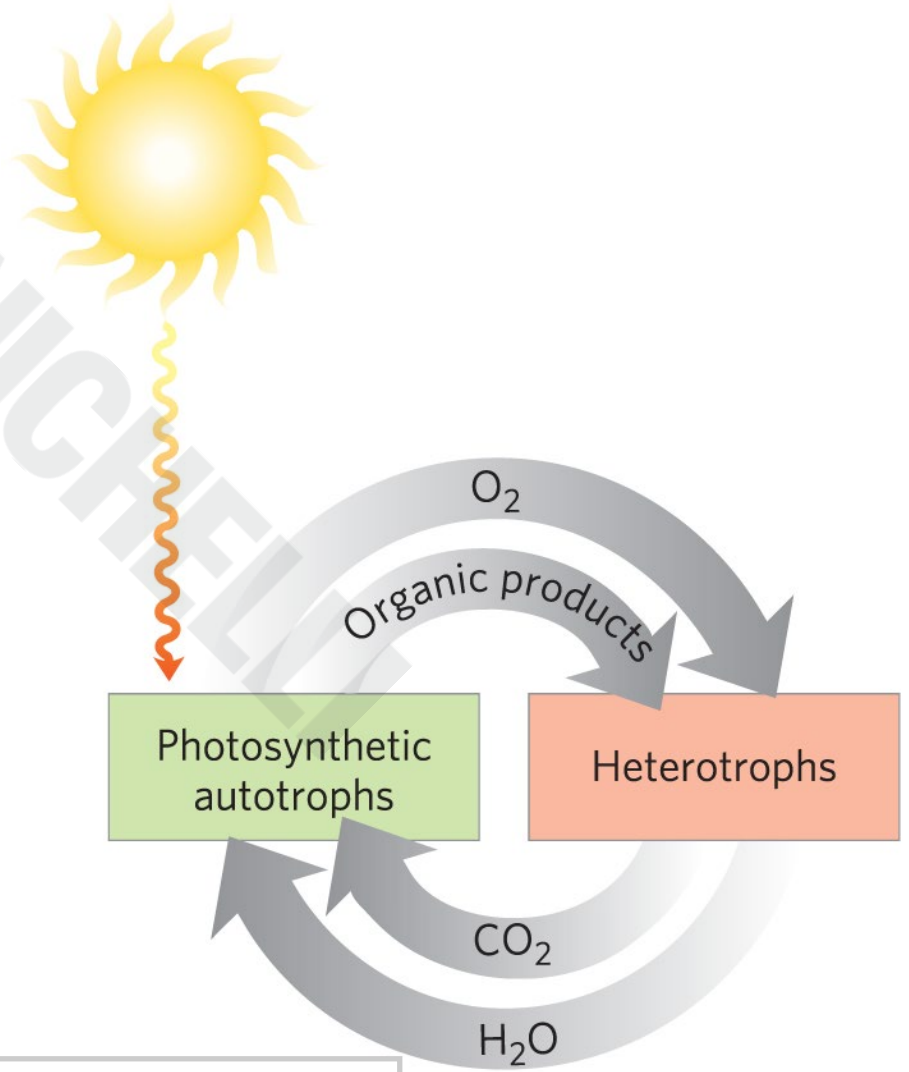
13 Introduction to Metabolism

Autotrophs and Heterotrophs

- **autotrophs** = use CO_2 from the atmosphere as their sole source of carbon

- **heterotrophs** = cannot use atmospheric CO_2 and must obtain carbon from their environment



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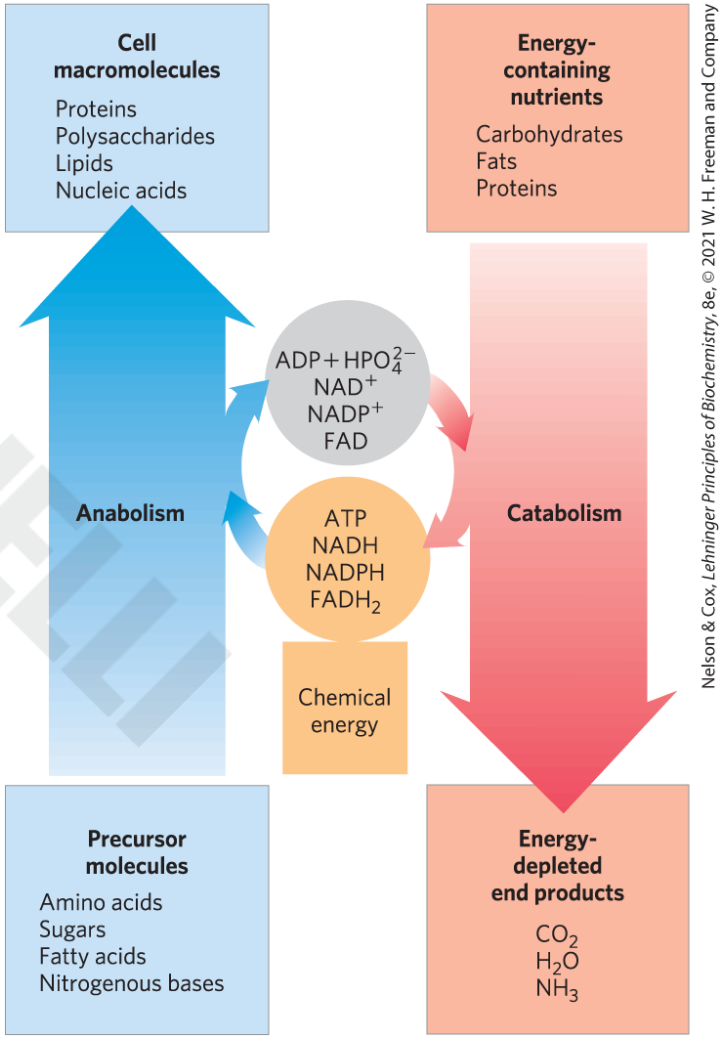
Metabolites and Intermediary Metabolism

- **metabolites** = series of intermediates through which precursors are converted to products

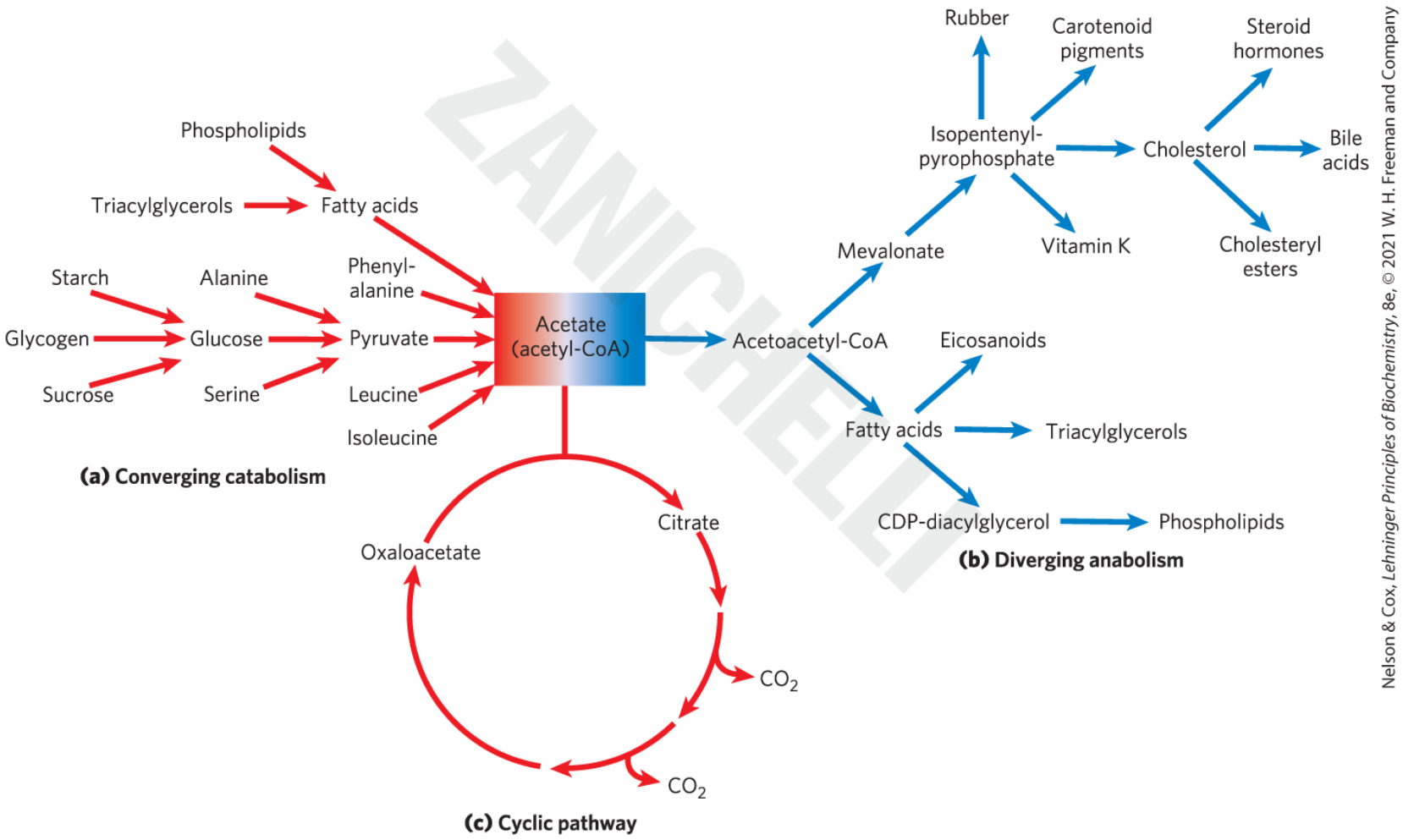
- **intermediary metabolism** = the combined activities of all the metabolic pathways that interconvert precursors, metabolites, and products of low molecular weight

The Relationship Between Catabolic and Anabolic Pathways

- **catabolism** = the degradative phase of metabolism
  - releases energy
- **anabolism** (biosynthesis) = the building phase of metabolism
  - requires energy



Catabolic Pathways Converge and Anabolic Pathways Diverge



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P1 Principle 1 (1 of 4)

The chemical changes and energy transductions in living organisms follow the laws of thermodynamics.

P2 Principle 2 (1 of 3)

The free-energy change is the maximum energy made available to do work when a chemical reaction occurs. If two reactions can be combined to yield a third reaction, the overall free energy change is the sum of the two. Cells accomplish energy-requiring chemical work by coupling an energy-releasing (exergonic) reaction, such as the cleavage of ATP, to an endergonic reaction (which requires energy input).

P3 Principle 3 (1 of 4)

Although thousands of different chemical reactions occur in the biosphere, most of them fall within a small set of reaction types.

P4 Principle 4 (1 of 5)

ATP is the universal energy currency in living organisms. Transfer of its phosphoryl group to a water molecule or metabolic intermediates provides the energetic push for muscle contraction, the pumping of solutes against concentration gradients, and the synthesis of complex molecules.

P5 Principle 5 (1 of 5)

Oxidation-reduction reactions indirectly provide much of the energy needed to make ATP. Reduced substrates such as glucose are oxidized in several steps, with the energy of oxidation steps conserved in the form of a reduced cofactor, NADH. Energy stored in NADH is used to drive the synthesis of ATP.

P6 Principle 6 (1 of 5)

To respond to changes in external circumstances, cells must regulate enzyme activities by changing either the number of enzyme molecules or the catalytic activity of preexisting enzyme molecules.

13.1 Bioenergetics and Thermodynamics

Bioenergetics Is the Quantitative Study of Energy Transductions

- **energy transductions** = changes of one form of energy into another

Biological Energy Transformations Obey the Laws of Thermodynamics

- the first law of thermodynamics is the principle of the conservation of energy

- the second law of thermodynamics says that the universe always tends toward increasing disorder

Systems and Their Surroundings

- *reacting system* = the collection of matter that is undergoing a particular chemical or physical process
  - can be closed or open

- *universe* = the reacting system and its *surroundings* together

- living cells and organisms are open systems, exchanging material and energy with their surroundings

Free Energy, *G*

- **free energy**, ***G*** = expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure
  - when ΔG is negative, free energy is released and the reaction is **exergonic**
  - when ΔG is positive, free energy is gained and the reaction is **endergonic**

Enthalpy, *H*

- **enthalpy**, ***H*** = the heat content of the reacting system
  - reflects the number and kinds of chemical bonds (covalent and noncovalent) in the reactants and products
  - when ΔH is negative, the reaction releases heat and the reaction is **exothermic**
  - when ΔH is positive, the reaction takes up heat and the reaction is **endothermic**

Entropy, *S*

- **entropy**, ***S*** = a quantitative expression for the randomness or disorder in a system
  - when ΔS is negative, the reactants are less complex and more disordered than the products
  - when ΔS is positive, the products are less complex and more disordered than the reactants

Physical Constants and Units Used in Thermodynamics

Table 13-1 Some Physical Constants and Units Used in Thermodynamics

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

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

Faraday constant, 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)

Free Energy Change, ΔG

- free-energy change, $\Delta G = \Delta H - T\Delta S$ (13-1)
  - ΔH is negative for a reaction that releases heat
  - ΔS is positive for a reaction that increases the system's randomness

- spontaneous reactions occur when ΔG is negative

Living Cells and the Second Law of Thermodynamics

- the order produced within cells as they grow and divide is compensated for by the disorder they create in their surroundings
  - living organisms take free energy from, and return heat and entropy to, their surroundings

Standard Free-Energy Change Is Directly Related to the Equilibrium Constant

- for the general reaction

$$aA + bB \rightarrow cC + dD$$

the equilibrium constant, K_{eq}, is given by

$$K_{eq} = \frac{[C]_{eq}^{c}[D]_{eq}^{d}}{[A]_{eq}^{a}[B]_{eq}^{d}} \qquad (13\text{-}2)$$

Standard Transformed Constants

- **standard transformed constants** = physical constants based on the biochemical standard state ($[H^+]$ is 10^{-7} M, $[H_2O]$ is 55.5 M, $[Mg^{2+}]$ is 1 mM)
  - **standard free-energy change,** $\Delta G'°$
  - **standard equilibrium constant,** K'_{eq}

P1 Principle 1 (3 of 4)

The chemical changes and energy transductions in living organisms follow the laws of thermodynamics.

The Relationship Between K'_{eq} and $\Delta G'°$

- the relationship between K'_{eq} and $\Delta G'°$ is:

$$\Delta G'° = -RT \ln K'_{eq} \qquad (13\text{-}3)$$

- the standard free-energy change of a chemical reaction is simply an alternative mathematical way of expressing its equilibrium constant

Relationship between Equilibrium Constants and Standard Free-Energy Changes

- if K'_{eq} is > 1.0, its $\Delta G'°$ is negative
- if K'_{eq} is < 1.0, its $\Delta G'°$ is positive
- the relationship is exponential

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

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

Effects of K'_{eq} and $\Delta G'^{\circ}$ on the Direction of Chemical Reactions

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

Standard Free-Energy Changes of Some Chemical Reactions

- to describe the energy released under the conditions existing in cells, an expression for the *actual* free-energy change is needed

Reaction type	$\Delta G'^{\circ}$ (kJ/mol)	$\Delta G'^{\circ}$ (kcal/mol)
Hydrolysis reactions		
Acid anhydrides		
Acetic anhydride + $H_2O \rightarrow$ 2 acetate	−91.1	−21.8
ATP + $H_2O \rightarrow$ ADP + P_i	−30.5	−7.3
ATP + $H_2O \rightarrow$ AMP + PP_i	−45.6	−10.9
PP_i + $H_2O \rightarrow 2P_i$	−19.2	−4.6
UDP-glucose + $H_2O \rightarrow$ UMP + glucose 1-phosphate	−43.0	−10.3
Esters		
Ethyl acetate + $H_2O \rightarrow$ ethanol + acetate	−19.6	−4.7
Glucose 6-phosphate + $H_2O \rightarrow$ glucose + P_i	−13.8	−3.3
Amides and peptides		
Glutamine + $H_2O \rightarrow$ glutamate + NH^+_4	−14.2	−3.4
Glycylglycine + $H_2O \rightarrow$ 2 glycine	−9.2	−2.2
Glycosides		
Maltose + $H_2O \rightarrow$ 2 glucose	−15.5	−3.7
Lactose + $H_2O \rightarrow$ glucose + galactose	−15.9	−3.8
Rearrangements		
Glucose 1-phosphate→glucose 6-phosphate	−7.3	−1.7
Fructose 6-phosphate→glucose 6-phosphate	−1.7	−0.4
Elimination of water		
Malate →fumarate + H_2O	3.1	0.8
Oxidations with molecular oxygen		
Glucose + $6O_2 \rightarrow 6CO_2 + 6H_2O$	−2,840	−686
Palmitate + $23O_2 \rightarrow 16CO_2 + 16H_2O$	−9,770	−2,338

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

P1 Principle 1 (4 of 4)

The chemical changes and energy transductions in living organisms follow the laws of thermodynamics.

Actual Free-Energy Changes Depend on Reactant and Product Concentrations

- for any reaction $aA + bB \rightleftarrows cC + dD$, ΔG and $\Delta G'^\circ$ are related by the equation:

$$\Delta G = \Delta G'^\circ + RT \ln \frac{[C]^c [D]^d}{[A]^a [B]^b} \quad (13\text{-}4)$$

mass-action ratio, *Q*

- when $\Delta G = 0$, the system is at equilibrium

Free-Energy Changes and Enzymes

- the free-energy change for a reaction is independent of the pathway by which the reaction occurs

- enzymes cannot change equilibrium constants
  - can increase the rate at which a reaction proceeds

Standard Free-Energy Changes Are Additive

- for two sequential chemical reactions, A ⇄ B and B ⇄ C, each chemical reaction has its own equilibrium constant and standard free-energy change $\Delta G_1'°$ and $\Delta G_2'°$

- for the overall reaction, A ⇄ C, the standard free-energy change is the sum of the individual standard free-energy changes

(1)	$A \longrightarrow B$	$\Delta G_1'°$
(2)	$B \longrightarrow C$	$\Delta G_2'°$
Sum:	$A \longrightarrow C$	$\Delta G_1'° + \Delta G_2'°$

P2 Principle 2 (2 of 3)

The free-energy change is the maximum energy made available to do work when a chemical reaction occurs. If two reactions can be combined to yield a third reaction, the overall free energy change is the sum of the two. Cells accomplish energy-requiring chemical work by coupling an energy-releasing (exergonic) reaction, such as the cleavage of ATP, to an endergonic reaction (which requires energy input).

Thermodynamically Unfavorable Reactions Can Be Coupled to Favorable Reactions

- a thermodynamically unfavorable (endergonic) reaction can be driven in the forward direction by coupling it to a highly exergonic reaction
  - often involves coupling to ATP hydrolysis ($\Delta G'° = -30.5$ kJ/mol)

Equilibrium Constants Are Multiplicative

- the K'_{eq} for the overall reaction is the product of the individual K'_{eq} values for the two reactions

13.2 Chemical Logic and Common Biochemical Reactions

P3 Principle 3 (2 of 4)

Although thousands of different chemical reactions occur in the biosphere, most of them fall within a small set of reaction types.

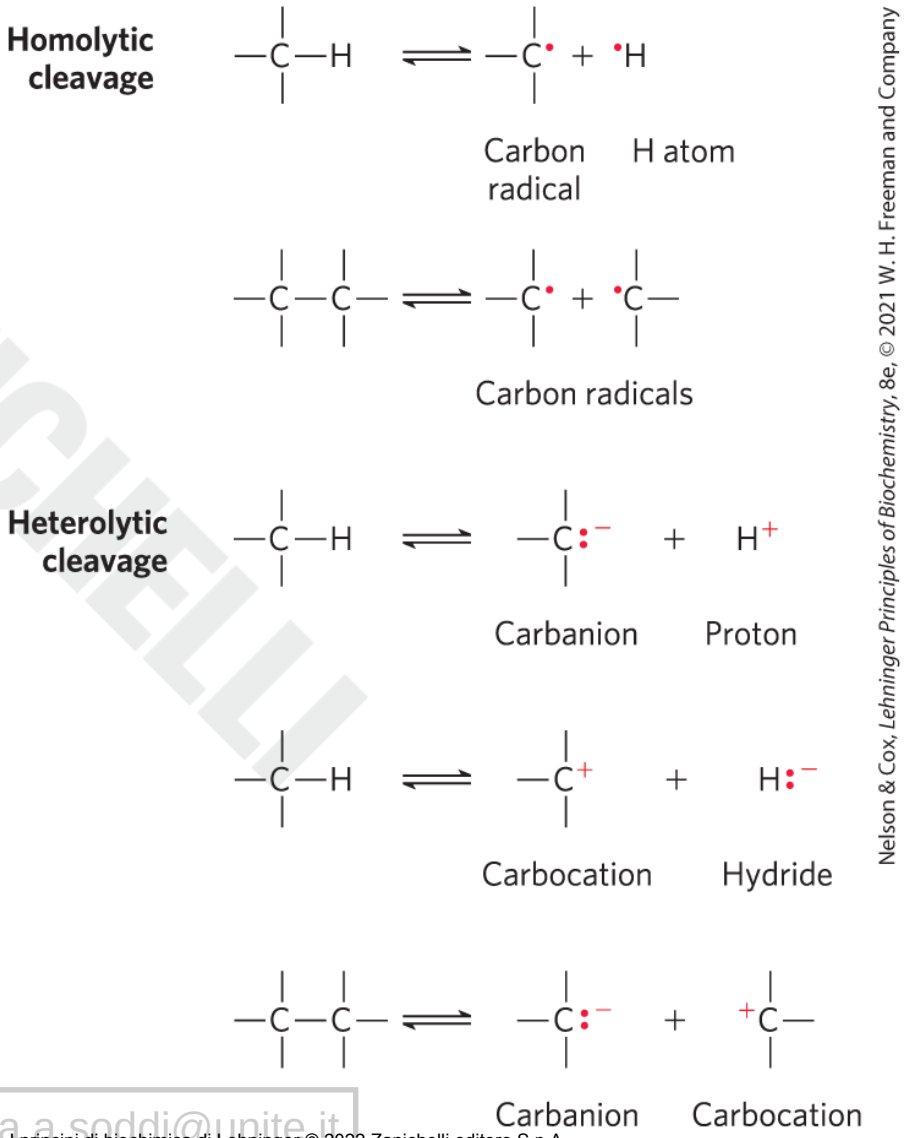
Biochemical Reactions Occur in Repeating Patterns

- five general categories of reactions in living cells:
  - reactions that make or break carbon–carbon bonds
  - internal rearrangements, isomerizations, and eliminations
  - free-radical reactions
  - group transfers
  - oxidation-reductions

Homolytic and Heterolytic Cleavage

- **homolytic cleavage** = cleavage of a covalent bond where each atom leaves the bond as a **radical**, carrying one unpaired electron

- **heterolytic cleavage** = cleavage of a covalent bond where one atom retains both bonding electrons
  - more common



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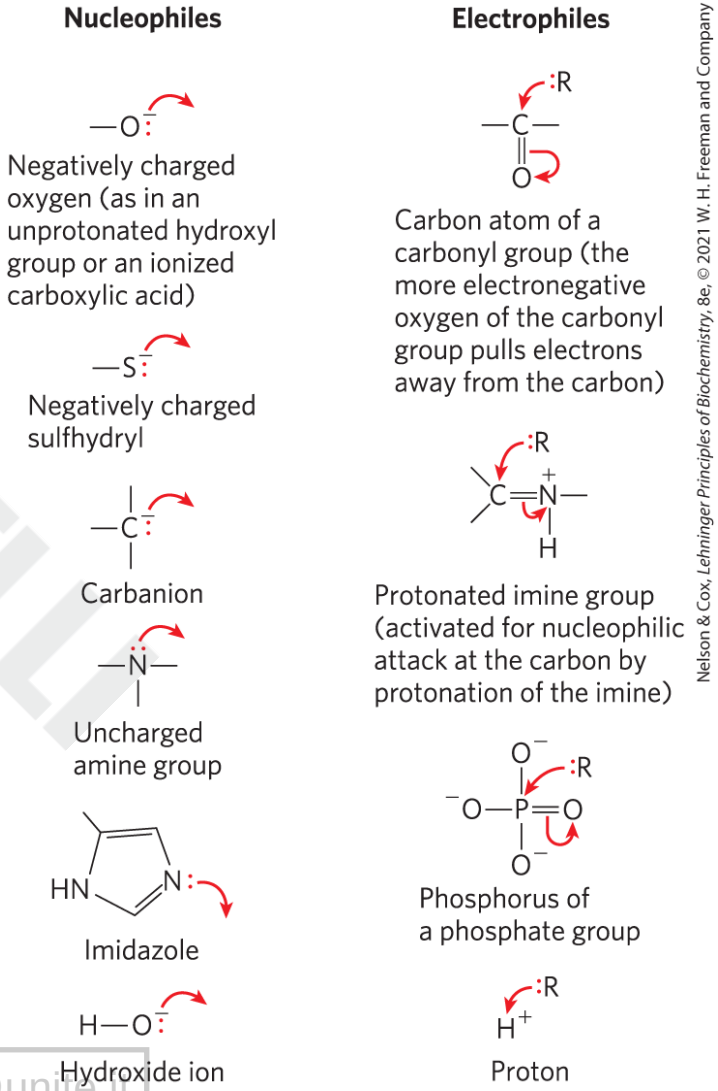
Nucleophiles and Electrophiles

- **nucleophiles** = functional groups rich in and capable of donating electrons
  - combine with and give up electrons to electrophiles
- **electrophiles** = electron-deficient functional groups that seek electrons
- carbon can act as either a nucleophile or electrophile



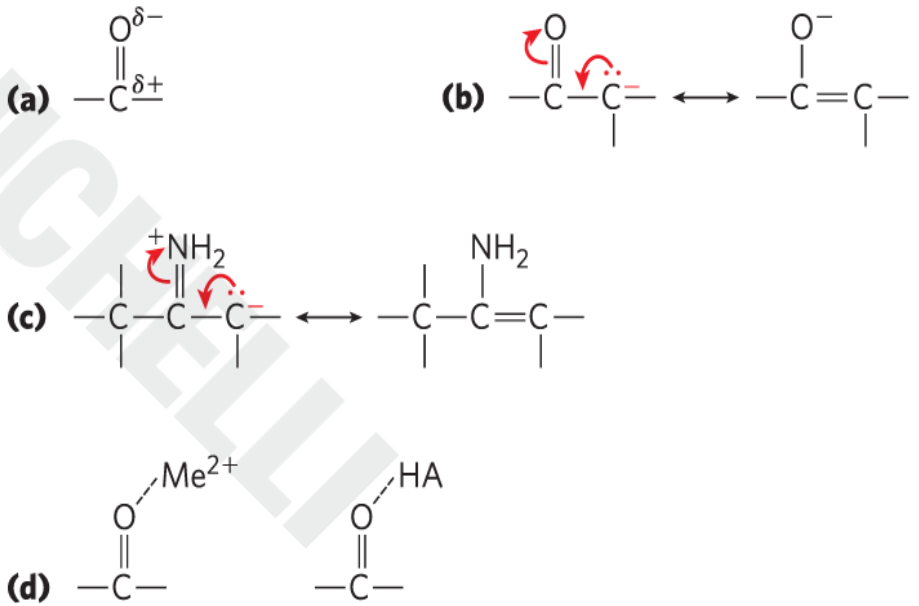
Reactions That Make or Break Carbon–Carbon Bonds

- heterolytic cleavage of a C–C bond yields a **carbanion** and a **carbocation**
- combination of a nucleophilic carbanion and an electrophilic carbocation forms a C–C bond
- impossible reactions for the purposes of biochemistry because carbanions and carbocations are generally unstable

Chemical Properties of Carbonyl Groups

- the carbon of a carbonyl group is an electrophilic carbon

- carbonyl and imine groups form carbanions on adjoining carbons by delocalizing electrons
  - general acid catalysts or metal ions (Me^{2+}) further enhance



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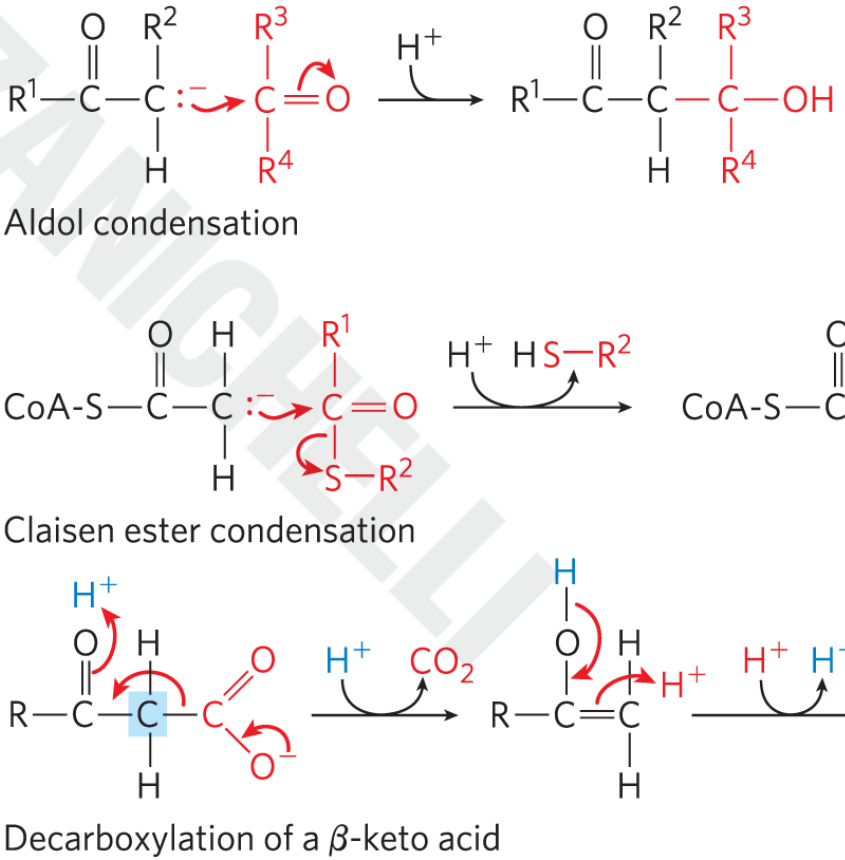
P3 Principle 3 (3 of 4)

Although thousands of different chemical reactions occur in the biosphere, most of them fall within a small set of reaction types.

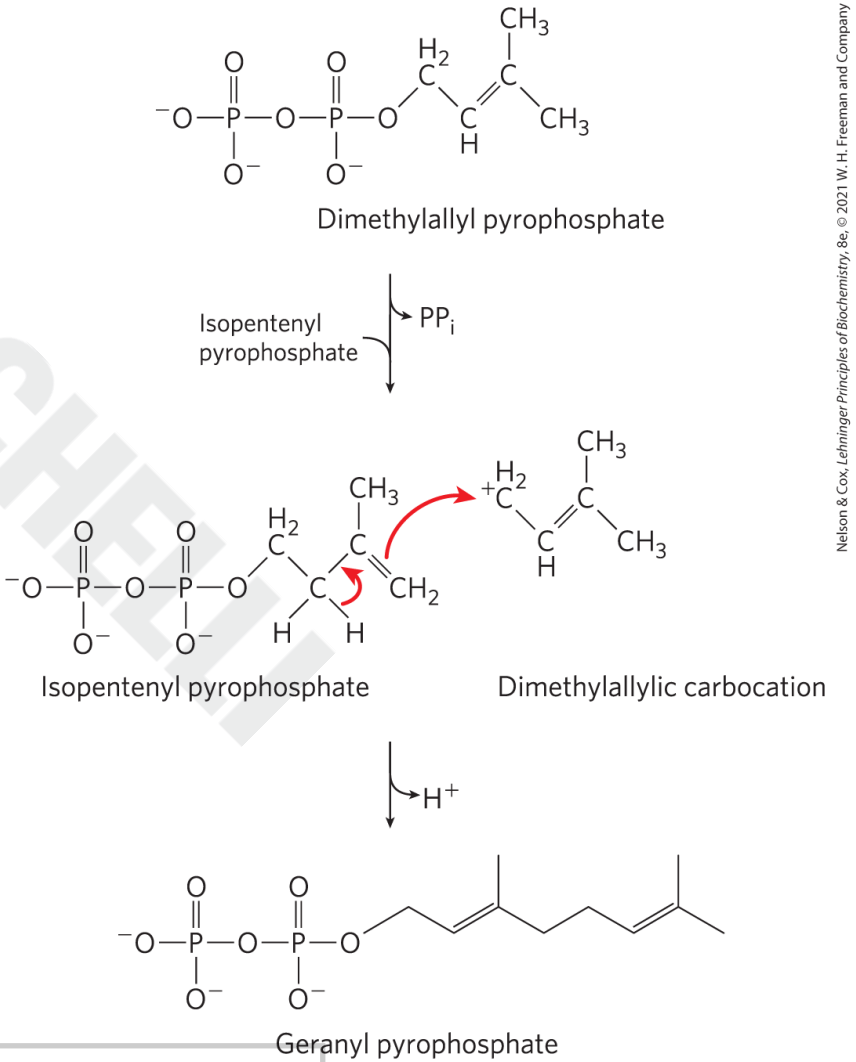
Common Reactions that Form and Break Carbon–Carbon Bonds

- in **aldol condensation, Claisen condensation,** and decarboxylation reactions, a carbanion intermediate is stabilized by a carbonyl group



Carbocations in Carbon–Carbon Bond Formation

- carbocation intermediates are generated by the elimination of an excellent leaving group, such as pyrophosphate



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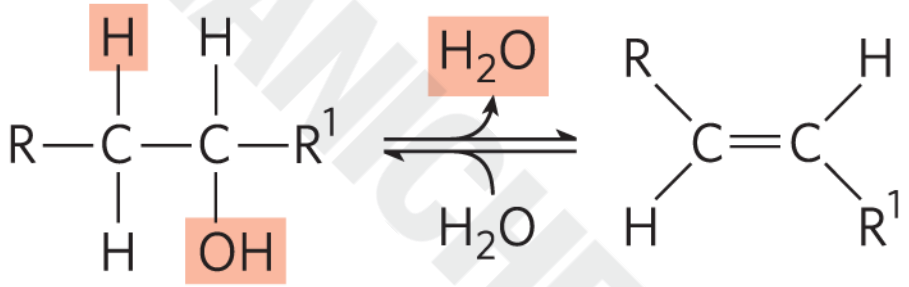
Internal Rearrangements, Isomerizations, and Eliminations

- redistribution of electrons results in alterations without changes in the overall oxidation state of the molecule

- reactions include:
  - intramolecular oxidation-reduction
  - change in cis-trans arrangement at a double bond
  - transposition of double bonds

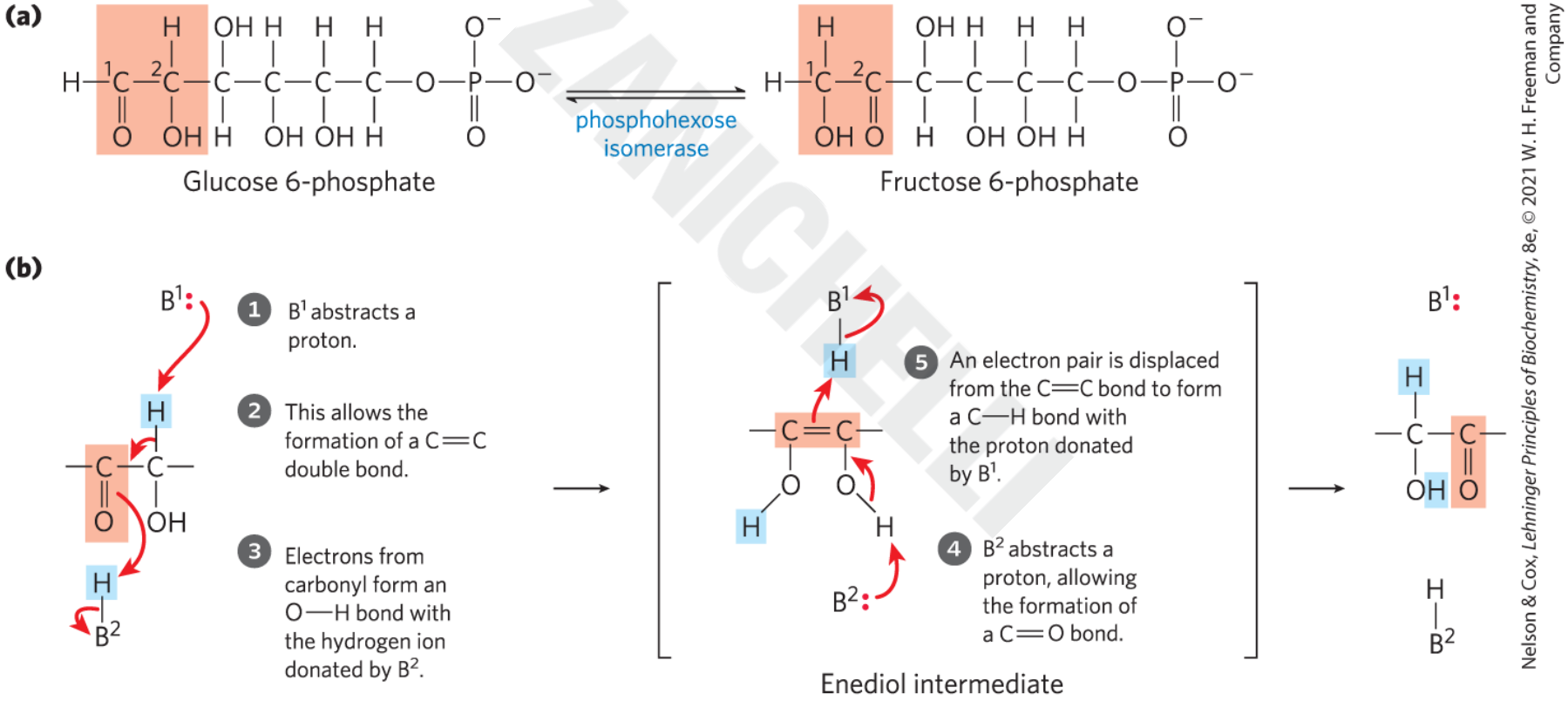
Elimination Reactions

- loss of water from an alcohol introduces a C=C bond



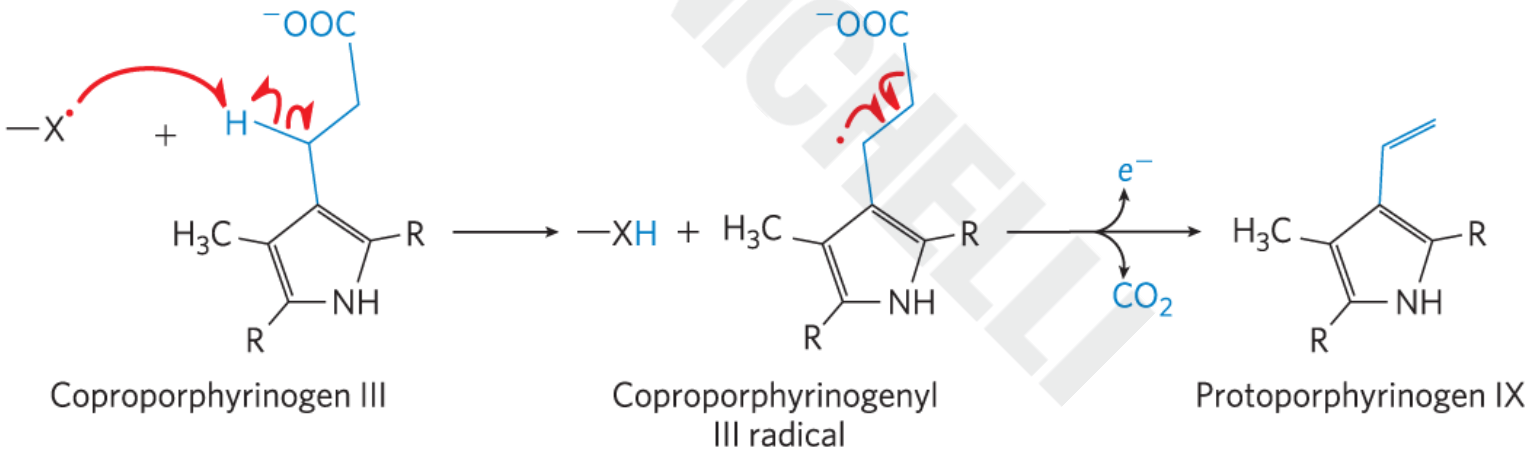
- similar reactions result from eliminations in amines

Isomerization and Elimination Reactions



Free-Radical Reactions

- homolytic cleavage of covalent bonds to generate free radicals occurs in some pathways



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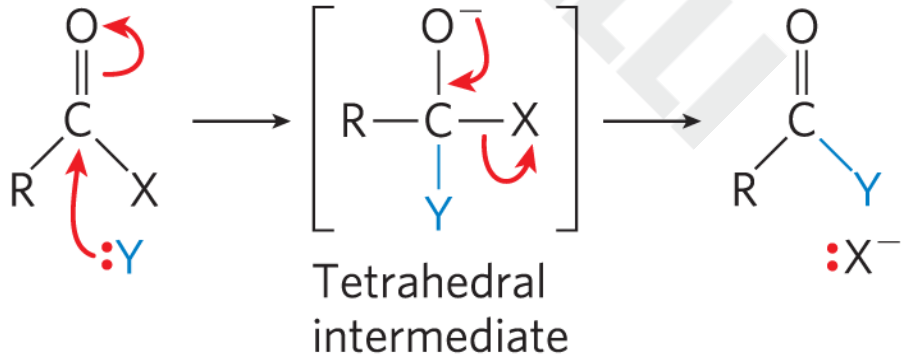
Group Transfer Reactions

- the transfer of acyl, glycosyl, and phosphoryl groups from one nucleophile to another is common

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



Glycosyl Group Transfers

- glycosyl group transfers involve nucleophilic substitution at C-1 of a sugar ring
  - substitution can proceed by an S_N1 or S_N2 pathway

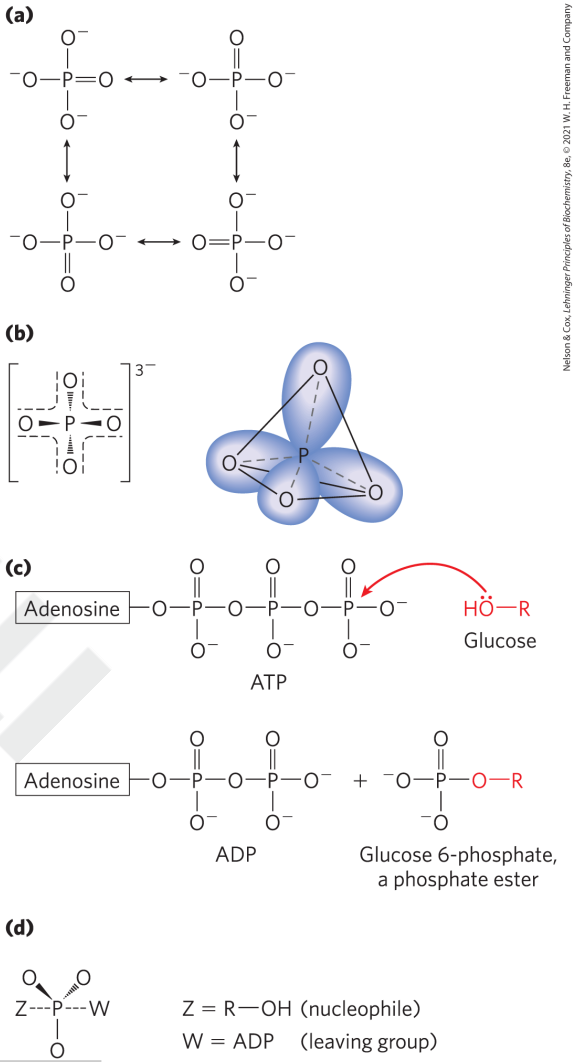
P3 Principle 3 (4 of 4)

Although thousands of different chemical reactions occur in the biosphere, most of them fall within a small set of reaction types.

Phosphoryl Group Transfers

- attachment of a good leaving group to a metabolic intermediate "activates" the intermediate for subsequent reaction
  - phosphoryl groups ($-PO_3^{2-}$) commonly serve as leaving groups

- **kinases** = enzymes that catalyze phosphoryl group transfers with ATP as a donor



Kinases, Phosphorylases, and Phosphatases

- **kinases** = transfer a phosphoryl group from ATP to an acceptor molecule
  - catalyze a phosphorylation reaction

- **phosphorylases** = catalyze a displacement reaction where phosphate attacks and becomes covalently attached at the point of bond breakage
  - catalyze a phosphorolysis reaction

- phosphatases = catalyze the removal of a phosphoryl group from a phosphate ester
  - catalyze dephosphorylation reactions

Synthases, Synthetases, Ligases, and Lyases

- **synthases** = catalyze condensation reactions in which no nucleotide triphosphate is required
- **synthetases** = catalyze condensation reactions that require a nucleotide triphosphate
- **ligases** = catalyze condensation reactions in which two atoms are joined using ATP or another energy source
- **lyases** = catalyze cleavages or additions in which electronic rearrangements occur

Oxidases and Oxygenases

- **oxidases** = catalyze biological oxidation reactions where oxygen is the electron acceptor and oxygen does not appear in the oxidized product
  - **mixed function oxidases** = oxidize two different substrates simultaneously

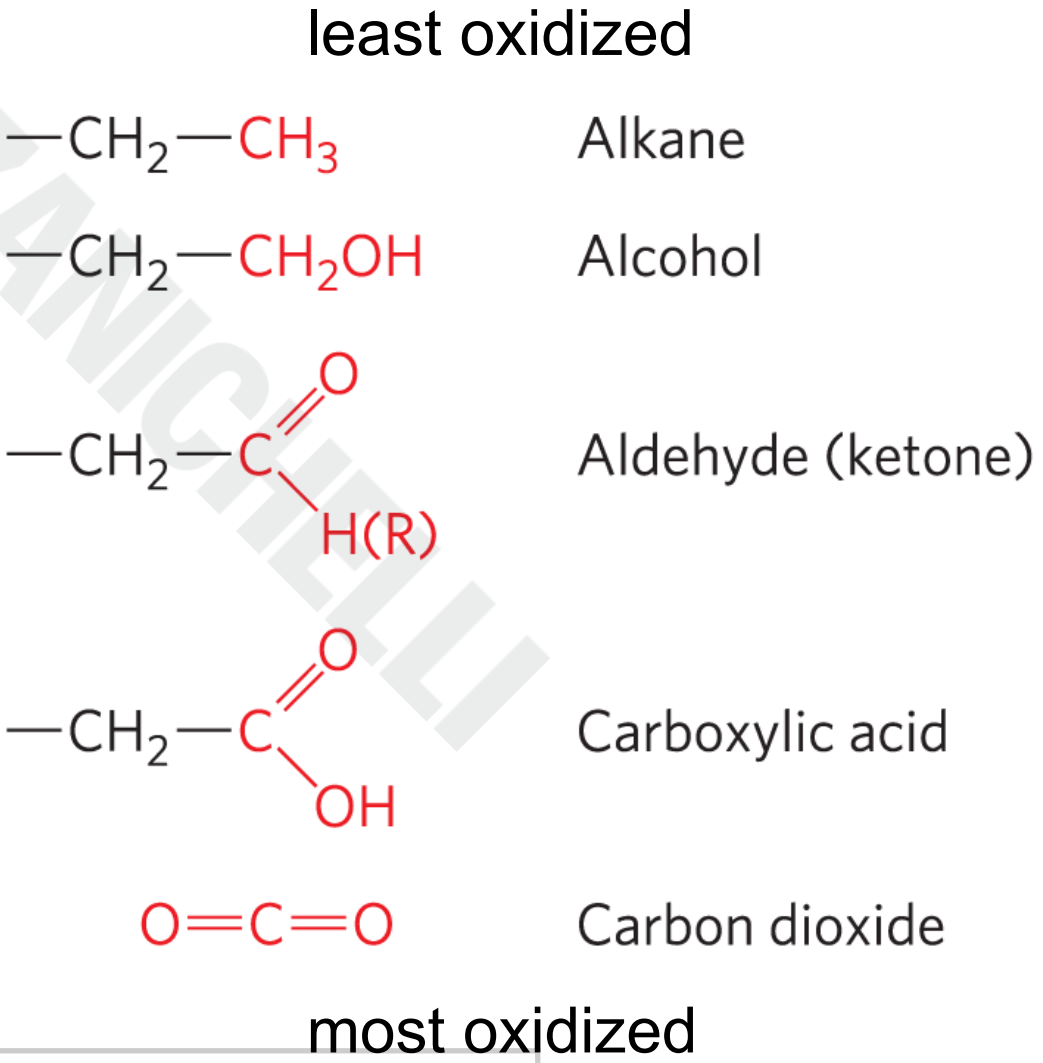
- **oxygenases** = catalyze biological oxidation reactions where oxygen is the electron acceptor and oxygen does appear in the oxidized product
  - **monooxygenases** = one oxygen atom is incorporated in the product
  - **dioxygenases** = two oxygen atoms are incorporated in the product

Dehydrogenases

- **dehydrogenases** = catalyze oxidation-reductase reactions in which NAD^+ is the electron acceptor and molecular oxygen is not involved

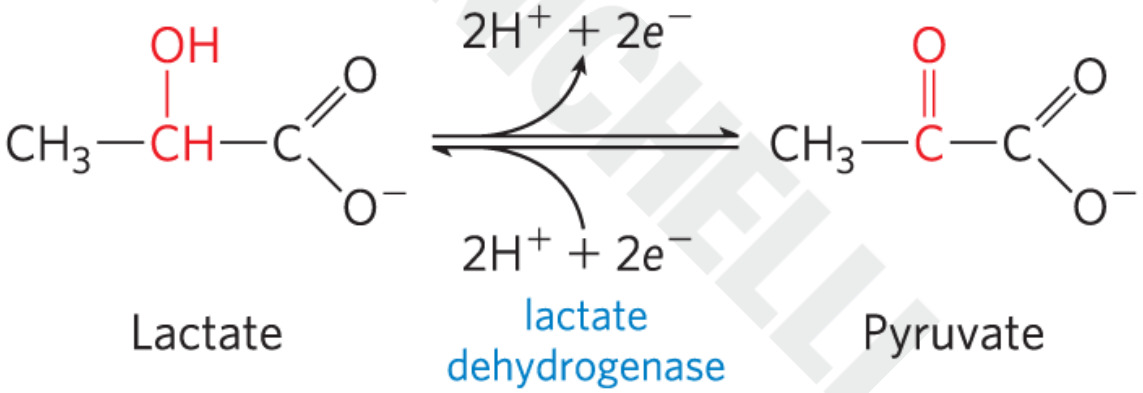
Oxidation-Reduction Reactions

- carbon atoms exist in different oxidation states, depending on the elements with which they share electrons



An Oxidation-Reduction Reaction

- in many biological oxidations, a compound loses two electrons and two hydrogen ions
  - catalyzed by dehydrogenases



- in some biological oxidations, a carbon atom becomes bonded to an oxygen
  - catalyzed by oxidases or oxygenases

P5 Principle 5 (2 of 5)

Oxidation-reduction reactions indirectly provide much of the energy needed to make ATP. Reduced substrates such as glucose are oxidized in several steps, with the energy of oxidation steps conserved in the form of a reduced cofactor, NADH. Energy stored in NADH is used to drive the synthesis of ATP.

Oxidations Are Accompanied by Reductions

- oxidation-reduction reactions involve the loss or gain of electrons
  - one reactant gains electrons and is reduced
  - the other loses electrons and is oxidized

- oxidation reactions generally release energy
  - living cells oxidize metabolic fuels (carbohydrates, fat)

Biochemical and Chemical Equations Are Not Identical

- biochemical equations:
  - do not balance for H^+ or Mg^{2+}
  - do not attempt to describe the state of phosphate ionization

- chemical equations account for all atoms and charges in a reaction

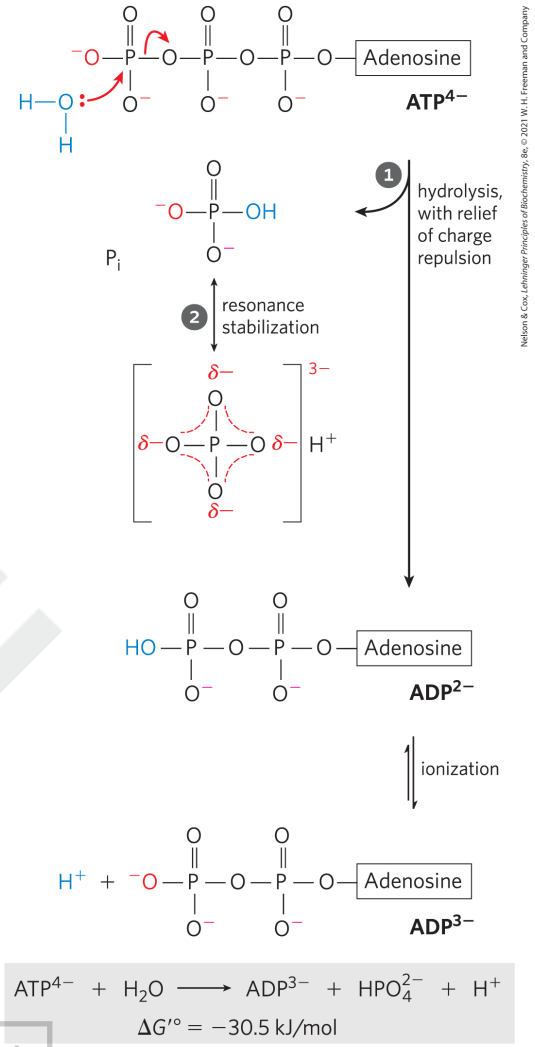
13.3 Phosphoryl Group Transfers and ATP

The Special Role of ATP

- ATP is the chemical link between catabolism and anabolism
  - energy obtained from catabolism of nutrient molecules is used to make ATP from ADP and P_i
  - the exergonic conversion of ATP to ADP and P_i (or to AMP and PP_i) is coupled to many endergonic reactions and processes

The Free-Energy Change for ATP Hydrolysis Is Large and Negative

- the hydrolytic cleavage of the terminal phosphoanhydride bond in ATP relieves some of the internal electrostatic repulsion in ATP



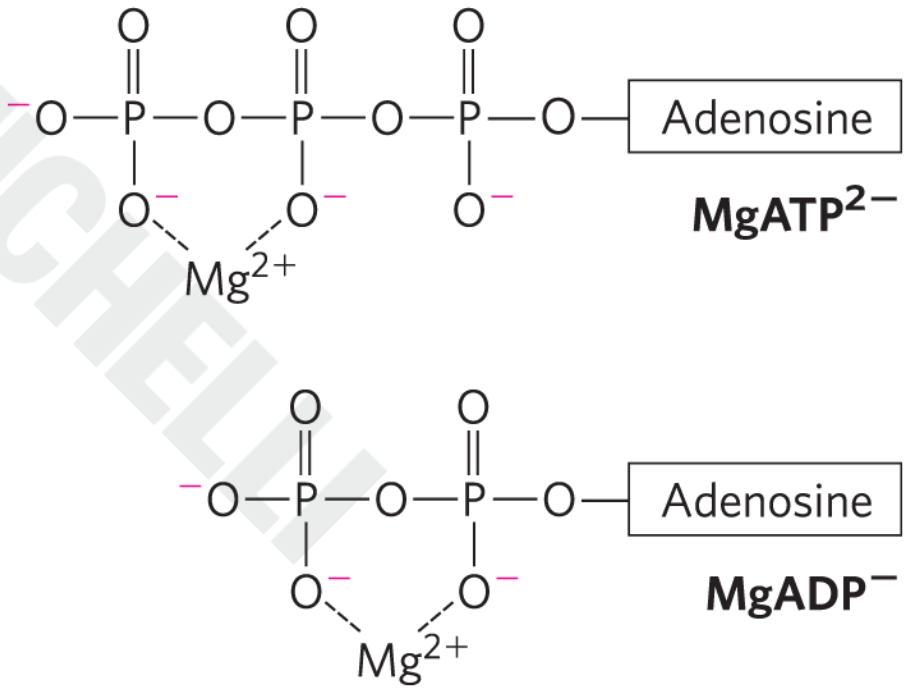
Cellular Conditions of ATP, ADP, and P_i

Table 13-5 Total Concentrations of Adenine Nucleotides, Inorganic Phosphate, and Phosphocreatine in Some Cells

Cell type	Concentration (mM): ATP	Concentration (mM): ADP	Concentration (mM): AMP	Concentration (mM): P_i	Concentration (mM): PCr
Rat hepatocyte	3.38	1.32	0.29	4.8	0
Rat myocyte	8.05	0.93	0.04	8.05	27
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	9.6	0.56	0.28	—	—

Mg^{2+} and ATP

- Mg^{2+} in the cytosol binds to ATP and ADP
- for most enzymatic reactions involving ATP, the true substrate is $MgATP^{2-}$



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The Free-Energy Change for ATP Hydrolysis Under Cellular Conditions

- **phosphorylation potential,** ΔG_p = the actual free energy of hydrolysis of ATP under intracellular conditions
  - varies from cell to cell and over time

- in vivo, the energy released by ATP hydrolysis is greater than the standard free-energy change, $\Delta G'°$

P4 Principle 4 (2 of 5)

ATP is the universal energy currency in living organisms. Transfer of its phosphoryl group to a water molecule or metabolic intermediates provides the energetic push for muscle contraction, the pumping of solutes against concentration gradients, and the synthesis of complex molecules.

Cellular ATP Levels

- due to strong selective pressure over the course of evolution, regulatory mechanisms hold cellular [ATP] far above the equilibrium concentrations for the hydrolysis reaction

- when the ATP level drops:
  - the *amount* of fuel decreases
  - the fuel itself *loses its potency*

Other Phosphorylated Compounds and Thioesters Also Have Large, Negative Free Energies of Hydrolysis

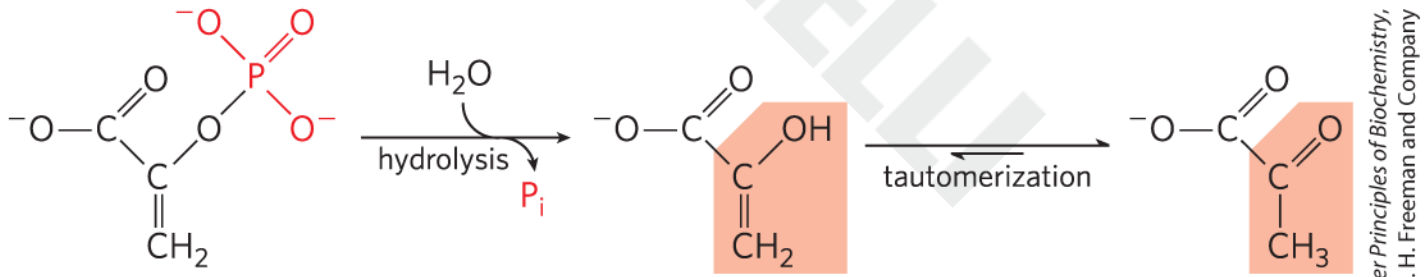
- in these phosphate-releasing reactions, the several resonance forms available to P_i stabilize this product relative to the reactant

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

Compounds and Acetyl-CoA	$\Delta G'°$: (kJ/mol)	$\Delta G'°$: (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

Hydrolysis of Phosphoenolpyruvate (PEP)

- high standard free energy of hydrolysis because:
  - the reaction occurs with a gain in entropy
  - greater resonance stabilization in the P_i released by PEP cleavage



$$PEP^{3-} + H_2O \longrightarrow pyruvate^- + HPO_4^{2-}$$
$$\Delta G'^\circ = -61.9 \text{ kJ/mol}$$

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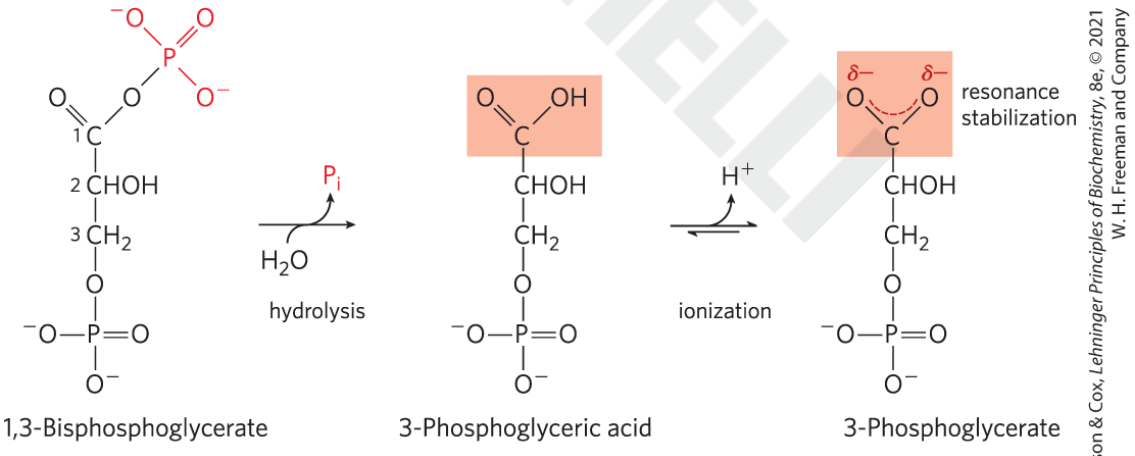
Hydrolysis of 1,3-Bisphosphoglycerate

- large standard free energy of hydrolysis because:
  - 3-phosphoglycerate has two resonance forms
  - removal of 3-phosphoglyceric acid by further metabolism and formation of the ion favor the forward reaction



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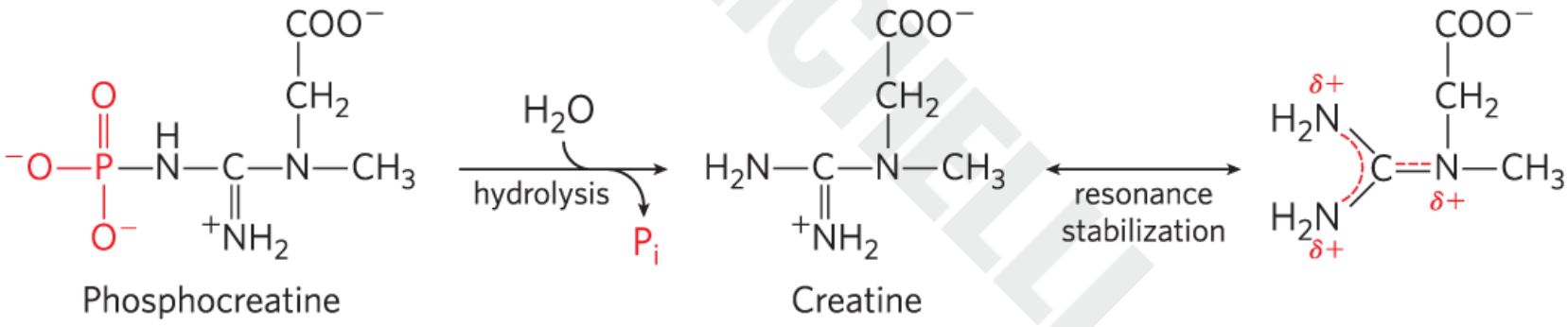
$$1,3\text{-Bisphosphoglycerate}^{4-} + H_2O \longrightarrow 3\text{-phosphoglycerate}^{3-} + HPO_4^{2-} + H^+$$
$$\Delta G'^{\circ} = -49.3\ \text{kJ/mol}$$

Hydrolysis of Phosphocreatine

- large standard free energy of hydrolysis because the release of P_i and the resonance stabilization of creatine favor the forward reaction



$$\text{Phosphocreatine}^{2-} + H_2O \longrightarrow \text{creatine} + HPO_4^{2-}$$
$$\Delta G'^{\circ} = -43.0 \text{ kJ/mol}$$

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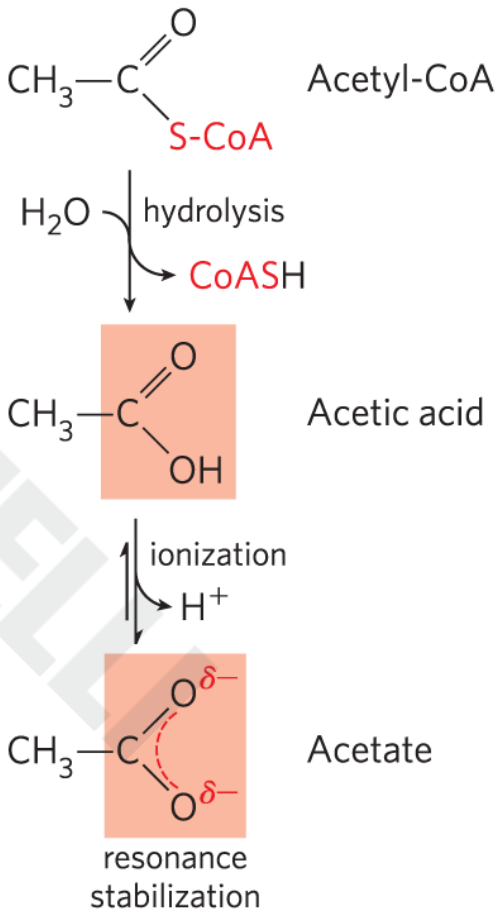
Hydrolysis of Acetyl-Coenzyme A

- **thioesters** = have a sulfur atom that has replaced the oxygen in the ester bond



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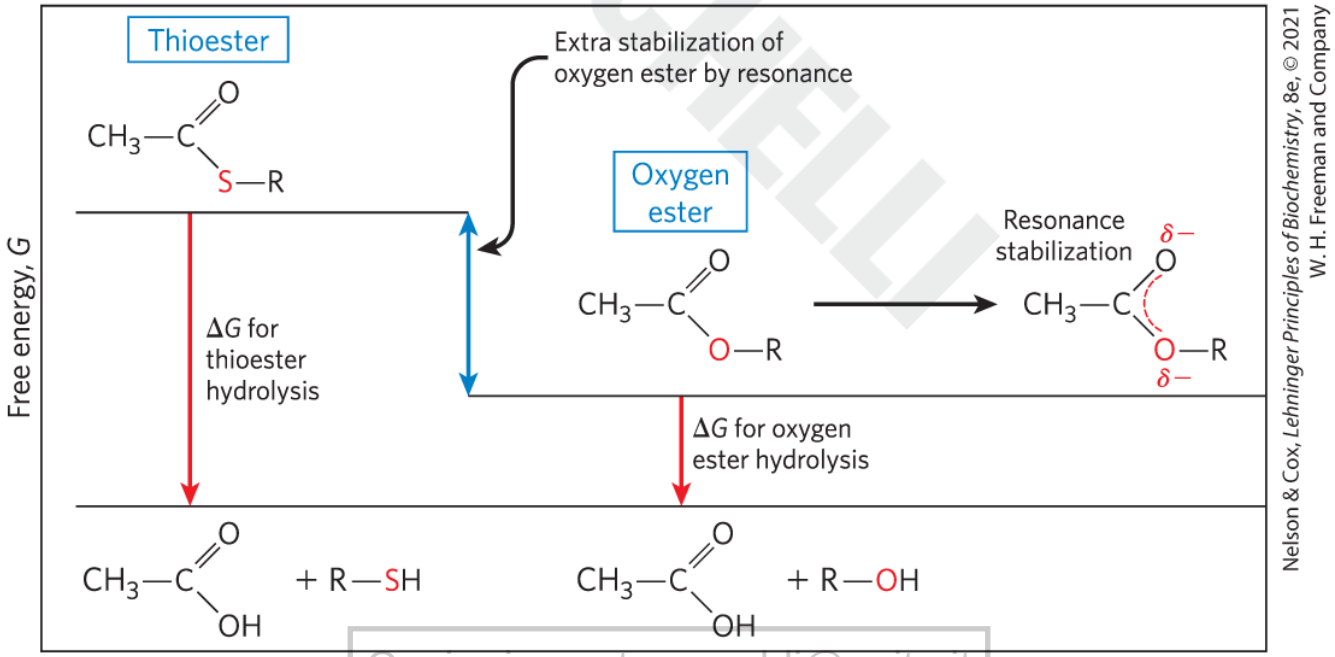
$$\text{Acetyl-CoA} + H_2O \longrightarrow \text{acetate}^- + \text{CoA-SH} + H^+$$
$$\Delta G'^\circ = -31.4\ \text{kJ/mol}$$

Free Energy of Hydrolysis for Thioesters and Oxygen Esters

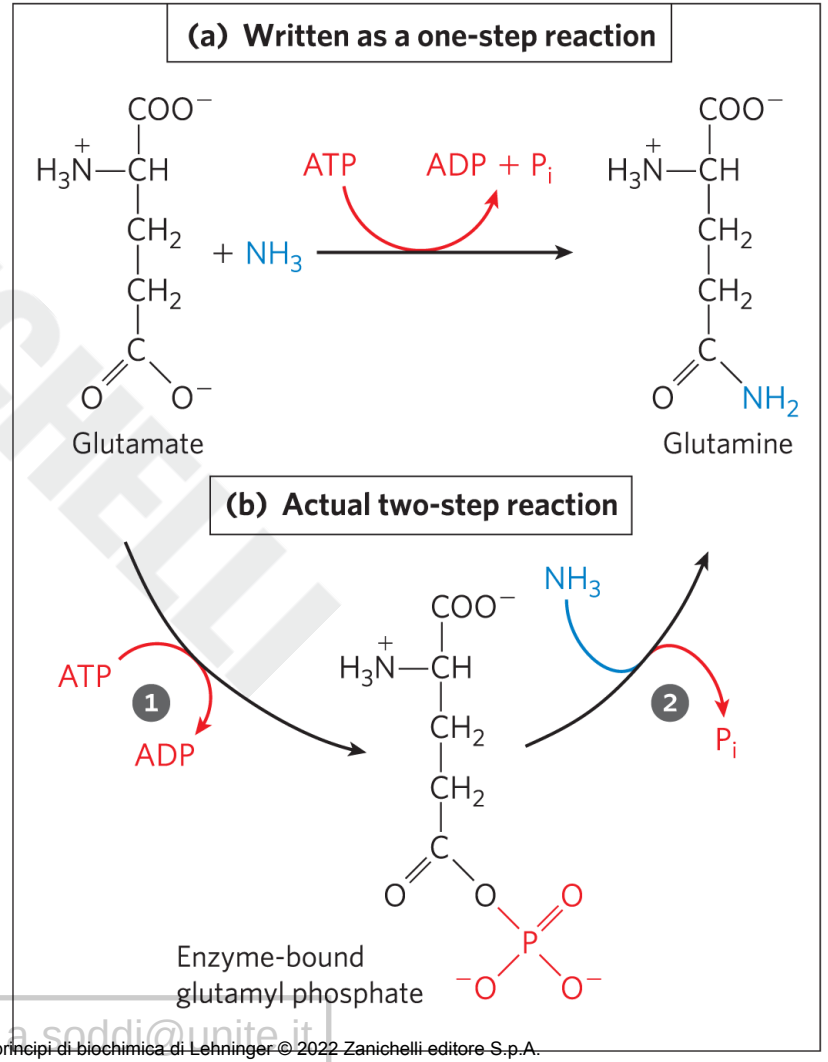
- thioesters undergo much less resonance stabilization than oxygen esters
  - difference in free energy between reactant and products is greater for thioesters



ATP Provides Energy by Group Transfers, Not by Simple Hydrolysis

- first step = transfer of part of the ATP molecule to a substrate molecule or to an amino acid residue, activating it

- second step = displacement of the phosphate-containing moiety, generating P_i, PP_i, or AMP as the leaving group



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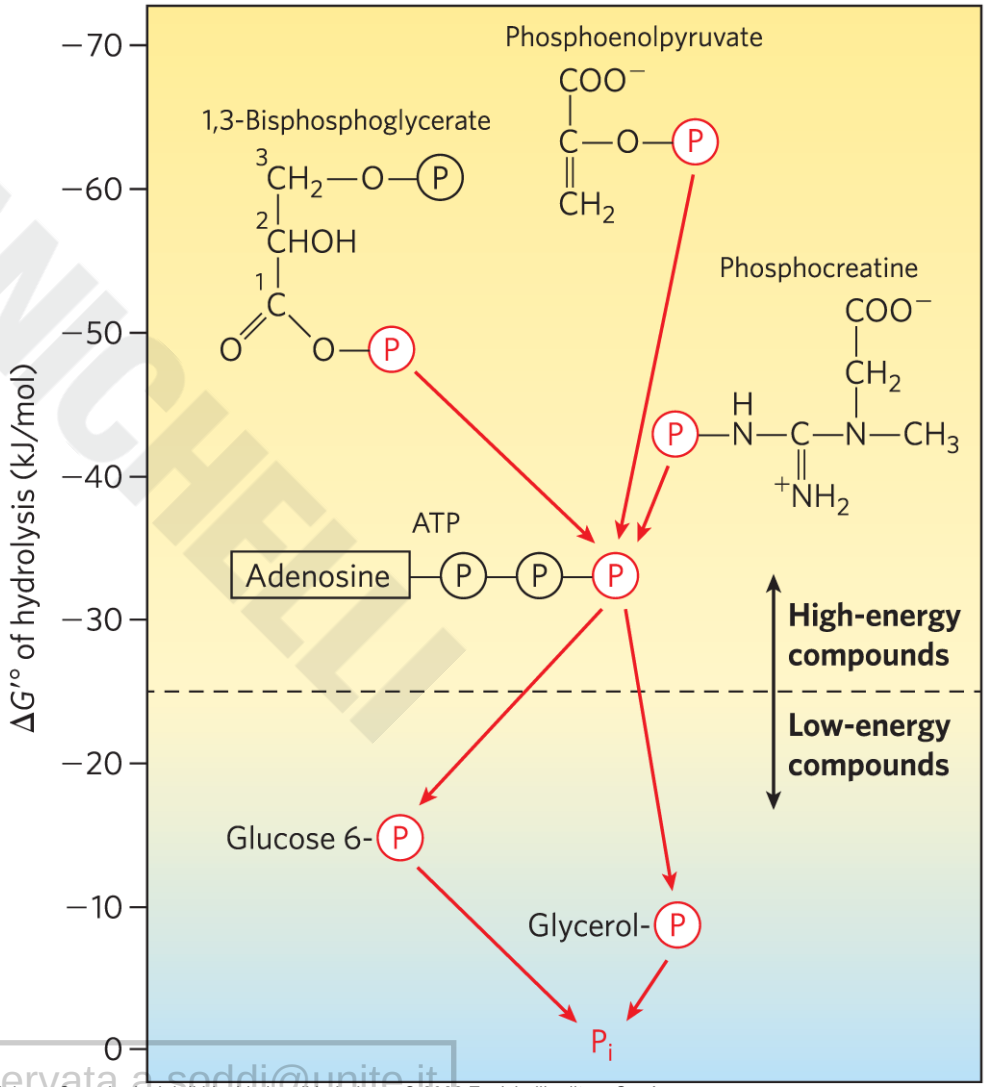
P4 Principle 4 (3 of 5)

ATP is the universal energy currency in living organisms. Transfer of its phosphoryl group to a water molecule or metabolic intermediates provides the energetic push for muscle contraction, the pumping of solutes against concentration gradients, and the synthesis of complex molecules.

Ranking of Biological Phosphate Compounds by $\Delta G'°$

- "high-energy" compounds have a $\Delta G'°$ more negative than -25 kJ/mol
- "low-energy" compounds have a less negative $\Delta G'°$



P2 Principle 2 (3 of 3)

The free-energy change is the maximum energy made available to do work when a chemical reaction occurs. If two reactions can be combined to yield a third reaction, the overall free energy change is the sum of the two. Cells accomplish energy-requiring chemical work by coupling an energy-releasing (exergonic) reaction, such as the cleavage of ATP, to an endergonic reaction (which requires energy input).

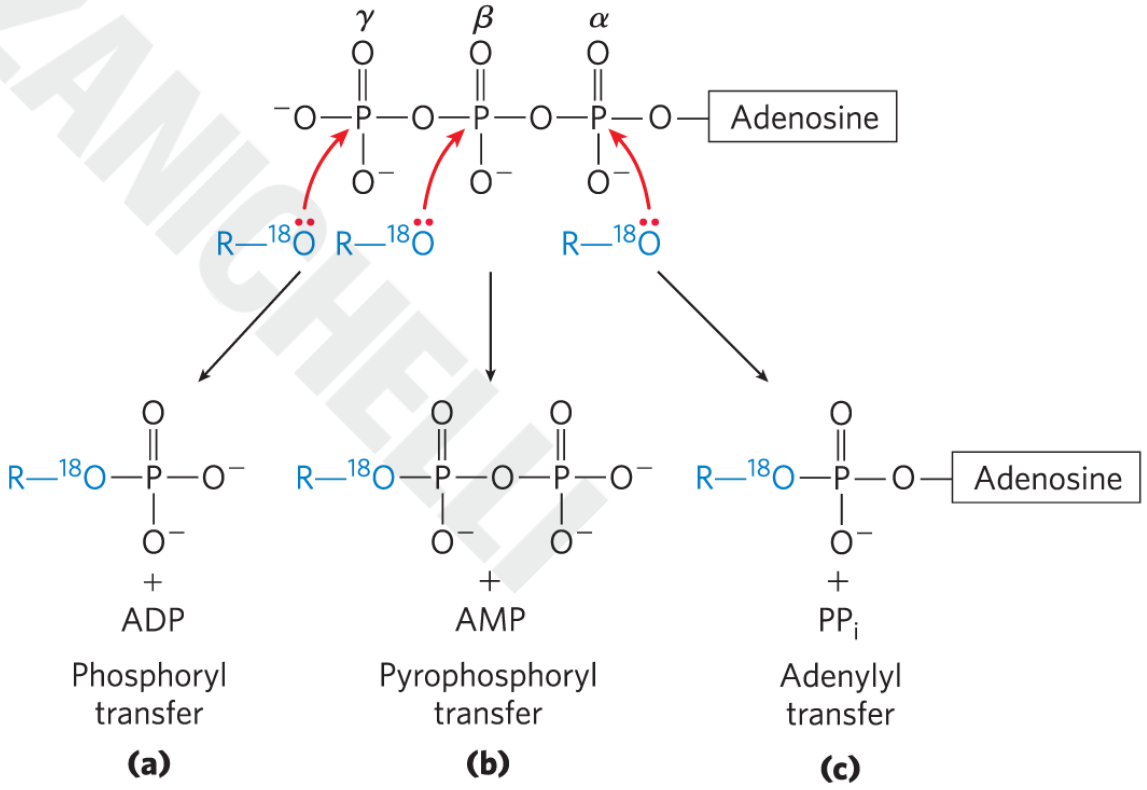
Phosphate Compounds with High $\Delta G'°$ of Hydrolysis Donate their Phosphoryl Group

- any phosphorylated compound can be synthesized by coupling the synthesis to the breakdown of another phosphorylated compound with a more negative free energy of hydrolysis

		$\Delta G'°$ (kJ/mol)
(1)	$PEP + H_2O \longrightarrow Pyruvate + P_i$	−61.9
(2)	$ADP + P_i \longrightarrow ATP + H_2O$	+30.5
Sum:	$PEP + ADP \longrightarrow Pyruvate + ATP$	−31.4

ATP Donates Phosphoryl, Pyrophosphoryl, and Adenylyl Groups

- each of the three phosphates of ATP is susceptible to nucleophilic attack



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P4 Principle 4 (4 of 5)

ATP is the universal energy currency in living organisms. Transfer of its phosphoryl group to a water molecule or metabolic intermediates provides the energetic push for muscle contraction, the pumping of solutes against concentration gradients, and the synthesis of complex molecules.

Adenylylation Reactions

- **adenylylation** = reaction that transfers adenylate (5′-AMP) as an adenyl group
  - often the method of energy coupling

- thermodynamically very favorable
  - hydrolysis of the *α*–*β* phosphoanhydride bond releases more energy than hydrolysis of the *β*–*γ* bond
  - PP_i formed as a byproduct is hydrolyzed to two P_i by **inorganic pyrophosphatase**, releasing additional energy

Assembly of Informational Macromolecules Requires Energy

- through group transfer reactions, ATP provides the energy for:
  - the synthesis of informational macromolecules (DNA, RNA, protein)
  - the transport of molecules and ions across membranes against gradients
- through hydrolysis, ATP provides the energy for muscle contraction

P4 Principle 4 (5 of 5)

ATP is the universal energy currency in living organisms. Transfer of its phosphoryl group to a water molecule or metabolic intermediates provides the energetic push for muscle contraction, the pumping of solutes against concentration gradients, and the synthesis of complex molecules.

Transphosphorylations between Nucleotides Occur in All Cell Types

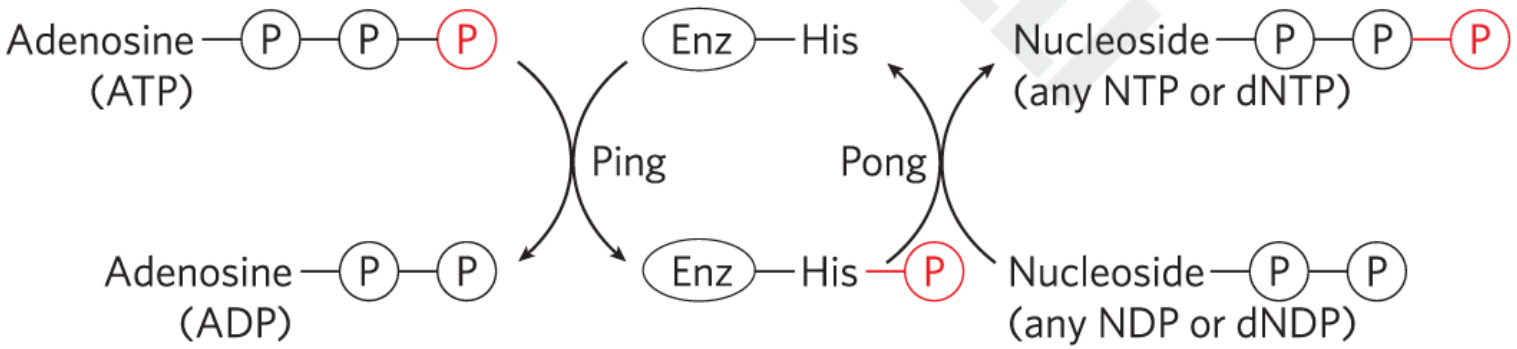
- the other nucleoside triphosphates (GTP, UTP, and CTP) and all deoxynucleoside triphosphates (dATP, dGTP, dTTP, and dCTP) are energetically equivalent to ATP

- **nucleoside diphosphate kinase** = carries phosphoryl groups from ATP to other nucleotides
  - high [ATP]/[ADP] ratio normally drives the reaction forward

Ping-Pong Mechanism of Nucleoside Diphosphate Kinase

- first step = phosphoryl group transfer from ATP to an active-site His residue produces a phosphoenzyme intermediate

- second step = transfer of the phosphoryl group is from the His residue to an NDP acceptor



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Adenylate Kinase and Creatine Kinase

- **adenylate kinase** = lowers the ADP concentration and replenishes ATP during periods of intense demand for ATP
  - guanylate kinase is a similar enzyme

- **creatine kinase** = uses the phosphocreatine reservoir to replenish ATP at a rapid rate
  - lower phyla organisms employ **phosphagens** (phosphocreatine-like molecules) as phosphoryl reservoirs

13.4 Biological Oxidation-Reduction Reactions

P5 Principle 5 (3 of 5)

Oxidation-reduction reactions indirectly provide much of the energy needed to make ATP. Reduced substrates such as glucose are oxidized in several steps, with the energy of oxidation steps conserved in the form of a reduced cofactor, NADH. Energy stored in NADH is used to drive the synthesis of ATP.

The Flow of Electrons in Oxidation-Reduction Reactions

- electron flow in oxidation-reduction reactions is responsible for all work done by living organisms

- electrons move from metabolic intermediates to electron carriers to acceptors with higher electron affinities
  - release energy

P5 Principle 5 (4 of 5)

Oxidation-reduction reactions indirectly provide much of the energy needed to make ATP. Reduced substrates such as glucose are oxidized in several steps, with the energy of oxidation steps conserved in the form of a reduced cofactor, NADH. Energy stored in NADH is used to drive the synthesis of ATP.

The Flow of Electrons Can Do Biological Work

- **electromotive force (emf)** = force proportional to the difference in electron affinity between chemical species
  - drives electron flow spontaneously through a circuit

- biological "circuit" = electrons from glucose flow through a series of electron-carrier intermediates to another chemical species, such as O_2

Oxidation-Reductions Can Be Described as Half-Reactions

- the oxidation of ferrous ion by cupric ion,

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

can be describe in terms of two half-reactions:

(1) $Fe^{2+} \rightleftarrows Fe^{3+} + e^-$

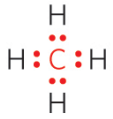
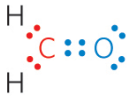
(2) $Cu^{2+} + e^- \rightleftarrows Cu^+$

Conjugate Redox Pairs

- reducing agent or reductant = electron-donating molecule
- oxidizing agent or oxidant = electron-accepting molecule
- **conjugate redox pair** = consists of an electron donor and an electron acceptor

Biological Oxidations Often Involve Dehydrogenation

- the more electro-negative atom “owns” the bonding electrons it shares with another atom

Methane		8
Ethane (alkane)		7
Ethene (alkene)		6
Ethanol (alcohol)		5
Acetylene (alkyne)		5
Formaldehyde		4

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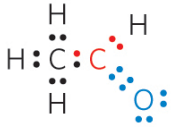
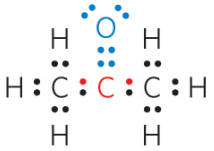
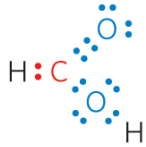
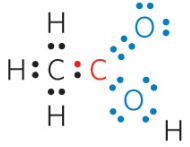
Acetaldehyde (aldehyde)		3
Acetone (ketone)		2
Formic acid (carboxylic acid)		2
Carbon monoxide		2
Acetic acid (carboxylic acid)		1
Carbon dioxide		0

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Oxidation Is Often Synonymous With Dehydrogenation

- **dehydrogenation** = reaction where a compound loses two electrons and two hydrogen ions
  - catalyzed by **dehydrogenases**

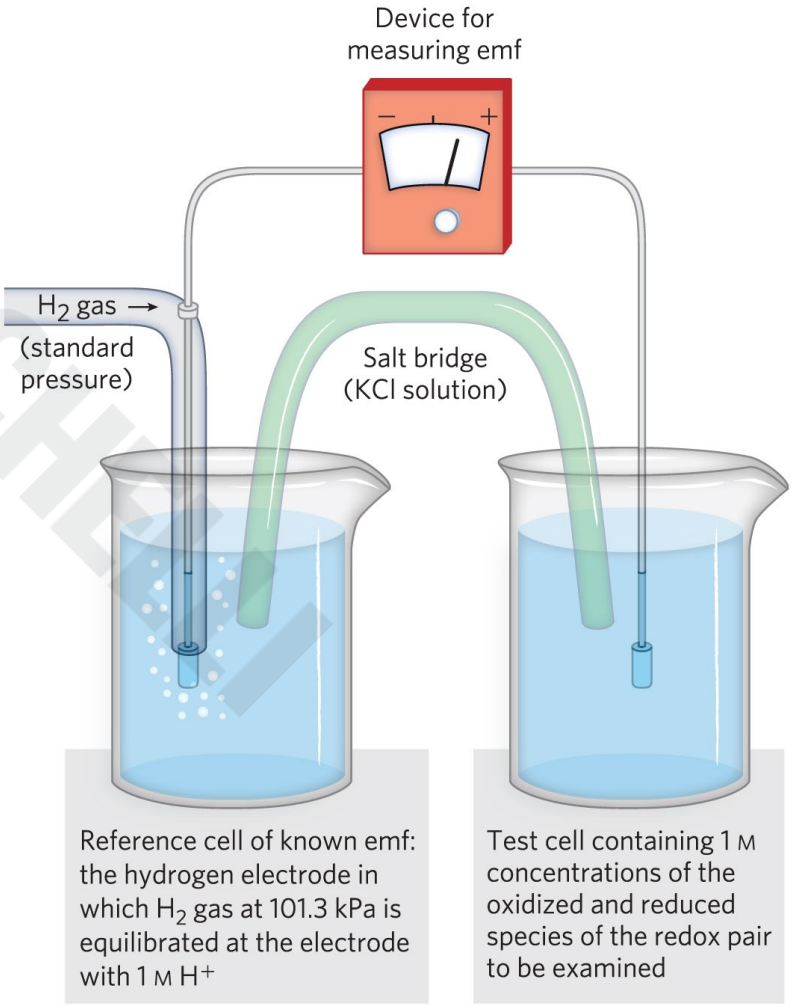
- electrons are transferred from the electron donor to the electron acceptor:
  - directly as electrons
  - as hydrogen atoms
  - as a hydride ion ($:H^-$)
  - through direct combination with oxygen

Reducing Equivalent

- **reducing equivalent** = designates a single electron equivalent participating in an oxidation-reduction reaction regardless of the transfer method

Reduction Potentials Measure Affinity for Electrons

- **standard reduction potential, *E*°** = a measure (in volts) of the relative affinity of the electron acceptor of each redox pair for electrons
  - positive value = takes electrons
  - negative value = donates electrons



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Electrons Tend to Flow From Low to High Reduction Potential

- standard of reference is the half-reaction $H^+ + e^- \longrightarrow \frac{1}{2} H_2$
- when any two half-cells are connected, the half-cell with the more positive $E°$ is reduced
  - this half-cell has the greater reduction potential

The Nernst Equation

- the Nernst equation relates standard reduction potential to actual reduction potential:

$$E = E^\circ + \frac{RT}{nF} + \frac{[\text{electron acceptor}]}{[\text{electron donor}]} \qquad (13\text{-}5)$$

where R and T have their usual meanings, n is the number of electrons transferred per molecule, and F is the Faraday constant

The Simplified Nernst Equation

- at 298 K (25 °C), the Nernst equation reduces to:

$$E = E^\circ + \frac{0.026\ \text{V}}{n} + \frac{[\text{electron acceptor}]}{[\text{electron donor}]} \qquad (13\text{-}6)$$

Standard Reduction Potentials

- standard reduction potentials are valid only for systems at neutral pH
- by convention, $\Delta E'°$ for any redox reaction is given as $E'°$ of the electron acceptor minus $E'°$ of the electron donor

Half-reaction	E'° (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.816
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.771
$NO^-_3 + 2H^+ + 2e^- \rightarrow NO^-_2 + H_2O$	0.421
Cytochrome *f* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *f* (Fe^{2+})	0.365
$Fe(CN)^{3-}_6$ (ferricyanide) + $e^- \rightarrow Fe(CN)^{4-}_6$	0.36
Cytochrome a_3 (Fe^{3+}) + $e^- \rightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	0.295
Cytochrome *a* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome *c* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + $e^- \rightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *b* (Fe^{3+}) + $e^- \rightarrow$ cytochrome *b* (Fe^{2+})	0.077
Ubiquinone + $2H^+ + 2e^- \rightarrow$ ubiquinol	0.045
Fumarate^{2-} + $2H^+ + 2e^- \rightarrow$ succinate^{2-}	0.031
$2H^+ + 2e^- \rightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2H^+ + 2e^- \rightarrow$ butyryl-CoA	−0.015
Oxaloacetate^{2-} + $2H^+ + 2e^- \rightarrow$ lactate$^-$	−0.166
Pyruvate$^-$ + $2H^+ + 2e^- \rightarrow$ lactate$^-$	−0.185
Acetaldehyde + $2H^+ + 2e^- \rightarrow$ ethanol	−0.197
FAD + $2H^+ + 2e^- \rightarrow FADH_2$	−0.219
Glutathione + $2H^+ + 2e^- \rightarrow$ 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \rightarrow H_2S$	−0.243
Lipoic acid + $2H^+ + 2e^- \rightarrow$ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \rightarrow$ NADH	−0.320
$NADP^+ + H^+ + 2e^- \rightarrow$ NADPH	−0.324
Acetoacetate + $2H^+ + 2e^- \rightarrow$ β-hydroxybutyrate	−0.346
α-Ketoglutarate + $CO_2 + 2H^+ + 2e^- \rightarrow$ isocitrate	−0.38
$2H^+ + 2e^- \rightarrow H_2$ (at pH 7)	−0.414
Ferredoxin (Fe^{3+}) + $e^- \rightarrow$ ferredoxin (Fe^{2+})	−0.432

Table 13-7 Standard Reduction Potentials of Some Biologically Important Half-Reactions

P5 Principle 5 (5 of 5)

Oxidation-reduction reactions indirectly provide much of the energy needed to make ATP. Reduced substrates such as glucose are oxidized in several steps, with the energy of oxidation steps conserved in the form of a reduced cofactor, NADH. Energy stored in NADH is used to drive the synthesis of ATP.

Standard Reduction Potentials Can Be Used to Calculate Free-Energy Change

- electrons tend to flow to the half-cell with the more positive E (higher reduction potential)

- the strength of this tendency is:
  - proportional to ΔE
  - a function of the concentrations of oxidized and reduced species

$$\Delta G = -nF\Delta E \quad \text{or} \quad \Delta G'^\circ = -nF\Delta E'^\circ \qquad (13\text{-}7)$$

where n is the number of electrons transferred

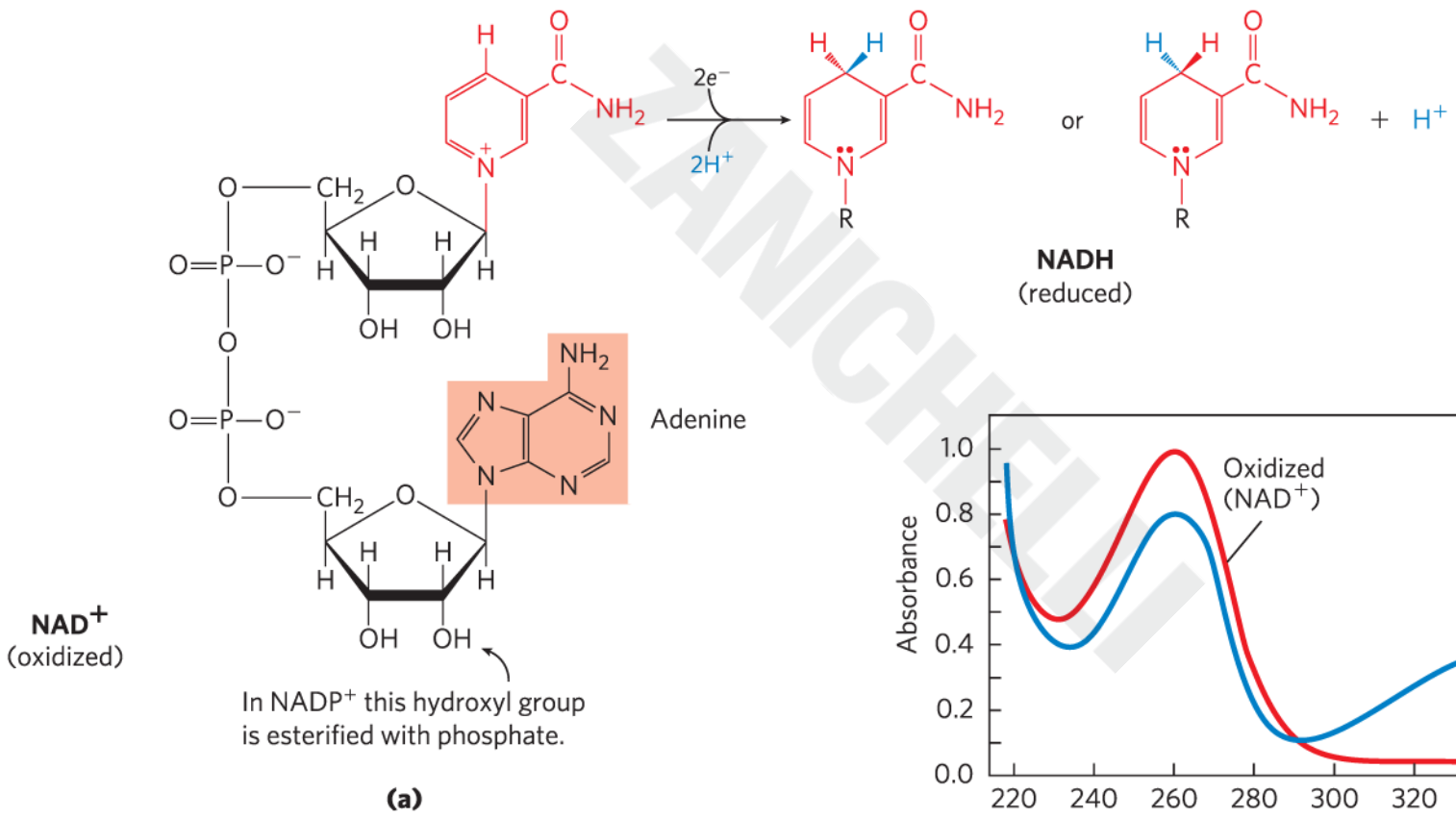
A Few Types of Coenzymes and Proteins Serve as Universal Electron Carriers

- NAD, NADP, FMN, and FAD are water-soluble coenzymes that undergo reversible oxidation and reduction in many of the electron-transfer reactions
  - NAD and NADP are freely diffusible
  - FMN and FAD are usually enzyme-bound

The Pyridine Nucleotides

- **pyridine nucleotides** = compounds with a nicotinamide ring that resembles pyridine
  - nicotinamide adenine dinucleotide (NAD; NAD^+ when oxidized)
  - nicotinamide adenine dinucleotide phosphate (NADP; $NADP^+$ when oxidized)

NAD and NADP Undergo Reversible Reduction of the Nicotinamide Ring



Half-Reactions for Nucleotide Cofactors

- both NAD+ and NADP+ accept two electrons and one proton

$$NAD^+ + 2e^- + 2H^+ \longrightarrow NADH + H^+$$

$$NADP^+ + 2e^- + 2H^+ \longrightarrow NADPH + H^+$$

General Reactions for Nucleotide Cofactors

- accepting a hydride ion from a reduced substrate:

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

$$AH_2 + NADP^+ \longrightarrow A + NADPH + H^+$$

- donating a hydride ion to an oxidized substrate:

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

$$A + NADPH + H^+ \longrightarrow AH_2 + NADP^+$$

where AH_2 is the reduced substrate and A is the oxidized substrate

Oxidoreductase (Dehydrogenases)

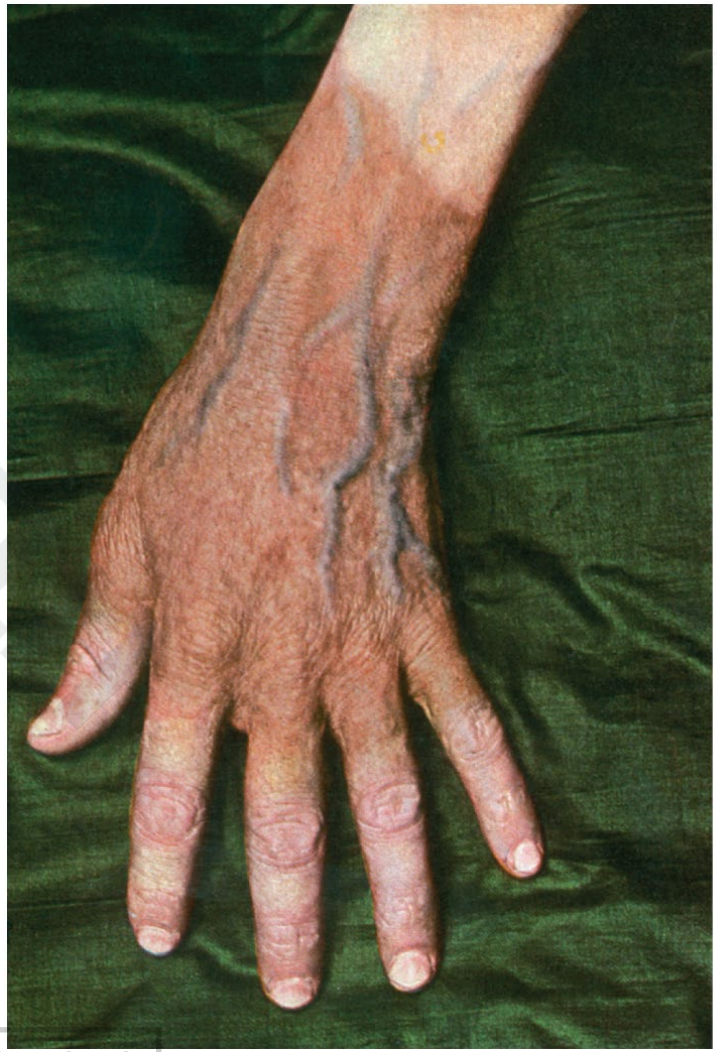
- **oxidoreductases** = also called dehydrogenases = catalyze oxidation-reductase reactions using NAD^+ (or $NADP^+$) as coenzymes

NAD Has Important Functions in Addition to Electron Transfer

- NAD^+ is:
  - the source of the activating AMP group in the bacterial DNA ligase reaction
  - the source of ADP-ribose in the cholera toxin reaction
  - hydrolyzed in the deacetylation of the ε-amino group of an acetylated Lys residue by sirtuin proteins
    - regulates inflammation, apoptosis, aging, and DNA transcription (through histones)

Insufficient Niacin in the Diet Causes Pellagra

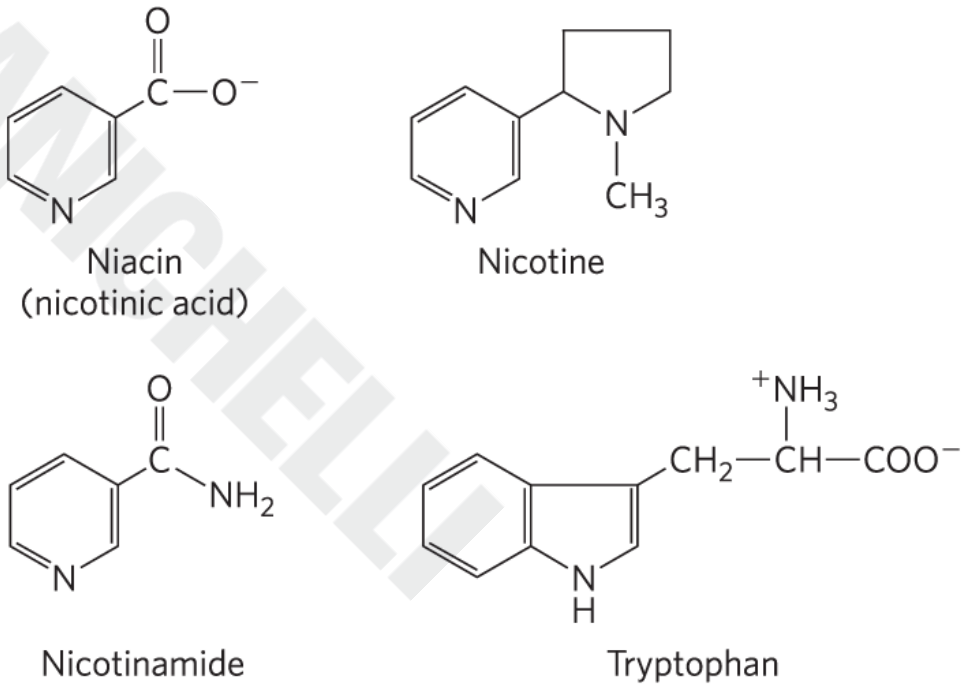
- dietary deficiency of niacin (the vitamin form of NAD and NADP) prevents NAD synthesis and causes pellagra
  - characterized by the “three Ds”: dermatitis, diarrhea, and dementia
- black tongue = related disease in dogs



Double Vision/Science Source

Niacin (Nicotinic Acid) and Its Derivative Nicotin-Amide

- tryptophan = the biosynthetic precursor
- nicotinic acid and nicotinamide cure pellagra
- nicotine (from cigarettes) has no curative activity



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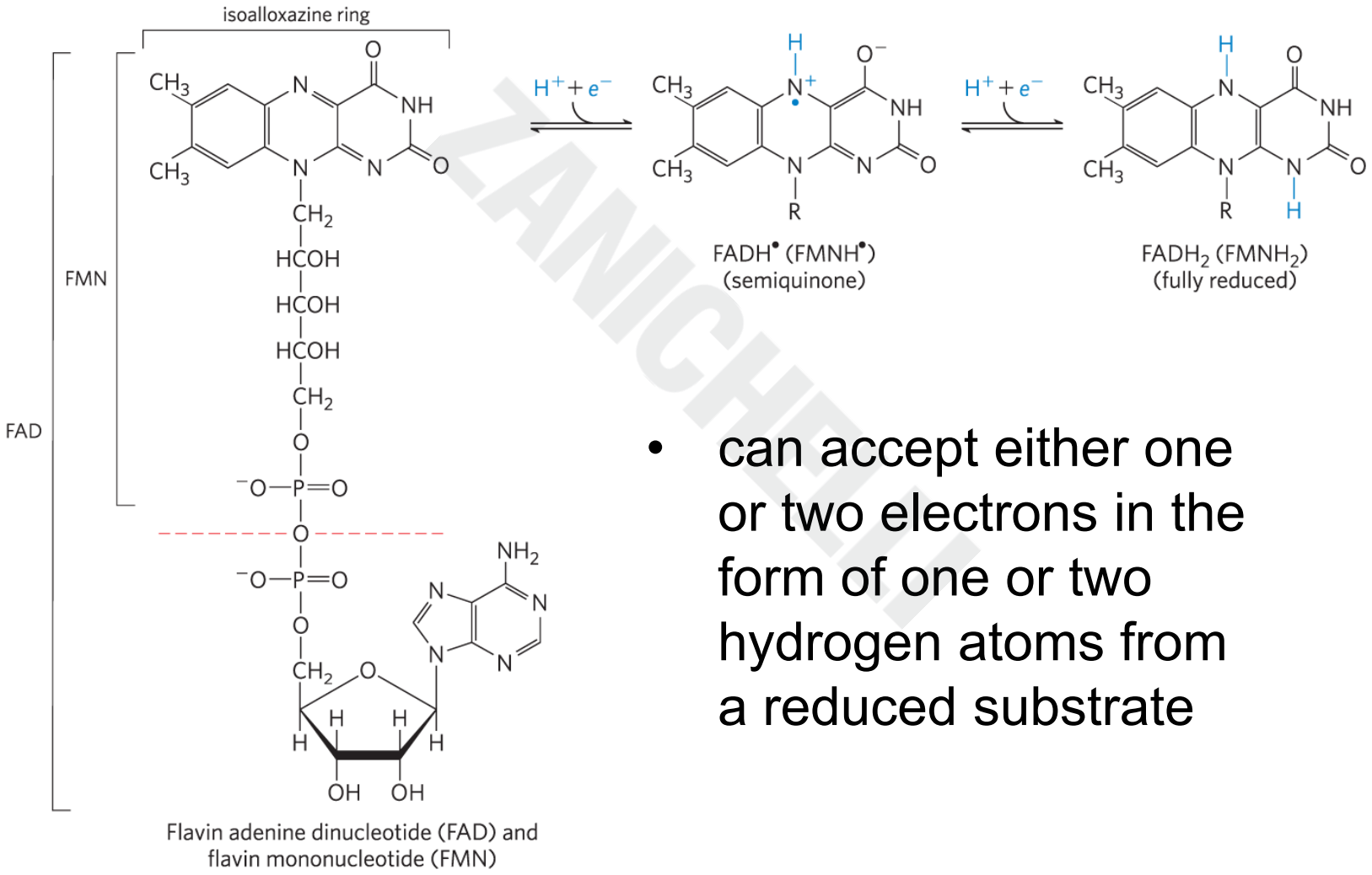
Flavin Nucleotides Are Tightly Bound in Flavoproteins

- **flavoproteins** = enzymes that catalyze oxidation-reduction reactions using either flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as coenzyme

- **flavin nucleotides** = enzymes derived from the vitamin riboflavin
  - reduction potentials depend on the flavoprotein with which they are associated

Oxidized and Reduced FAD and FMN



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

- can accept either one or two electrons in the form of one or two hydrogen atoms from a reduced substrate

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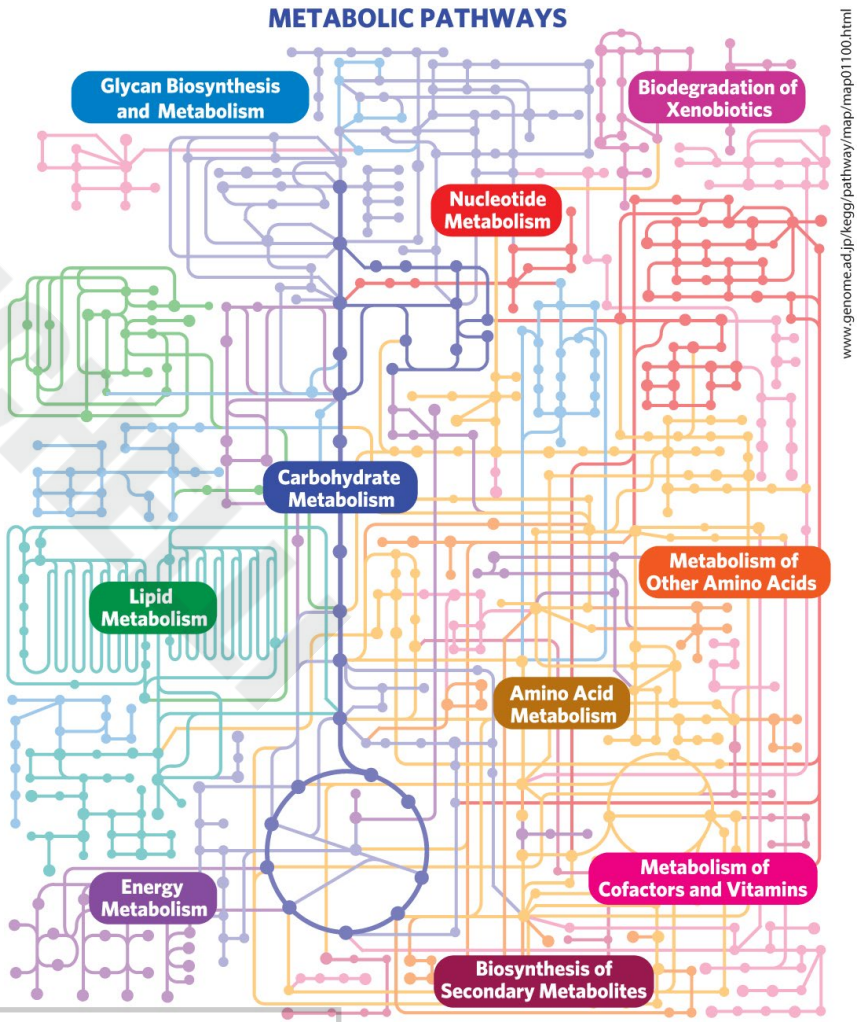
13.5 Regulation of Metabolic Pathways

Metabolism as a Three-Dimensional Meshwork

- pathways are inextricably intertwined with all the other cellular pathways in a multidimensional network of reactions



P6 Principle 6 (2 of 5)

To respond to changes in external circumstances, cells must regulate enzyme activities by changing either the number of enzyme molecules or the catalytic activity of preexisting enzyme molecules.

Cells and Organisms Maintain a Dynamic Steady State

- in a metabolically active cell in a steady state, intermediates are formed and consumed at equal rates:

$$A \xrightarrow{v_1} S \xrightarrow{v_2} P$$

when $v_1 = v_2$, [S] is constant (**homeostasis**)

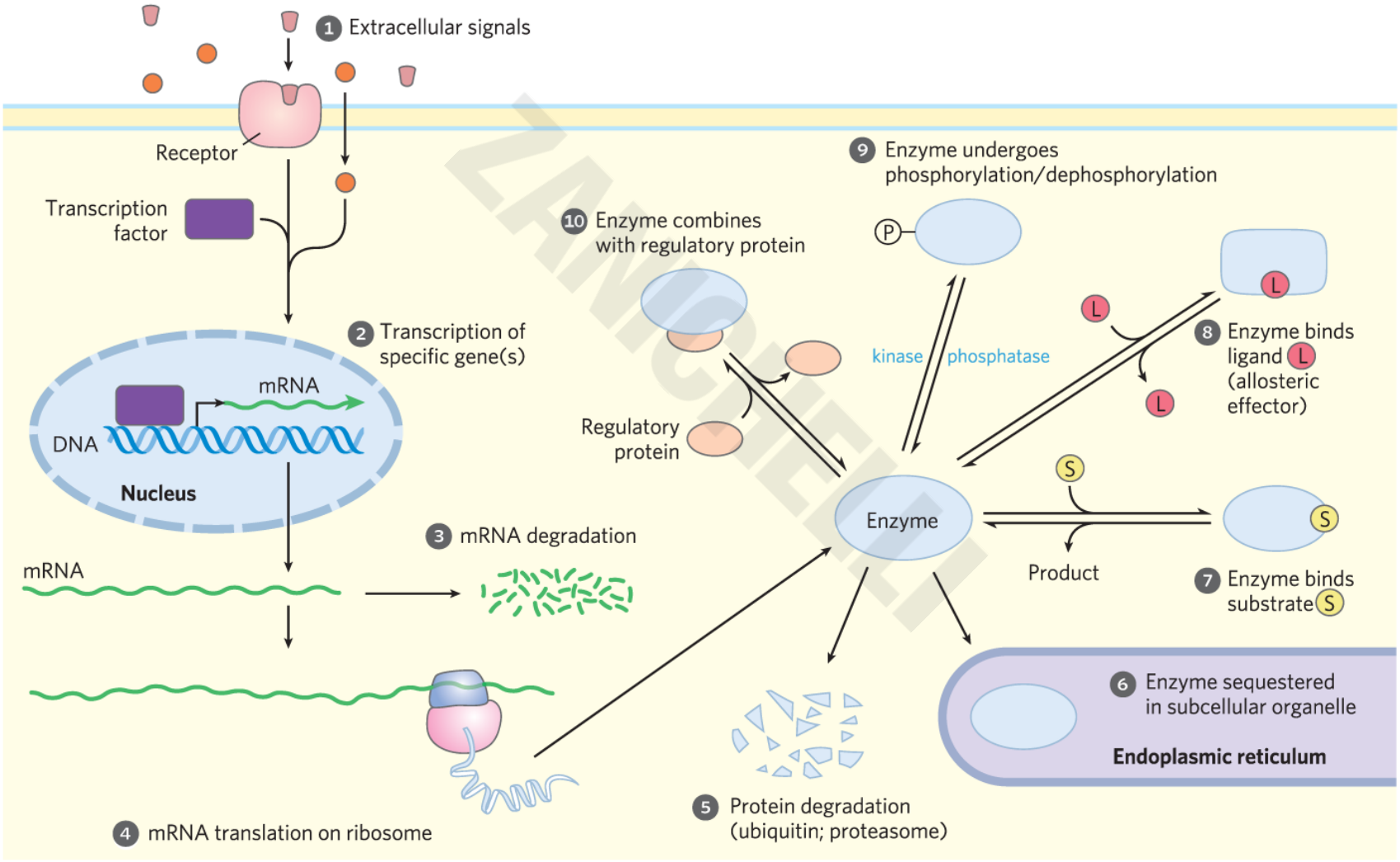
- **flux** = rate (v) of metabolite flow

Both the Amount and the Catalytic Activity of an Enzyme Can Be Regulated

- to return a system to the steady state, flux can be modulated by changes in the:
  - number of enzyme molecules
  - catalytic activity of each present enzyme molecule

- time scale = less than a millisecond to days

Factors Affecting Enzyme Activity



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Extracellular Signals

- extracellular signals may be:
  - hormonal (insulin, epinephrine)
  - neuronal (acetylcholine)
  - growth factors
  - cytokines

Transcription Factors

- **transcription factors** = nuclear proteins that, when activated, bind specific DNA regions (**response elements)** near a gene's promoter
  - can activate or repress gene transcription

mRNA Degradation and Translation

- stability of mRNA varies
  - depends on their resistance to ribonuclease degradation

- rate of mRNA translation is regulated

P6 Principle 6 (3 of 5)

To respond to changes in external circumstances, cells must regulate enzyme activities by changing either the number of enzyme molecules or the catalytic activity of preexisting enzyme molecules.

Protein Degradation

- **turnover** = synthesis followed by degradation

- rapid turnover is energetically expensive, but proteins with a short half-life reach new steady-state levels faster than those with a long half-life

Table 13-8 Average Half-Life of Proteins in Mammalian Tissues	
Tissue	**Average half-life (days)**
Liver	0.9
Kidney	1.7
Heart	4.1
Brain	4.6
Muscle	10.7

Enzyme Sequestration

- sequestering enzyme and its substrate in different compartments alters the *effective* activity of an enzyme

Changes in the Transcriptome Affect the Proteome and the Metabolome

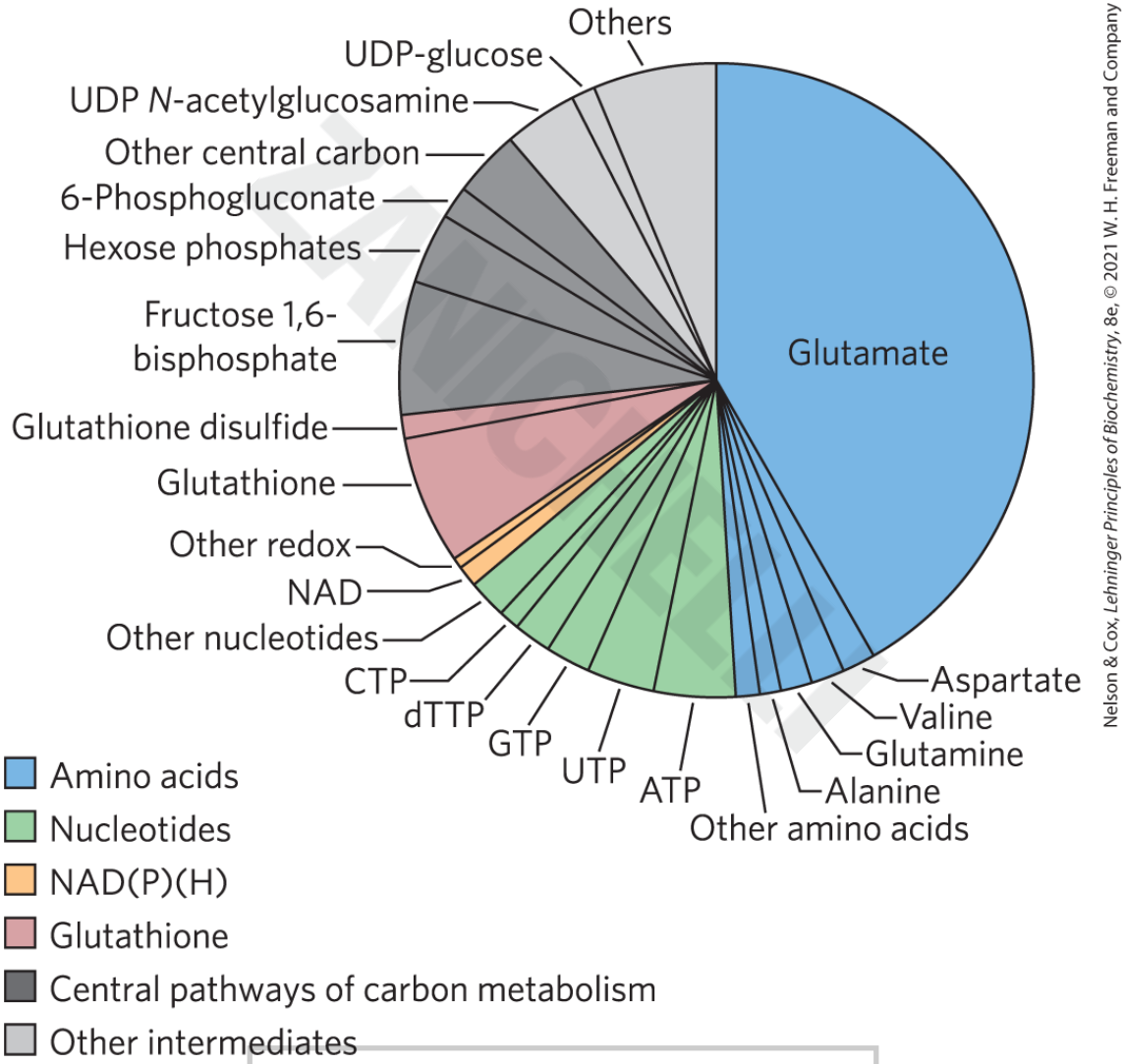
- changes in the transcriptome lead to changes in the proteome and, ultimately, in the metabolome of a cell or tissue

- **transcriptome** = entire complement of mRNAs

- **proteome** = entire complement of protein

- **metabolome** = entire complement of low molecular weight metabolites

The Metabolome of *E. coli* Growing on Glucose



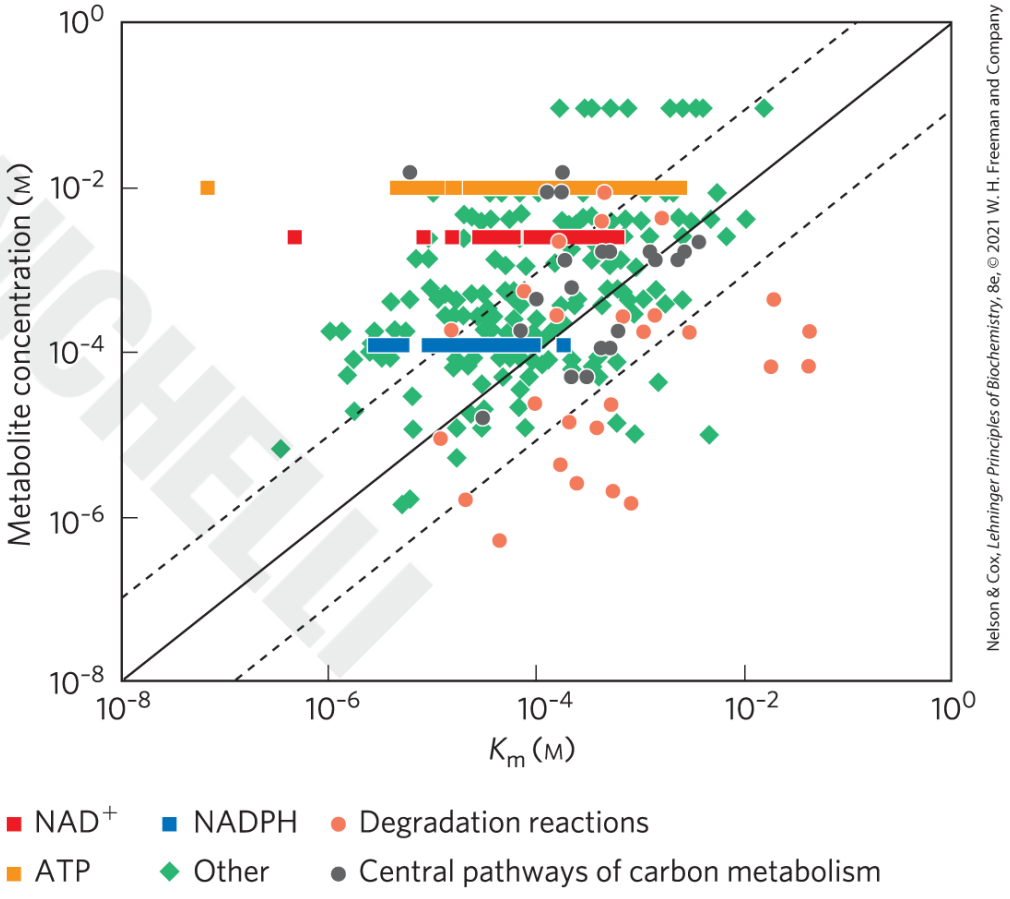
Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2021 W. H. Freeman and Company

Regulating the Catalytic Activity of Individual Enzyme Molecules

- can be regulated by:
  - the concentration of substrate
  - the presence of allosteric effectors
  - covalent modifications
  - binding of regulatory proteins

Enzymes Are Sensitive to Substrate Concentration

- for enzymes that follow Michaelis-Menten kinetics:
  - the initial rate of the reaction is half-max when [S] = K_m
  - activity drops off at lower [S]
  - when [S] << K_m, the reaction rate is linearly dependent on [S]



Allosteric Effectors Can Increase or Decrease Enzyme Activity

- typically convert hyperbolic kinetics to sigmoid kinetics

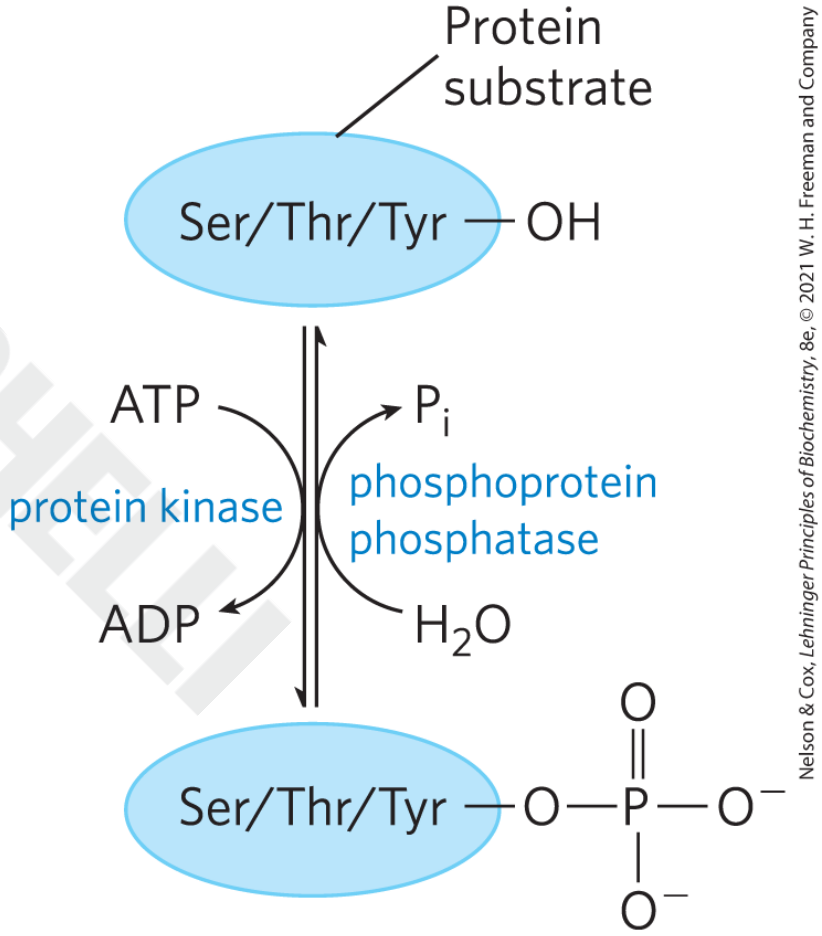
- cooperativity of an allosteric protein can be expressed as a Hill coefficient, with higher coefficients meaning greater cooperativity

Table 13-9 Relationship between Hill Coefficient and the Effect of Substrate Concentration on Reaction Rate for Allosteric Enzymes

Hill coefficient (n_H)	Required change in [S] to increase V_0 from 10% to 90% V_{max}
0.5	× 6,600
1.0	× 81
2.0	× 9
3.0	× 4.3
4.0	× 3

Covalent Modifications and Binding of Regulatory Proteins

- covalent modifications:
  - occur within seconds or minutes of a regulatory signal
  - phosphorylation and dephosphorylation are most common
- association with and dissociation from regulatory proteins regulates enzymes



P6 Principle 6 (4 of 5)

To respond to changes in external circumstances, cells must regulate enzyme activities by changing either the number of enzyme molecules or the catalytic activity of preexisting enzyme molecules.

The Mechanisms for Altering Flux Are Not Mutually Exclusive

- enzymes are commonly regulated at the level of transcription and by both allosteric and covalent mechanisms

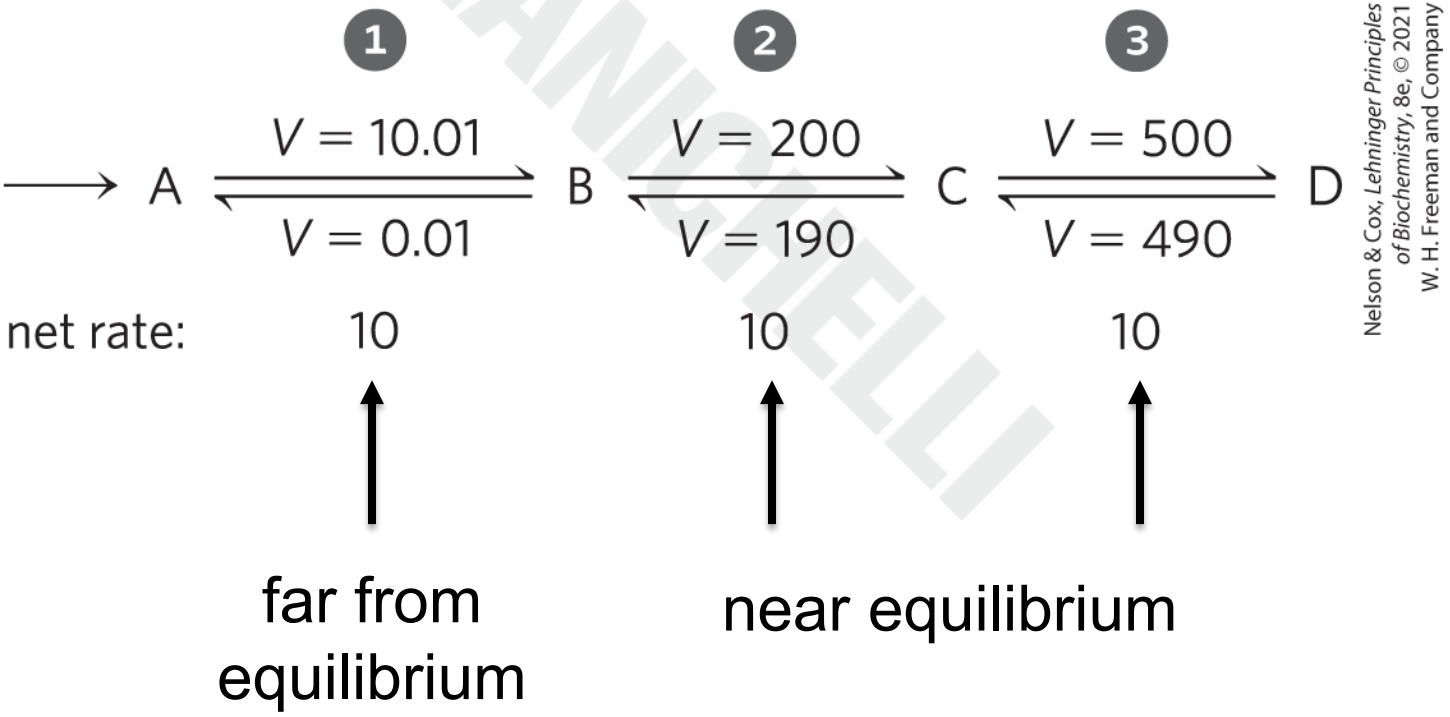
- allow for fast, smooth, effective regulation

Metabolic Regulation and Metabolic Control

- **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

Reactions Far from Equilibrium in Cells Are Common Points of Regulation



Mass-Action Ratio, *Q*

- **mass-action ratio, *Q*** = [C][D]/[A][B] for the reaction
  A + B ⟶ C + D

- when *Q* and K'_{eq} are within 1 to 2 orders of magnitude of each other, the reaction is near equilibrium

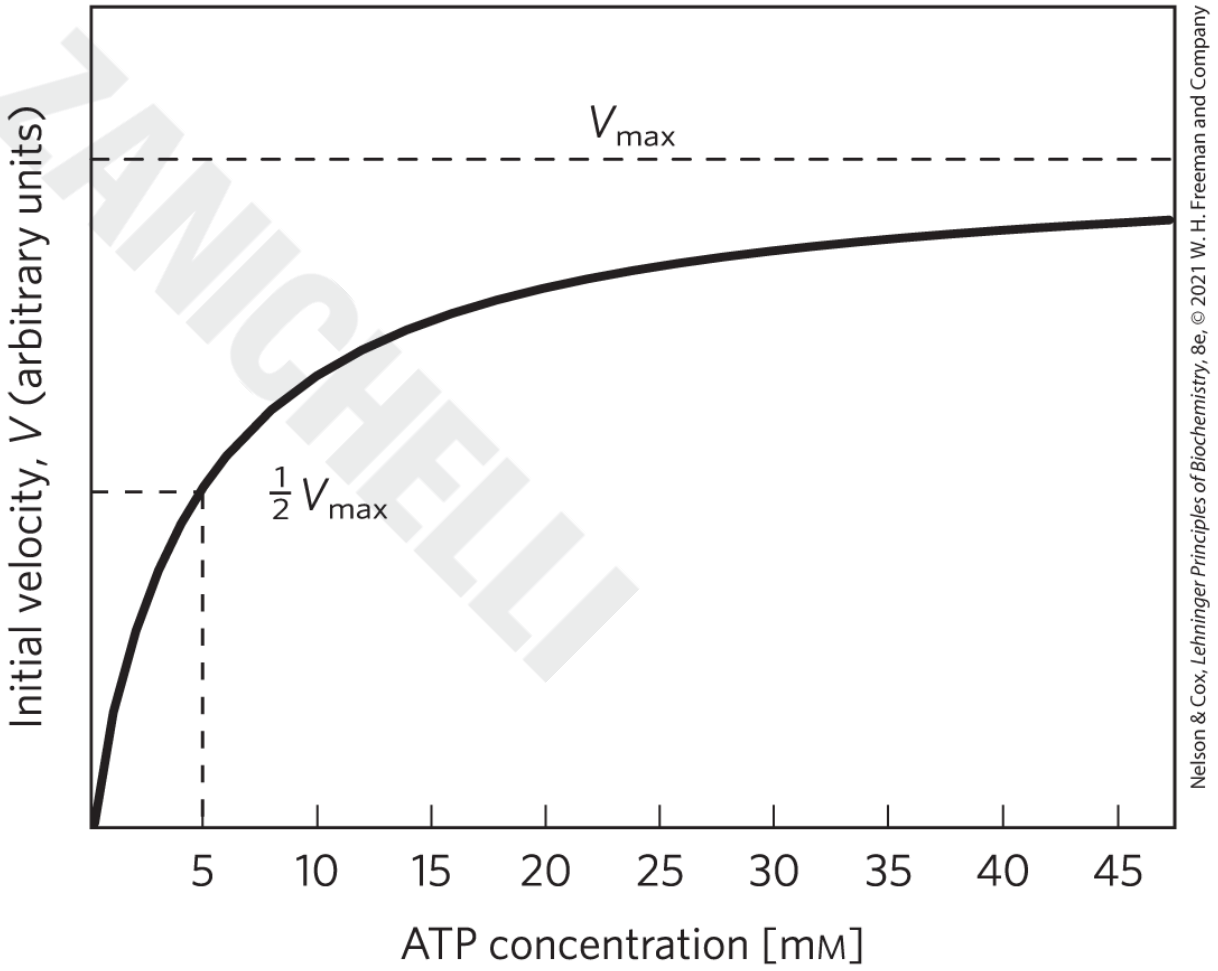
Enzymes of Carbohydrate Metabolism

Table 13-10 Equilibrium Constants, Mass-Action Ratios, and Free-Energy Changes for Enzymes of Carbohydrate Metabolism

Enzyme	K'_{eq}	Mass-action ratio, Q: Liver	Mass-action ratio, Q: Heart	Reaction near equilibrium in vivo?	$\Delta G'^{\circ}$ (kJ/mol)	$\Delta G'^{\circ}$ (kJ/mol) in heart
Hexokinase	1×10^{3}	2×10^{-2}	8×10^{-2}	No	−17	−27
PFK-1	1.0×10^{3}	9×10^{-2}	3×10^{-2}	No	−14	−23
Aldolase	1.0×10^{-4}	1.2×10^{-6}	9×10^{-6}	Yes	+24	−6.0
Triose phosphate isomerase	4×10^{-2}	—	2.4×10^{-1}	Yes	+7.5	+3.8
Glyceraldehyde 3-phosphate dehydrogenase + phosphoglycerate kinase	2×10^{3}	6×10^{2}	9.0	Yes	−13	+3.5
Phosphoglycerate mutase	1×10^{-1}	1×10^{-1}	1.2×10^{-1}	Yes	+4.4	+0.6
Enolase	3	2.9	1.4	Yes	−3.2	−0.5
Pyruvate kinase	2×10^{4}	7×10^{-1}	40	No	−31	−17
Phosphohexose isomerase	4×10^{-1}	3.1×10^{-1}	2.4×10^{-1}	Yes	+2.2	−1.4
Pyruvate carboxylase + PEP carboxykinase	7	1×10^{-3}	—	No	−5.0	−23
Glucose 6-phosphatase	8.5×10^{2}	1.2×10^{2}	—	Yes	−17	−5.0

Adenine Nucleotides Play Special Roles in Metabolic Regulation

- if [ATP] were to drop, enzymes would be less than fully saturated by their substrate (ATP), and the reaction rates would decrease



P6 Principle 6 (5 of 5)

To respond to changes in external circumstances, cells must regulate enzyme activities by changing either the number of enzyme molecules or the catalytic activity of preexisting enzyme molecules.

Adenylate Kinase and AMP-Activated Protein Kinase (AMPK)

- **adenylate kinase** = catalyzes the production of AMP in the reaction 2ADP ⟶ AMP + ATP

- **AMP-activated protein kinase (AMPK)** = responds to a decrease in the [ATP]/[AMP] ratio by phosphorylating key proteins and regulating their activities