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CHAPTER 14

Introduction to Metabolism

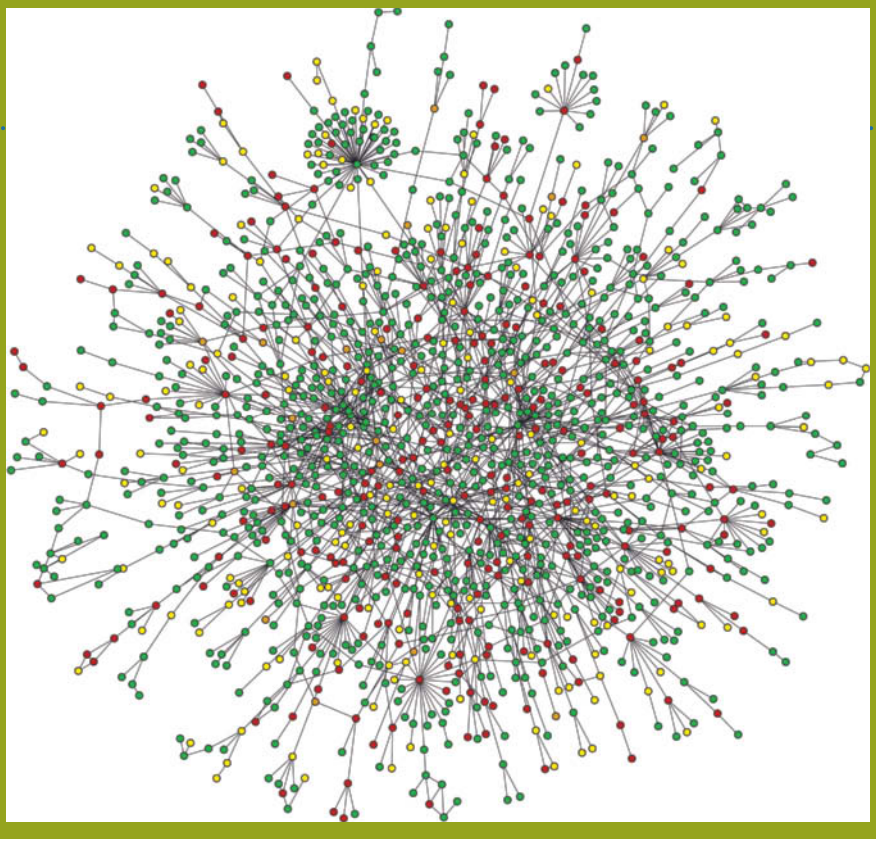
Chapter Contents

1 **Overview of Metabolism**
- A Nutrition Involves Food Intake and Use
- B Vitamins and Minerals Assist Metabolic Reactions
- C Metabolic Pathways Consist of Series of Enzymatic Reactions
- D Thermodynamics Dictates the Direction and Regulatory Capacity of Metabolic Pathways
- E Metabolic Flux Must Be Controlled

2 **"High-Energy" Compounds**
- A ATP Has a High Phosphoryl Group-Transfer Potential
- B Coupled Reactions Drive Endergonic Processes
- C Some Other Phosphorylated Compounds Have High Phosphoryl Group-Transfer Potentials
- D Thioesters Are Energy-Rich Compounds

3 **Oxidation–Reduction Reactions**
- A NAD^+ and FAD Are Electron Carriers
- B The Nernst Equation Describes Oxidation–Reduction Reactions
- C Spontaneity Can Be Determined by Measuring Reduction Potential Differences

4 **Experimental Approaches to the Study of Metabolism**
- A Labeled Metabolites Can Be Traced
- B Studying Metabolic Pathways Often Involves Perturbing the System
- C Systems Biology Has Entered the Study of Metabolism



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Modern approaches to understanding metabolism include the use of network theory to probe the functional importance of interacting cellular components, such as the yeast proteins shown here.

Understanding the chemical compositions and three-dimensional structures of biological molecules is not sufficient to understand how they are assembled into organisms or how they function to sustain life. We must therefore examine the reactions in which biological molecules are built and broken down. We must also consider how free energy is consumed in building cellular materials and carrying out cellular work and how free energy is generated from organic or other sources. **Metabolism**, the overall process through which living systems acquire and use free energy to carry out their various functions, is traditionally divided into two parts:

1. **Catabolism**, or degradation, in which nutrients and cell constituents are broken down to salvage their components and/or to make energy available.
2. **Anabolism**, or biosynthesis, in which biomolecules are synthesized from simpler components.

In general, catabolic reactions carry out the exergonic oxidation of nutrient molecules. The free energy thereby released is used to drive such endergonic processes as anabolic reactions, the performance of mechanical work, and the active transport of molecules against concentration gradients. Exergonic and endergonic processes are often coupled through the intermediate synthesis of a "high-energy" compound such as ATP. This simple principle underlies many of the chemical reactions presented in the following chapters. In this chapter, we introduce the general features of metabolic reactions and the roles of ATP and other compounds as energy carriers. Because many metabolic reactions are also oxidation–reduction reactions, we review the thermodynamics of these processes. Finally, we examine some approaches to studying metabolic reactions.

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1 Overview of Metabolism

KEY CONCEPTS

- Different organisms use different strategies for capturing free energy from their environment and can be classified by their requirement for oxygen.
- Mammalian nutrition involves the intake of macronutrients (proteins, carbohydrates, and lipids) and micronutrients (vitamins and minerals).
- A metabolic pathway is a series of enzyme-catalyzed reactions, often located in a specific part of a cell.
- The flux of material through a metabolic pathway varies with the activities of the enzymes that catalyze irreversible reactions.
- These flux-controlling enzymes are regulated by allosteric mechanisms, covalent modification, substrate cycling, and changes in gene expression.

A bewildering array of chemical reactions occur in any living cell. Yet the principles that govern metabolism are the same in all organisms, a result of their common evolutionary origin and the constraints of the laws of thermodynamics. In fact, many of the specific reactions of metabolism are common to all organisms, with variations due primarily to differences in the sources of the free energy that supports them.

A Nutrition Involves Food Intake and Use

Nutrition, the intake and utilization of food, affects health, development, and performance. Food supplies the energy that powers life processes and provides the raw materials to build and repair body tissues. The nutritional requirements of an organism reflect its source of metabolic energy. For example, some prokaryotes are **autotrophs** (Greek: *autos,* self + *trophos,* feeder), which can synthesize all their cellular constituents from simple molecules such as H_2O, CO_2, NH_3, and H_2S. There are two possible free energy sources for this process. **Chemolithotrophs** (Greek: *lithos,* stone) obtain their energy through the oxidation of inorganic compounds such as NH_3, H_2S, or even Fe^{2+}:

$$2\,NH_3 + 4\,O_2 \rightarrow 2\,HNO_3 + 2\,H_2O$$
$$H_2S + 2\,O_2 \rightarrow H_2SO_4$$
$$4\,FeCO_3 + O_2 + 6\,H_2O \rightarrow 4\,Fe(OH)_3 + 4\,CO_2$$

Photoautotrophs do so via photosynthesis, a process in which light energy powers the transfer of electrons from inorganic donors to CO_2 to produce carbohydrates, $(CH_2O)_n$, which are later oxidized to release free energy. **Heterotrophs** (Greek: *hetero,* other) obtain free energy through the oxidation of organic compounds (carbohydrates, lipids, and proteins) and hence ultimately depend on autotrophs for those substances.

Organisms can be further classified by the identity of the oxidizing agent for nutrient breakdown. **Obligate aerobes** (which include animals) must use O_2, whereas **anaerobes** employ oxidizing agents such as sulfate or nitrate. **Facultative anaerobes,** such as *E. coli,* can grow in either the presence or the absence of O_2. **Obligate anaerobes,** in contrast, are poisoned by the presence of O_2. Their metabolisms are thought to resemble those of the earliest life-forms, which arose more than 3.5 billion years ago when the earth's atmosphere lacked O_2. Most of our discussion of metabolism will focus on aerobic processes.

Animals are obligate **aerobic** heterotrophs, whose nutrition depends on a balanced intake of the **macronutrients** proteins, carbohydrates, and lipids. These are broken down by the digestive system to their component amino acids, monosaccharides, fatty acids, and glycerol—the major nutrients involved in cellular metabolism—which are then transported by the circulatory system to the tissues. The metabolic utilization of the latter substances also requires the intake of O_2 and water, as well as **micronutrients** composed of **vitamins** and **minerals**.

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TABLE 14-1 Characteristics of Common Vitamins

Vitamin	Coenzyme Product	Reaction Mediated	Human Deficiency Disease
Water-Soluble			
Biotin (B_7)	Biocytin	Carboxylation	*a*
Pantothenic acid (B_5)	Coenzyme A	Acyl transfer	*a*
Cobalamin (B_{12})	Cobalamin coenzymes	Alkylation	Pernicious anemia
Riboflavin (B_2)	Flavin coenzymes	Oxidation–reduction	*a*
—	Lipoic acid	Acyl transfer	*a*
Nicotinamide (niacin; B_3)	Nicotinamide coenzymes	Oxidation–reduction	Pellagra
Pyridoxine (B_6)	Pyridoxal phosphate	Amino group transfer	*a*
Folic acid (B_9)	Tetrahydrofolate	One-carbon group transfer	Megaloblastic anemia
Thiamine (B_1)	Thiamine pyrophosphate	Aldehyde transfer	Beriberi
Ascorbic acid (C)	Ascorbate	Hydroxylation	Scurvy
Fat-Soluble			
Vitamin A		Vision	Night blindness
Vitamin D		Ca^{2+} absorption	Rickets
Vitamin E		Antioxidant	*a*
Vitamin K		Blood clotting	Hemorrhage

[a]No specific name; deficiency in humans is rare or unobserved.

TABLE 14-2 Major Essential Minerals and Trace Elements

Major Minerals	Trace Elements
Sodium	Iron
Potassium	Copper
Chlorine	Zinc
Calcium	Selenium
Phosphorus	Iodine
Magnesium	Chromium
Sulfur	Fluorine

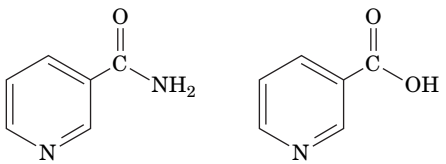
? **Which of the elements listed here occur as covalently bonded components of biological molecules?**

B Vitamins and Minerals Assist Metabolic Reactions

Vitamins are organic molecules that an animal is unable to synthesize and must therefore obtain from its diet. Vitamins can be divided into two groups: **water-soluble vitamins** and **fat-soluble vitamins.** Table 14-1 lists many common vitamins and the types of reactions or processes in which they participate (we will consider the structures of these substances and their reaction mechanisms in the appropriate sections of the text).

Table 14-2 lists the essential minerals and trace elements necessary for metabolism. They participate in metabolic processes in many ways. Mg^{2+}, for example, is involved in nearly all reactions that involve ATP and other nucleotides, including the synthesis of DNA, RNA, and proteins. Zn^{2+} is a cofactor in a variety of enzymes, including carbonic anhydrase (Section 11-3C). Ca^{2+}, in addition to being the major mineral component of bones and teeth, is a vital participant in signal transduction processes (Section 13-4).

Most Water-Soluble Vitamins Are Converted to Coenzymes. Many coenzymes (Section 11-1C) were discovered as growth factors for microorganisms or as substances that cure nutritional deficiency diseases in humans and/or animals. For example, the NAD^+ component **nicotinamide,** or its carboxylic acid analog **nicotinic acid** (**niacin;** Fig. 14-1), relieves the ultimately fatal



Nicotinamide (niacinamide) **Nicotinic acid (niacin)**

FIG. 14-1 The structures of nicotinamide and nicotinic acid. These vitamins form the redox-active components of the nicotinamide coenzymes NAD^+ and $NADP^+$ (compare with Fig. 11-4).

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dietary deficiency disease in humans known as **pellagra**. Pellagra (Italian: *pelle,* skin + *agra,* sour), which is characterized by dermatitis, diarrhea, and dementia, was endemic in the rural southern United States in the early twentieth century. Most animals, including humans, can synthesize nicotinamide from the amino acid tryptophan. However, the corn (maize)-rich diet that was prevalent in the rural South contained little available nicotinamide or tryptophan from which to synthesize it. (Corn actually contains significant quantities of niacin but in a form that requires treatment with base before it can be intestinally absorbed. The Mexican Indians, who domesticated the corn plant but did not suffer from pellagra, customarily soak corn meal in lime water—dilute $Ca(OH)_2$ solution—before using it to make their staple food, tortillas.) Dietary supplementation with nicotinamide or niacin has all but eliminated pellagra in the developed world.

The water-soluble vitamins in the human diet are all coenzyme precursors. In contrast, the fat-soluble vitamins, with the exception of vitamin K (Section 9-1F), are not components of coenzymes, although they are also required in small amounts in the diets of many higher animals.

The distant ancestors of humans probably had the ability to synthesize the various vitamins, as do many modern plants and microorganisms. Yet since vitamins are normally available in the diets of animals, which all eat other organisms, or are synthesized by the bacteria that normally inhabit their digestive systems, it seems likely that the superfluous cellular machinery to synthesize them was lost through evolution. For example, vitamin C (ascorbic acid) is required in the diets of only humans, apes, and guinea pigs (Section 6-1C and Box 6-2) because, in what is apparently a recent evolutionary loss, they lack a key enzyme for ascorbic acid biosynthesis.

C Metabolic Pathways Consist of Series of Enzymatic Reactions

Metabolic pathways are series of connected enzymatic reactions that produce specific products. Their reactants, intermediates, and products are referred to as **metabolites**. There are around 4000 known metabolic reactions, each catalyzed by a distinct enzyme. The types of enzymes and metabolites in a given cell vary with the identity of the organism, the cell type, its nutritional status, and its developmental stage. Many metabolic pathways are branched and interconnected, so delineating a pathway from a network of thousands of reactions is somewhat arbitrary and is driven by tradition as much as by chemical logic.

In general, degradative and biosynthetic pathways are related as follows (**Fig. 14-2**): In degradative pathways, the major nutrients, referred to as complex metabolites, are exergonically broken down into simpler products. The free energy released in the degradative process is conserved by the synthesis of ATP

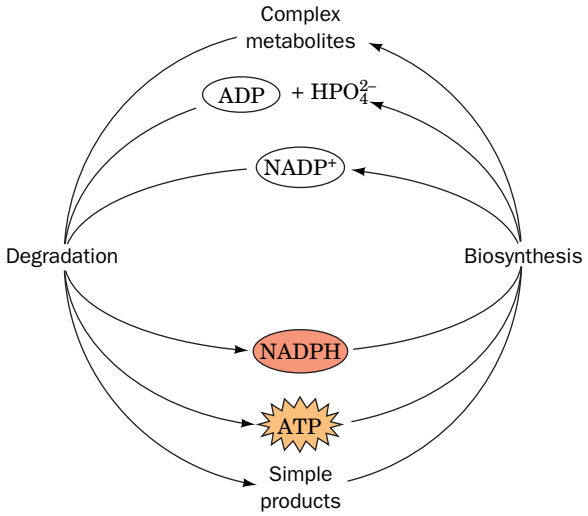


FIG. 14-2 Roles of ATP and NADP$^+$ in metabolism. ATP and NADPH, generated through the degradation of complex metabolites such as carbohydrates, lipids, and proteins, are sources of free energy for biosynthetic and other reactions.

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from ADP + P_i or by the reduction of a coenzyme such as $NADP^+$ (Fig. 11-4) to NADPH. ATP and NADPH are the major free energy sources for biosynthetic reactions. We will consider the thermodynamic properties of ATP and NADPH later in this chapter.

A striking characteristic of degradative metabolism is that *the pathways for the catabolism of a large number of diverse substances (carbohydrates, lipids, and proteins) converge on a few common intermediates,* in many cases, a two-carbon acetyl unit linked to **coenzyme A** to form **acetyl-coenzyme A** (**acetyl-CoA;** Section 14-2D). These intermediates are then further metabolized in a central oxidative pathway. **Figure 14-3** outlines the breakdown of various foodstuffs to their monomeric units and then to acetyl-CoA. This is followed by the oxidation of the acetyl carbons to CO_2 by the **citric acid cycle** (Chapter 17). When one substance is **oxidized** (loses electrons), another must be **reduced** (gain electrons; Box 14-1). The citric acid cycle thus produces the reduced coenzymes NADH and $FADH_2$ (Section 14-3A), which then pass their electrons to O_2 to produce H_2O in the processes of **electron transport** and **oxidative phosphorylation** (Chapter 18).

Biosynthetic pathways carry out the opposite process. *Relatively few metabolites serve as starting materials for a host of varied products*. In the next several chapters, we discuss many catabolic and anabolic pathways in detail.

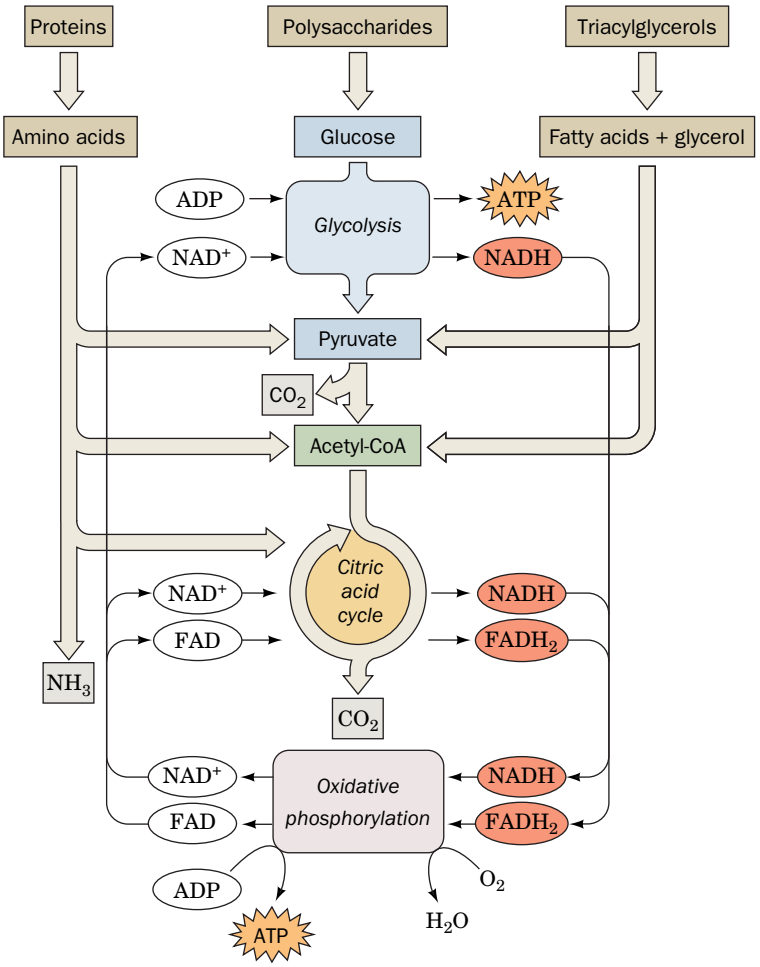


FIG. 14-3 Overview of catabolism. Complex metabolites such as carbohydrates, proteins, and lipids are degraded first to their monomeric units, chiefly glucose, amino acids, fatty acids, and glycerol, and then to the common intermediate, acetyl-CoA. The acetyl group is oxidized to CO_2 via the citric acid cycle with concomitant reduction of NAD^+ and **FAD** to NADH and **$FADH_2$**. Reoxidation of NADH and $FADH_2$ by O_2 during electron transport and oxidative phosphorylation yields H_2O and ATP.

? Identify the three major waste products of catabolism.

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Box 14-1 Perspectives in Biochemistry Oxidation States of Carbon

The carbon atoms in biological molecules can assume different oxidation states depending on the atoms to which they are bonded. For example, a carbon atom bonded to less electronegative hydrogen atoms is more reduced than a carbon atom bonded to highly electronegative oxygen atoms.

The simplest way to determine the oxidation number (and hence the oxidation state) of a particular carbon atom is to examine each of its bonds and assign the electrons to the more electronegative atom. In a C—O bond, both electrons "belong" to O; in a C—H bond, both electrons "belong" to C; and in a C—C bond, each carbon "owns" one electron. An atom's oxidation number is the number of valence electrons on the free atom (4 for carbon) minus the number of its lone pair and assigned electrons. For example, the oxidation number of carbon in CO_2 is $4 - (0 + 0) = +4$, and the oxidation number of carbon in CH_4 is $4 - (0 + 8) = -4$. Keep in mind, however, that oxidation numbers are only accounting devices; actual atomic charges are much closer to neutrality.

The following compounds are listed according to the oxidation state of the highlighted carbon atom. In general, the more oxidized compounds have fewer electrons per C atom and are richer in oxygen, and the more reduced compounds have more electrons per C atom and are richer in hydrogen. But note that not all reduction events (gain of electrons) or oxidation events (loss of electrons) are associated with bonding to oxygen. For example, when an alkane is converted to an alkene, the formation of a carbon–carbon double bond involves the loss of electrons and therefore is an oxidation reaction although no oxygen is involved. Knowing the oxidation number of a carbon atom is seldom required. However, it is useful to be able to determine whether the oxidation state of a given atom increases or decreases during a chemical reaction.

Compound	Formula	Oxidation Number
Carbon dioxide	$O{=}C{=}O$	+4 (most oxidized)
Acetic acid	$H_3C{-}C(=O){-}OH$	+3
Carbon monoxide	$:C{\equiv}O:$	+2
Formic acid	$H{-}C(=O){-}OH$	+2
Acetone	$H_3C{-}C(=O){-}CH_3$	+2
Acetaldehyde	$H_3C{-}C(=O){-}H$	+1
Formaldehyde	$H{-}C(=O){-}H$	0
Acetylene	$HC{\equiv}CH$	−1
Ethanol	$H_3C{-}C(H)(H){-}OH$	−1
Ethene	$H_2C{=}C(H)(H)$	−2
Ethane	$H_3C{-}C(H)(H){-}H$	−3
Methane	$H{-}C(H)(H){-}H$	−4 (least oxidized)

Enzymes Catalyze the Reactions of Metabolic Pathways. With a few exceptions, the interconversions of metabolites in degradative and biosynthetic pathways are catalyzed by enzymes. In the absence of enzymes, the reactions would occur far too slowly to support life. In addition, the specificity of enzymes guarantees the efficiency of metabolic reactions by preventing the formation of useless or toxic by-products. Most importantly, enzymes provide a mechanism for coupling an endergonic chemical reaction (which would not occur on its own) with an energetically favorable reaction, as discussed below.

We will see examples of reactions catalyzed by all six classes of enzymes introduced in Section 11-1A. These reactions fall into four major types: **oxidations and reductions** (catalyzed by oxidoreductases), **group-transfer reactions** (catalyzed by transferases and hydrolases), **eliminations, isomerizations, and**

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rearrangements (catalyzed by isomerases and mutases), and **reactions that make or break carbon–carbon bonds** (catalyzed by hydrolases, lyases, and ligases).

Metabolic Pathways Occur in Specific Cellular Locations. The compartmentation of the eukaryotic cytoplasm allows different metabolic pathways to operate in different locations. For example, electron transport and oxidative phosphorylation occur in the mitochondria, whereas **glycolysis** (a carbohydrate degradation pathway) and fatty acid biosynthesis occur in the cytosol. **Figure 14-4** shows the major metabolic functions of eukaryotic organelles. Metabolic processes in prokaryotes, which lack organelles, may be localized to particular areas of the cytosol.

The synthesis of metabolites in specific membrane-bounded compartments in eukaryotic cells requires mechanisms to transport these substances between compartments. Accordingly, transport proteins (Chapter 10) are essential components of many metabolic processes. For example, a transport protein is required to move ATP, which is generated in the mitochondria, to the cytosol, where most of it is consumed (Section 18-1B).

In multicellular organisms, compartmentation is carried a step further to the level of tissues and organs. The mammalian liver, for example, is largely responsible for the synthesis of glucose from noncarbohydrate precursors (**gluconeogenesis**; Section 16-4) so as to maintain a relatively constant level of glucose in the circulation, whereas adipose tissue is specialized for storage of triacylglycerols. The interdependence of the metabolic functions of the various organs is the subject of Chapter 22.

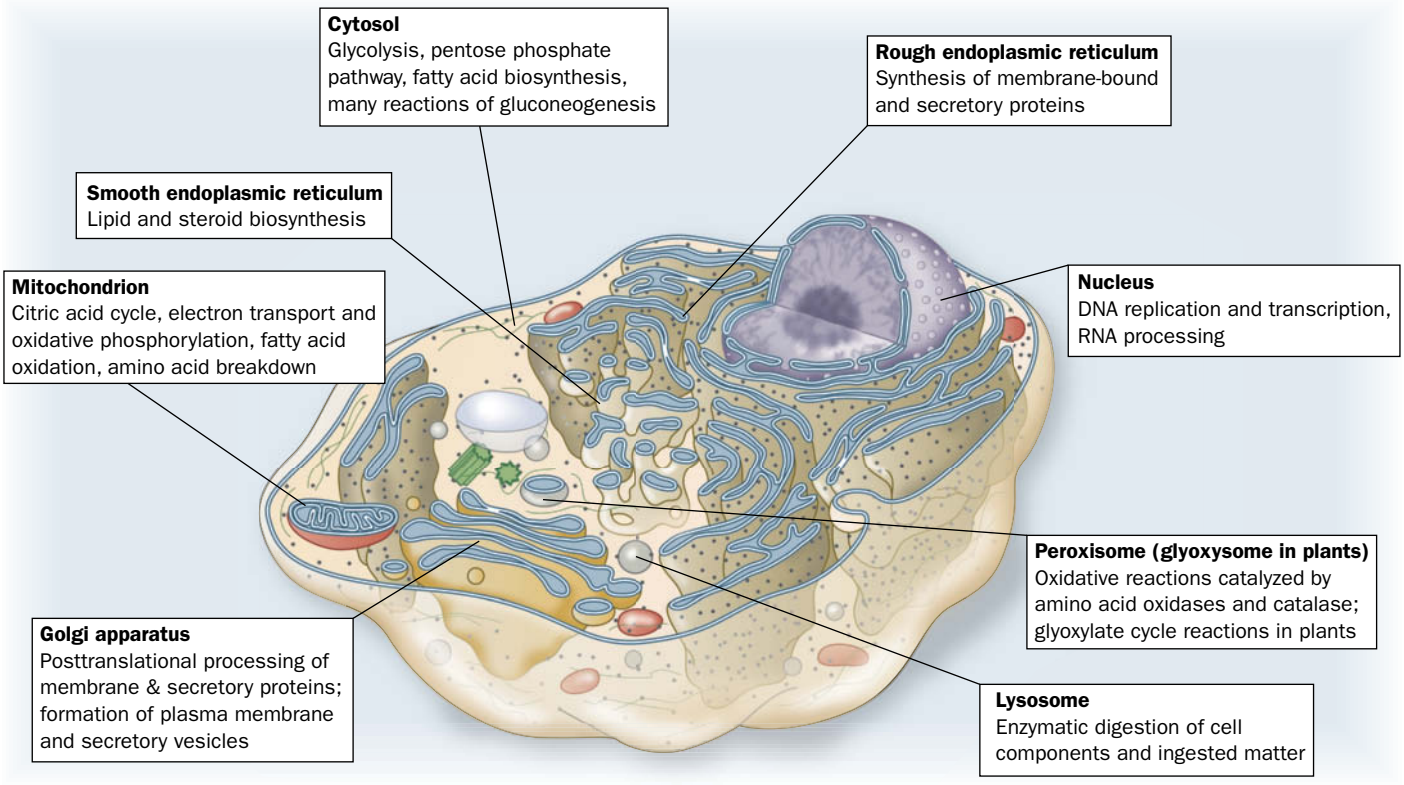


FIG. 14-4 Metabolic functions of eukaryotic organelles. Degradative and biosynthetic processes may occur in specialized compartments in the cell, or may involve several compartments.

? Without looking at the figure, summarize the major function of each cellular compartment. Identify which compartments carry out degradative versus synthetic processes.

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An intriguing manifestation of specialization of tissues and subcellular compartments is the existence of **isozymes**, enzymes that catalyze the same reaction but are encoded by different genes and have different kinetic or regulatory properties. For example, we have seen that mammals have three isozymes of glycogen phosphorylase, those expressed in muscle, brain, and liver (Section 12-3B). Similarly, vertebrates possess two homologs of the enzyme **lactate dehydrogenase:** the M type, which predominates in tissues subject to anaerobic conditions such as skeletal muscle and liver, and the H type, which predominates in aerobic tissues such as heart muscle. Lactate dehydrogenase catalyzes the interconversion of **pyruvate,** a product of glycolysis, and **lactate** (Section 15-3A). The M-type isozyme appears mainly to function in the reduction by NADH of pyruvate to lactate, whereas the H-type enzyme appears to be better adapted to catalyze the reverse reaction.



The existence of isozymes allows for the testing of various illnesses. For example, heart attacks cause the death of heart muscle cells, which consequently rupture and release H-type LDH into the blood. A blood test indicating the presence of H-type LDH is therefore diagnostic of a heart attack.

D Thermodynamics Dictates the Direction and Regulatory Capacity of Metabolic Pathways

Knowing the location of a metabolic pathway and enumerating its substrates and products does not necessarily reveal how that pathway functions as part of a larger network of interrelated biochemical processes. It is also necessary to appreciate how fast end product can be generated by the pathway, as well as how pathway activity is regulated as the cell's needs change. Conclusions about a pathway's output and its potential for regulation can be gleaned from information about the thermodynamics of each enzyme-catalyzed step.

Recall from Section 1-3D that the free energy change ΔG of a biochemical process, such as the reaction

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

is related to the standard free energy change ($\Delta G^{\circ\prime}$) and the concentrations of the reactants and products (Eq. 1-15):

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \left(\frac{[C][D]}{[A][B]} \right) \qquad [14\text{-}1]$$

At equilibrium, $\Delta G = 0$ and the equation becomes

$$\Delta G^{\circ\prime} = -RT \ln K_{eq} \qquad [14\text{-}2]$$

Thus, the value of $\Delta G^{\circ\prime}$ can be calculated from the equilibrium constant and vice versa (see Sample Calculation 14-1).

When the reactants are present at values close to their equilibrium values, $[C]_{eq}[D]_{eq}/[A]_{eq}[B]_{eq} \approx K_{eq}$, and $\Delta G \approx 0$. This is the case for many metabolic reactions, which are said to be **near-equilibrium reactions**. Because their ΔG values are close to zero, they can be relatively easily reversed by changing the ratio of products to reactants. When the reactants are in excess of their equilibrium concentrations, the net reaction proceeds in the forward direction until the excess reactants have been converted to products and equilibrium is attained. Conversely, when products are in excess, the net reaction proceeds in the reverse direction to convert products to reactants until the equilibrium concentration ratio is again achieved. *Enzymes that catalyze near-equilibrium reactions tend to act quickly to restore equilibrium concentrations, and the net rates of such reactions are effectively controlled by the relative concentrations of substrates and products.*

SAMPLE CALCULATION 14-1

Calculate the equilibrium constant for the hydrolysis of glucose-1-phosphate at 37°C, using the information in Table 14-3 (see Section 14-2A).

$\Delta G^{\circ\prime}$ for the reaction

$$\text{Glucose-1-phosphate} + H_2O \rightarrow \text{glucose} + P_i$$

is $-20.9\ \text{kJ} \cdot \text{mol}^{-1}$. At equilibrium, $\Delta G = 0$ and Eq. 14-1 becomes

$$\Delta G^{\circ\prime} = -RT \ln K$$

(Eq. 14-2). Therefore,

$$K = e^{-\Delta G^{\circ\prime}/RT}$$

$$K = e^{-(-20{,}900\ \text{J}\cdot\text{mol}^{-1})/(8.3145\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1})(310\ \text{K})}$$

$$K = 3.3 \times 10^3$$

WPLS See Sample Calculation Videos.

GATEWAY CONCEPT Le Châtelier's Principle

Recall from Chapter 1 that adding or removing components from a reaction at equilibrium causes the reaction to proceed in one direction or the other until a new equilibrium is established.

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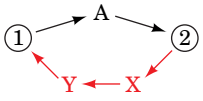
GATEWAY CONCEPT Free Energy Change

You can think of the free energy change (ΔG) for a reaction in terms of an urge or a force pushing the reactants toward equilibrium. The larger the free energy change, the farther the reaction is from equilibrium and the stronger is the tendency for the reaction to proceed. At equilibrium, of course, the reactants undergo no net change and $\Delta G = 0$.

Other metabolic reactions function far from equilibrium; that is, they are irreversible. This is because an enzyme catalyzing such a reaction has insufficient catalytic activity (the rate of the reaction it catalyzes is too slow) to allow the reaction to come to equilibrium under physiological conditions. Reactants therefore accumulate in large excess of their equilibrium amounts, making $\Delta G \ll 0$. Changes in substrate concentrations therefore have relatively little effect on the rate of an irreversible reaction; the enzyme is essentially saturated. Only changes in the activity of the enzyme—through allosteric interactions, for example—can significantly alter the rate. The enzyme is therefore analogous to a dam on a river: *It controls the flow of substrate through the reaction by varying its activity, much as a dam controls the flow of a river by varying the opening of its floodgates.*

Understanding the **flux** (rate of flow) of metabolites through a metabolic pathway requires knowledge of which reactions are functioning near equilibrium and which are far from it. Most enzymes in a metabolic pathway operate near equilibrium and therefore have net rates that vary with their substrate concentrations. However, certain enzymes that operate far from equilibrium are strategically located in metabolic pathways. This has several important implications:

1. ***Metabolic pathways are irreversible.*** A highly exergonic reaction (one with $\Delta G \ll 0$) is irreversible; that is, it goes to completion. If such a reaction is part of a multistep pathway, it confers directionality on the pathway; that is, it makes the entire pathway irreversible.
2. ***Every metabolic pathway has a first committed step.*** Although most reactions in a metabolic pathway function close to equilibrium, there is generally an irreversible (exergonic) reaction early in the pathway that "commits" its product to continue down the pathway (likewise, water that has gone over a dam cannot spontaneously return).
3. ***Catabolic and anabolic pathways differ.*** If a metabolite is converted to another metabolite by an exergonic process, free energy must be supplied to convert the second metabolite back to the first. This energetically "uphill" process requires a different pathway for at least one of the reaction steps.



The existence of independent interconversion routes, as we will see, is an important property of metabolic pathways because it allows independent control of the two processes. If metabolite 2 is required by the cell, it is necessary to "turn off" the pathway from 2 to 1 while "turning on" the pathway from 1 to 2. Such independent control would be impossible without different pathways.

E Metabolic Flux Must Be Controlled

GATEWAY CONCEPT The Steady State

Although many reactions are near equilibrium, an entire metabolic pathway—and the cell's metabolism as a whole—never reaches equilibrium. This is because materials and energy are constantly entering and leaving the system, which is in a steady state. Metabolic pathways proceed, as if trying to reach equilibrium (Le Châtelier's principle), but they cannot get there because new reactants keep arriving and products do not accumulate.

Living organisms are thermodynamically open systems that tend to maintain a steady state rather than reaching equilibrium (Section 1-3E). This is strikingly demonstrated by the observation that, over a 40-year time span, a normal human adult consumes literally tons of nutrients and imbibes more than 20,000 L of water but does so without major weight change. *The flux of intermediates through a metabolic pathway in a steady state is more or less constant; that is, the rates of synthesis and breakdown of each pathway intermediate maintain it at a constant concentration.* A steady state far from equilibrium is thermodynamically efficient, because only a nonequilibrium process ($\Delta G \neq 0$) can perform useful work. Indeed, living systems that have reached equilibrium are dead.

Since a metabolic pathway is a series of enzyme-catalyzed reactions, it is easiest to describe the flux of metabolites through the pathway by considering its

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reaction steps individually. The flux of metabolites, J, through each reaction step is the rate of the forward reaction, v_f, less that of the reverse reaction, v_r:

$$J = v_f - v_r \qquad [14\text{-}3]$$

At equilibrium, by definition, there is no flux ($J = 0$), although v_f and v_r may be quite large. In reactions that are far from equilibrium, $v_f \gg v_r$, the flux is essentially equal to the rate of the forward reaction ($J \approx v_f$).

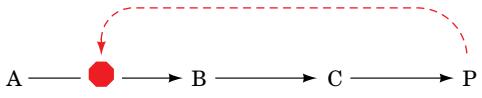
For the pathway as a whole, flux is set by the rate-determining step of the pathway. By definition, this step is the pathway's slowest step, which is often the first committed step of the pathway. In some pathways, flux control is distributed over several enzymes, all of which help determine the overall rate of flow of metabolites through the pathway. Because a rate-determining step is slow relative to other steps in the pathway, its product is removed by succeeding steps in the pathway before it can equilibrate with reactant. Thus, *the rate-determining step functions far from equilibrium and has a large negative free energy change.* In an analogous manner, a dam creates a difference in water levels between its upstream and downstream sides, and a large negative free energy change results from the hydrostatic pressure difference. The dam can release water to generate electricity, varying the water flow according to the need for electrical power.

Reactions that function near equilibrium respond rapidly to changes in substrate concentration. For example, upon a sudden increase in the concentration of a reactant for a near-equilibrium reaction, the enzyme catalyzing it would increase the net reaction rate to rapidly achieve the new equilibrium level. Thus, a series of near-equilibrium reactions downstream from the rate-determining step all have the same flux. Likewise, the flux of water in a river is the same at all points downstream from a dam.

In practice, it is often possible to identify flux control points for a pathway by identifying reactions that have large negative free energy changes. The relative insensitivity of the rates of these nonequilibrium reactions to variations in the concentrations of their substrates permits the establishment of a steady state flux of metabolites through the pathway. Of course, flux through a pathway must vary in response to the organism's requirements to reach a new steady state. Altering the rates of the rate-determining steps can alter the flux of material through the entire pathway, often by an order of magnitude or more.

Cells use several mechanisms to control flux through the rate-determining steps of metabolic pathways:

1. ***Allosteric control.*** Many enzymes are allosterically regulated (Section 12-3A) by effectors that are often substrates, products, or coenzymes of the pathway but not necessarily of the enzyme in question. For example, in negative feedback regulation, the product of a pathway inhibits an earlier step in the pathway:

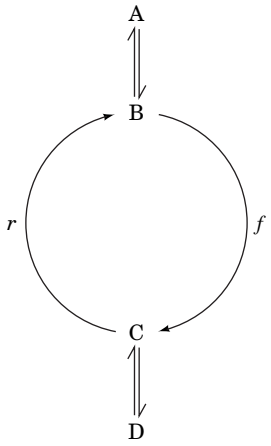


   Thus, as we have seen, CTP, a product of pyrimidine biosynthesis, inhibits ATCase, which catalyzes the rate-determining step in the pathway (Fig. 12-11).

2. ***Covalent modification.*** Many enzymes that control pathway fluxes have specific sites that may be enzymatically phosphorylated and dephosphorylated (Section 12-3B) or covalently modified in some other way. Such enzymatic modification processes, which are themselves subject to control by external signals such as hormones (Section 13-1), greatly alter the activities of the modified enzymes. The signaling methods involved in such flux control mechanisms are discussed in Chapter 13.

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3. ***Substrate cycles.*** If v_f and v_r represent the rates of two opposing nonequilibrium reactions that are catalyzed by different enzymes, v_f and v_r may be independently varied.



For example, flux ($v_f - v_r$) can be increased not just by accelerating the forward reaction but by slowing the reverse reaction. The flux through such a **substrate cycle**, as we will see in Section 15-4, is more sensitive to the concentrations of allosteric effectors than is the flux through a single unopposed nonequilibrium reaction.

4. ***Genetic control.*** Enzyme concentrations, and hence enzyme activities, may be altered by protein synthesis in response to metabolic needs. The processes of transcribing a gene to messenger RNA and then translating the RNA to a polypeptide chain offer numerous points for regulation. Mechanisms of genetic control of enzyme concentrations are a major concern of Part V of this text.

Mechanisms 1 to 3 can respond rapidly (within seconds or minutes) to external stimuli and are therefore classified as "short-term" control mechanisms. Mechanism 4 responds more slowly to changing conditions (within hours or days in higher organisms) and is therefore regarded as a "long-term" control mechanism.

Control of most metabolic pathways involves several nonequilibrium steps. Hence, the flux of material through a pathway that supplies intermediates for use by an organism may depend on multiple effectors whose relative importance reflects the overall metabolic demands of the organism at a given time. Thus, a metabolic pathway is part of a **supply–demand process.**

CHECKPOINT

- Describe the differences between autotrophs and heterotrophs.
- Use the words obligate, facultative, aerobic, anaerobic, autotroph, and heterotroph to describe the metabolism of a human, oak tree, *E. coli,* and *Methanococcus jannaschii* (an organism that lives in deepwater anoxic sediments).
- List the categories of macronutrients and micronutrients required for mammalian metabolism and provide examples of each.
- What is the relationship between vitamins and coenzymes?
- Explain the roles of ATP and NADPH in catabolic and anabolic reactions.
- Give some reasons why enzymes are essential for the operation of metabolic pathways.
- Why might different tissues express different isozymes?
- How are free energy changes and equilibrium constants related?
- Explain the metabolic significance of reactions that function near equilibrium and reactions that function far from equilibrium.
- Discuss the mechanisms by which the flux through a metabolic pathway can be controlled. Which mechanisms can rapidly alter flux?

2 "High-Energy" Compounds

KEY CONCEPTS

- Organisms capture the free energy released on degradation of nutrients as "high-energy" compounds such as ATP, whose subsequent breakdown is used to power otherwise endergonic reactions.
- The "high energy" of ATP is related to the large negative free energy change for hydrolysis of its phosphoanhydride bonds.
- ATP hydrolysis can be coupled to an endergonic reaction such that the net reaction is favorable.
- Phosphoryl groups are transferred from compounds with high phosphoryl group-transfer potentials to those with low phosphoryl group-transfer potentials.
- The thioester bond in acetyl-CoA is a "high-energy" bond.

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The complete oxidation of a metabolic fuel such as glucose

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O$$

releases considerable energy ($\Delta G^{\circ\prime} = -2850\ kJ \cdot mol^{-1}$). The complete oxidation of palmitate, a typical fatty acid,

$$C_{16}H_{32}O_2 + 23\,O_2 \rightarrow 16\,CO_2 + 16\,H_2O$$

is even more exergonic ($\Delta G^{\circ\prime} = -9781\ kJ \cdot mol^{-1}$). Oxidative metabolism proceeds in a stepwise fashion, so the released free energy can be recovered in a manageable form at each exergonic step of the overall process. *These "packets" of energy are conserved by the synthesis of a few types of* ***"high-energy" intermediates*** *whose subsequent exergonic breakdown drives endergonic processes.* These intermediates therefore form a sort of free energy "currency" through which free energy–producing reactions such as glucose oxidation or fatty acid oxidation "pay for" the free energy–consuming processes in biological systems (Box 14-2).

The cell uses several forms of energy currency, including phosphorylated compounds such as the nucleotide ATP (the cell's primary energy currency), compounds that contain thioester bonds, and reduced coenzymes such as NADH. Each of these represents a source of free energy that the cell can use in various ways, including the synthesis of ATP. We will first examine ATP and then discuss the properties of other forms of energy currency.

GATEWAY CONCEPT Energy Transformation

Energy cannot be created or destroyed, but it can be transformed. The metabolic reactions that occur in cells convert one form of energy to another. Most often, the energy of chemical bonds is involved, but cells can also deal with thermal energy, light energy, mechanical energy, electrical energy, the energy of concentration gradients, and so on.

Box 14-2 Pathways of Discovery — Fritz Lipmann and "High-Energy" Compounds



Fritz Albert Lipmann (1899–1986) Among the many scientists who fled Europe for the United States in the 1930s was Fritz Lipmann, a German-born physician-turned-biochemist. During the first part of the twentieth century, scientists were interested primarily in the structures and compositions of biological molecules, so not much was known about their biosynthesis. Lipmann's contribution to this field centers on his understanding of "energy-rich" phosphates and other "active" compounds.

Lipmann began his research career by studying creatine phosphate, a compound that could provide energy for muscle contraction. He, like many of his contemporaries, was puzzled by the absence of an obvious link between this phosphorylated compound and the known metabolic activity of a contracting muscle, namely, converting glucose to lactate. One link was discovered by Otto Warburg (Box 15-1), who showed that one of the steps of glycolysis was accompanied by the incorporation of inorganic phosphate. The resulting acyl phosphate (1,3-bisphosphoglycerate) could then react with ADP to form ATP.

Lipmann wondered whether other phosphorylated compounds might behave in a similar manner. Because the purification of such labile (prone to degradation) compounds from whole cells was impractical, Lipmann synthesized them himself. He was able to show that cell extracts used synthetic acetyl phosphate to produce ATP. Lipmann went on to propose that cells contain two classes of phosphorylated compounds, which he termed "energy-poor" and "energy-rich," by which he meant compounds with low and high negative free energies of hydrolysis (the "squiggle," ~, which is still used, was his symbol for an "energy-rich" bond). Lipmann described a sort of "phosphate current" in which photosynthesis or breakdown of food molecules generates "energy-rich" phosphates that lead to the synthesis of ATP. The ATP, in turn, can power mechanical work such as muscle contraction or drive biosynthetic reactions.

Until this point (1941), biochemists studying biosynthetic processes were largely limited to working with whole animals or relatively intact tissue slices. Lipmann's insight regarding the role of ATP freed researchers from their cumbersome and poorly reproducible experimental systems. Biochemists could simply add ATP to their cell-free preparations to reconstitute the biosynthetic process.

Lipmann was intrigued by the discovery that a two-carbon group, an "active acetate," served as a precursor for the synthesis of fatty acids and steroids. Was acetyl phosphate also the "active acetate"? This proved not to be the case, although Lipmann was able to show that the addition of a two-carbon unit to another molecule (acetylation) required acetate, ATP, and a heat-stable factor present in pigeon liver extracts. He isolated and determined the structure of this factor, which he named coenzyme A. For this seminal discovery, Lipmann was awarded the 1953 Nobel Prize in Physiology or Medicine.

Even after "high-energy" thioesters (as in acetyl-CoA) came on the scene, Lipmann remained a staunch advocate of "high-energy" phosphates. For example, he realized that carbamoyl phosphate ($H_2N—COO—PO_3^{2-}$) could function as an "active" carbamoyl group donor in biosynthetic reactions. He also helped identify more obscure compounds, mixed anhydrides between phosphate and sulfate, as "active" sulfates that function as sulfate group donors.

Kleinkauf, H., von Döhren, H., and Jaenicke, L. (Eds.), *The Roots of Modern Biochemistry. Fritz Lipmann's Squiggle and Its Consequences,* Walter de Gruyter (1988).

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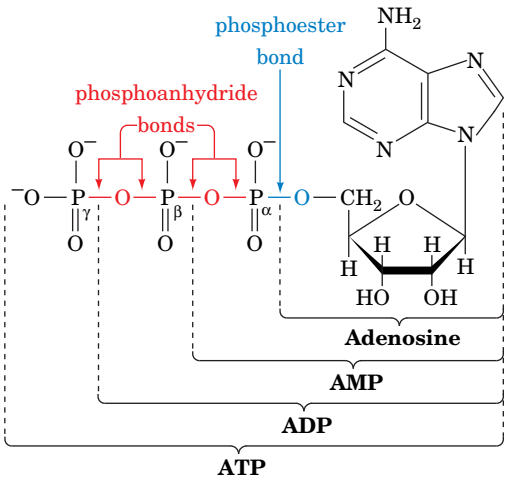


FIG. 14-5 **The structure of ATP indicating its relationship to ADP, AMP, and adenosine.** The phosphoryl groups, starting from AMP, are referred to as the α-, β-, and γ-phosphates. Note the differences between phosphoester and phosphoanhydride bonds.

? **Describe the products of hydrolysis of each of the indicated bonds.**

A ATP Has a High Phosphoryl Group-Transfer Potential

The "high-energy" intermediate adenosine triphosphate (ATP; Fig. 14-5) occurs in all known life-forms. ATP consists of an **adenosine** moiety (adenine + ribose) to which three phosphoryl ($—PO_3^{2-}$) groups are sequentially linked via a **phosphoester** bond followed by two **phosphoanhydride** bonds.

The biological importance of ATP rests in the large free energy change that accompanies cleavage of its phosphoanhydride bonds. This occurs when either a phosphoryl group is transferred to another compound, leaving ADP, or a nucleotidyl (AMP) group is transferred, leaving **pyrophosphate** ($P_2O_7^{4-}$; **PP_i**). When the acceptor is water, the process is known as hydrolysis:

$$ATP + H_2O \rightleftharpoons ADP + P_i$$
$$ATP + H_2O \rightleftharpoons AMP + PP_i$$

Most biological group-transfer reactions involve acceptors other than water. However, knowing the free energy of hydrolysis of various phosphoryl compounds allows us to calculate the free energy of transfer of phosphoryl groups to other acceptors by determining the difference in free energy of hydrolysis of the phosphoryl donor and acceptor.

The $\Delta G^{\circ\prime}$ values for hydrolysis of several phosphorylated compounds of biochemical importance are tabulated in Table 14-3. The negatives of these values are often referred to as **phosphoryl group-transfer potentials**; they are a measure of the tendency of phosphorylated compounds to transfer their phosphoryl groups to water. Note that ATP has an intermediate phosphate group-transfer potential. Under standard conditions, the compounds above ATP in Table 14-3 can spontaneously transfer a phosphoryl group to ADP to form ATP, which can, in turn, spontaneously transfer a phosphoryl group to the appropriate groups to form the compounds listed below it. Note that a favorable free energy change for a reaction does not indicate how quickly the reaction occurs. Despite their high group-transfer potentials, ATP and related phosphoryl compounds are **kinetically stable** and do not react at a significant rate unless acted upon by an appropriate enzyme.

What Is the Nature of the "Energy" in "High-Energy" Compounds? Bonds whose hydrolysis proceeds with large negative values of $\Delta G^{\circ\prime}$ (customarily less than $-25\ kJ \cdot mol^{-1}$) are often referred to as **"high-energy" bonds** or **"energy-rich" bonds** and are frequently symbolized by the squiggle (~). Thus, ATP can be represented as AR—P~P~P, where A, R, and P symbolize adenyl, ribosyl, and phosphoryl groups, respectively. Yet the phosphoester bond joining the adenosyl group of ATP to its α-phosphoryl group appears to be not greatly different in electronic character from the "high-energy" bonds bridging its α- and β- and its β- and γ-phosphoryl groups. In fact, none of these bonds has any unusual properties, so the term "high-energy" bond is somewhat of a misnomer (in any case, it should not be confused with the term "bond energy," which is defined as the energy required to break, not hydrolyze, a covalent bond). Why, then, are the phosphoryl group-transfer reactions of ATP so exergonic? Several factors appear to be responsible for the "high-energy" character of phosphoanhydride bonds such as those in ATP (Fig. 14-6):

1. The resonance stabilization of a phosphoanhydride bond is less than that of its hydrolysis products. This is because a phosphoanhydride's two strongly electron-withdrawing groups must compete for the lone pairs of electrons of its bridging oxygen atom, whereas this competition is absent in the hydrolysis products. In other words, the electronic requirements of the phosphoryl groups are less satisfied in a phosphoanhydride than in its hydrolysis products.
2. Of perhaps greater importance is the destabilizing effect of the electrostatic repulsions between the charged groups of a phosphoanhydride compared to those of its hydrolysis products. In the physiological pH range, ATP has three to four negative charges whose mutual electrostatic repulsions are partially relieved by ATP hydrolysis.

TABLE 14-3 Standard Free Energies of Phosphate Hydrolysis of Some Compounds of Biological Interest

Compound	$\Delta G^{\circ\prime}$ ($kJ \cdot mol^{-1}$)
Phosphoenolpyruvate	−61.9
1,3-Bisphosphoglycerate	−49.4
ATP (→ AMP + PP_i)	−45.6
Acetyl phosphate	−43.1
Phosphocreatine	−43.1
ATP (→ ADP + P_i)	−30.5
Glucose-1-phosphate	−20.9
PP_i	−19.2
Fructose-6-phosphate	−13.8
Glucose-6-phosphate	−13.8
Glycerol-3-phosphate	−9.2

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

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3. Another destabilizing influence, which is difficult to assess, is the smaller solvation energy of a phosphoanhydride compared to that of its hydrolysis products. Some estimates suggest that this factor provides the dominant thermodynamic driving force for the hydrolysis of phosphoanhydrides.

Of course, the free energy change for any reaction, including phosphoryl group transfer from a "high-energy" compound, depends in part on the concentrations of the reactants and products (Eq. 14-1). Furthermore, because ATP and its hydrolysis products are ions, ΔG also depends on pH and ionic strength (Box 14-3).

B Coupled Reactions Drive Endergonic Processes

The hydrolysis of a "high-energy" compound, while releasing considerable free energy, is not in itself a useful reaction. However, the exergonic reactions of "high-energy" compounds can be coupled to endergonic processes to drive them to completion. The thermodynamic explanation for the coupling of an exergonic and an endergonic process is based on the additivity of free energy. Consider the following two-step reaction pathway:

$$(1)\quad A + B \rightleftharpoons C + D \qquad \Delta G_1$$
$$(2)\quad D + E \rightleftharpoons F + G \qquad \Delta G_2$$

If $\Delta G_1 \geq 0$, Reaction 1 will not occur spontaneously. However, if ΔG_2 is sufficiently exergonic so $\Delta G_1 + \Delta G_2 < 0$, then although the equilibrium concentration of D in Reaction 1 will be relatively small, it will be larger than that in Reaction 2. As Reaction 2 converts D to products, Reaction 1 will operate in the forward direction to replenish the equilibrium concentration of D. The highly exergonic Reaction 2 therefore "drives" or "pulls" the endergonic Reaction 1, and the two reactions are said to be coupled through their common intermediate, D. That these coupled reactions proceed spontaneously can also be seen by summing Reactions 1 and 2 to yield the overall reaction where $\Delta G_3 = \Delta G_1 + \Delta G_2 < 0$. *As long as the overall pathway is exergonic, it will operate in the forward direction.*

$$(1 + 2)\quad A + B + E \rightleftharpoons C + F + G \qquad \Delta G_3$$

To illustrate this concept, let us consider two examples of phosphoryl group-transfer reactions. The initial step in the metabolism of glucose is its conversion to **glucose-6-phosphate** (Section 15-2A). Yet the direct reaction of glucose and

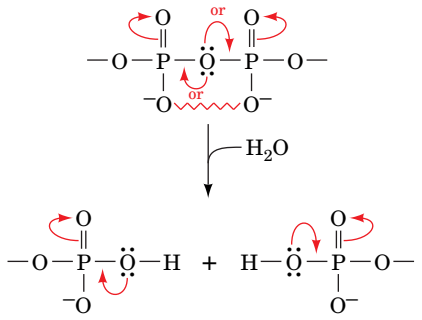


FIG. 14-6 Resonance and electrostatic stabilization in a phosphoanhydride and its hydrolytic products. The competing resonances (*curved arrows* from the central O) and charge–charge repulsions (*zigzag lines*) between phosphoryl groups decrease the stability of a phosphoanhydride relative to its hydrolysis products.

GATEWAY CONCEPT Resonance

Resonance refers to the delocalization of electrons in a chemical structure. Compounds are stabilized by resonance, which can be roughly assessed by the number of different ways to draw the structure.



Box 14-3 Perspectives in Biochemistry **ATP and ΔG**

The standard conditions reflected in $\Delta G°'$ values never occur in living organisms. Furthermore, other compounds that are present at high concentrations and that can potentially interact with the substrates and products of a metabolic reaction may dramatically affect ΔG values. For example, Mg^{2+} ions in cells partially neutralize the negative charges on the phosphate groups in ATP and its hydrolysis products, thereby diminishing the electrostatic repulsions that make ATP hydrolysis so exergonic. Similarly, changes in pH alter the ionic character of phosphorylated compounds and therefore alter their free energies.

In a given cell, the concentrations of many ions, coenzymes, and metabolites vary with both location and time, often by several orders of magnitude. Intracellular ATP concentrations are maintained within a relatively narrow range, usually 2–10 mM, but the concentrations of ADP and P_i are more variable. Consider a typical cell with [ATP] = 3.0 mM, [ADP] = 0.8 mM, and $[P_i]$ = 4.0 mM. Using Eq. 14-1, the actual free energy of ATP hydrolysis at 37°C is calculated as follows:

$$\Delta G = \Delta G°' + RT \ln\left(\frac{[\text{ADP}]\,[P_i]}{[\text{ATP}]}\right)$$
$$= -30.5\ \text{kJ}\cdot\text{mol}^{-1} + (8.3145\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1})(310\ \text{K})$$
$$\ln\left(\frac{(0.8\times10^{-3}\ \text{M})(4.0\times10^{-3}\ \text{M})}{(3.0\times10^{-3}\ \text{M})}\right)$$
$$= -30.5\ \text{kJ}\cdot\text{mol}^{-1} - 17.6\ \text{kJ}\cdot\text{mol}^{-1}$$
$$= -48.1\ \text{kJ}\cdot\text{mol}^{-1}$$

This value is even greater than the standard free energy of ATP hydrolysis. However, because of the difficulty in accurately measuring the concentrations of particular chemical species in a cell or organelle, the ΔGs for most *in vivo* reactions are little more than estimates. For the sake of consistency, we will, for the most part, use $\Delta G°'$ values in this textbook.

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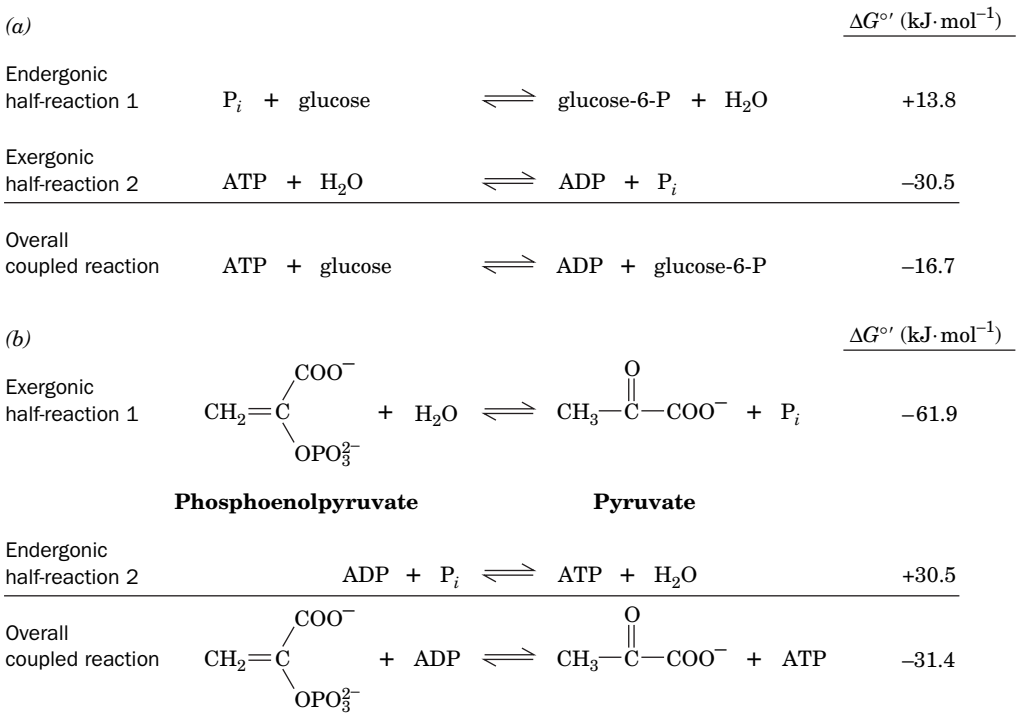


FIG. 14-7 Some coupled reactions involving ATP. (*a*) The phosphorylation of glucose to form glucose-6-phosphate and ADP. (*b*) The phosphorylation of ADP by phosphoenolpyruvate to form ATP and pyruvate. Each reaction has been conceptually decomposed into a direct phosphorylation step (half-reaction 1) and a step in which ATP is hydrolyzed (half-reaction 2). Both half-reactions proceed in the direction that makes the overall reaction exergonic ($\Delta G < 0$).

? In theory, would the transfer of a phosphoryl group from phosphoenolpyruvate to glucose be spontaneous? Would the transfer of a phosphoryl group from glucose-6-phosphate to pyruvate be spontaneous?

P_i is thermodynamically unfavorable ($\Delta G^{\circ\prime} = +13.8$ kJ · mol^{-1}; Fig. 14-7*a*). In cells, however, this reaction is coupled to the exergonic cleavage of ATP (for ATP hydrolysis, $\Delta G^{\circ\prime} = -30.5$ kJ · mol^{-1}), so the overall reaction is thermodynamically favorable ($\Delta G^{\circ\prime} = +13.8 - 30.5 = -16.7$ kJ · mol^{-1}). ATP can be similarly regenerated ($\Delta G^{\circ\prime} = +30.5$ kJ · mol^{-1}) by coupling its synthesis from ADP and P_i to the even more exergonic cleavage of **phosphoenolpyruvate** ($\Delta G^{\circ\prime} = -61.9$ kJ · mol^{-1}; Fig. 14-7*b* and Section 15-2J).

Note that the half-reactions shown in Fig. 14-7 do not actually occur as written in an enzyme active site. **Hexokinase,** the enzyme that catalyzes the formation of glucose-6-phosphate (Fig. 14-7*a*), does not catalyze ATP hydrolysis but instead catalyzes the transfer of a phosphoryl group from ATP directly to glucose. Likewise, **pyruvate kinase,** the enzyme that catalyzes the reaction shown in Fig. 14-7*b,* does not add a free phosphoryl group to ADP but transfers a phosphoryl group from phosphoenolpyruvate to ADP to form ATP.

Phosphoanhydride Hydrolysis Drives Some Biochemical Processes. The free energy of the phosphoanhydride bonds of "high-energy" compounds such as ATP can be used to drive reactions even when the phosphoryl groups are not transferred to another organic compound. For example, ATP hydrolysis (i.e., phosphoryl group transfer directly to H_2O) provides the free energy for the operation of molecular chaperones (Section 6-5B), muscle contraction (Section 7-2B), and transmembrane active transport (Section 10-3). In these processes, proteins undergo conformational changes in response to binding ATP. *The exergonic hydrolysis of ATP and release of ADP and P_i renders these changes irreversible and thereby drives the processes forward.* GTP hydrolysis functions similarly to drive some of the reactions of signal transduction (Section 13-3B) and protein synthesis (Section 27-4).

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In the absence of an appropriate enzyme, phosphoanhydride bonds are stable; that is, they hydrolyze quite slowly, despite the large amount of free energy released by these reactions. This is because these hydrolysis reactions have unusually high free energies of activation ($\Delta G^{\ddagger}$; Section 11-2). Consequently, *ATP hydrolysis is thermodynamically favored but kinetically disfavored.* For example, consider the reaction of glucose with ATP that yields glucose-6-phosphate (Fig. 14-7*a*). $\Delta G^{\ddagger}$ for the nonenzymatic transfer of a phosphoryl group from ATP to glucose is greater than that for ATP hydrolysis, so the hydrolysis reaction predominates (although neither reaction occurs at a biologically significant rate). However, in the presence of the appropriate enzyme, **hexokinase** (Section 15-2A), glucose-6-phosphate is formed far more rapidly than ATP is hydrolyzed. This is because the catalytic influence of the enzyme reduces the activation energy for phosphoryl group transfer from ATP to glucose to less than the activation energy for ATP hydrolysis. This example underscores the point that a thermodynamically favored reaction ($\Delta G < 0$) may not occur at a significant rate in a living system in the absence of a specific enzyme that catalyzes the reaction (i.e., lowers $\Delta G^{\ddagger}$ to increase the rate of product formation; Box 12-2).

Inorganic Pyrophosphatase Catalyzes Additional Phosphoanhydride Bond Cleavage. Although many reactions involving ATP yield ADP and P_i (**orthophosphate cleavage**), others yield AMP and PP_i (**pyrophosphate cleavage**). In these latter cases, the PP_i is rapidly hydrolyzed to 2 P_i by **inorganic pyrophosphatase** ($\Delta G^{\circ\prime} = -19.2$ kJ · mol^{-1}) so that *the pyrophosphate cleavage of ATP ultimately consumes two "high-energy" phosphoanhydride bonds.* The attachment of amino acids to tRNA molecules for protein synthesis is an example of this phenomenon (**Fig. 14-8** and Section 27-2B). The two steps of the reaction are readily reversible because the free energies of hydrolysis of the bonds formed are comparable to that of ATP hydrolysis. The overall reaction is driven to completion by the irreversible hydrolysis of PP_i. Nucleic acid biosynthesis from nucleoside triphosphates also releases PP_i (Sections 25-1 and 26-1). The standard free energy changes of these reactions are around 0, so the subsequent hydrolysis of PP_i is also essential for the synthesis of nucleic acids.

C Some Other Phosphorylated Compounds Have High Phosphoryl Group-Transfer Potentials

"High-energy" compounds other than ATP are essential for energy metabolism, in part because they help maintain a relatively constant level of cellular ATP. *ATP is continually being hydrolyzed and regenerated.* Indeed, experimental

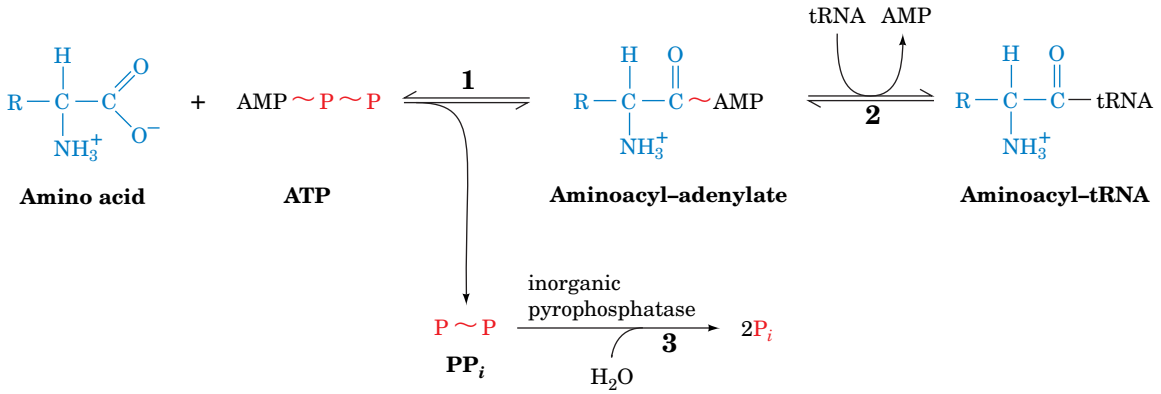


FIG. 14-8 Pyrophosphate cleavage in the synthesis of an aminoacyl-tRNA. (1) In the first reaction step, the amino acid is **adenylated** by ATP. (**2**) In the second step, a tRNA molecule displaces the AMP moiety to form an aminoacyl–tRNA. (**3**) The exergonic hydrolysis of pyrophosphate ($\Delta G^{\circ\prime} = -19.2$ kJ · mol^{-1}) drives the reaction forward.

? **Write the net reaction for this process.**

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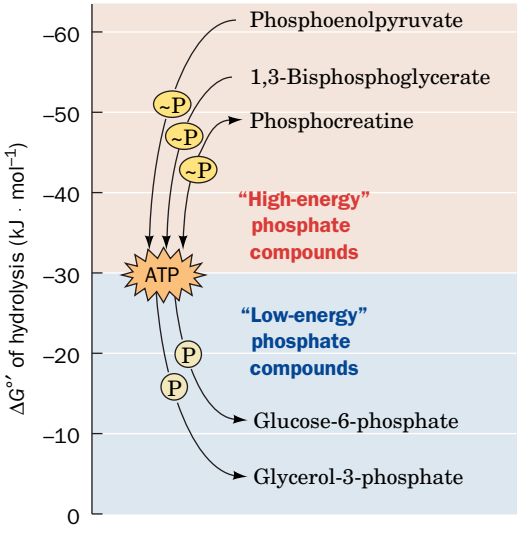


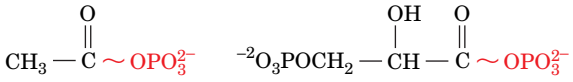
FIG. 14-9 Position of ATP relative to "high-energy" and "low-energy" phosphate compounds. Phosphoryl groups flow from the "high-energy" donors, via the ATP–ADP system, to "low-energy" acceptors.

evidence indicates that the metabolic half-life of an ATP molecule varies from seconds to minutes, depending on the cell type and its metabolic activity. For instance, brain cells have only a few seconds supply of ATP (which partly accounts for the rapid deterioration of brain tissue by oxygen deprivation). An average person at rest consumes and regenerates ATP at a rate of ~3 mol (1.5 kg) per hour and as much as an order of magnitude faster during strenuous activity.

Just as ATP drives endergonic reactions through the exergonic process of phosphoryl group transfer and phosphoanhydride hydrolysis, *ATP itself can be regenerated by coupling its formation to a more highly exergonic metabolic process.* As Table 14-3 indicates, in the thermodynamic hierarchy of phosphoryl-transfer agents, ATP occupies the middle rank. ATP can therefore be formed from ADP by direct transfer of a phosphoryl group from a "high-energy" compound (e.g., phosphoenolpyruvate; Fig. 14-7*b* and Section 15-2J). Such a reaction is referred to as a **substrate-level phosphorylation**. Other mechanisms generate ATP indirectly, using the energy supplied by transmembrane proton concentration gradients. In oxidative metabolism, this process is called **oxidative phosphorylation** (Section 18-3), whereas in photosynthesis, it is termed **photophosphorylation** (Section 19-2D).

The flow of energy from "high-energy" phosphate compounds to ATP and from ATP to "low-energy" phosphate compounds is diagrammed in **Fig. 14-9**. These reactions are catalyzed by enzymes known as **kinases**, which transfer phosphoryl groups from ATP to other compounds or from phosphorylated compounds to ADP. We will revisit these processes in our discussions of carbohydrate metabolism in Chapters 15 and 16.

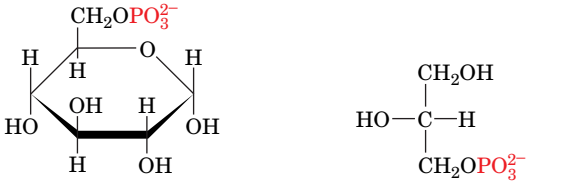
The compounds whose phosphoryl group-transfer potentials are greater than that of ATP have additional stabilizing effects. For example, the hydrolysis of **acyl phosphates** (mixed phosphoric–carboxylic anhydrides), such as **acetyl phosphate** and **1,3-bisphosphoglycerate,**



Acetyl phosphate **1,3-Bisphosphoglycerate**

is driven by the same competing resonance and differential solvation effects that influence the hydrolysis of phosphoanhydrides (Fig. 14-6). Apparently, these effects are more pronounced for acyl phosphates than for phosphoanhydrides, as the rankings in Table 14-3 indicate.

In contrast, compounds such as glucose-6-phosphate and **glycerol-3-phosphate,**



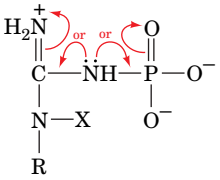
α-D-Glucose-6-phosphate **L-Glycerol-3-phosphate**

which are below ATP in Table 14-3, have no significantly different resonance stabilization or charge separation compared to their hydrolysis products. Their free energies of hydrolysis are therefore much less than those of the preceding "high-energy" compounds.

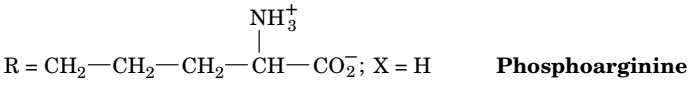
The high phosphoryl group-transfer potentials of **phosphoguanidines,** such as **phosphocreatine** and **phosphoarginine,** largely result from the competing

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resonances in the **guanidino** group, which are even more pronounced than they are in the phosphate group of phosphoanhydrides:



R = $CH_2—CO_2^-$; X = CH_3 **Phosphocreatine**



Phosphoarginine

Consequently, phosphocreatine can transfer its phosphoryl group to ADP to form ATP.

Phosphocreatine Provides a "High-Energy" Reservoir for ATP Formation. Muscle and nerve cells, which have a high ATP turnover, rely on phosphoguanidines to regenerate ATP rapidly. In vertebrates, phosphocreatine is synthesized by the reversible phosphorylation of creatine by ATP catalyzed by **creatine kinase:**

$$\text{ATP} + \text{creatine} \rightleftharpoons \text{phosphocreatine} + \text{ADP} \qquad \Delta G^{\circ\prime} = +12.6\ \text{kJ} \cdot \text{mol}^{-1}$$

Note that this reaction is endergonic under standard conditions; however, *the intracellular concentrations of its reactants and products are such that it operates close to equilibrium* ($\Delta G \approx 0$). Accordingly, when the cell is in a resting state, so [ATP] is relatively high, the reaction proceeds with net synthesis of phosphocreatine, whereas at times of high metabolic activity, when [ATP] is low, the equilibrium shifts so as to yield net synthesis of ATP from phosphocreatine and ADP. *Phosphocreatine thereby acts as an ATP "buffer" in cells that contain creatine kinase.* A resting vertebrate skeletal muscle normally has sufficient phosphocreatine to supply its free energy needs for several minutes (but for only a few seconds at maximum exertion). In the muscles of some invertebrates, such as lobsters, phosphoarginine performs the same function. These phosphoguanidines are collectively named **phosphagens**.

Nucleoside Triphosphates Are Freely Interconverted. Many biosynthetic processes, such as the synthesis of proteins and nucleic acids, require nucleoside triphosphates other than ATP. For example, RNA synthesis requires the ribonucleotides CTP, GTP, and UTP, along with ATP, and DNA synthesis requires dCTP, dGTP, dTTP, and dATP (Section 3-1). All these nucleoside triphosphates (**NTPs**) are synthesized from ATP and the corresponding nucleoside diphosphate (**NDP**) in a reaction catalyzed by the nonspecific enzyme **nucleoside diphosphate kinase:**

$$\text{ATP} + \text{NDP} \rightleftharpoons \text{ADP} + \text{NTP}$$

The $\Delta G^{\circ\prime}$ values for these reactions are nearly 0, as might be expected from the structural similarities among the NTPs. These reactions are driven by the depletion of the NTPs through their exergonic utilization in subsequent reactions.

Other kinases reversibly convert nucleoside monophosphates to their diphosphate forms at the expense of ATP. One of these phosphoryl group-transfer reactions is catalyzed by **adenylate kinase:**

$$\text{AMP} + \text{ATP} \rightleftharpoons 2\ \text{ADP}$$

This enzyme is present in all tissues, where it functions to maintain equilibrium concentrations of the three nucleotides. When AMP accumulates, it is converted to ADP, which can be used to synthesize ATP through substrate-level phosphorylation, oxidative phosphorylation, or photophosphorylation. The reverse reaction helps restore cellular ATP because rapid consumption of ATP increases the level of ADP.

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