6: Bioenergetics and Oxidative Phosphorylation

Overview

Listen

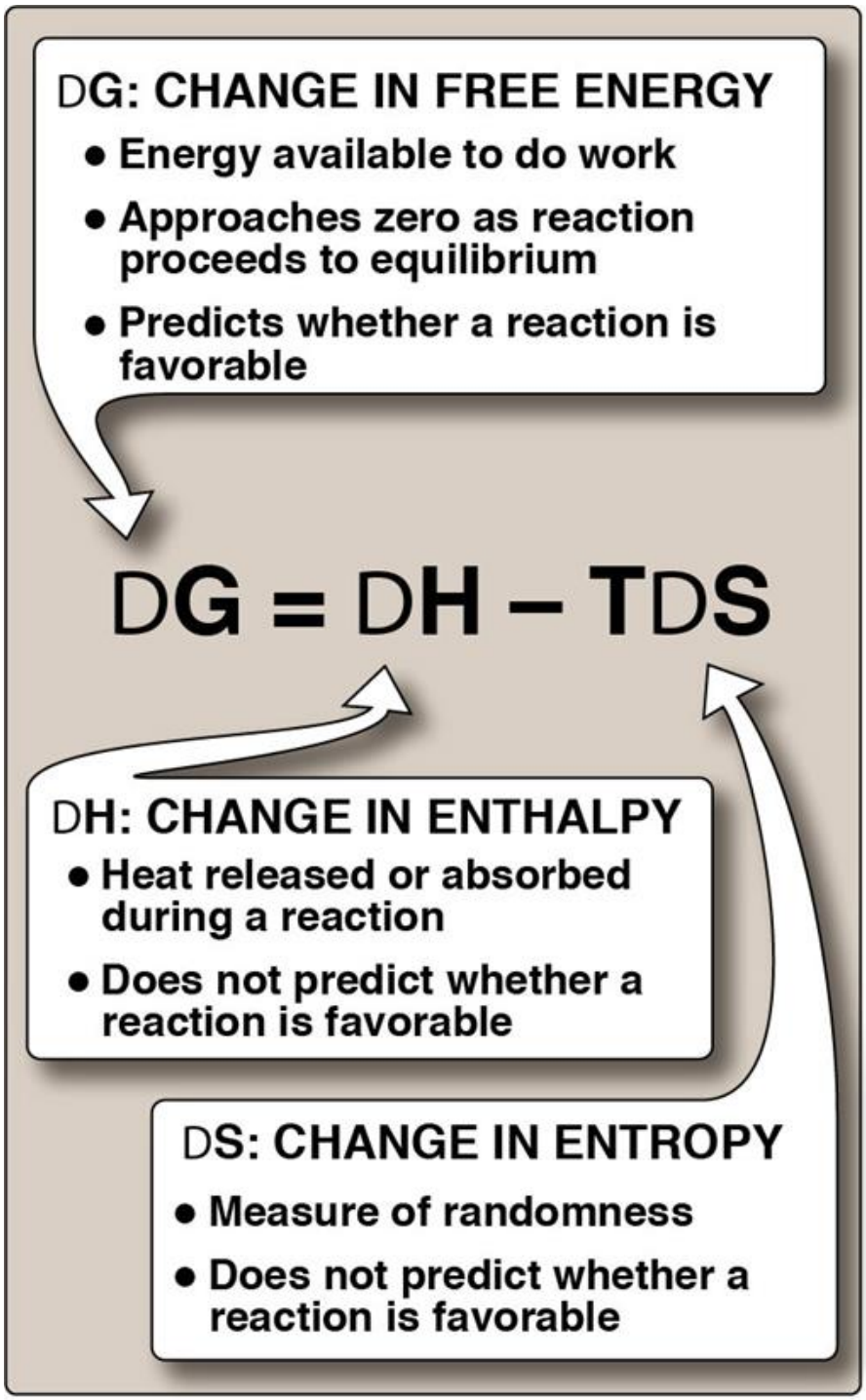
Bioenergetics describes the transfer and utilization of energy in biologic systems and concerns the initial and final energy states of the reaction components. Bioenergetics makes use of a few basic ideas from the field of thermodynamics, particularly the concept of free energy. Because changes in free energy provide a measure of the energetic feasibility of a chemical reaction, they allow prediction of whether a reaction or process can take place. In short, bioenergetics predicts if a process is possible, whereas kinetics measures the reaction rate.

Free energy

Listen

The direction and extent to which a chemical reaction proceeds are determined by the degree to which two factors change during the reaction. These are enthalpy (ΔH, a measure of the change [Δ] in heat content of the reactants and products) and entropy (ΔS, a measure of the change in randomness or disorder of the reactants and products), as shown in Figure 6.1. Neither of these thermodynamic quantities by itself is sufficient to determine whether a chemical reaction will proceed spontaneously in the direction it is written. However, when combined mathematically (see Fig. 6.1), enthalpy and entropy can be used to define a third quantity, free energy (G), which predicts the direction in which a reaction will spontaneously proceed.

FIGURE 6.1



ınd entropy (S).

Free Energy Change

Listen

The **change in free energy** is represented in two ways, ΔG and ΔG^0. The first, ΔG (without the superscript "0"), represents the change in free energy and, thus, the direction of a reaction at any specified concentration of products and reactants. ΔG, then, is a variable. This contrasts with the standard free energy change, ΔG^0 (with the superscript "0"), which is the energy change when reactants and products are at a concentration of 1 mol/l. (Note: The concentration of protons $[H^+]$ is assumed to be 10^{-7}mol/l [i.e., pH = 7]. This may be shown by a prime sign [′], e.g., $\Delta G^{0\prime}$.) Although ΔG^0, a constant, represents energy changes at these nonphysiologic concentrations of reactants and products, it is nonetheless useful in comparing the energy changes of different reactions. Furthermore, ΔG° can readily be determined from measurement of the equilibrium constant.

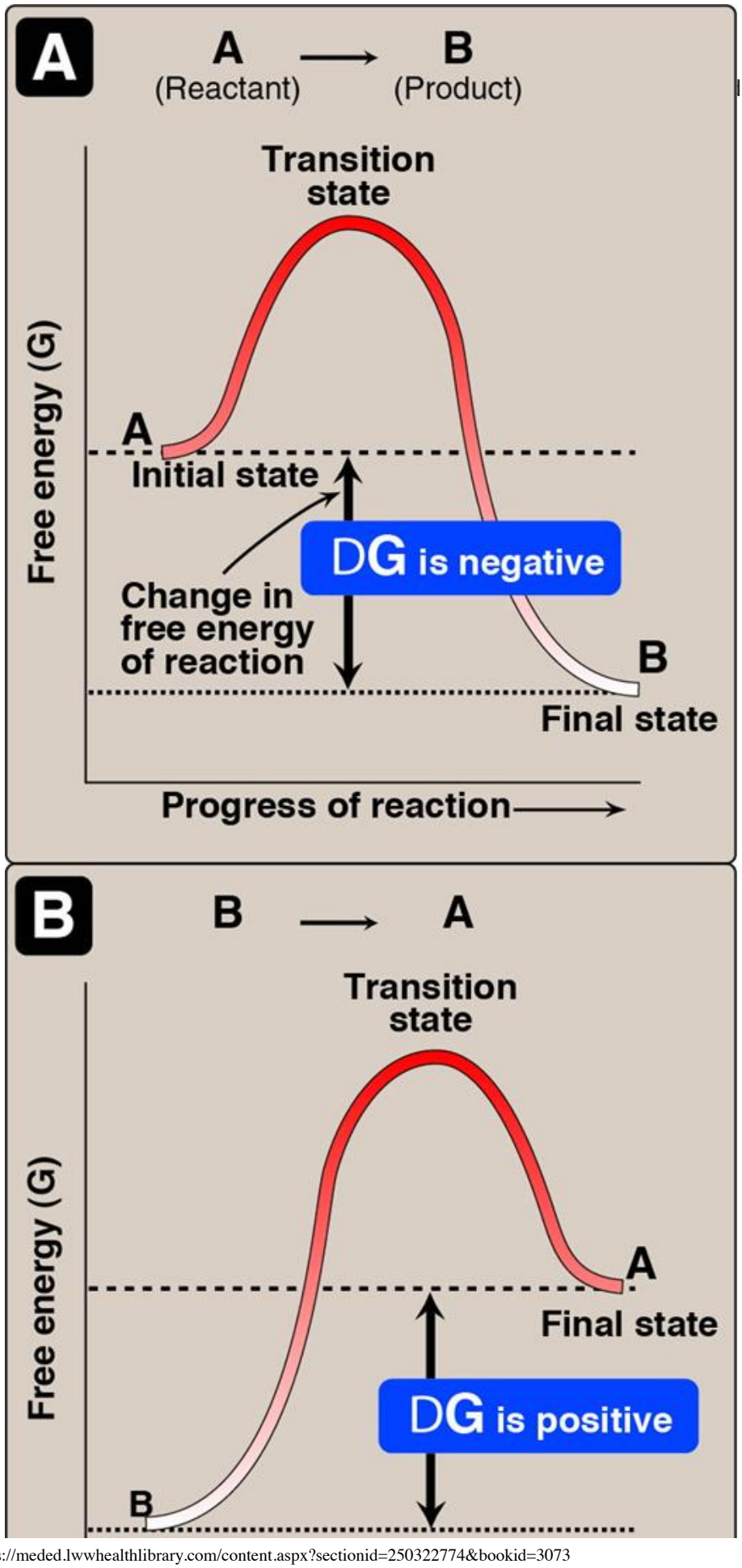
ΔG and reaction direction

The sign of ΔG can be used to predict the direction of a reaction at constant temperature and pressure. Consider the reaction:

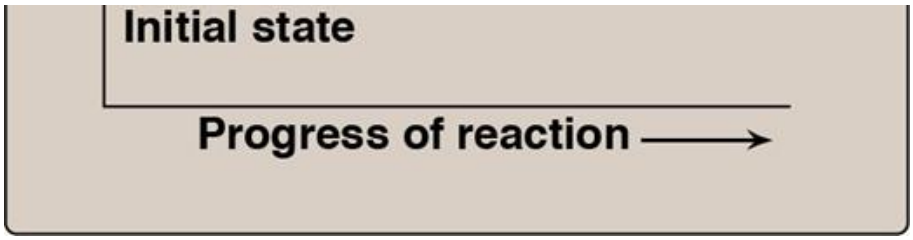
$$A \rightleftarrows B$$

If ΔG is negative, the reaction is considered exergonic with a net loss of energy. In this case, the reaction proceeds spontaneously as written, with A converted to B (Fig. 6.2A). If ΔG is positive, the reaction is endergonic with a net gain of energy. Energy must be added to the system in order for the reaction from B to A to take place (Fig. 6.2B). In cases where ΔG = 0, the reaction is in equilibrium. Note that when a reaction is proceeding spontaneously (ΔG is negative), the reaction will continue until ΔG reaches zero and equilibrium is established.

FIGURE 6.2



luct has a higher free energy than the



ΔG of the forward and reverse reactions

The free energy of the forward reaction (A → B) is equal in magnitude but opposite in sign to that of the reverse reaction (B → A). For example, if ΔG of the forward reaction is −5 kcal/mol, then that of the back reaction is +5 kcal/mol. (Note: ΔG can also be expressed in kilojoules per mole or kJ/mol[1 kcal = 4.2 kJ].)

ΔG and reactant and product concentrations

The ΔG of the reaction A → B depends on the concentrations of the reactant and of the product. At constant temperature and pressure, the following relationship can be derived:

$$\Delta G = \Delta G^0 + RT \ln \frac{[B]}{[A]}$$

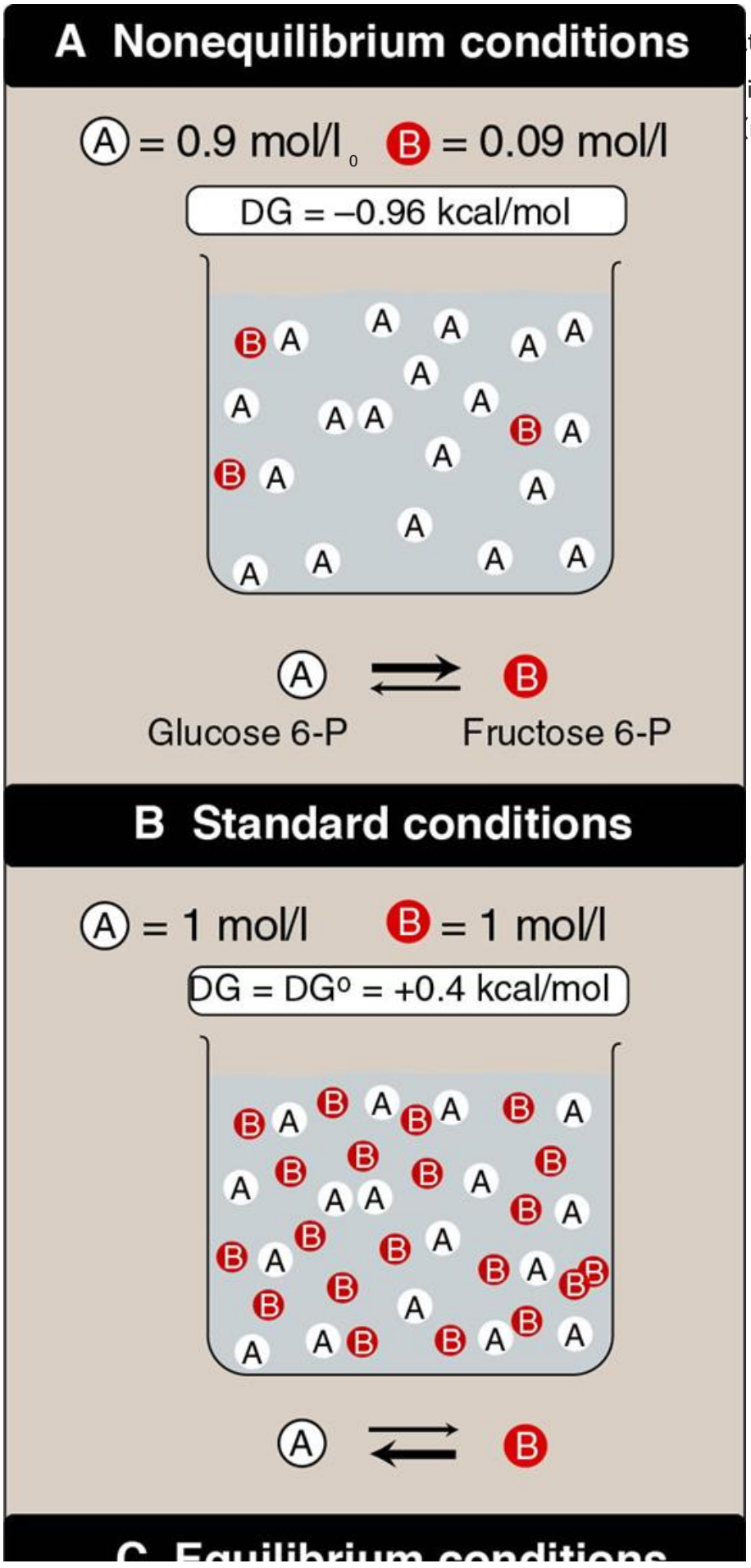
where ΔG^0 is the standard free energy change (see D. below)
R is the gas constant (1.987 cal/mol K)
T is the absolute temperature (K)
[A] and [B] are the actual concentrations of the reactant and product
ln represents the natural logarithm.

A reaction with a positive ΔG° can proceed in the forward direction if the ratio of products to reactants ([B]/[A]) is sufficiently small (i.e., the ratio of reactants to products is large) to make ΔG negative. For example, consider the reaction:

Glucose 6-phosphate ⇄ fructose 6-phosphate

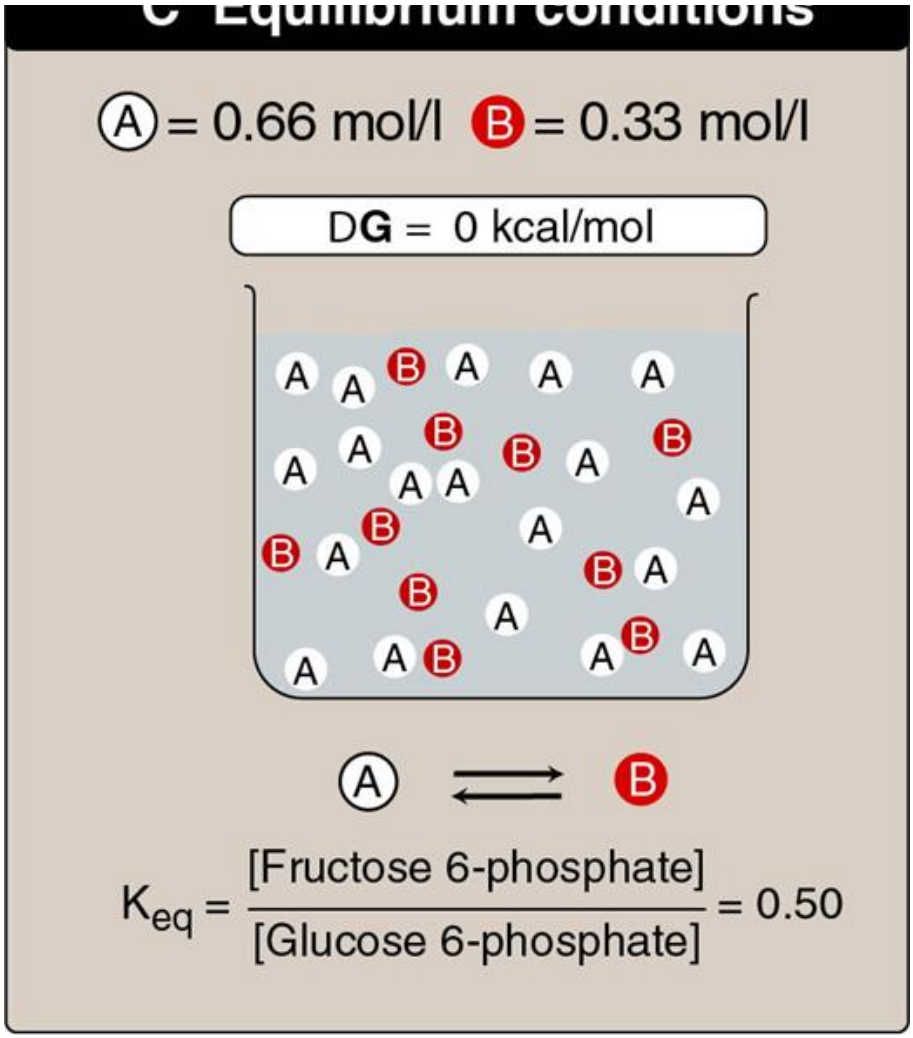
Figure 6.3A shows reaction conditions in which the concentration of reactant, glucose 6-phosphate, is high compared with the concentration of product, fructose 6-phosphate. This means that the ratio of the product to reactant is small, and RT ln([fructose 6-phosphate]/[glucose 6-phosphate]) is large and negative, causing ΔG to be negative despite ΔG^0 being positive. Thus, the reaction can proceed in the forward direction.

FIGURE 6.3



tion of reactant and product.

is negative when the ratio of reactant (middle, **panel B**), and is zero at



Standard free energy change

The standard free energy change, ΔG^0, is equal to the free energy change, ΔG, under standard conditions, when reactants and products are at 1 mol/l concentrations (Fig. 6.3B). Under these conditions, the natural logarithm of the ratio of products to reactants is zero (ln1 = 0), and, therefore, the equation shown at the bottom of the previous page becomes:

$$\Delta G = \Delta G^0 + 0$$

ΔG^0 and reaction direction

Under standard conditions, ΔG° can be used to predict the direction a reaction proceeds because, under these conditions, ΔG^0 is equal to ΔG. However, ΔG° cannot predict the direction of a reaction under physiologic conditions because it is composed solely of constants (R, T, and K_{eq} [see 2. below]) and is not, therefore, altered by changes in product or substrate concentrations.

Relationship between ΔG^0 and K_{eq}

In a reaction A B, a point of equilibrium is reached at which no further net chemical change takes place. In this state, the ratio of [B] to [A] is constant, regardless of the actual concentrations of the two compounds:

$$K_{eq} = \frac{[B]_{eq}}{[A]_{eq}}$$

where K_{eq} is the equilibrium constant, and $[A]_{eq}$ and $[B]_{eq}$ are the concentrations of A and B at equilibrium. If the reaction A B is allowed to reach equilibrium at constant temperature and pressure, then, at equilibrium, the overall ΔG is zero (Fig. 6.3C). Therefore,

$$\Delta G = 0 = \Delta G^0 + RT \ln \frac{[B]_{eq}}{[A]_{eq}}$$

where the actual concentrations of A and B are equal to the equilibrium concentrations of reactant and product ($[A]_{eq}$ and $[B]_{eq}$), and their ratio is equal to the K_{eq}. Thus,

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

This equation allows some simple predictions:

If $K_{eq} = 1$, then $\Delta G^0 = 0$
If $K_{eq} > 1$, then $\Delta G^0 < 0$
If $K_{eq} < 1$, then $\Delta G^0 > 0$

ΔG^0s of two consecutive reactions

The ΔG^0s are additive in any sequence of consecutive reactions, as are the ΔGs. For example:

Glucose + ATP	→ glucose 6-phosphate + adenosine diphosphate (ADP)	ΔG^0 = −4,000 cal/mol
Glucose 6-phosphate	→ fructose 6-phosphate	ΔG^0 = +400 cal/mol
Glucose + ATP	→ fructose 6-phosphate + ADP	ΔG^0 = −3,600 cal/mol

ΔGs of a pathway

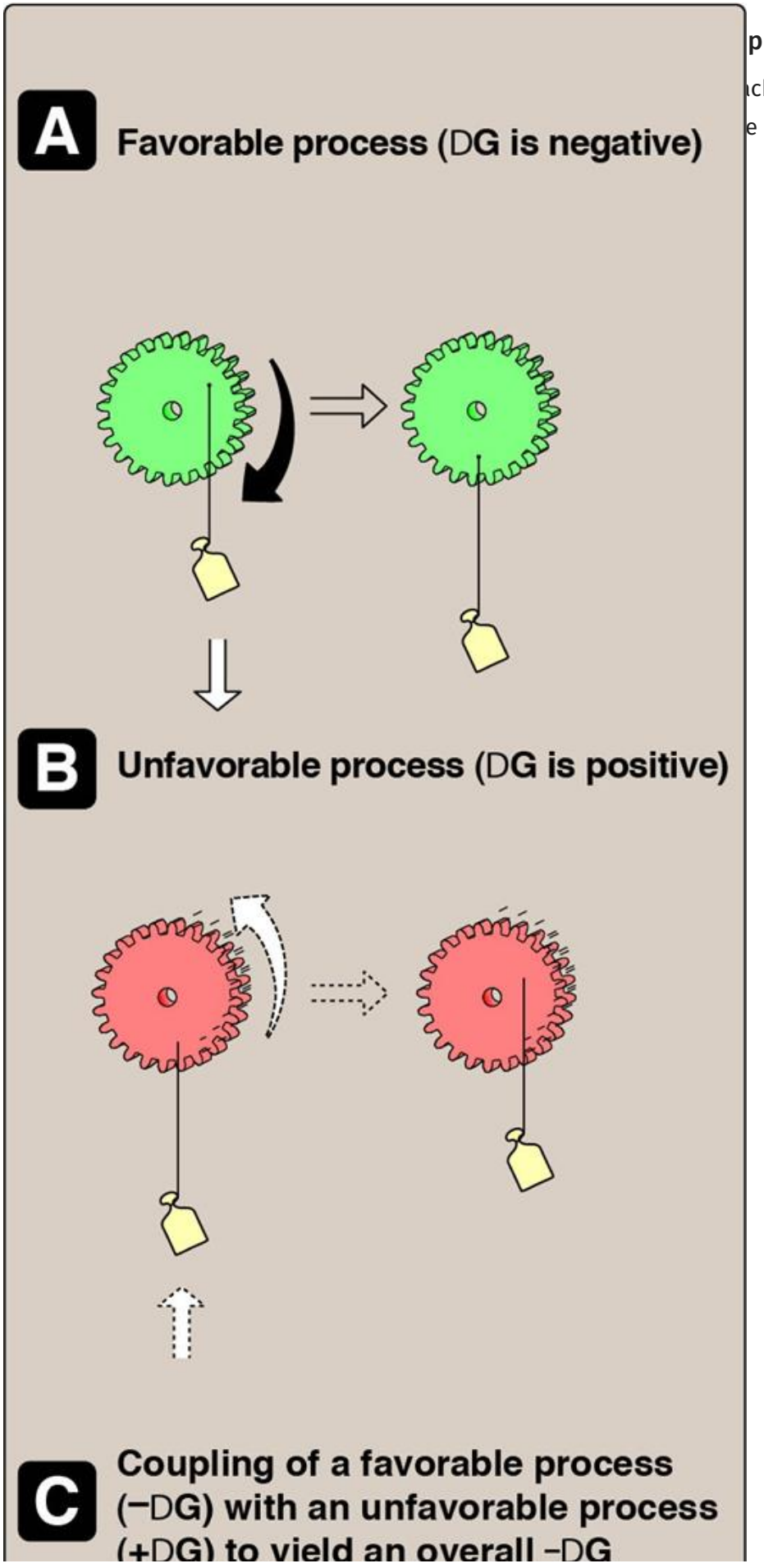
The additive property of ΔG is very important in biochemical pathways through which substrates must pass in a particular direction (e.g., A → B → C → D → ...). As long as the sum of the ΔGs of the individual reactions is negative, the pathway can proceed as written, even if some of the individual reactions of the pathway have a positive ΔG. However, the actual rates of the reactions depend on the lowering of activation energies (E_a) by the enzymes that catalyze the reactions.

ATP: An Energy Carrier

Listen

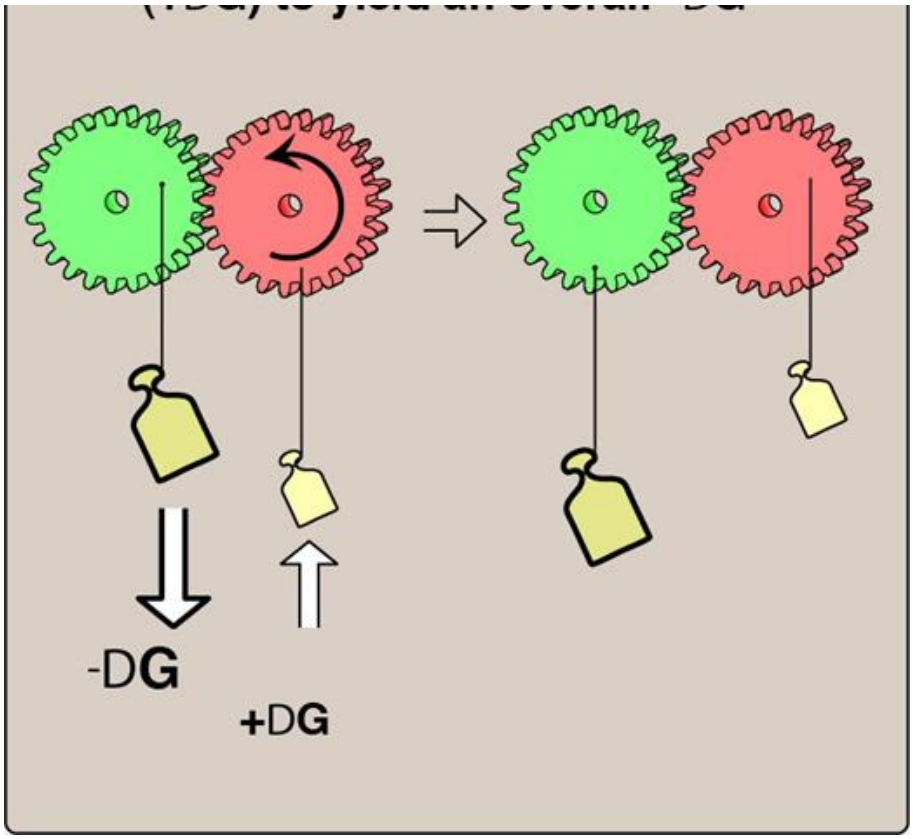
Reactions with a large positive ΔG, are made possible by coupling the endergonic movement of ions with a second, spontaneous process with a large negative ΔG such as the exergonic hydrolysis of ATP (see p. 96). Figure 6.4 shows a mechanical model of energy coupling. The simplest example of energy coupling in biologic reactions occurs when the energy-requiring and the energy-yielding reactions share a common intermediate.

FIGURE 6.4



processes.

achieves the lowest energy state. **B:** The
e energetically favorable movement



Common intermediates

Two chemical reactions have a common intermediate when they occur sequentially in that the product of the first reaction is a substrate for the second. For example, given the reactions

$$A + B \rightarrow C + D$$

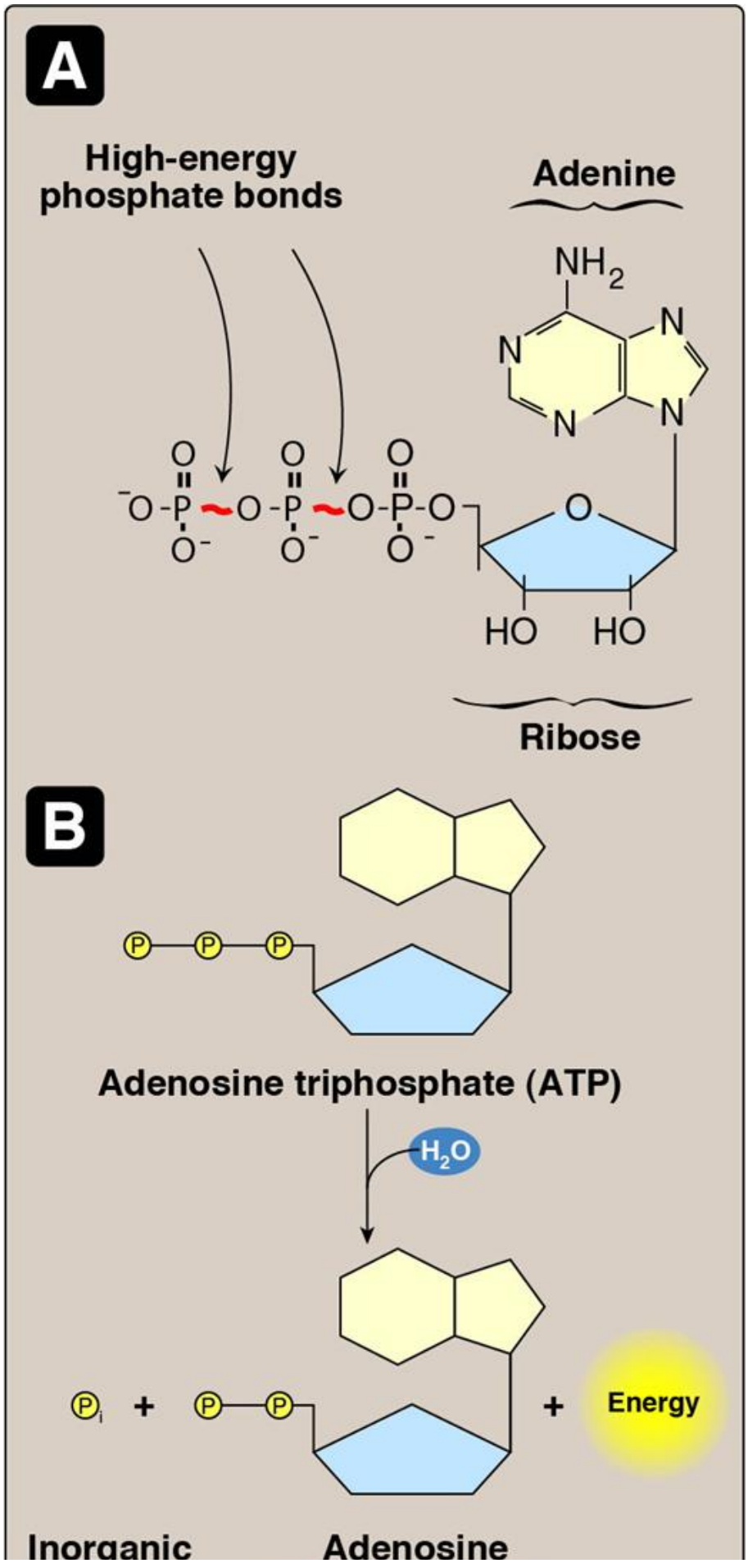
$$D + X \rightarrow Y + Z$$

D is the common intermediate and can serve as a carrier of chemical energy between the two reactions. (Note: The intermediate may be linked to an enzyme.) Many coupled reactions use ATP to generate a common intermediate. These reactions may involve the transfer of a phosphate group from ATP to another molecule. Other reactions involve the transfer of phosphate from an energy-rich intermediate to ADP, forming ATP.

Energy carried by ATP

ATP consists of a molecule of adenosine to which three phosphate groups are attached (Fig. 6.5). Removal of one phosphate produces ADP, and removal of two phosphates produces adenosine monophosphate (AMP). For ATP, the ΔG^0 of hydrolysis is approximately –7.3 kcal/mol for each of the two terminal phosphate groups. Because of this large negative ΔG^0 of hydrolysis, ATP is called a high-energy phosphate compound. (Note: Adenine nucleotides are interconverted [2 ADP ⇄ ATP + AMP] by adenylate kinase.)

FIGURE 6.5


A
High-energy phosphate bonds
Adenine
NH2
N
N
N
N
O
O
O
-O-P~O-P~O-P-O
O-
O-
O-
O
HO
HO
Ribose
B
P
P
P
Adenosine triphosphate (ATP)
H2O
Pi
+
P
P
+
Energy
Inorganic
Adenosine

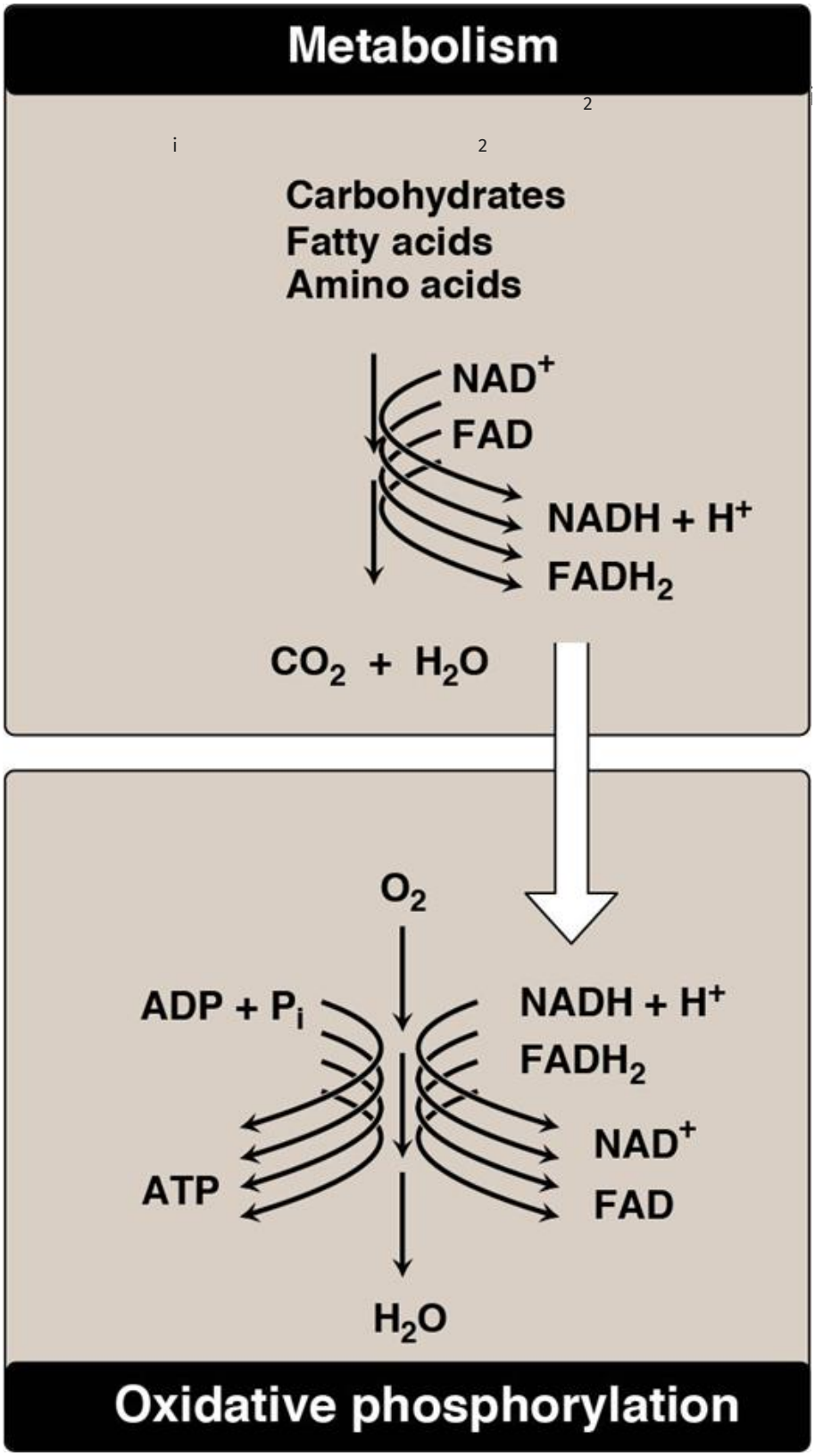
phosphate diphosphate (ADP)

Electron Transport Chain

Listen

Energy-rich molecules, such as glucose, are metabolized by a series of oxidation reactions ultimately yielding carbon dioxide and water (H_2O) (Fig. 6.6). The metabolic intermediates of these reactions donate electrons to specific coenzymes, nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD), to form the energy-rich reduced forms, NADH and flavin adenine dinucleotide ($FADH_2$). These reduced coenzymes can, in turn, each donate a pair of electrons to a specialized set of electron carriers, collectively called the electron transport chain (ETC), described in this section. As electrons are passed down the ETC, they lose much of their free energy. This energy is used to move H^+ across the inner mitochondrial membrane, creating a H^+ gradient that drives the production of ATP from ADP and inorganic phosphate (P_i). The coupling of electron transport with ATP synthesis is called oxidative phosphorylation, sometimes denoted as OXPHOS. It proceeds continuously in all tissues that contain mitochondria. Note that the free energy not trapped as ATP is used to drive ancillary reactions such as transport of calcium ions into mitochondria and to generate heat.

FIGURE 6.6



inucleotide; ADP = adenosine

2

i 2

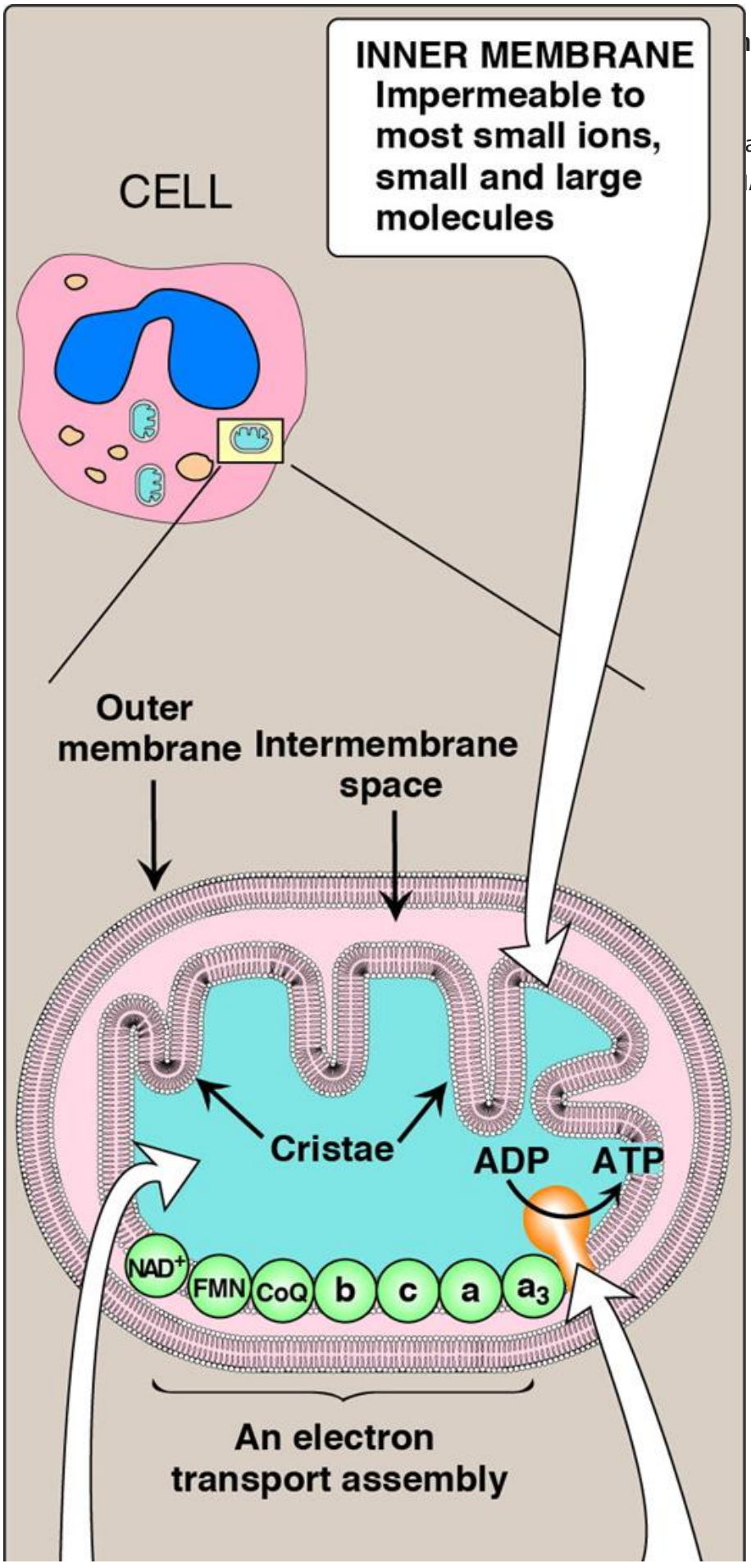
Mitochondrial electron transport chain

The ETC (except for cytochrome c) is located in the inner mitochondrial membrane and is the final common pathway by which electrons derived from different fuels of the body flow to oxygen (O_2), reducing it to H_2O (see Fig. 6.6).

Mitochondrial membranes

Mitochondria have an outer and an inner membrane separated by the intermembrane space. The outer membrane contains specialized channels formed by the protein porin, making it freely permeable to most ions and small molecules. The inner membrane is a specialized structure that is impermeable to most small ions, including H^+, and small molecules such as ATP, ADP, pyruvate, and other metabolites important to mitochondrial function (Fig. 6.7). Transport proteins are required to move ions or molecules across this membrane. The inner mitochondrial membrane is unusually rich in proteins, over half of which are directly involved in oxidative phosphorylation. It also contains convolutions, called cristae, which greatly increase its surface area.

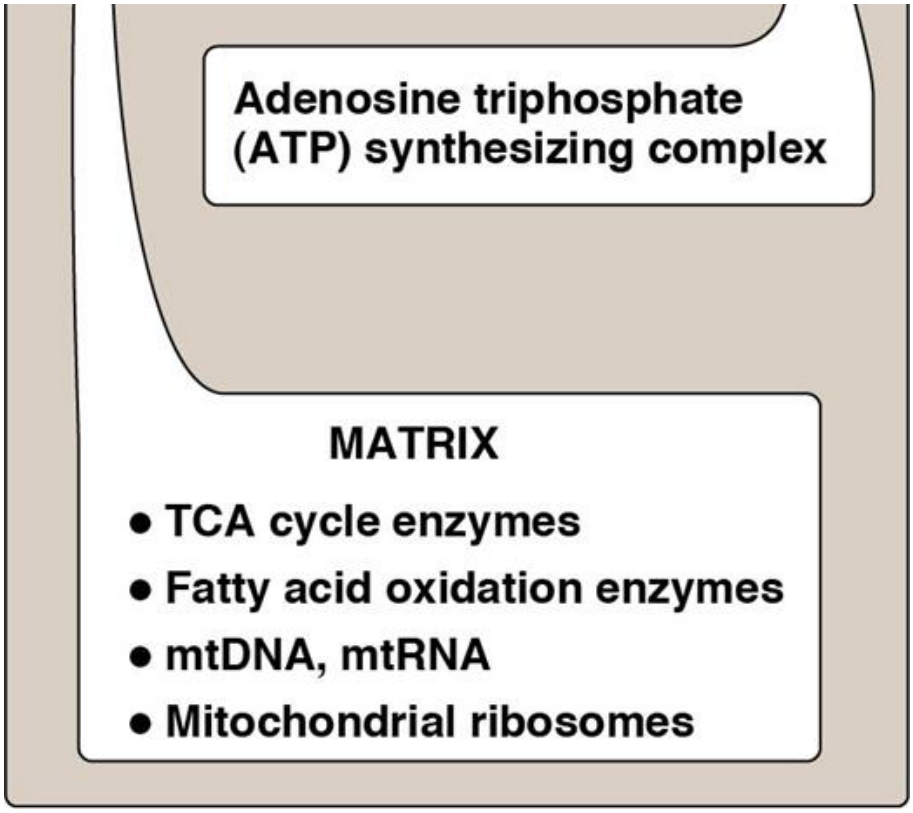
FIGURE 6.7



of the electron transport chain

able, and the milieu of the

A = ribonucleic acid; ADP = adenosine



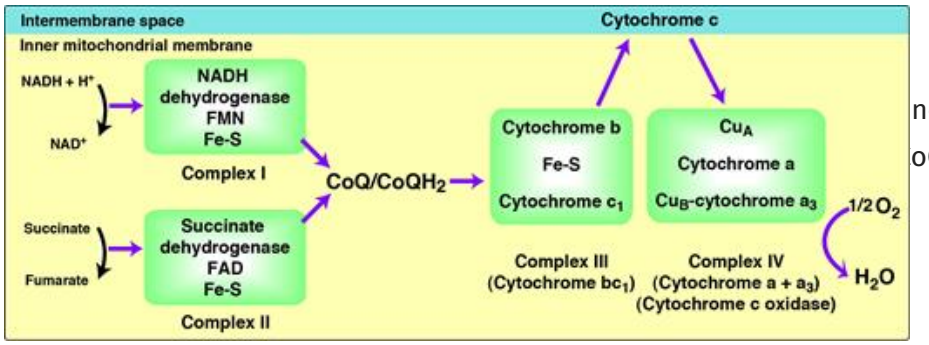
Mitochondrial matrix

The gel-like solution of the interior of the mitochondria is called the matrix and it is also rich in proteins. These include the enzymes responsible for the oxidation of pyruvate, amino acids, and fatty acids (by β-oxidation) as well as those of the tricarboxylic acid (TCA) cycle. The synthesis of glucose, urea, and heme occurs partially in the matrix of mitochondria. In addition, the matrix contains NAD^+ and FAD (the oxidized forms of the two coenzymes that are required as electron acceptors), and ADP and P_i, which are used to produce ATP. (Note: The matrix also contains mitochondrial deoxyribonucleic acid [mtDNA], ribonucleic acid [mtRNA], and ribosomes.)

Organization

The inner mitochondrial membrane contains four separate protein complexes, called Complexes I, II, III, and IV that each contain part of the ETC (Fig. 6.8). These complexes accept or donate electrons to the relatively mobile electron carrier coenzyme Q (CoQ) and **cytochrome c**. Each carrier in the ETC can receive electrons from an electron donor and can subsequently donate electrons to the next acceptor in the chain. The electrons ultimately combine with O_2 and H^+ to form H_2O. This requirement for O_2 makes the electron transport process the respiratory chain, which accounts for the greatest portion of the body's use of O_2.

FIGURE 6.8



ne dinucleotide; FMN = flavin
oQ = coenzyme Q; Cu = copper.

Reactions

With the exception of CoQ, which is a lipid-soluble quinone, all members of the ETC are proteins. These may function as enzymes as is the case with the flavin-containing dehydrogenases, may contain iron as part of an iron–sulfur (Fe–S) center, may contain iron as part of the porphyrin prosthetic group of heme as in the cytochromes, or may contain copper (Cu) as does the cytochrome a + a_3 complex.

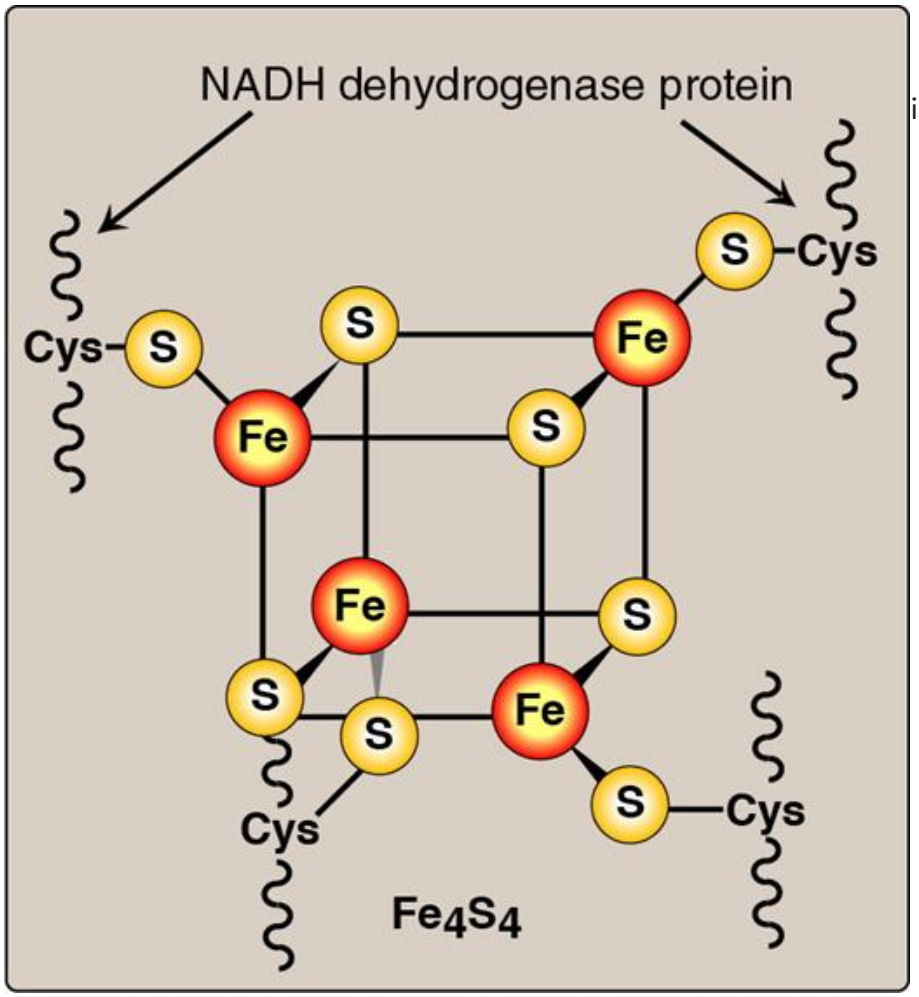
NADH formation

NAD^+ is reduced to NADH by dehydrogenases that remove two hydrogen atoms from their substrate. (Note: For examples of these reactions, see the discussion of the dehydrogenases of the TCA cycle, p. 123.) Both electrons but only one H^+ (i.e., a hydride ion [$:H^-$]) are transferred to the NAD^+, forming NADH plus a free H^+.

NADH dehydrogenase

The free H^+ plus the hydride ion carried by NADH are transferred to NADH dehydrogenase, a protein complex (Complex I) embedded in the inner mitochondrial membrane. Complex I has a tightly bound molecule of flavin mononucleotide (FMN), a coenzyme structurally related to FAD that accepts the two hydrogen atoms (two electrons + two H^+), becoming $FMNH_2$. NADH dehydrogenase also contains peptide subunits with Fe–S centers (Fig. 6.9). At Complex I, electrons move from NADH to FMN to the iron of the Fe–S centers and then to CoQ. As electrons flow, they lose energy. This energy is used to pump four H^+ across the inner mitochondrial membrane, from the matrix to the intermembrane space.

FIGURE 6.9



ide adenine dinucleotide; Cys =

Succinate dehydrogenase

At Complex II, electrons from the succinate dehydrogenase–catalyzed oxidation of succinate to fumarate move from the coenzyme, **$FADH_2$**, to an Fe–S protein, and then to CoQ. (Note: Because no energy is lost in this process, no H^+ are pumped at Complex II.)

Coenzyme Q

CoQ, also known as ubiquinone, is a quinone derivative with a long, hydrophobic isoprenoid tail, made from an intermediate of cholesterol synthesis (see Chapter 18). CoQ is a mobile electron carrier and can accept electrons from NADH dehydrogenase (Complex I), from succinate dehydrogenase (Complex II) and from other mitochondrial dehydrogenases, such as glycerol 3-phosphate dehydrogenase and acyl CoA dehydrogenases. CoQ transfers electrons to Complex III (cytochrome bc_1). Thus, a function of CoQ is to link the flavoproteindehydrogenases to the cytochromes.

Cytochromes

The remaining members of the ETC are cytochrome proteins. Each contains a heme group which is a porphyrin ring plus iron. Unlike the heme groups of hemoglobin, the cytochrome iron is reversibly converted from its ferric (Fe^{3+}) to its ferrous (Fe^{2+}) form as a normal part of its function as an acceptor and donor of electrons. Electrons are passed along the chain from cytochrome bc_1 (Complex III), to cytochrome c, and then to cytochromes a + a_3 ([Complex IV], see Fig. 6.8). As electrons flow, four H^+ are pumped across the inner mitochondrial membrane at Complex III and two at Complex IV. (Note: Cytochrome c is located in the intermembrane space, loosely associated with the outer face of the inner membrane. As seen with CoQ, cytochrome c is a mobile electron carrier.)

Cytochrome a + a_3

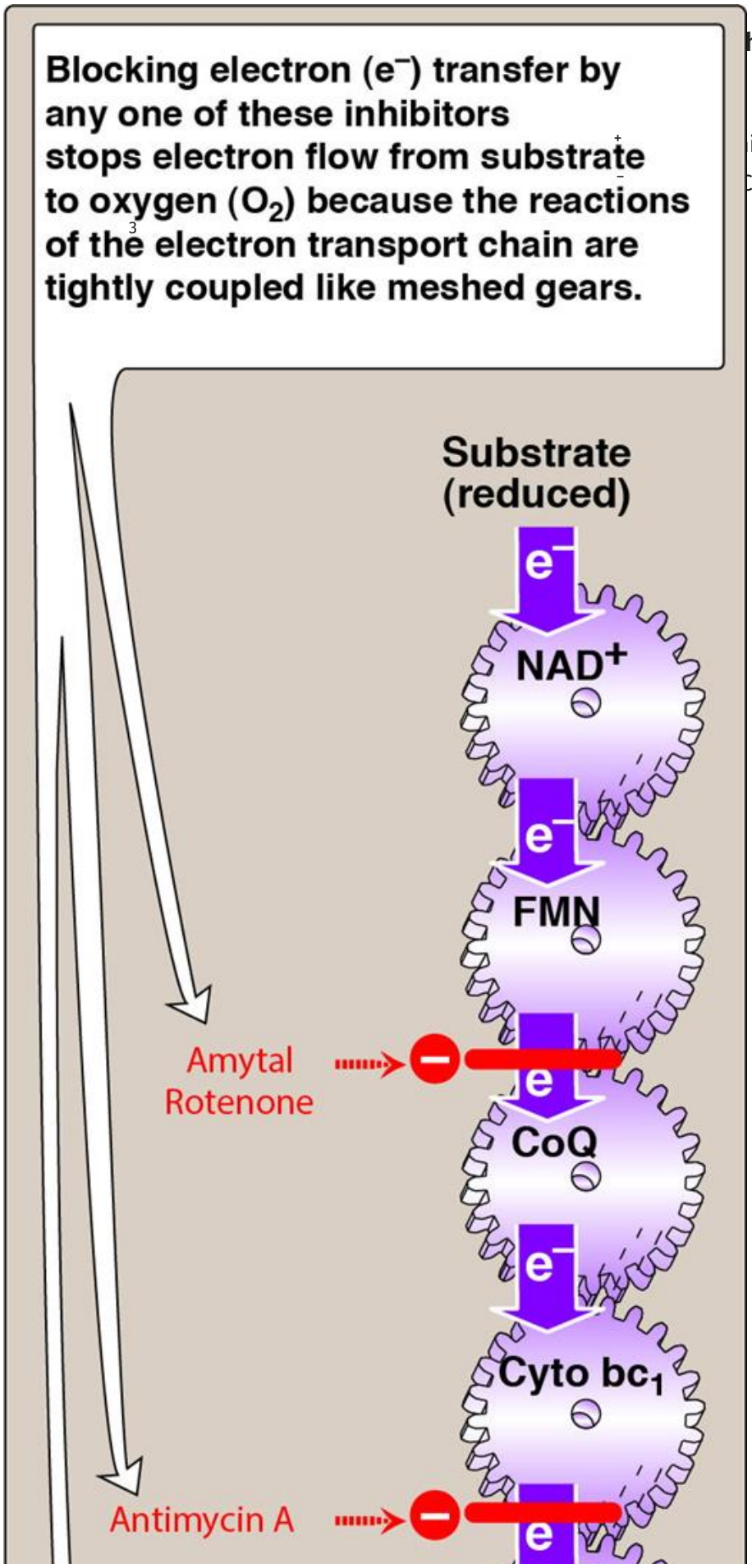
Because this cytochrome complex (Complex IV) is the only electron carrier in which the heme iron has an available coordination site that can react directly with O_2, it also is called cytochrome c oxidase. At Complex IV, the transported electrons, O_2, and free H^+ are brought together, and O_2 is reduced to H_2O (see Fig. 6.8). (Note: Four electrons are required to reduce one molecule of O_2 to two molecules of H_2O.) Cytochrome c oxidase contains Cu atoms that are required for this complicated reaction to occur. Electrons move from Cu_A to cytochrome a to cytochrome a_3 (in association with Cu_B) to O_2.

Site-specific inhibitors

Inhibitors of specific sites in the ETC have been identified and are illustrated in Figure 6.10. These respiratory inhibitors prevent the passage of electrons by binding to a component of the chain, blocking the oxidation–reduction reaction. Therefore, all electron carriers before the block are fully reduced, whereas those located after the block are oxidized. (Note: Inhibition of the ETC inhibits ATP synthesis because these processes are tightly coupled.)

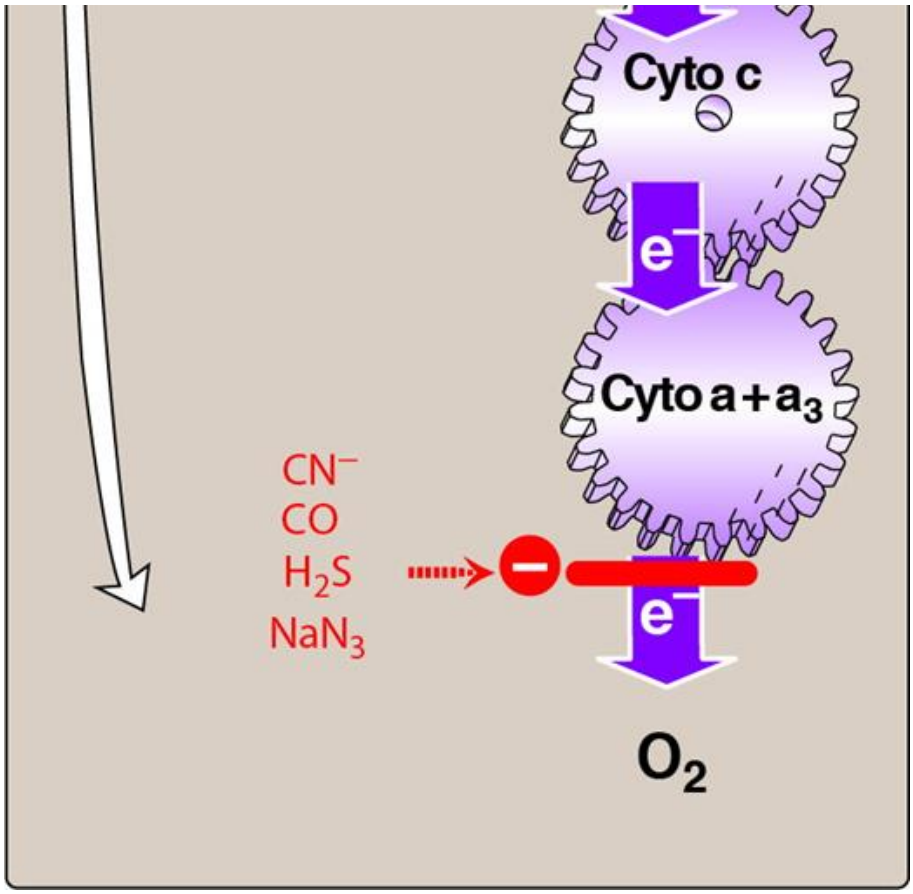
Leakage of electrons from the ETC produces reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). ROS damages DNA and proteins and cause lipid peroxidation. Enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase are cellular defenses against ROS (see p. 163).

FIGURE 6.10



hanical model for the coupling of

ide adenine dinucleotide; FMN = flavin
O = carbon monoxide; H_2S = hydrogen



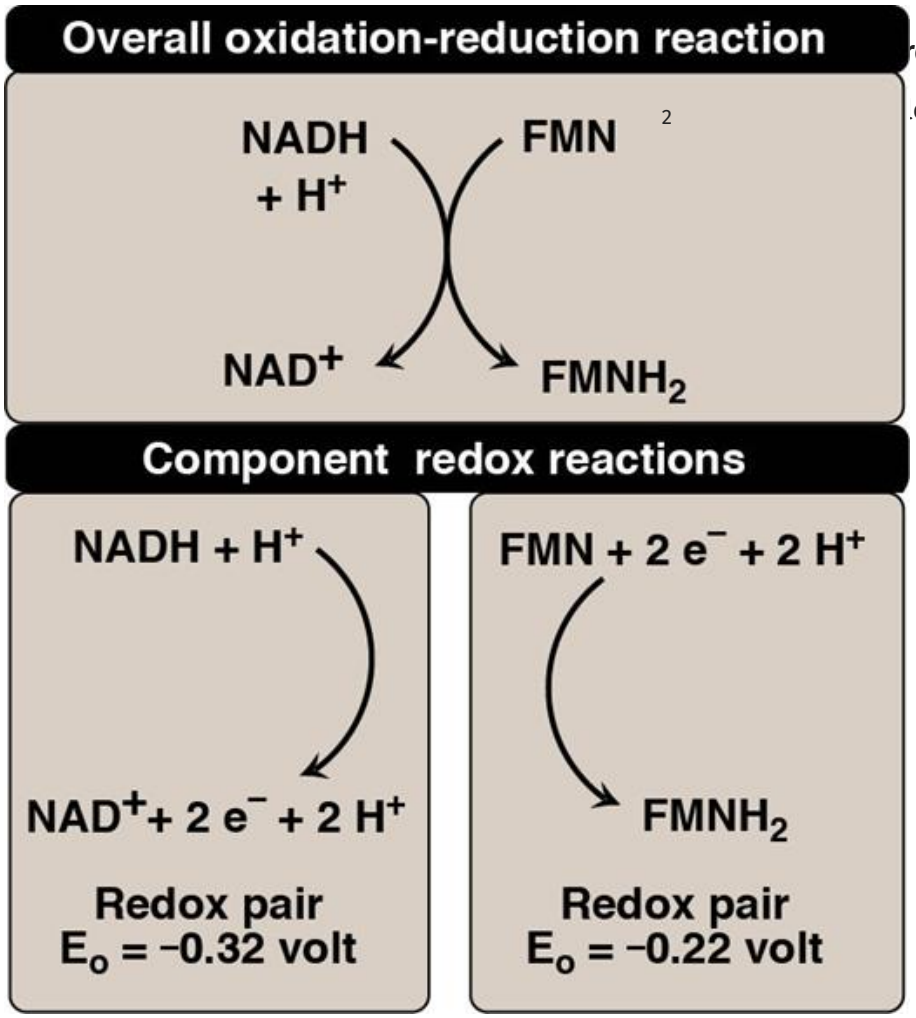
Free energy release during electron transport

The free energy released as electrons is transferred along the ETC from an electron donor (reducing agent or reductant) to an electron acceptor (oxidizing agent or oxidant) is used to pump H^+ at Complexes I, III, and IV. (Note: The electrons can be transferred as hydride ions to NAD^+; as hydrogen atoms to FMN, CoQ, and FAD; or as electrons to cytochromes.)

Redox pairs

Oxidation (loss of electrons) of one substance is always accompanied by reduction (gain of electrons) of a second. For example, Figure 6.11 shows the oxidation of NADH to NAD^+ by NADH dehydrogenase at Complex I, accompanied by the reduction of FMN, the prosthetic group, to $FMNH_2$. Such redox reactions can be written as the sum of two separate half reactions, one an oxidation and the other a reduction (see Fig. 6.11). NAD^+ and NADH form a redox pair, as do FMN and $FMNH_2$. Redox pairs differ in their tendency to lose electrons. This tendency is a characteristic of a particular redox pair and can be quantitatively specified by a constant, E_0 (the standard reduction potential), with units in volts.

FIGURE 6.11



reactions.

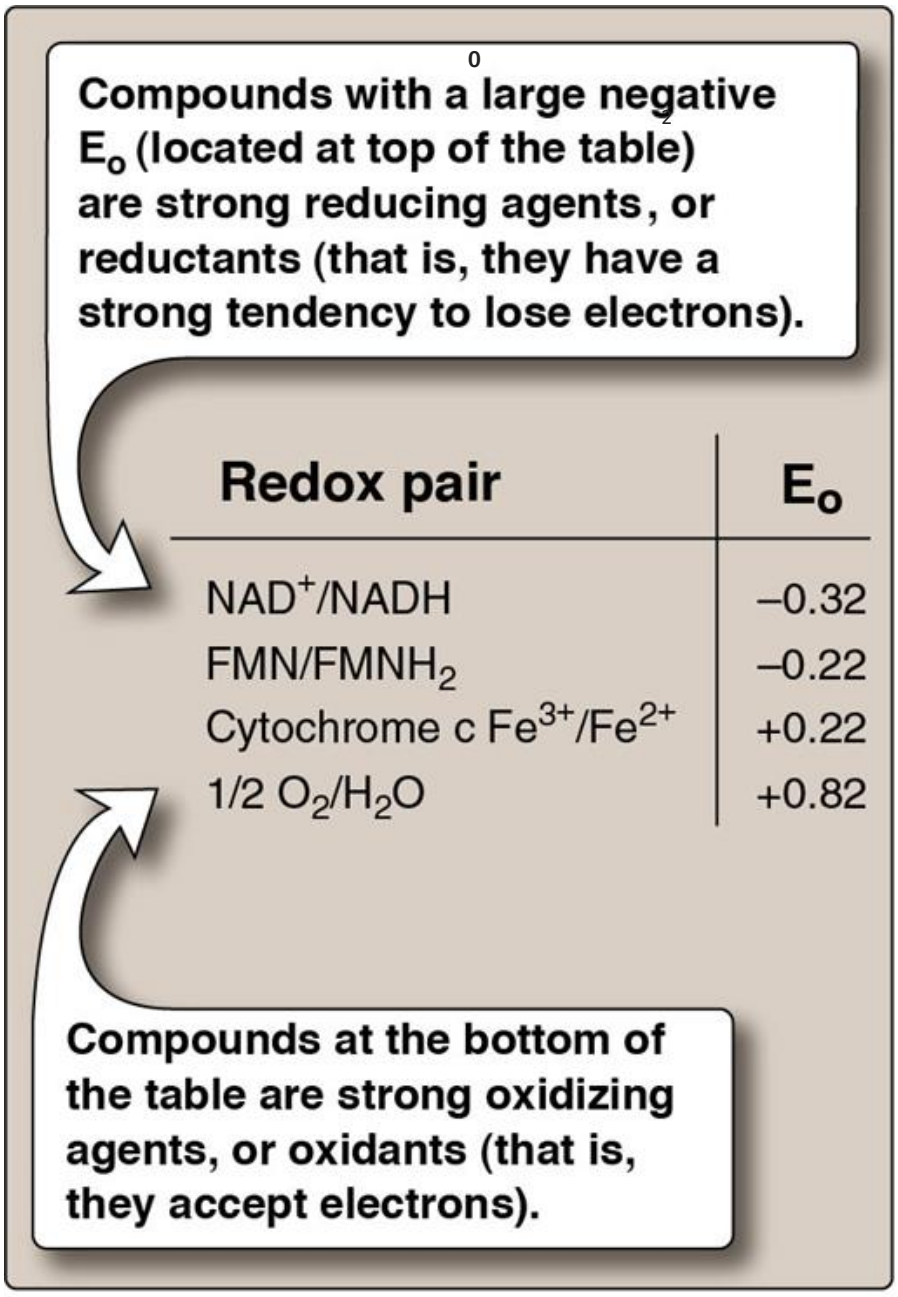
2

eotide; e^- = electron; H^+ = proton; E0 =

Standard reduction potential

The E_0 of various redox pairs can be ordered from the most negative E_0 to the most positive. The more negative the E_0 of a redox pair, the greater the tendency of the reductant member of that pair to lose electrons. The more positive the E_0, the greater the tendency of the oxidant member of that pair to accept electrons. Therefore, electrons flow from the pair with the more negative E_0 to that with the more positive E_0. The E_0 values for some members of the ETC are shown in Figure 6.12. (Note: The components of the chain are arranged in order of increasingly positive E_0 values.)

FIGURE 6.12



.eotide; Fe = iron.

Relationship of ΔG^0 to ΔE_0

The ΔG^0 is related directly to the magnitude of the change in E_0:

$$\Delta G^0 = -nF\,\Delta E_0,$$

where n = number of electrons transferred (1 for a cytochrome, 2 for NADH, $FADH_2$, and CoQ)
F = Faraday constant (23.1 kcal/volt mol)
ΔE_0 = E_0 of the electron-accepting pair minus the E_0 of the electron-donating pair
ΔG^0 = change in the standard free energy

ΔG^0 of ATP

The ΔG° for the phosphorylation of ADP to ATP is +7.3 kcal/mol. The transport of a pair of electrons from NADH to O_2 through the ETC releases 52.6 kcal. Therefore, more than sufficient energy is available to produce three ATP from three ADP and three P_i (3 × 7.3 = 21.9 kcal/mol), sometimes expressed as a P/O ratio (ATP made per O atom reduced) of 3:1. The remaining calories are used for ancillary reactions or released as heat. (Note: The P:O for $FADH_2$ is 2:1 because Complex I is bypassed.)

Phosphorylation of ADP to ATP

Listen

The transfer of electrons down the ETC is energetically favored because NADH is a strong electron donor and O_2 is an avid electron acceptor. However, the flow of electrons does not directly result in ATP synthesis.

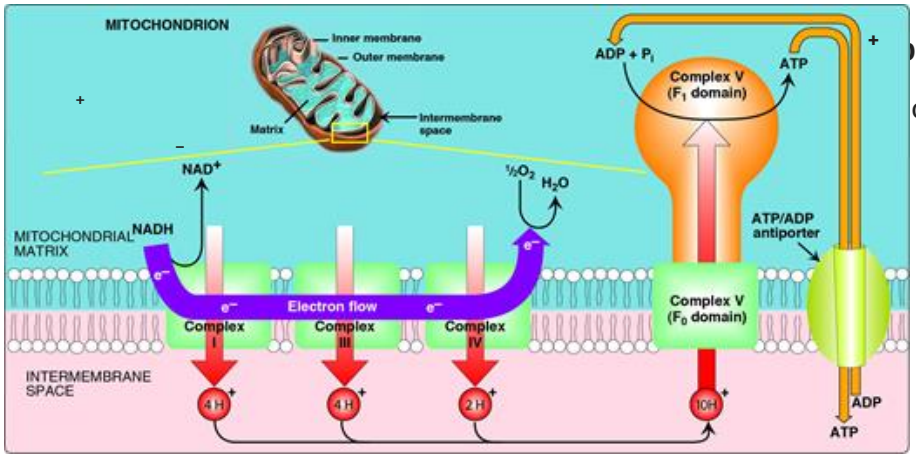
Chemiosmotic hypothesis

The chemiosmotic hypothesis (also known as the Mitchell hypothesis) explains how the free energy generated by the transport of electrons by the ETC is used to produce ATP from ADP + P_i.

Proton pump: Electron transport

is coupled to ADP phosphorylation by the pumping of H^+ across the inner mitochondrial membrane, from the matrix to the intermembrane space, at Complexes I, III, and IV. For each pair of electrons transferred from NADH to O_2, 10 H^+ are pumped. This creates an electrical gradient (with more positive charges on the cytosolic side of the membrane than on the matrix side) and a pH (chemical) gradient (the cytosolic side of the membrane is at a lower pH than the matrix side), as shown in Figure 6.13. The energy (proton-motive force) generated by these gradients is sufficient to drive ATP synthesis. Thus, the H^+ gradient serves as the common intermediate that couples oxidation to phosphorylation.

FIGURE 6.13



umping.

oxidized. (Note: $H^+$are not pumped at

ATP synthase

The multisubunit enzyme ATP synthase ([Complex V], Fig. 6.14) synthesizes ATP using the energy of the H^+ gradient. It contains a membrane domain (F_o) that spans the inner mitochondrial membrane and an extramembranous domain (F_1) that appears as a sphere that protrudes into the mitochondrial matrix (see Fig. 6.13). The chemiosmotic hypothesis proposes that after H^+ have been pumped to the cytosolic side of the inner mitochondrial membrane, they reenter the matrix by passing through a H^+ channel in the F_o domain, driving rotation of the c ring of F_o and, at the same time, dissipating the pH and electrical gradients. Rotation in F_o causes conformational changes in the three β subunits of F_1 that allow them to bind ADP + P_i, phosphorylate ADP to ATP, and release ATP. One complete rotation of the c ring produces three ATP. (Note: ATP synthase is also called F_1/F_o-ATPase because the enzyme can also catalyze the hydrolysis of ATP to ADP and P_i.)

Coupling in oxidative phosphorylation

In normal mitochondria, ATP synthesis is coupled to electron transport through the H^+ gradient. Increasing (or decreasing) one process has the same effect on the other. For example, hydrolysis of ATP to ADP and P_i in energy-requiring reactions increases the availability of substrates for ATP synthase and, thus, increases H^+ flow through the enzyme. Electron transport and H^+ pumping by the ETC increase to maintain the H^+ gradient and allow ATP synthesis.

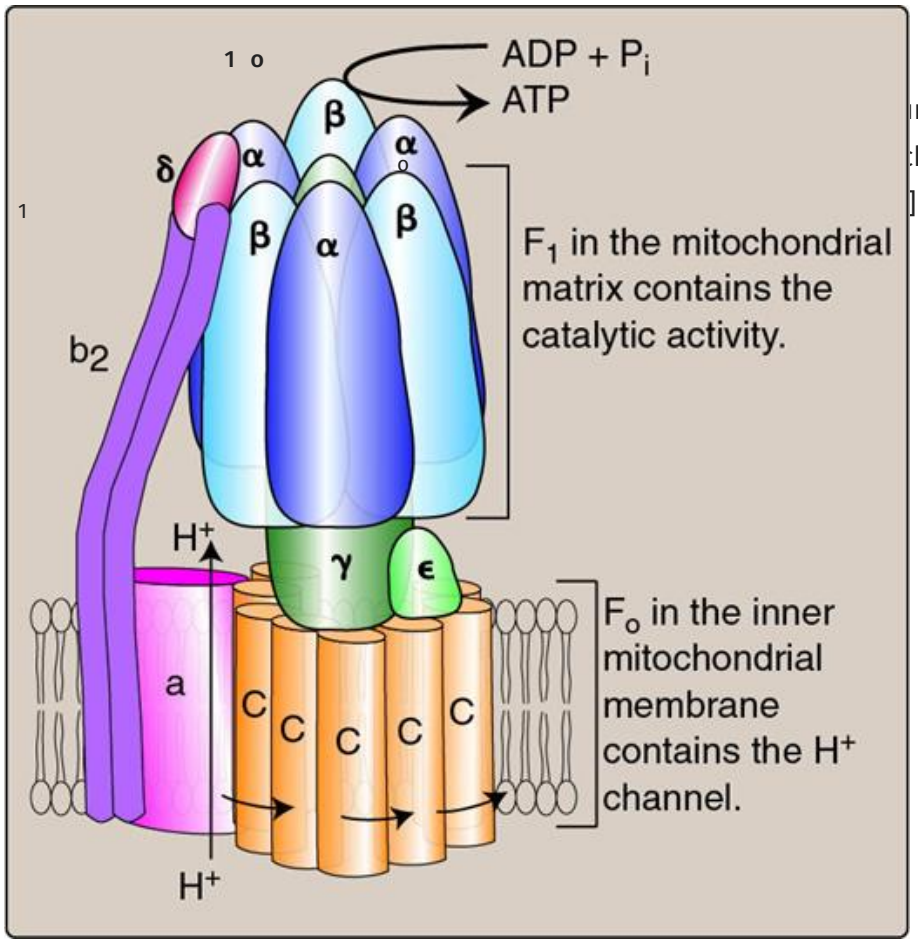
Oligomycin

This drug binds to the F_o (hence the letter "o") domain of ATP synthase, closing the H^+ channel and preventing reentry of H^+ into the matrix, thereby inhibiting phosphorylation of ADP to ATP. Because the pH and electrical gradients cannot be dissipated in the presence of this phosphorylation inhibitor, electron transport stops because of the difficulty of pumping any more H^+ against the steep concentration gradient. This dependency of cellular respiration on the ability to phosphorylate ADP to ATP is known as respiratory control and is the consequence of the tight coupling of these processes.

Uncoupling proteins

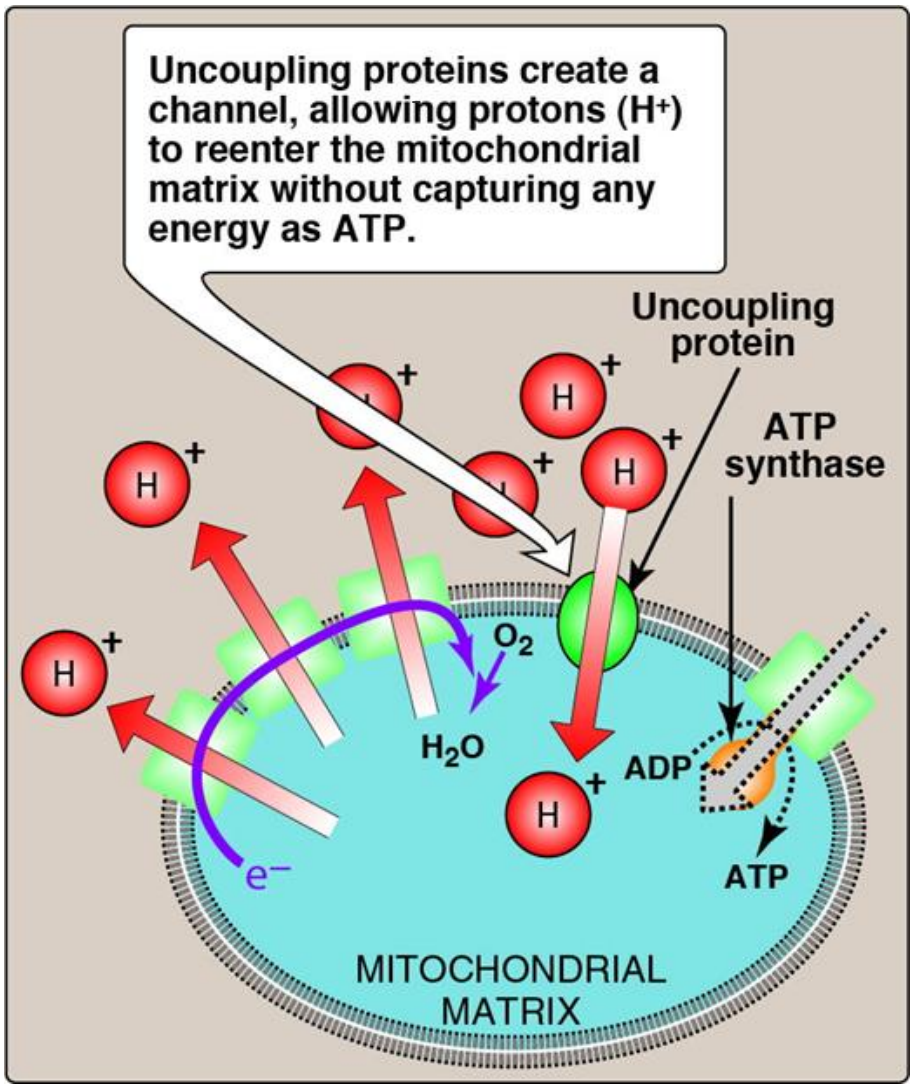
Uncoupling proteins (UCPs) occur in the inner mitochondrial membrane of mammals, including humans. These proteins form channels that allow H^+ to reenter the mitochondrial matrix without energy being captured as ATP (Fig. 6.15). The energy is released as heat, and the process is called nonshivering thermogenesis. UCP1, also called thermogenin, is responsible for heat production in the mitochondria-rich brown adipocytes of mammals. (Note: Cold causes catecholamine-dependent activation of UCP1 expression.) In brown fat, unlike the more abundant white fat, ~90% of its respiratory energy is used for thermogenesis in infants in response to cold. Thus, brown fat is involved in energy expenditure, whereas white fat is involved in energy storage. (Note: Brown fat depots have recently been shown to be present in adults.)

FIGURE 6.14



ırn of the ring is driven by eight H^+ hanges in the three β subunits of the] to three ATP.) P_i = inorganic

FIGURE 6.15

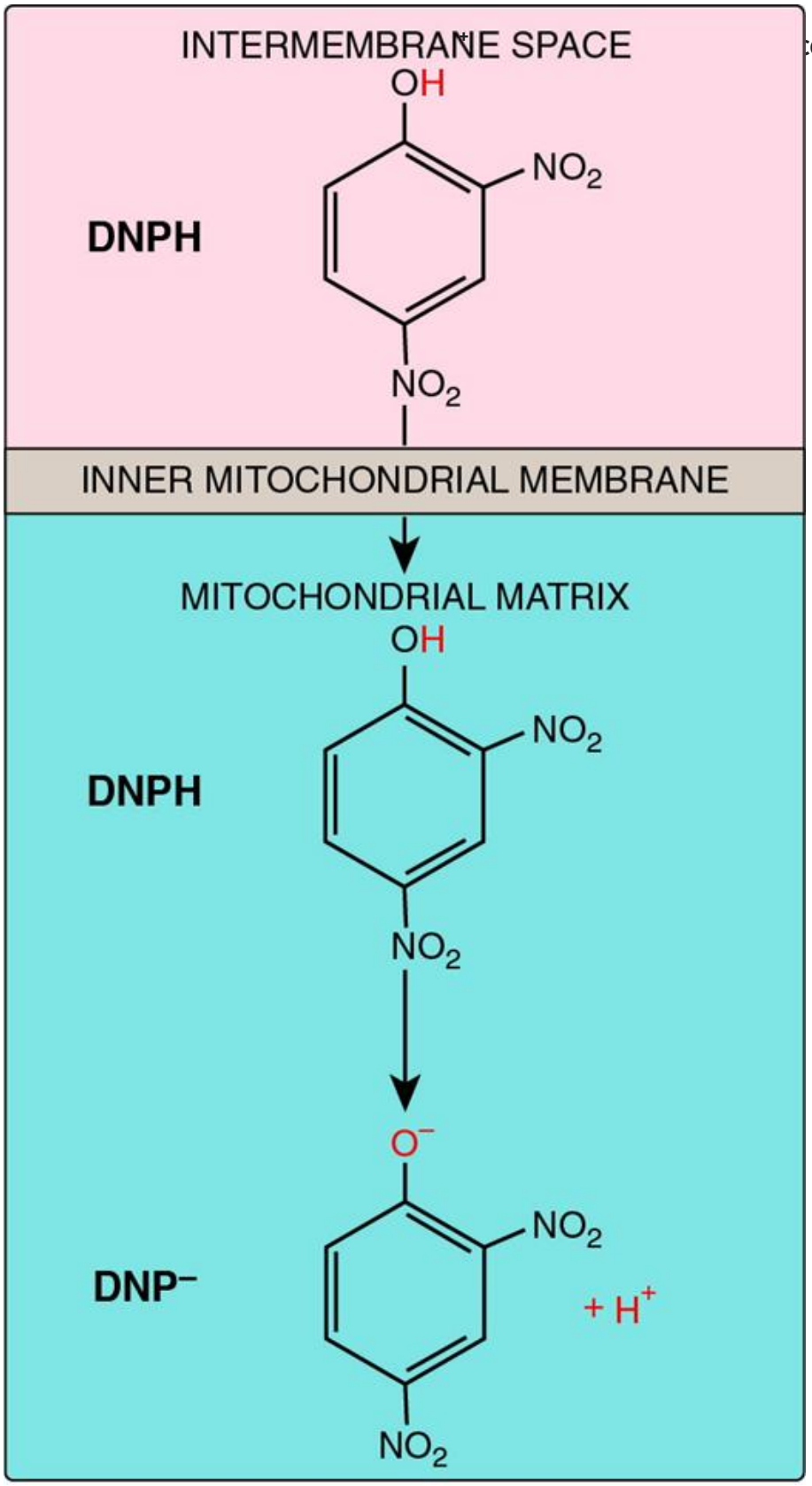


uncoupling protein.

Synthetic uncouplers

Electron transport and phosphorylation of ADP can also be uncoupled by compounds that shuttle H^+ across the inner mitochondrial membrane, dissipating the gradient. The classic example is 2,4-dinitrophenol, a lipophilic H^+ carrier (ionophore) that readily diffuses through the mitochondrial membrane (Fig. 6.16). This uncoupler causes electron transport to proceed at a rapid rate without establishing a H^+ gradient, much as do the UCP. Again, energy is released as heat rather than being used to synthesize ATP. (Note: In high doses, aspirin and other salicylates uncouple oxidative phosphorylation, explaining the fever that accompanies toxic overdoses of these drugs.)

FIGURE 6.16



ted (DNPH) and oxidized (DNP^-)

Membrane transport systems

The inner mitochondrial membrane is impermeable to most charged or hydrophilic substances. However, it contains numerous transport proteins that permit passage of certain molecules from the cytosol to the mitochondrial matrix.

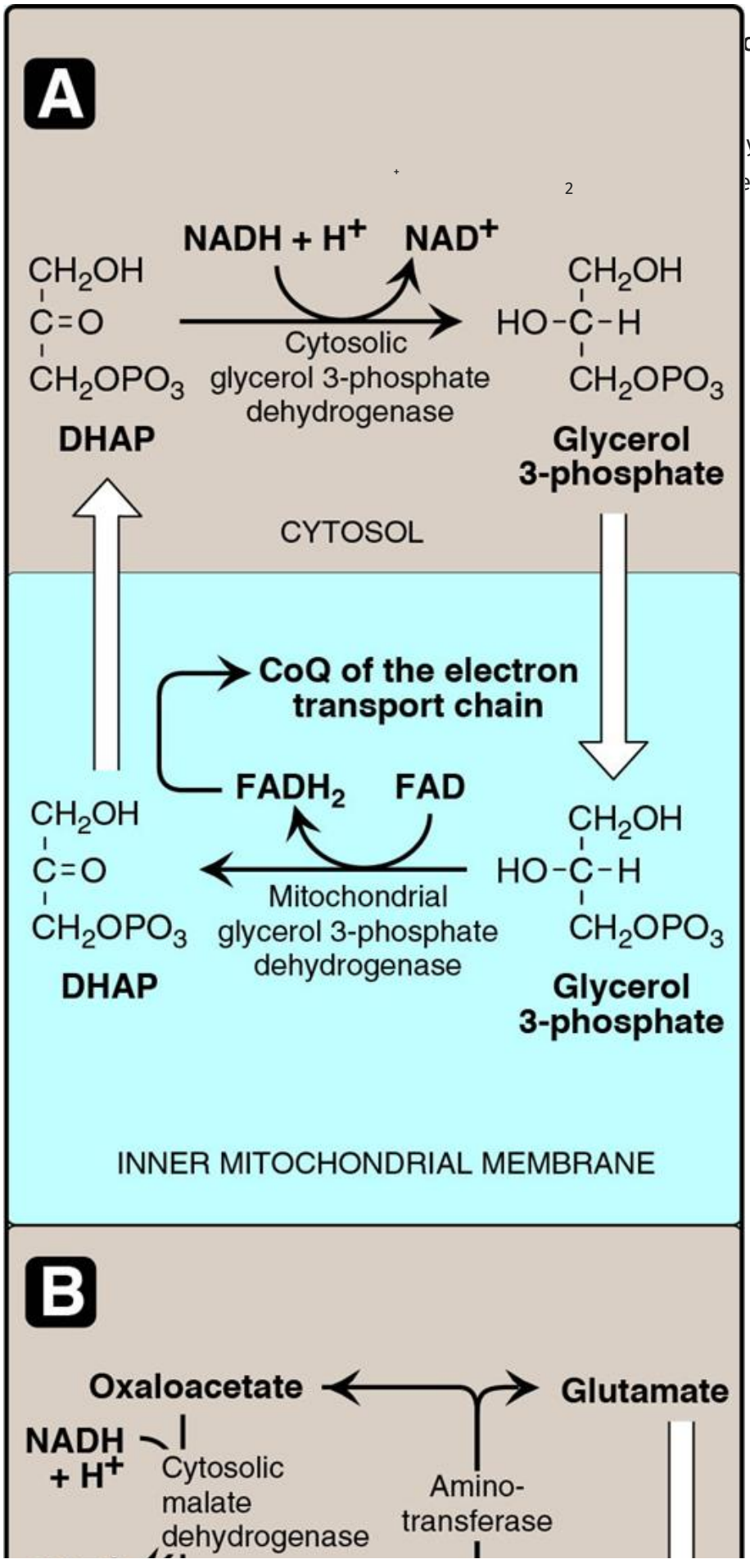
ATP and ADP transport

The inner membrane requires specialized carriers to transport ADP and P_i from the cytosol (where ATP is hydrolyzed to ADP in many energy-requiring reactions) into mitochondria, where ATP can be resynthesized. An adenine nucleotide antiporter imports one ADP from the cytosol into the matrix, while exporting one ATP from the matrix into the cytosol (see Fig. 6.13). A symportercotransports P_i and H^+ from the cytosol into the matrix.

Reducing equivalent transport

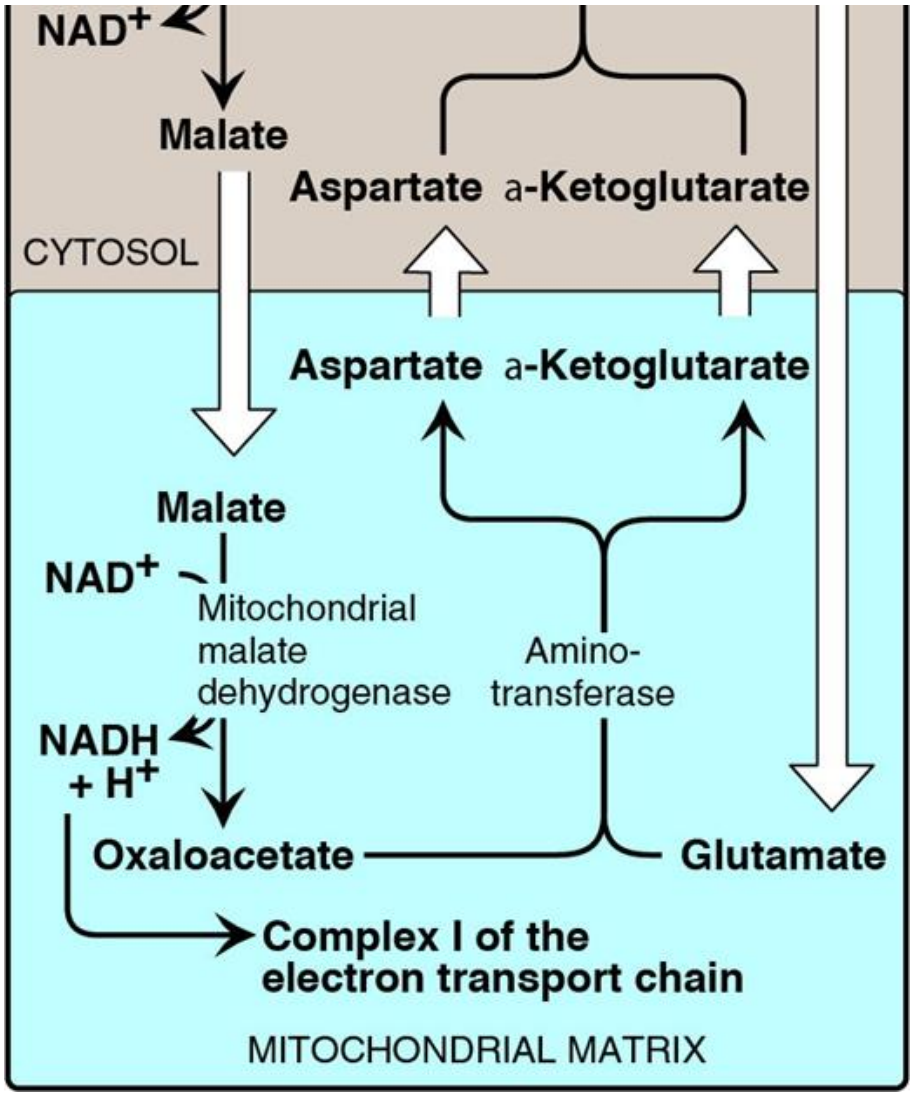
The inner mitochondrial membrane lacks an NADH transporter, and NADH produced in the cytosol (e.g., in glycolysis; see p. 111) cannot directly enter the mitochondrial matrix. However, reducing equivalents of NADH are transported from the cytosol into the matrix using substrate shuttles. In the glycerol 3-phosphate shuttle (Fig. 6.17A), two electrons are transferred from NADH to dihydroxyacetone phosphate by cytosolic glycerol 3-phosphate dehydrogenase. The glycerol 3-phosphate produced is oxidized by the mitochondrial isozyme as FAD is reduced to $FADH_2$. CoQ of the ETC oxidizes the $FADH_2$. Therefore, the glycerol 3-phosphate shuttle results in the synthesis of two ATP for each cytosolic NADH oxidized. This contrasts with the malate–aspartate shuttle (Fig. 6.17B), which produces NADH (rather than $FADH_2$) in the mitochondrial matrix, thereby yielding three ATP for each cytosolic NADH oxidized by malate dehydrogenase as oxaloacetate is reduced to malate. A transport protein moves malate into the mitochondrial matrix.

FIGURE 6.17



oss the inner mitochondrial

ydroxyacetone phosphate; NAD(H) =
e dinucleotide; CoQ = coenzyme Q.



Inherited defects in oxidative phosphorylation

Thirteen of the ~90 polypeptides required for oxidative phosphorylation are encoded by mtDNA and synthesized in mitochondria, whereas the remaining proteins are encoded by nuclear DNA, synthesized in the cytosol, and then transported into mitochondria. Defects in oxidative phosphorylation are more likely a result of alterations in mtDNA, which has a mutation rate about 10 times greater than that of nuclear DNA. Cells in tissues with high ATP requirements include those in brain, nerves, retina, skeletal and heart muscle, and the liver are particularly vulnerable. Impairments in oxidative phosphorylation usually cause lactic acidosis, particularly in the muscles, central nervous system, and retina. Clinical manifestations of oxidative phosphorylation disorders include seizures, ophthalmoplegia, muscle weakness, and cardiomyopathy (Table 6.1). Some medications are known to affect mitochondrial function and these should be avoided in persons with mitochondrial disorders.

TABLE 6.1

Disorders of Mitochondrial Oxidative Phosphorylation

Disease	Characteristics
Kearns–Sayre syndrome	• Weakness or paralysis of eye muscles with drooping eyelids (ptosis), vision loss, cardiac conduction defects, unsteadiness when walking (ataxia), muscle weakness in limbs, kidney problems, deterioration of cognitive function (dementia), and short stature • Features appear before age 20 • Caused by mutation in mtDNA
Leber hereditary optic neuropathy (LHON)	• Bilateral central vision loss caused by retinal detachment • Onset usually in the patient's 20s or 30s • Caused by mitochondrial inheritance along maternal line, however four times more males are affected than females
Leigh disease	• Severe neurologic disorder that manifests in first year of life. Progressive swallowing problems, poor weight gain, hypotonia, weakness, ataxia, nystagmus, and optic atrophy accompany lactic acidosis • Death usually occurs between ages 2 and 3 yrs from respiratory failure • Caused by mutations in nuclear or mtDNA
Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS)	• Progressive neurodegeneration • Repeated episodes of lactic acidosis and myopathy • Cells often contain mutant and wild-typemtDNA and expression is variable
Disease	**Characteristics**
Myoclonic epilepsy	• Progressive condition

Myoclonic epilepsy with ragged-red fibers (MERRF)	• Uncontrolled muscle contractions, dementia, ataxia, and myopathy • Caused by mutation in mtDNA; expression of disease is variable
Neuropathy, ataxia, and retinitis pigmentosa (NARP)	• Progressive condition • Sensory neuropathy with numbness or tingling in the extremities, muscle weakness, ataxia and vision loss, cognitive decline, and seizures • Caused by mutation in mtDNA altering ATP synthase and reducing ability to make ATP

(Note: mtDNA is maternally inherited because mitochondria from the sperm do not survive the fertilization process and only those from the oocyte survive in the developing embryo and into the adult individual.)

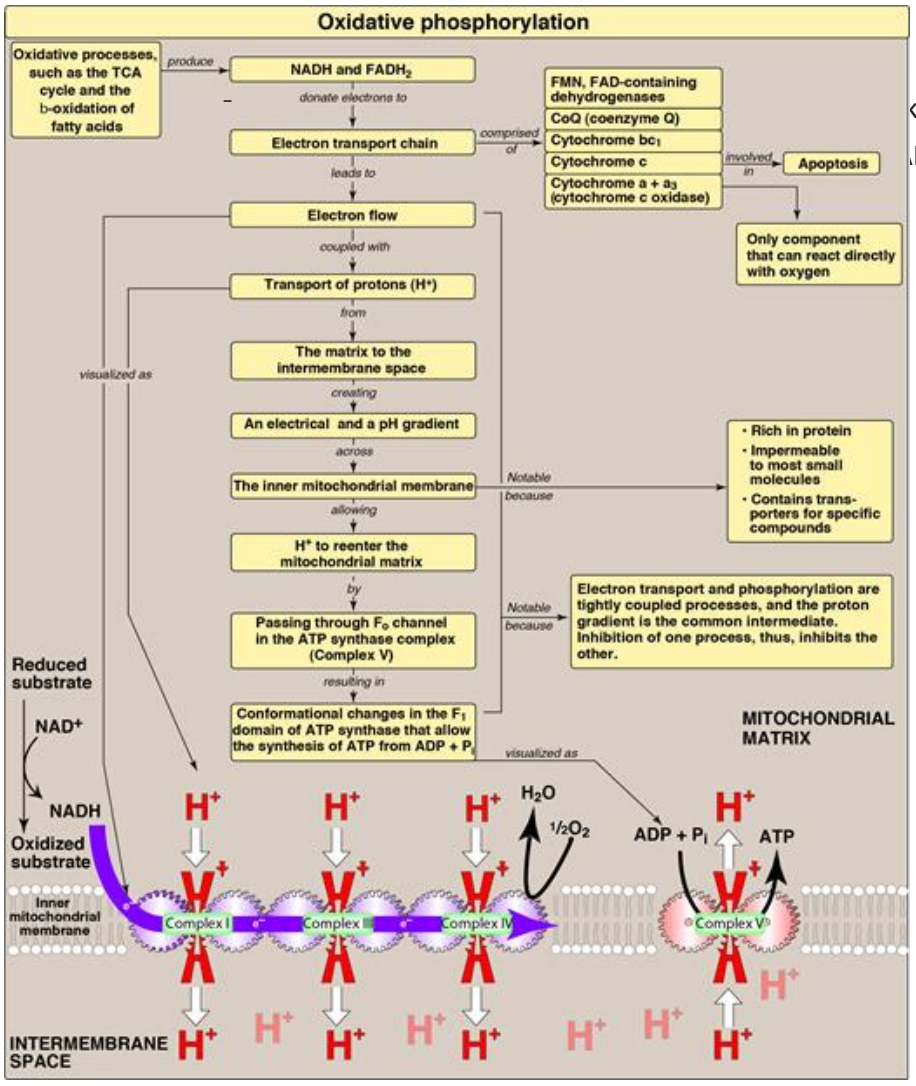
Mitochondria and apoptosis

The process of apoptosis or programmed cell death may be initiated through the intrinsic or mitochondrial-mediated pathway in response to irreparable damage within the cell. During this process, channel proteins (Bax or Bak) are inserted in the outer mitochondrial membrane and allow cytochrome c to leave the intermembrane space and enter the cytosol. There, cytochrome c, in association with proapoptotic factors to form a structure called the apoptosome which then activates a family of proteolytic enzymes (the caspases), causing cleavage of key proteins and resulting in the morphologic and biochemical changes characteristic of apoptosis.

Chapter Summary

- The **change** in **free energy** (**ΔG**) occurring during a reaction predicts the **direction** in which that reaction will spontaneously proceed (Fig. 6.18).

FIGURE 6.18



king gears to emphasize coupling.)
D(H_2) = flavin adenine dinucleotide;

- If ΔG is **negative**, then the reaction is **spontaneous** as written. If ΔG is **positive**, then the reaction is **not spontaneous**. If ΔG **= 0**, then the reaction is in **equilibrium**.
- The ΔG of the forward reaction is equal in magnitude but opposite in sign to that of the reverse reaction.
- Reactions with a large, positive ΔG are made possible by **coupling** with those that have a large, negative ΔG such as **ATP hydrolysis**.
- The reduced coenzymes **NADH** and **$FADH_2$** each donate a pair of electrons to a specialized set of **electron carriers**, consisting of **FMN**, Fe–S center**s**, **CoQ**, and a series of heme-containing **cytochromes**, collectively called the **ETC**.
- This pathway is present in the **inner mitochondrial membrane** and is the final common pathway by which electrons derived from different fuels of the body flow to O_2, which has a large, positive **reduction potential** (**E_0**), reducing it to water.
- The terminal cytochrome, **cytochrome c oxidase**, is the only cytochrome able to bind O_2.

- Electron transport results in the **pumping of protons** (H^+) across the inner mitochondrial membrane from the matrix to the intermembrane space, 10 H^+ per NADH oxidized.
- This process creates **electrical** and **pH gradients** across the inner mitochondrial membrane. After H^+ have been transferred to the cytosolic side of the membrane, they reenter the matrix by passing through the $\mathbf{F_o}$ H^+ channel in **ATP synthase** (**Complex V**), dissipating the pH and electrical gradients and causing conformational changes in the $\mathbf{F_1}$ β subunits of the synthase that result in the synthesis of ATP from ADP + inorganic phosphate.
- **Electron transport** and **phosphorylation** are tightly coupled in **oxidative phosphorylation**. These processes can be uncoupled by **UCP1** of the inner mitochondrial membrane of brown adipocytes and by synthetic compounds such as **2,4-dinitrophenol** and **aspirin**, all of which dissipate the H^+ gradient.
- In uncoupled mitochondria, the energy produced by electron transport is released as **heat** rather than being used to synthesize ATP.
- Defects in oxidative phosphorylation are usually a result of alterations in mtDNA. Impairments in oxidative phosphorylation usually cause **lactic acidosis**, particularly in the muscles, central nervous system, and retina. Clinical manifestations of oxidative phosphorylation disorders include seizures, ophthalmoplegia, muscle weakness, and cardiomyopathy.
- The release of **cytochrome c** from mitochondria into the cytosol stimulates generation of the apoptosome and subsequent activation of proteolyticcaspases that results in **apoptotic cell death**.

Study Questions

Listen

Choose the ONE best answer.

6.1. 2,4-Dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, was used as a weight-loss agent in the 1930s. Reports of fatal overdoses led to its discontinuation in 1939. Which of the following would most likely be true concerning individuals taking 2,4-DNP?

A. ATP levels in the mitochondria are greater than normal.

B. Body temperature is elevated as a result of hypermetabolism.

C. Cyanide has no effect on electron flow.

D. The H+ gradient across the inner mitochondrial membrane is greater than normal.

E. The rate of electron transport is abnormally low.

Correct answer = B. When phosphorylation is uncoupled from electron flow, a decrease in the proton gradient across the inner mitochondrial membrane and, therefore, impaired ATP synthesis are expected. In an attempt to compensate for this defect in energy capture, metabolism and electron flow to oxygen are increased. This hypermetabolism will be accompanied by elevated body temperature because the energy in fuels is largely wasted, appearing as heat. The electron transport chain will still be inhibited by cyanide.

6.2. Which of the following has the strongest tendency to gain electrons?

A. Coenzyme Q

B. Cytochrome c

C. Flavin adenine dinucleotide

D. Nicotinamide adenine dinucleotide

E. Oxygen

Correct answer = E. Oxygen is the terminal acceptor of electrons in the electron transport chain (ETC). Electrons flow down the ETC to oxygen because it has the highest (most positive) reduction potential (E_0). The other choices precede oxygen in the ETC and have lower E_0 values.

6.3. Explain why and how the malate–aspartate shuttle moves nicotinamide adenine dinucleotide reducing equivalents from the cytosol to the mitochondrial matrix.

There is no transporter for nicotinamide adenine dinucleotide (NADH) in the inner mitochondrial membrane. However, cytoplasmic NADH can be oxidized to NAD^+ by malate dehydrogenase as oxaloacetate (OAA) is reduced to malate. The malate is transported across the inner membrane to the matrix where the mitochondrial isozyme of malate dehydrogenase oxidizes it to OAA as mitochondrial NAD^+ is reduced to NADH. This NADH can be oxidized by Complex I of the electron transport chain, generating three ATP through the coupled processes of oxidative phosphorylation.

6.4. Carbon monoxide (CO) binds to and inhibits Complex IV of the electron transport chain. What effect, if any, should this respiratory inhibitor have on phosphorylation of adenosine diphosphate (ADP) to ATP?

Inhibition of electron transport by respiratory inhibitors such as CO results in an inability to maintain the proton (H^+) gradient. Therefore, phosphorylation of ADP to ATP is inhibited, as are ancillary reactions such as calcium uptake by mitochondria, because they also require the H^+ gradient.

6.5. Persons with defects in oxidative phosphorylation most often developed their condition from

A. acquired damage to autosomal genes.

B. inheritance of a mutation on mtDNA.

C. mutations inherited from their father.

D. X-linked inheritance from their mother.

Correct answer = B. Defects in oxidative phosphorylation are more likely a result of alterations in mtDNA, which has a mutation rate about 10 times greater than that of nuclear DNA. Mitochondria and mtDNA are inherited exclusively from the mother. X-linked inheritance is of nuclear DNA, not mtDNA.