CHAPTER 14
ENERGY GENERATION IN MITOCHONDRIA AND CHLOROPLASTS

Mitochondria and Oxidative Phosphorylation

14-1 The link between bond-forming reactions and membrane transport processes in the mitochondria is called __________________.
- (a) chemiosmotic coupling
- (b) proton pumping
- (c) electron transfer
- (d) ATP synthesis

14-2 Describe how a standard flashlight battery can convert energy into useful work and explain how this is similar to the energy conversions in the mitochondria.

14-3 Modern eucaryotes depend on mitochondria to generate most of the cell's ATP. How many molecules of ATP can a single molecule of glucose generate?
- (a) 30
- (b) 2
- (c) 20
- (d) 36

14-4 The citric acid cycle generates NADH and $FADH_2$, which are then used in the process of oxidative phosphorylation to make ATP. If the citric acid cycle (which does not use oxygen) and oxidative phosphorylation are separate processes, as they are, then why is it that the citric acid cycle stops almost immediately when O_2 is removed?

14-5 Indicate whether the following statements are true or false. If a statement is false, explain why it is false.
- A. The number and location of mitochondria within a cell can change, depending on the both the cell type and the amount of energy required.
- B. The inner mitochondrial membrane contains porins, which allow pyruvate to enter for use in the citric acid cycle.
- C. The inner mitochondrial membrane is actually a series of discrete flattened membrane-enclosed compartments called cristae, similar to what is seen in the Golgi apparatus.
- D. The intermembrane space of the mitochondria is chemically equivalent to the cytosol with respect to pH and the small molecules present.

14-6 In which of the four compartments of a mitochondrion are each of the following located?
- A. porin

B. the mitochondrial genome
C. citric acid cycle enzymes
D. proteins of the electron-transport chain
E. ATP synthase
F. membrane transport protein for pyruvate

14-7 NADH contains a high-energy bond that, when cleaved, donates a pair of electrons to the electron-transport chain. What are the immediate products of this bond cleavage?

(a) $NAD^+ + OH^-$
(b) $NAD^+ + H^-$
(c) $NAD^- + H^+$
(d) NAD + H

14-8 For each of the following sentences, fill in the blanks with the best word or phrase selected from the list below. Not all words or phrases will be used; each word or phrase should be used only once.

Mitochondria can use both __________________ and __________________ directly as fuel. __________________ produced in the citric acid cycle donates electrons to the electron-transport chain. The citric acid cycle oxidizes __________________ and produces __________________ as a waste product. __________________ acts as the final electron acceptor in the electron-transport chain. The synthesis of ATP in mitochondria is also known as __________________.

acetyl groups	NADH
carbon dioxide	$NADP^+$
chemiosmosis	NADPH
fatty acids	oxidative phosphorylation
glucose	oxygen
NAD^+	pyruvate

14-9 Electron transport is coupled to ATP synthesis in mitochondria, in chloroplasts, and in the thermophilic bacterium *Methanococcus.* Which of the following is likely to affect the coupling of electron transport to ATP synthesis in *all* of these systems?

(a) a potent inhibitor of cytochrome oxidase
(b) the removal of oxygen
(c) the absence of light
(d) an ADP analogue that inhibits ATP synthase

14-10 Stage 1 of oxidative phosphorylation requires the movement of electrons along the electron-transport chain coupled to the pumping of protons into the intermembrane space. What is the final result of these electron transfers?

(a) OH^- is oxidized to O_2.
(b) Pyruvate is oxidized to CO_2.
(c) O_2 is reduced to H_2O.

(d) H^- is converted to H_2.

14-11 Which component of the electron-transport chain is required to combine the pair of electrons with molecular oxygen?

(a) cytochrome *c*
(b) cytochrome b-c_1 complex
(c) ubiquinone
(d) cytochrome *c* oxidase

14-12 For each of the following sentences, fill in the blanks with the best word or phrase selected from the list below. Not all words or phrases will be used; each word or phrase should be used only once.

NADH donates electrons to the ___________________ of the three respiratory enzyme complexes in the mitochondrial electron-transport chain. ___________________ is a small protein that acts as a mobile electron carrier in the respiratory chain. ___________________ transfers electrons to oxygen. Electron transfer in the chain occurs in a series of ___________________ reactions. The first mobile electron carrier in the respiratory chain is ___________________.

cytochrome *c*	plastoquinone
cytochrome oxidase	reduction
first	second
NADH dehydrogenase	the cytochrome b-c_1 complex
oxidation	third
oxidation–reduction	ubiquinone
phosphorylation	

14-13 In oxidative phosphorylation, ATP production is coupled to the events in the electron-transport chain. What is accomplished in the final electron transfer event in the electron-transport chain?

(a) OH^- is oxidized to O_2.
(b) Pyruvate is oxidized to CO_2.
(c) O_2 is reduced to H_2O.
(d) NAD^+ is reduced to NADH.

14-14 Which of the following statements is *true*?

(a) Because the electrons in NADH are at a higher energy than the electrons in reduced ubiquinone, the NADH dehydrogenase complex can pump more protons than can the cytochrome b-c_1 complex.
(b) The pH in the mitochondrial matrix is higher than the pH in the intermembrane space.
(c) The proton concentration gradient and the membrane potential across the inner mitochondrial membrane tend to work against each other in driving protons from the intermembrane space into the matrix.

(d) The difference in proton concentration across the inner mitochondrial membrane has a much larger effect than the membrane potential on the total proton-motive force.

14-15 Some bacteria can live both aerobically and anaerobically. How does the ATP synthase in the plasma membrane of the bacterium help such bacteria to keep functioning in the absence of oxygen?

14-16 Which of the following types of ion movement might be expected to require co-transport of protons from the intermembrane space to the matrix, inasmuch as it could not be driven by the membrane potential across the inner membrane? (Assume that each ion being moved is moving against its concentration gradient.)

(a) import of Ca^{2+} into the matrix from the intermembrane space
(b) import of acetate ions into the matrix from the intermembrane space
(c) exchange of Fe^{2+} in the matrix for Fe^{3+} in the intermembrane space
(d) exchange of ATP from the matrix for ADP in the intermembrane space

14-17 The F_1 portion of the mitochondrial ATP synthase comprises several different protein subunits. Which subunit binds to ADP + P_i and catalyzes the synthesis of ATP as a result of a conformational change?

(a) α
(b) β
(c) δ
(d) ε

14-18 The F_0 portion of the ATP synthase is a multisubunit complex that spans the inner mitochondrial membrane.

A. What are the designations for the subunits, and which are present in multiple copies in the assembled complex?
B. Explain how the F_0 complex harnesses the proton-motive force to help synthesize ATP. What would happen if the proton gradient were reversed?

14-19 Indicate whether the following statements are true or false. If a statement is false, explain why it is false.

A. The driving force that pulls protons into the matrix is called the proton-motive force, which is a combination of the large force due to the pH gradient and the smaller force that results from the voltage gradient across the inner mitochondrial membrane.
B. Under anaerobic conditions, the ATP synthase can hydrolyze ATP instead of synthesizing it.
C. ATP is moved out of the matrix, across the inner mitochondrial membrane, in a co-transporter that also brings ADP into the matrix.
D. Brown fat cells make less ATP because they have an inefficient ATP synthase.

14-20 Bongkrekic acid is an antibiotic that inhibits the ATP/ADP transport protein in the inner mitochondrial membrane. Which of the following will allow electron transport to occur in mitochondria treated with bongkrekic acid?

(a) placing the mitochondria in anaerobic conditions
(b) adding $FADH_2$
(c) making the inner membrane permeable to protons
(d) inhibiting the ATP synthase

14-21 The relationship of free-energy change (δG) to the concentrations of reactants and products is important because it predicts the direction of spontaneous chemical reactions. In the hydrolysis of ATP to ADP and inorganic phosphate (P_i), the standard free-energy change ($\delta G°$) is -7.3 kcal/mole. The free-energy change depends on concentrations according to the following equation:

$\delta G = \delta G° + 1.42 \log_{10} ([ADP] [P_i]/[ATP])$

In a resting muscle, the concentrations of ATP, ADP, and P_i are approximately 0.005 M, 0.001 M, and 0.010 M, respectively. What is the δG for ATP hydrolysis in resting muscle?

(a) -11.1 kcal/mole
(b) -8.72 kcal/mole
(c) 6.01 kcal/mole
(d) -5.88 kcal/mole

14-22 The relationship of free-energy change (δG) to the concentrations of reactants and products is important because it predicts the direction of spontaneous chemical reactions. In the hydrolysis of ATP to ADP and inorganic phosphate (P_i), the standard free-energy change ($\delta G°$) is -7.3 kcal/mole. The free-energy change depends on concentrations according to the following equation:

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In a resting muscle, the concentrations of ATP, ADP, and P_i are approximately 0.005 M, 0.001 M, and 0.010 M, respectively. What is the δG for ATP synthesis in resting muscle?

(a) -6.01 kcal/mole
(b) 5.88 kcal/mole
(c) 8.72 kcal/mole
(d) 11 kcal/mole

14-23 The relationship of free-energy change (δG) to the concentrations of reactants and products is important because it predicts the direction of spontaneous chemical reactions. Consider, for example, the hydrolysis of ATP to ADP and inorganic phosphate (P_i). The standard free-energy change ($\delta G°$) for this reaction is -7.3 kcal/mole. The free-energy change depends on concentrations according to the following equation:

$\delta G = \delta G° + 1.42 \log_{10} ([ADP] [P_i]/[ATP])$

In a resting muscle, the concentrations of ATP, ADP, and P_i are approximately 0.005 M, 0.001 M, and 0.010 M, respectively. At $[P_i]$ = 0.010 M, what will be the ratio of [ATP] to [ADP] at equilibrium?

(a) 1.38×10^6
(b) 1
(c) 7.2×10^{-8}
(d) 5.14

14-24 NADH and $FADH_2$ carry high-energy electrons that are used to power the production of ATP in the mitochondria. These cofactors are generated during glycolysis, the citric acid cycle, and the fatty acid oxidation cycle. Which molecuale below can produce the most ATP? Explain your answer.

(a) NADH from glycolysis
(b) $FADH_2$ from the fatty acid cycle
(c) NADH from the citric acid cycle
(d) $FADH_2$ from the citric acid cycle

How We Know: How Chemiosmotic Coupling Drives ATP Synthesis

14-25 Experimental evidence supporting the chemiosmotic hypothesis was gathered by using artificial vesicles containing a protein that can pump protons in one direction across the vesicle membrane to create a proton gradient. Which protein was used to generate the gradient in a highly controlled manner?

(a) cytochrome *c* oxidase
(b) NADH dehydrogenase
(c) cytochrome *c*
(d) bacteriorhodopsin

14-26 Explain how scientists used artificial vesicles to prove that the generation of ATP by the ATP synthase was not powered by a single high-energy intermediate but rather by a proton gradient. Be sure to describe the two experiments that were negative controls (no ATP generated), the positive control (ATP generated as expected), and a fourth experiment proving that the gradient is the required energy source.

14-27 The respiratory chain is relatively inaccessible to the experimental manipulation of intact mitochondria. After disrupting mitochondria with ultrasound, however, it is possible to isolate functional submitochondrial particles, which consist of broken cristae that have resealed inside-out into small closed vesicles. In these vesicles the components that originally faced the matrix are now exposed to the surrounding medium.

A. How might such an arrangement aid in the study of electron transport and ATP synthesis?

B. Consider an anaerobic preparation of such submitochondrial particles. If a small amount of oxygen is added, do you predict that the preparation will consume oxygen in respiration reactions? Will the medium outside the particles become more acidic or more basic? What, if anything, will change if the flow of protons through ATP synthase is blocked by an inhibitor? Explain your answer.

Molecular Mechanisms of Electron Transport and Proton Pumping

14-28 A. Match each equation in column A with the corresponding standard redox potential in column B.

Column A	**Column B**
1. $H_2O \leftrightarrow \frac{1}{2}O_2 + 2H^+ + 2\ e^-$	A) +30 mV
2. reduced ubiquinone $\leftrightarrow$ oxidized ubiquinone + $2H^+ + 2\ e^-$	B) +820 mV
3. NADH $\leftrightarrow$ $NAD^+ + H^+ + 2\ e^-$	C) +230 mV
4. reduced cytochrome *c* $\leftrightarrow$ oxidized cytochrome *c* + e^-	D) -320 mV

B. How do these standard redox potentials support our understanding of the stepwise electron transfers that occur in the electron-transport chain?

C. Why would it not be advantageous for living systems to evolve a mechanism for the direct transfer of electrons from NADH to O_2?

14-29 Which ratio of NADH to NAD^+ in solution will generate the largest, positive redox potential?

(a) 1:10
(b) 10:1
(c) 1:1
(d) 5:1

14-30 Which of the following statements is *true*?

(a) Only compounds with negative redox potentials can donate electrons to other compounds under standard conditions.
(b) Compounds that donate one electron have higher redox potentials than those of compounds that donate two electrons.
(c) The $\delta E'_0$ of a redox pair does not depend on the concentration of each member of the pair.
(d) The free-energy change, δG, for an electron transfer reaction does not depend on the concentration of each member of a redox pair.

14-31 Indicate whether the following statements are true or false. If a statement is false, explain why it is false.

A. Ubiquinone is associated with the inner mitochondrial membrane as a protein-bound electron carrier molecule.
B. Ubiquinone can transfer only one electron in each cycle.
C. The iron–sulfur centers in NADH dehydrogenase are relatively poor electron acceptors.
D. Cytochrome oxidase binds O_2 using an iron–heme group, where four electrons are shuttled one at a time.

14-32 Which of the following reactions have a large enough free-energy change to enable it to be used, in principle, to provide the energy needed to synthesize one molecule of ATP from ADP and P_i under standard conditions? See Table 14-23. Recall that

$\delta G° = -n\ (0.023)\ \delta E'_0$ and $\delta E'_0 = E'_0$ (acceptor) - E'_0 (donor).

(a) the reduction of a molecule of pyruvate by NADH
(b) the reduction of a molecule of cytochrome *b* by NADH
(c) the reduction of a molecule of cytochrome *b* by reduced ubiquinone
(d) the oxidation of a molecule of reduced ubiquinone by cytochrome *c*

Reaction	$E'0$
$NADH \rightarrow NAD^+ + H^+ + 2e^-$	−320 mV
Lactate → pyruvate + $2H^+ + 2e^-$	−190 mV
Reduced ubiquinone → ubiquinone + $2H^+ + 2e^-$	30 mV
Cytochrome b (Fe^{2+}) → cytochrome b (Fe^{3+}) + e^-	70 mV
Cytochrome c (Fe^{2+}) → cytochrome c (Fe^{3+}) + e^-	230 mV
$H_2O \rightarrow 1/2O_2 + 2H^+ + 2e^-$	820 mV

Table 14-32

14-33 Cytochrome oxidase is an enzyme complex that uses metal ions to help coordinate the transfer of four electrons to O_2. Which metal atoms are found in the active site of this complex?
(a) two iron atoms
(b) one iron atom and one copper atom
(c) one iron atom and one zinc atom
(d) one zinc atom and one copper atom

14-34 Consider a redox reaction between molecules A and B. Molecule A has a redox potential of -100 mV and molecule B has a redox potential of +100 mV. For the transfer of electrons from A to B, is the $\delta G°$ positive or negative or zero? Under what conditions will the reverse reaction, transfer of electrons from B to A, occur?

14-35 For each of the following sentences, choose one of the options enclosed in square brackets to make a correct statement.

> "An electron bound to a molecule with low affinity for electrons is a [high/low]-energy electron. Transfer of an electron from a molecule with low affinity to one with higher affinity has a [positive/negative] $\delta G°$ and is thus [favorable/unfavorable] under standard conditions. If the reduced form of a redox pair is a strong electron donor with a [high/low] affinity for electrons, it is easily oxidized; the oxidized member of such a redox pair is a [weak/strong] electron acceptor."

14-36 Which of the following statements is *true*?

(a) Ubiquinone is a small hydrophobic protein containing a metal group that acts as an electron carrier.
(b) A 2Fe2S iron–sulfur center carries one electron, whereas a 4Fe4S center carries two.
(c) Iron–sulfur centers generally have a higher redox potential than do cytochromes.
(d) Mitochondrial electron carriers with the highest redox potential generally contain copper ions and/or heme groups.

14-37 Which of the following is not an electron carrier that participates in the electron-transport chain?
(a) cytochrome
(b) quinone
(c) rhodopsin
(d) copper ion

Chloroplasts and Photosynthesis

14-38 Photosynthesis is a process that takes place in chloroplasts and uses light energy to generate high-energy electrons, which are passed along an electron-transport chain. Where are the proteins of the electron-transport chain located in chloroplasts?
(a) thylakoid space
(b) stroma
(c) inner membrane
(d) thylakoid membrane

14-39 In stage 1 of photosynthesis, a proton gradient is generated and ATP is synthesized. Where do protons become concentrated in the chloroplast?
(a) thylakoid space
(b) stroma
(c) inner membrane
(d) thylakoid membrane

14-40 The ATP synthase found in chloroplasts is structurally similar to the ATP synthase in mitochondria. Given that ATP is being synthesized in the stroma, where will the F_0 portion of the ATP synthase be located?
(a) thylakoid space
(b) stroma
(c) inner membrane
(d) thylakoid membrane

14-41 Stage 2 of photosynthesis, sometimes referred to as the dark reactions, involves the reduction of CO_2 to produce organic compounds such as sucrose. What cofactor is the electron donor for carbon fixation?
(a) H_2O
(b) NADH
(c) $FADH_2$

(d) NADPH

14-42 In the electron-transport chain in chloroplasts, ________-energy electrons are taken from __________.

(a) high; H_2O
(b) low; H_2O
(c) high; NADPH
(d) low; NADPH

14-43 The photosystems in chloroplasts contain hundreds of chlorophyll molecules, most of which are part of _______________.

(a) plastoquinone
(b) the antenna complex
(c) the reaction center
(d) the ferredoxin complex

14-44 Use the terms provided below to fill in the blanks. Not all words or phrases will be used; each word or phrase may be used more than once.

Photons from sunlight that are in the ______________ wavelength range are preferentially absorbed by chlorophyll molecules to raise the energy levels of electrons in the __________ ring. The __________ emitted are lower in energy, which is reflected in the ________, green wavelengths detected by the human eye.

red	benzene	heme	blue	shorter	electrons
longer	photons	porphyrin	orange		

14-45 If you shine light on chloroplasts and measure the rate of photosynthesis as a function of light intensity, you get a curve that reaches a plateau at a fixed rate of photosynthesis, *x*, as shown in Figure Q14-45.

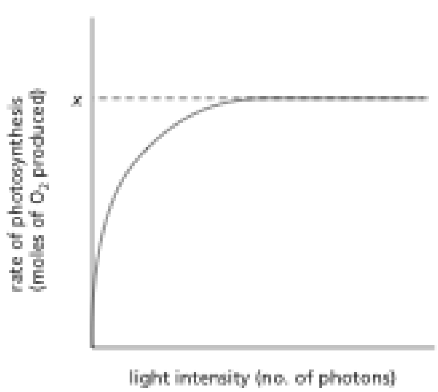

Figure Q14-45

Which of the following conditions will increase the value of *x*?

(a) increasing the number of chlorophyll molecules in the antennae complexes

(b) increasing the number of reaction centers
(c) adding a powerful oxidizing agent
(d) decreasing the wavelength of light used

14-46 If you add a compound to illuminated chloroplasts that inhibits the $NADP^+$ reductase, NADPH generation ceases, as expected. However, ferredoxin does not accumulate in the reduced form because it is able to donate its electrons not only to $NADP^+$ (via $NADP^+$ reductase) but also back to the cytochrome b_6-*f* complex. Thus, in the presence of the compound, a "cyclic" form of photosynthesis occurs in which electrons flow in a circle from ferredoxin, to the cytochrome b_6-*f* complex, to plastocyanin, to photosystem I, to ferredoxin. What will happen if you now also inhibit photosystem II?
(a) Less ATP will be generated per photon absorbed.
(b) ATP synthesis will cease.
(c) Plastoquinone will accumulate in the oxidized form.
(d) Plastocyanin will accumulate in the oxidized form.

14-47 The enzyme ribulose bisphosphate carboxylase (rubisco) normally adds carbon dioxide to ribulose 1,5-bisphosphate. However, it will also catalyze a competing reaction in which O_2 is added to ribulose 1,5-bisphosphate to form 3-phosphoglycerate and phosphoglycolate. Assume that phosphoglycolate is a compound that cannot be used in any further reactions. If O_2 and CO_2 have the same affinity for rubisco, which of the following is the lowest ratio of CO_2 to O_2 at which a net synthesis of sugar can occur?
(a) 1:3
(b) 1:2
(c) 3:1
(d) 2:1

14-48 Indicate whether the following statements are true or false. If a statement is false, explain why it is false.
A. The dark reactions of photosynthesis occur only in the absence of light.
B. Much of the glyceraldehyde 3-phosphate made in the chloroplast ends up producing the molecules needed by the mitochondria to produce ATP.
C. Ribulose 1,5-bisphosphate is similar to oxaloacetate in the Krebs cycle in that they are both regenerated at the end of their respective cycles.
D. Each round of the Calvin cycle uses five molecules of CO_2 to produce one molecule of glyceraldehyde 3-phosphate and one of pyruvate.

14-49 In 1925, David Keilin used a simple spectroscope to observe the characteristic absorption bands of the cytochromes that participate in the electron-transport chain in mitochondria. A spectroscope passes a very bright light through the sample of interest and then through a prism to display the spectrum from red to blue. If molecules in the sample absorb light of particular wavelengths, dark bands will interrupt the colors of the rainbow. His key discovery was that the absorption bands disappeared when oxygen was introduced and then reappeared when the samples became anoxic. Subsequent findings demonstrated that different cytochromes absorb light of different frequencies. When light of a characteristic wavelength shines on a mitochondrial sample, the amount of light absorbed is proportional to the amount of a particular cytochrome present in its reduced form. Thus, spectrophotometric methods can be used to measure how the amounts of reduced cytochromes change over time in response to various treatments. If isolated mitochondria are incubated with a source of electrons such as succinate, but without oxygen, electrons enter the respiratory chain, reducing each of the electron carriers almost completely. When oxygen is then introduced, the carriers oxidize at different rates, as can be seen from the decline in the amount of reduced cytochrome (see Figure Q14-49). Note that cytochromes *a* and a_3 cannot be distinguished and thus are listed as cytochrome ($a + a_3$). How does this result allow you to order the electron carriers in the respiratory chain? What is their order?

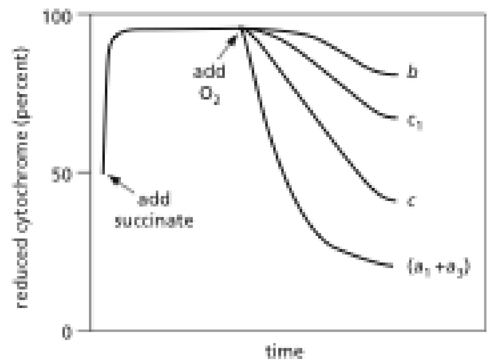

Figure Q14-49

14-50 For each of the following sentences, fill in the blanks with the best word or phrase selected from the list below. Not all words or phrases will be used; each word or phrase should be used only once.

In the carbon fixation process in chloroplasts, carbon dioxide is initially added to the sugar ___________________. The final product of carbon fixation in chloroplasts is the three-carbon compound ___________________. This is converted into ___________________ (which can be used directly by the mitochondria), into ___________________ (which is exported to other cells), and into ___________________ (which is stored in the stroma). The carbon fixation cycle requires energy in the form of ___________________ and reducing power in the form of ___________________.

3-phosphoglycerate	pyruvate
ATP	ribose 1,5-bisphosphate
glyceraldehyde 3-phosphate	ribulose 1,5-bisphosphate
NADH	starch
NADPH	sucrose

The Origins of Chloroplasts and Mitochondria

14-51 Oxidative phosphorylation, as it occurs in modern eucaryotes, is a complex process that probably arose in simple stages in primitive bacteria. Which mechanism is proposed to have arisen first as this complex system evolved?

(a) electron transfers coupled to a proton pump
(b) the reaction of oxygen with an ancestor of cytochrome oxidase
(c) ATP-driven proton pumps
(d) the generation of ATP from the energy of a proton gradient

14-52 Below is a list of breakthroughs in energy metabolism in living systems. Which is the correct order in which they are thought to have evolved?

A. H_2O-splitting enzyme activity
B. light-dependent transfer of electrons from H_2S to NADPH
C. the consumption of fermentable organic acids
D. oxygen-dependent ATP synthesis

(a) A, C, D, B
(b) C, A, B, D
(c) B, C, A, D
(d) C, B, A, D

14-53 Which of the phylogenetic trees in Figure Q14-53 is the most accurate? (The mitochondria and chloroplasts are from maize, but they are treated as independent "organisms" for the purposes of this question.)

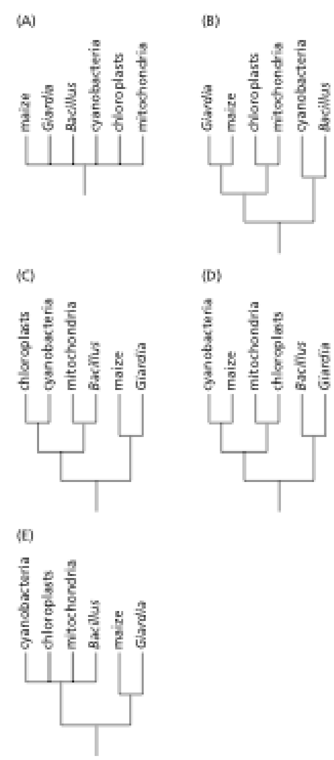

Figure Q14-53

Answers

14-1 (a). Choices (b), (c), and (d) are individual parts of the overall process of chemiosmotic coupling.

14-2 A battery contains chemicals that generate negatively charged ions at one pole, and it is able to cause the continuous transfer of electrons along a metal wire if that pole is connected to the other end of the battery. The energy released by the electron transfer process driven by the battery can be harnessed to do useful work, as when it is used to run an electric motor.. Likewise, the energy released by the electron transfers that occur between the protein complexes in the electron transport chain does useful work when it drives the movement of protons to one side of the membrane, since the resulting proton gradient is then used to generate chemical energy in the form of ATP.

14-3 (a). Glycolysis of a single glucose molecule generates 2 ATP molecules. Oxidative phosphorylation in the mitochondria generates an additional 28 ATP molecules, making a total of 30 ATP molecules for each glucose molecule.

14-4 The citric acid cycle stops almost immediately when oxygen is removed, because several steps in the cycle require the oxidized forms of NAD^+ and FAD. In the absence of oxygen, these electron carriers can be reduced by the reactions of the citric acid cycle but cannot be reoxidized by the electron-transport chain that participates in oxidative phosphorylation.

14-5
A. True.
B. False. The outer mitochondrial membrane contains porins, allowing the passage of all molecules with a mass of less than 5000 daltons. Although pyruvate must pass through the inner membrane, it does so in a highly regulated manner via specific transporter channels.
C. False. Although the cristae do look like individual compartments on the basis of the images of the inner structure of the mitochondria, the inner membrane is a single, albeit highly convoluted, membrane.
D. True.

14-6
A. Porin is in the outer membrane.
B. The mitochondrial genome is in the matrix.
C. The citric acid cycle enzymes are in the matrix.
D. The proteins in the electron-transport chain are in the inner membrane.
E. ATP synthase is in the inner membrane.
F. The transport protein for pyruvate is in the inner membrane.

14-7 (b)

14-8 Mitochondria can use both **pyruvate** and **fatty acids** directly as fuel. **NADH** produced in the citric acid cycle donates electrons to the electron-transport chain. The citric acid cycle oxidizes **acetyl groups** and produces **carbon dioxide** as a waste product. **Oxygen** acts as the final electron acceptor in the electron-transport chain. The synthesis of ATP in mitochondria is also known as **oxidative phosphorylation**.

14-9 (d). All chemiosmotic coupling systems involve a proton gradient that ATP synthase uses to bind to ADP and phosphorylate it. Hence agents that prevent ADP from binding the synthase or that dissipate the proton gradient affect all chemiosmotic systems. Cytochrome oxidase and oxygen are required only for mitochondria and aerobic bacteria (not *Methanococcus*); light is required only for chloroplasts and photosynthetic bacteria (not *Methanococcus*).

14-10 (c). Contrary to what the term "oxidative phosphorylation" may imply, the phosphorylation event does not depend on an oxidative reaction but rather on the reduction of molecular oxygen, converting it to water.

14-11 (d)

14-12 NADH donates electrons to the **first** of the three respiratory enzyme complexes in the mitochondrial electron-transport chain. **Cytochrome *c*** is a small protein that acts as a mobile electron carrier in the respiratory chain. **Cytochrome oxidase** transfers electrons to oxygen. Electron transfer in the chain occurs in a series of **oxidation–reduction** reactions. The first mobile electron carrier in the respiratory chain is **ubiquinone**.

14-13 (c)

14-14 Choice (b) is the correct answer. The pumping of protons out of the matrix into the intermembrane space creates a difference in proton concentration between the two sides of the membrane, with the matrix at a higher pH (i.e., more alkaline) than the intermembrane space, which tends to equilibrate with the cytosol (which has a neutral pH). The electrons in NADH are at a higher energy than the electrons in reduced ubiquinone, but proton pumping is not determined simply by the energy of the electron donors (choice (a)). Instead, the number of protons that can be pumped by each complex is determined by the difference in energy between the electrons in each substrate/product pair (i.e., the difference between the electrons in NADH and reduced ubiquinone, compared with that between reduced ubiquinone and reduced cytochrome *c*). The proton concentration gradient and the membrane potential generated by the electron-transport chain work in the same direction (choice (c)), creating a steep electrochemical gradient for protons across the membrane. For choice (d), the difference in proton concentration has a smaller effect than the membrane potential on the total proton-motive force.

14-15 In the absence of oxygen, the respiratory chain no longer pumps protons, and thus no proton electrochemical gradient is generated across the bacterial membrane. In these conditions the ATP synthase uses some of the ATP generated by glycolysis in the cytosol to pump protons out of the bacterium, thus forming the proton gradient across the membrane that the bacterium requires for importing vital nutrients by coupled transport.

14-16 Choice (b) is the correct answer. Because the inside of the membrane (the mitochondrial matrix) is more negative than the outside, in principle any traffic resulting in an increase in the positive charge in the matrix can be driven by the membrane potential. Hence, exchange of Fe^{2+} (or ATP) in the matrix for Fe^{3+} (or ADP) in the intermembrane space can be driven by the membrane potential and need not require the co-transport of protons down the pH gradient. The same applies to the import of Ca^{2+}. But import of acetate ions into the matrix and exchange of Ca^{2+} in the matrix for Na^{+} in the intermembrane space result in an increase in the amount of negative charge in the matrix and it therefore cannot be driven by the charge difference between the two mitochondrial compartments.

14-17 (b)

14-18 A. The individual subunits are a, b, and c. The a and b subunits are present in the complex as single polypeptides. However, there are between 10 and 14 polypeptides of subunit c that form off a ring in the F_0 complex.

B. Protons flow through a channel that exists between each of the c subunits in the ring: these form part of the "stalk." The flow of protons makes the entire stalk rotate, which causes a conformational change in each of the three β subunits in the F_1 portion of the synthase. If the proton gradient is reversed, the flow of protons is reversed and the stalk rotates in the opposite direction, causing ATP hydrolysis rather than ATP synthesis.

14-19 A. False. Although it is true that both the pH gradient and the voltage gradient are components of the proton-motive force, it is the voltage gradient (also referred to as the membrane potential) that is the greater of the two.

B. True.

C. True.

D. False. The inner mitochondrial membranes in brown fat cells contain a transport protein that allows protons to move down their gradient without passing through the ATP synthase. As a result, less ATP is made and most of the energy from the proton gradient is released as heat.

14-20 (c). Inhibition of the ATP/ADP translocase prevents the export of ATP generated by oxidative phosphorylation in exchange for an import of ADP into the matrix. The ensuing buildup of ATP at the expense of ADP inhibits the ATP synthase. Because protons are no longer being used to power the ATP synthase, the proton gradient is not dissipated; the increasingly steep proton gradient makes it increasingly difficult for the electron-transport proteins to pump protons out of the matrix, and electron transport quickly stops. Hence, the inner membrane becomes permeable to protons, allowing electron transport to resume (although no ATP will be synthesized).

14-21 (a). The δG for hydrolysis is -11.1 kcal/mole. This result is calculated by substituting values into the equation given: δG = -7.3 kcal/mole + 1.42 $\log_{10}$ ([0.001 M] [0.010 M]/[0.005 M]) = -7.3 kcal/mole + 1.42 $\log_{10}$ (0.002) = -11.1 kcal/mole.

14-22 (d). The δG for hydrolysis is -11.1 kcal/mole. This result is calculated by substituting values into the equation given: δG = -7.3 kcal/mole + 1.42 $\log_{10}$ ([0.001 M] [0.010 M]/[0.005 M]) = -7.3 kcal/mole + 1.42 $\log_{10}$ (0.002) = -11.1 kcal/mole. The δG for synthesis is +11.1 kcal/mole because the forward and reverse reactions always have the same numerical value for δG, but with the sign reversed.

14-23 (c). At equilibrium, the δG is equal to zero by definition. The ratio of [ATP] to [ADP] at equilibrium is less than $1{:}10^7$. This result is calculated by setting $\delta G = 0$, so that 1.42 $\log_{10}$ ([ADP] [P_i]/[ATP]) = $-\delta G°$ = 7.3 kcal/mole. Solving for [ADP]/[ATP], the equation becomes $\log_{10}$ ([ADP] [0.010]/[ATP]) = 7.3/1.42 = 5.14; then [ADP]/[ATP] = $(10^{5.14})/(0.010) = 13.8 \times 10^6$. Thus, the reciprocal [ATP]/[ADP] is 7.2×10^{-8}.

14-24 (c). NADH produced in glycolysis does not contribute directly to ATP production in the mitochondria because it cannot be imported into the matrix. If the energy is transferred to a different carrier, some of the stored energy is lost. $FADH_2$, from either the fatty acid cycle or the citric acid cycle, contributes less energy than NADH from the citric acid cycle because the electrons are donated further down the chain. Fewer electron transfers mean that fewer protons are pumped across the membrane.

14-25 (d). Bacteriorhodopsin is a transmembrane protein that pumps protons across the membrane when exposed to light. The other proteins pump protons but are part of the electron-transport chain, so they would not be good options to show that it is only the proton gradient that is required in this system rather than any specific intermediate in the electron-transport chain.

14-26 In all the experiments, artificial liposomes were generated and exposed to light, and the surrounding solution was checked for an increase in ATP. In the first experiment, the liposomal membranes contained only bacteriorhodopsin, a bacterial protein that pumps protons and is activated by light. In this negative control, ATP was not expected to be produced, and it was not. In the second experiment, also a negative control, the liposomes contained only ATP synthase. Again, if the chemiosmotic hypothesis was correct, ATP should not have been generated, which was what was observed. In the third experiment, both bacteriorhodopsin and ATP synthase were present in the liposomal membrane. When exposed to light, protons were pumped into the vesicle and ATP was generated. In the fourth experiment, to show that the ATP production was solely a result of the proton gradient, an uncoupling agent was added to the solution containing liposomes with bacteriorhodopsin and ATP synthase. In this case, even though the protons were being pumped into the liposomes, a gradient did not build up; this was because of the presence of the uncoupling agent, which made the membrane permeable to protons. No ATP was generated, proving that it was the proton gradient that was the energy source for ATP synthesis.

14-27 A. This arrangement of components within the vesicles allows the experimental manipulation of the medium surrounding the vesicles to permit the consequences of different conditions in the mitochondrial matrix to be examined. The medium can be altered by changing pH, adding electron carriers and oxygen, and providing ADP and P_i, for example. The oxidation of electron carriers, the consumption of oxygen, and the production of ATP can be measured in the medium. By changing the composition of the medium, it should be possible, for example, to identify the electron carriers that can donate electrons from the matrix to the transport chain (the side of the membrane that normally faces the matrix is now on the outside), to assess the redox potentials of various components of the transport chain, and to determine the dependence of ATP synthesis on the pH gradient across the membrane and on the ATP:ADP ratio.

B. Respiration reactions will rapidly consume at least some of the added oxygen. During the anaerobic conditions, the electron carriers in the electron-transport chain were reduced; on the addition of oxygen, electrons will be transferred to oxygen, thereby reducing the oxygen and oxidizing the carriers. Concomitantly with the electron flow, protons will be pumped from the medium into the vesicles, thereby making the medium slightly more basic and the inside of the vesicles acidic. Inhibition of the ATP synthase will not have an immediate effect on oxygen consumption or proton pumping. However, the proton concentration inside the vesicles will quickly become too high to continue the activity of the electron-transport-coupled proton pumping, and thus electron transport and oxygen consumption will cease.

14-28 A. 1—B; 2—A; 3—D; 4—C

B. Each successive member of the electron-transport chain is a better electron acceptor, which permits a unidirectional series of electron transfers until reaching O_2, which is the best electron acceptor and the final destination of the electrons, forming water as oxygen is consumed.

C. If NADH directly donated electrons to O_2, a large amount of energy would be released as heat and lost as a way for the cell to generate chemical energy in the form of ATP.

14-29 (d). NAD^+ is the electron acceptor; NADH is the electron donor. If there is an excess of NAD^+ in solution, there is less capacity to donate electrons (which would reflect a negative redox potential) and more capacity to accept electrons (which would reflect a positive redox potential).

14-30 Choice (c) is the correct answer. By definition, E'_0 refers to the standard state of equal concentrations of each member of the redox pair. Therefore $\delta E'_0$ does not vary with the actual concentrations. Compounds with positive redox potentials can donate electrons to other compounds under standard conditions, so long as the electron acceptor has a higher (more positive) redox potential; thus option (a) is incorrect. Compounds that are able to donate only one electron do not necessarily have higher redox potentials than compounds that are able to donate two electrons; thus option (b) is incorrect. (Water, for example, has a very high redox potential.) Although the $\delta E'_0$ of a reaction is directly proportional to the $\delta G°'$ of a reaction and both are independent of the concentrations of substrates and products, the δG depends on these concentrations; thus option (d) is incorrect.

14-31 A. False. Ubiquinone is an aromatic compound that uses its long hydrocarbon tail to associate with the inner mitochondrial membrane.

B. False. Ubiquinone can transfer one or two electrons. In the case in which only one electron is transferred, the molecule contains an unpaired electron, which is highly reactive.

C. True.

D. True.

14-32 (b). For a reaction to drive ATP synthesis under standard conditions, the $\delta G°'$ of the reaction must be less than -7.3 kcal/mol. Because $\delta G°' = -n\,(0.023)\,\delta E'_0$, the value of $\delta E'_0$ must be greater than 317 mV/n, where n is the number of electrons transferred. $\delta E'_0$ is 130 mV for the reduction of a molecule of pyruvate by NADH, 390 mV for the reduction of a molecule of cytochrome *b* by NADH, 40 mV for the reduction of a molecule of cytochrome *b* by ubiquinone, 200 mV for the oxidation of a molecule of ubiquinone by cytochrome *c*, and 590 mV for the oxidation of cytochrome *c* by oxygen. The numbers of electrons transferred in each of the above reactions are two, one, one, one, and one, respectively. Thus, only reactions (b) and (e) are sufficient to drive ATP synthesis.

14-33 (b)

14-34 The $\delta G°$ is negative. The sign of $\delta G°$ is the opposite of that of $\delta E'_0 = E'_0$ (acceptor) - E'_0 (donor). The acceptance of electrons by B from A has a $\delta E'_0 = 100 + 100 = 200$. The reverse reaction, the donation of electrons from B to A, has a positive $\delta G°$ and is therefore unfavorable under standard conditions. Remember that, by definition, the concentrations of A and its redox pair A′ are equal under standard conditions; similarly, the concentration of B is equal to the concentration of its redox pair B′. B will be able to donate electrons to A only when [B] > [B′] and/or [A] < [A′] to such an extent that the δG for electron transfer becomes negative.

14-35 An electron bound to a molecule with low affinity for electrons is a **high**-energy electron. Transfer of an electron from a molecule with low affinity to one with higher affinity has a **negative** $\delta G°$ and is thus **favorable** under standard conditions. If the reduced form of a redox pair is a strong electron donor with a **low** affinity for electrons, it is easily oxidized; the oxidized member of such a redox pair is a **weak** electron acceptor.

14-36 Choice (d) is the correct answer. Cytochrome oxidase, which is the last carrier in the mitochondrial electron-transport chain and therefore has the highest redox potential, contains copper ions and a heme group. Ubiquinone is not a protein and does not contain a metal group (choice (a)). Both 2Fe2S and 4Fe4S centers carry one electron (choice (b)). Iron–sulfur centers generally have a lower redox potential than do cytochromes (choice (c)). The heme group in cytochrome *c* contains a charged iron ion. The interiors of proteins are often hydrophobic, favoring a relatively high redox potential, because reduction of the iron ion decreases its charge, and charges are energetically unfavorable in a hydrophobic environment.

14-37 (c)

14-38 (d)

14-39 (a)

14-40 (d)

14-41 (d)

14-42 (b)

14-43 (b)

14-44 Photons from sunlight that are in the **red** wavelength range are preferentially absorbed by chlorophyll molecules to raise the energy levels of electrons in the **porphyrin** ring. The **photons** emitted are lower in energy, which is reflected in the **longer**, green wavelengths detected by the human eye.

14-45 (b). The rate of photosynthesis will increase with increasing light intensity until photons hit all of the reaction centers directly. At saturating levels of light, the number of reaction centers that are still capable of being excited limits the rate of photosynthesis, which can be increased only by increasing the number of reaction centers or by increasing the rate at which the reaction centers are restored to their low-energy state. Increasing the number of chlorophyll molecules in the antennae complexes, the energy per photon of light, or the rate at which chlorophyll molecules are able to transfer energy electrons to one another will have no effect on either of these parameters. Adding a powerful oxidizing agent might, if anything, interfere with the reduction of the reaction center back to its resting state.

14-46 (c). If you now inhibit photosystem II you will deprive plastoquinone, which can still donate its electrons to the cytochrome b_6-*f* complex, of an electron source. Hence, plastoquinone will accumulate in its oxidized form. In contrast, all of the other components downstream of plastoquinone will be able to cycle between their oxidized and reduced states. ATP synthesis will continue, because electrons are still being fed through the cytochrome b_6-*f* complex, and the same amount of ATP will be generated.

14-47 (c). Three molecules of O_2 are required to form three molecules of 3-phosphoglycerate and three molecules of phosphoglycolate. To break even (i.e., simply to keep the Calvin cycle going with no net sugar produced), you need to have enough 3-phosphoglycerate to synthesize ribulose 1,5-bisphosphate again. Therefore, for every three molecules of O_2 that react with ribulose 1,5-bisphosphate, you need to generate two additional molecules of 3-phosphoglycerate. For every three molecules of CO_2 that go into the Calvin cycle, one molecule of 3-phosphoglycerate is formed. So if you have at least six molecules of CO_2 per three molecules of O_2 going through the Calvin cycle, you will break even. Only if you have a ratio of CO_2 to O_2 higher than 6:3 (2:1) can you have a net synthesis of carbohydrate.

14-48 A. False. The dark reactions are those involved in carbon fixation and are named as such because they do not require light.

B. True.

C. True.

D. False. Three molecules of CO_2 are required for each round of the Calvin cycle, and the product is one molecule of glyceraldehyde 3-phosphate and the recycling of the ribulose 1,5-bisphosphate molecule.

14-49 This result allows you to order the electron carriers in the respiratory chain because when oxygen is added, the last carrier in the chain will be oxidized first. This is because oxygen is the final sink for the electrons that flow through the chain, and it participates directly in a redox reaction with the last electron carrier. The wave of oxidation will then proceed backward through the chain toward the first electron carrier in the chain; this is because the oxidation of each carrier will convert it to a form that can accept electrons from the "upstream" carrier in the chain, thereby oxidizing each upstream carrier sequentially. The order of cytochromes in the respiratory chain is the reverse of the order in which they are oxidized (i.e., the order in which the reduced form is lost). Listed from first to last, the cytochromes in the chain are b, c_1, c, ($a + a_3$).

14-50 In the carbon fixation process in chloroplasts, carbon dioxide is initially added to the sugar **ribulose 1,5-bisphosphate**. The final product of carbon fixation in chloroplasts is the three-carbon compound **glyceraldehyde 3-phosphate**. This is converted into **pyruvate** (which can be used directly by the mitochondria), into **sucrose** (which is exported to other cells), and into **starch** (which is stored in the stroma). The carbon fixation cycle requires energy in the form of **ATP** and reducing power in the form of **NADPH**.

14-51 (c)

14-52 (d)

14-53 (C). Mitochondria are most closely related to *Bacillus*, and chloroplasts to cyanobacteria. Maize (a eucaryote) is more closely related to *Giardia* (a simple eucaryote) than it is to bacteria (procaryotes).