# 12 Cellular Energetics

## *Review the Concepts*

1. The pmf is generated by a voltage and chemical (proton) gradient across the inner membrane of mitochondria and the thylakoid membrane of chloroplasts. Like ATP, the pmf is a form of stored energy, and the energy stored in the pmf may be converted to ATP by the action of ATP synthase.

2. The unique properties of the mitochondrial inner membrane include the presence of membrane invaginations (termed *cristae*), a higher than normal protein concentration, and an abundance of the lipid cardiolipin. The cristae increase the surface area of the inner membrane, thereby increasing the total amount of membrane and hence electron transport chain components, ATP synthase molecules, and transporters of reagents and products of the citric acid cycle and/or oxidative phosphorylation are all increased. The higher protein concentration, mostly proteins involved in electron transport and ATP synthesis, further increases the capacity of mitochondria to synthesize ATP. Finally, cardiolipin enhances the barrier properties of the inner membrane by reducing the membrane's permeability to protons.

3. Glycolysis does not require oxygen, but the citric acid cycle and the electron transport chain do require oxygen to function. In the case of the citric acid cycle, oxygen is not directly involved in any reaction, but the cycle will come to a halt as $NAD^+$ and FAD levels drop in the absence of oxygen. For electron transport, oxygen is required as an electron acceptor. In the absence of oxygen, certain eukaryotic organisms (facultative anaerobes) as well as certain cells (mammalian skeletal muscles during prolonged contraction) can produce limited amounts of ATP by glycolysis (a process known as fermentation).

4. Electrons produced by glycolysis are delivered to the electron transport chain via electron shuttles in the mitochondrial inner membrane. The most common shuttle is the malate-aspartate shuttle, which utilizes two different antiporters and various intermediate carriers to remove electrons from NADH in the cytosol and add them to $NAD^+$ in the matrix. The steps in this process are as follows. First, cytosolic malate dehydrogenase transfers electrons from cytosolic NADH to oxaloacetate to form malate. Second, the malate/α-ketoglutarate antiporter transports malate into the matrix in exchange for α-ketoglutarate. Third, mitochondrial malate dehydrogenase converts malate to oxaloacetate, reducing matrix $NAD^+$ to NADH in the process. Fourth, oxaloacetate is converted to the amino acid aspartate by addition of an amino group from the amino acid glutamate (the rest of the glutamate is converted to α-ketoglutarate). Fifth, the glutamate/aspartate antiporter exports aspartate to the cytosol in exchange for glutamate. Finally, aspartate is converted to oxaloacetate in the cytosol to complete the cycle. If a mutation inactivated the malate-aspartate shuttle, initially there would be a small reduction in the efficiency of oxidative phosphorylation as there would be a small reduction in the electrons delivered to the electron transport chain per glucose molecule. In the longer term, glycolysis would be inhibited as the availability of $NAD^+$ for glycolytic reactions would become limiting.

5. Fatty acids are oxidized in the mitochondria and the persoxisome, but unlike the mitochondria, oxidation in the peroxisome does not generate ATP. Oxidation of very long chain fatty acids in peroxisomes leads to their degradation. In the case of the human genetic disease X-linked adrenoleukodystrophy (ALD), however, this oxidation is defective because the ATP-binding cassette (ABC) ABCD1 transporter, localized to peroxisome membranes, is unable to import very long chain fatty acids into this organelle. ALD

patients have elevated levels of these fatty acids in their plasma and tissues, which is somehow associated with the degeneration of the adrenal gland and the myelin sheath surrounding nerve fibers in the brain.

6. Prosthetic groups are small nonpeptide organic molecules or metal ions that are tightly associated with a protein or protein complex. Several types of heme, an iron-containing prosthetic group, are associated with the cytochromes. The various cytochromes in the electron transport chain contain heme prosthetic groups with different axial ligands, and as a result, each cytochrome has a different reduction potential, so that electrons can move only in sequential order through the electron carriers.

7. The underlying reason for the difference in ATP yield for electrons donated by $FADH_2$ and NADH is that the electrons carried in $FADH_2$ have less potential energy (43.4 kcal/mol) than the electrons carried in NADH (52.6 kcal/mol). Thus, $FADH_2$ transfers electrons to the respiratory chain at a later point than does NADH, resulting in the translocation of fewer protons, a smaller change in pH, and fewer synthesized ATP molecules.

8. Aerobic bacteria carry out oxidative phosphorylation by the same processes that occur in mitochondria (and are simpler and easier to work with than mitochondria). Glycolysis and the citric acid cycle take place in the bacterial cell cytosol, while electron transport components are localized to the bacterial plasma membrane. Since electron transport takes place at the plasma membrane, the pmf is generated across the plasma membrane. In addition to using the pmf to synthesize ATP, aerobic bacteria also use the pmf to power uptake of certain nutrients and cell swimming.

9. In addition to providing energy to power ATP synthesis, the pmf also provides the energy used by several active transport proteins to move substrates into the mitochondria and products out of the mitochondria. The $OH^-$ gradient, which results from generation of the pmf by electron transport, is used to move $HPO_4^{2-}$ into the matrix, and the voltage gradient contribution of the pmf drives exchange of ADP for ATP.

10. The Q cycle functions to double the number of protons transported per electron pair moving through a specific complex of the electron transport chain and thereby maximizes the pmf across a membrane. In mitochondria, the specific complex is the $CoQH_2$-cytochrome *c* reductase complex, while in chloroplasts it is the cytochrome *bf* complex, and in purple bacteria it is the cytochrome $bc_1$ complex. Using mitochondria as an example, the Q cycle is believed to function as follows: $CoQH_2$ arrives at the $Q_o$ site on the intermembrane space side of the $CoQH_2$-cytochrome *c* reductase complex; it delivers two electrons to the complex, and releases two protons into the intermembrane space. Next, one electron is transported directly to cytochrome *c* while the other partially reduces a CoQ molecule bound to the $Q_i$ site on the inner side of the complex, forming a CoQ semiquinone anion. CoQ dissociates from the $Q_o$ site and is replaced by another $CoQH_2$, which delivers two more electrons and releases two protons to the intermembrane space. As before, one electron is transferred to cytochrome *c*, but the other combines with the CoQ semiquinone anion at the $Q_i$ site to produce $CoQH_2$, thus regenerating one $CoQH_2$. In sum, the net result of the Q cycle is that four protons are transported to the intermembrane space for every two electrons moving through the $CoQH_2$-cytochrome *c* reductase complex.

11. $6CO_2 + 6H_2O \rightarrow 6O_2 + C_6H_{12}O_6$

$O_2$-generating photosynthesis uses the energy of absorbed light to create, via electron donation to quinone, the powerful oxidant $P^+$ form of the reaction center chlorophyll. This, in turn, acts to remove electrons from $H_2O$, a poor electron donor. The electrons are then passed along an electron transport chain, and the stored energy is converted to other forms for subsequent use in ATP synthesis and carbon fixation. The $O_2$ is not used in subsequent reactions in this pathway and thus is a by-product of the removal of electrons from $H_2O$.

12. Photosynthesis consists of four stages. During stage 1, which occurs in the thylakoid membrane, light is absorbed by the reaction center chlorophyll, a charge separation is generated, and electrons are removed from water, forming oxygen. During stage 2, electrons are transported via carriers in the thylakoid membrane to the ultimate electron donor, $NADP^+$, reducing it to NADPH, and protons are pumped from the stroma into the thylakoid lumen, producing a proton gradient across the thylakoid membrane. During stage 3, protons move down their electrochemical gradient across the thylakoid membrane through $F_0F_1$ complexes and power ATP synthesis. Finally, during stage 4, the ATP and NADPH generated in the earlier stages are used to drive $CO_2$ fixation and carbohydrate synthesis. $CO_2$ fixation occurs in the stroma and carbohydrate (sucrose) synthesis occurs in the cytosol.

13. Chlorophyll *a* is present in both reaction centers and antenna. Additionally, antennas contain either chlorophyll *b* (vascular plants) or carotenoids (plants and photosynthetic bacteria). Antennas capture light energy and transmit it to the reaction center, where the primary reactions of photosynthesis occur. The primary evidence that these pigments are involved in photosynthesis is that the absorption spectrum of these pigments is similar to the action spectra of photosynthesis.

14. Photosynthesis in green and purple bacteria does not generate oxygen because these bacteria have only one photosystem, which cannot produce oxygen. These organisms still utilize photosynthesis to produce ATP by utilizing cyclic electron flow to produce a pmf (but no oxygen or reduced coenzymes), which can be utilized by $F_0F_1$ complexes. Alternatively, this photosystem can exhibit linear, noncyclic electron flow, which will generate both a pmf and NADH. For linear electron flow, hydrogen gas ($H_2$) or hydrogen sulfide ($H_2S$) rather than $H_2O$ donates electrons, so no oxygen is formed.

15. PSI is driven by light of 700 nm or less and its primary function is to transfer electrons to the final electron acceptor, $NADP^+$. PSII is driven by light of 680 nm or less, and its primary function is to split water to yield electrons, as well as protons and oxygen. During linear electron flow, electrons move as follows: PSII (water split to produce electrons) → plastoquinone (Q) → cytochrome *bf* complex → Plastocyanin → PSI → $NADP^+$. The energy stored as NADPH is used to fix $CO_2$ and ultimately synthesize carbohydrates.

16. The Calvin cycle reactions are inactivated in the dark to conserve ATP for the synthesis of other cell molecules. The mechanism of inactivation depends on the enzyme; examples include pH-dependent and $Mg^{2+}$-dependent enzyme regulation, as well as reversible reduction-oxidation of disulfide bonds within certain Calvin cycle enzymes.

17. Rubisco, or ribulose 1,5-bisphosphate carboxylase is a large enzyme present in the stromal space of the chloroplast. Rubisco is the enzyme responsible for adding (fixing) inorganic carbon in the form of $CO_2$ to the five-carbon sugar ribulose 1,5 bisphosphate, which is rapidly cleaved into two molecules of 3-phosphoglycerate that can be converted into starch and sugars.

## *Analyze the Data*

a. The electron transport system normally pumps protons out of the mitochondrial matrix, increasing the pH of the matrix; thus the fluorescence of matrix-trapped BCECF would increase in intensity. The observed decrease in intensity of BCECF trapped inside the vesicles suggests that the vesicles have an inverted (inside-out) orientation, so that protons were pumped from the outside to the inside of the vesicles.

b. The concentrations of ADP, $P_i$, and oxygen should decrease over time as the process of oxidative phosphorylation utilizes oxygen as an electron acceptor and uses ADP and $P_i$ to synthesize ATP.

c. Dinitrophenol compromises the pH gradient and the resulting equilibration of protons leads to an increase in the intravesicular pH and corresponding increase in emission intensity. Valinomycin, a potassium ionophore, affects the electric potential more than the pH gradient. Since

BCECF fluorescence reflects the pH of the milieu, it is largely unaffected by valinomycin-induced changes in the transmembrane electric potential.

d. The fluorescence intensity inside the vesicles should remain constant over time since inner mitochondrial membranes from brown fat tissue would likely contain thermogenin, a protein that functions as an uncoupler of oxidative phosphorylation. Since thermogenin is a proton transporter, its presence would prevent the generation of a proton gradient and thus no fluorescence change would be expected.