Instructor’s Manual and
Active Learning Guide

To accompany *The Cell* 9e

**Chapter 12: Mitochondria, Chloroplasts, and Peroxisome**

**Chapter Overview**

In addition to being involved in protein sorting and transport, cytoplasmic organelles provide specialized compartments in which a variety of metabolic activities take place. The generation of metabolic energy is a major activity of all cells, and two cytoplasmic organelles are specifically devoted to energy metabolism and the production of ATP. Mitochondria are responsible for generating most of the useful energy derived from the breakdown of lipids and carbohydrates, and chloroplasts use energy captured from sunlight to generate both ATP and the reducing power needed to synthesize carbohydrates from CO2 and H2O. The third organelle discussed in this chapter, the peroxisome, contains enzymes involved in a variety of different metabolic pathways, including the breakdown of fatty acids.

Mitochondria, chloroplasts, and peroxisomes differ from the organelles discussed in the preceding chapter not only in their functions but also in their mechanism of assembly. Rather than being synthesized on membrane-bound ribosomes and translocated into the endoplasmic reticulum, most proteins destined for mitochondria, chloroplasts, and peroxisomes are synthesized on free ribosomes in the cytosol and imported into their target organelles as completed polypeptide chains. Mitochondria and chloroplasts also contain their own genomes, which include some genes that are transcribed and translated within the organelle.

**Chapter Outline**

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Coupling of proton flow to ATP production by ATP synthase

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The genetic system of mitochondria

Import of nuclear-encoded mitochondrial proteins

Mitochondrial lipids

Transport of metabolites across the inner membrane

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Chloroplast structure and composition

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The chloroplast genome

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12.6 Peroxisomes

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**Section Reviews**

12.1 Mitochondrial Structure, Composition, and Dynamics

Mitochondria are surrounded by a double-membrane system. The matrix contains the enzymes of the citric acid cycle; the inner membrane contains protein complexes involved in electron transport and oxidative phosphorylation. In contrast to the inner membrane, the outer membrane is freely permeable to small molecules. Mitochondria also contain their own genomes, which encode rRNAs, tRNAs, and some of the proteins involved in oxidative phosphorylation. However, most mitochondrial proteins are encoded by the nuclear genome. These proteins are translated on free ribosomes and imported into mitochondria as completed polypeptide chains. Positively charged presequences target proteins for import to the mitochondrial matrix and inner membrane, with protein import driven by the electrochemical gradient across the inner membrane. The electrochemical gradient also drives the transport of ATP, ADP, and other metabolites into and out of mitochondria.

12.2 Oxidative Catabolism of Glucose and Fatty Acids

Aerobic organisms extract high-energy electrons from sugars, amino acids and lipids, use that energy to synthesize ATP, and ultimately deposit the now low-energy electrons on oxygen to produce water in a multistep process called cellular respiration. Glycolysis (the first stage) occurs in the cytoplasm and oxidizes glucose to two molecules of pyruvate and generates two reduced NADH and two ATP. The two pyruvates are decarboxylated, causing the loss of one CO2 per pyruvate. The resulting acetyl groups (two per glucose) are attached to coenzyme A (CoA) and transported into the mitochondrial matrix. There, enzymes of the citric acid cycle fully oxidize the molecules to four CO2 and use those electrons to reduce six NADH and two FADH2 (again, per glucose molecule). In all, one glucose molecule yields two substrate-level ATP (in glycolysis), six CO2, and 24 electrons upon full oxidation. Electrons may also enter the citric acid cycle via the oxidation of fatty acids. Two carbon atoms at a time are removed from a 14- to 16-carbon fatty acid. The resulting acetyl groups are ligated to CoA and enter the citric acid cycle as described above. Fatty acid oxidation produces 2.5 times more ATP per gram than the oxidation of glucose.

12.3 Oxidative Phosphorylation

The NADH and FADH2 derived from glycolysis and the citric acid cycle, and residing in the mitochondrial matrix, are used to reduce a series of proteins and cofactors in the inner mitochondrial membrane called the mitochondrial electron transport chain. As electrons move through the chain, protons are translocated from the matrix to the intermembrane space creating a transmembrane pH gradient. The electrons return to the matrix side and reduce oxygen to the level of water. Protons return to the matrix by passing through the ATP synthase, and ATP is made. Thus, the energy in the proton gradient is coupled to the synthesis of ATP—a process called chemiosmotic coupling. Because ATP is synthesized (ADP is phosphorylated) by oxidizing reduced carbon compounds (glucose or lipids), this process is termed oxidative phosphorylation.

12.4 Mitochondrial Assembly and Maintenance

Mitochondria contain their own DNA, which is prokaryotic-like due to the endosymbiotic nature of their origin. The DNA codes for a small minority of the organelle’s proteins (only 13 in mammals, 31 in *Arabidopsis*). The other approximately 1500 proteins are encoded on nuclear genes, synthesized in the cytoplasm, and imported into the organelle. Several classes of targeting signals direct the imported proteins to their final destination in the outer mitochondrial membrane, the intermembrane space, the inner membrane, or the matrix. Proteins are imported and sorted by the Tom (translocase of the outer membrane), Tim (translocase of the inner membrane), and SAM (sorting and assembly machinery) protein complexes. In animals, mitochondrial lipids are synthesized by the ER and imported via phospholipid transfer proteins. Numerous metabolites are transported into and out of mitochondria. The ouert membrane contains nonspecific porin proteins that allow for passive diffusion. However, the inner membrane uses the electrochemical gradient generated by the electron transport chain and specific transport proteins to facilitate the exchange of metabolites between the matrix and intermembrane space.

12.5 Chloroplasts and Other Plastids

Chloroplasts are large organelles that function in photosynthesis and other metabolic activities. Like mitochondria, chloroplasts are bounded by a double-membrane envelope. Unlike mitochondria, chloroplasts have a third, internal membrane system called the thylakoid membrane, which is the site of light-driven electron transport and ATP generation. Chloroplast genomes contain approximately 150 genes, which code for proteins involved in photosynthesis and metabolism. Most chloroplast proteins are synthesized on free ribosomes in the cytosol and targeted for import to chloroplasts by amino-terminal transit peptides. Proteins incorporated into the thylakoid membrane or thylakoid lumen are first imported into the chloroplast stroma and then targeted for transport into or across the thylakoid membrane. Other plastids store energy sources, such as starch and lipids, and function in diverse aspects of plant metabolism.

12.6 Peroxisomes

Peroxisomes are small organelles, bounded by a single membrane, that contain enzymes involved in a variety of metabolic reactions, including fatty acid oxidation, lipid biosynthesis, the glyoxylate cycle, and photorespiration. Most transmembrane proteins are transported to peroxisomes from the ER, whereas internal peroxisomal proteins are synthesized on free ribosomes in the cytosol and imported into peroxisomes as completed and folded polypeptide chains. Peroxisomes can be formed both *de novo* and by growth and division of existing peroxisomes.

**Key Terms**

adenine nucleotide translocator

amyloplast

ATP synthase

Calvin cycle

cardiolipin

catalase

chemiosmotic coupling

chlorophyll

chromoplast

citric acid cycle

coenzyme (CoA-SH)

coenzyme Q

complex V

cristae

cyclic electron flow

cytochrome *bf* complex

cytochrome *c*

elaioplast

mitochondrial replacement therapy

mitofusin1 (Mfn 1)

optic atrophy 1 (Opa1)

NADH dehydrogenase

NADP reductase

Oxa1

peroxin

peroxisomal ER

peroxisome biogenesis disorder

peroxisome targeting signal 1 (PTS1)

peroxisome targeting signal 2 (PTS2)

peroxisome

Pex protein

Pex13

Pex14

Pex17

Pex5

Pex7

phospholipid transfer protein

photocenter

photosynthesis

Photosystem I

Photosystem II

plasmalogen

plastid

plastoquinol

plastoquinone

porin

presequence

proplastid

SAM complex

stroma

stromal processing peptidase (SPP)

substrate-level phosphorylation

succinate reductase

thylakoid membrane

Tic complex

Tim complex

Tim22

Tim23

Tim9-Tim10

Toc complex

Tom complex

transit peptide

ubiquinol

ubiquinone

**Additional Media and Supplements for Use in the Classroom**

Additional instructor materials to help you and your students get the most out of this chapter can be found at [oup.com/he/cooper9e](http://www.oup.com/us/cooper8e). These include:

*Textbook Figures and Tables PowerPoint Presentation*

*Chapter Quiz*

*Chapter Test Bank*

*Data Analysis Problems with Answers*

The E-book contains a number of resources to help students understand and assess their learning:

*Animations and Videos*

*Flashcards*

*Self-Assessments*

*Micrographs*

Active Learning Activities for the Classroom

12.1 Mitochondrial Structure, Composition, and Dynamics

**Learning Objectives**
Students should be able to:

12.1.1 Illustrate the functional organization of mitochondria.

12.1.2 Compare and contrast the characteristics of the two mitochondrial membranes and how their characteristics relate to their roles.

12.1.3 Describe the dynamic nature of mitochondrial networks resulting from mitochondrial fusion and fission.

**Active Learning Exercises**

1. Have students prepare a **matrix** in which they compare and contrast the genomes of the following: a free-living *α*-proteobacterium, *Rickettsia prowazekii*, *A. thaliana* mitochondrion, and *H. sapiens* mitochondrion. (LO 12.1.1 Illustrate the functional organization of mitochondria.)

*Answer: (Numbers in bold should be provided to students, as they do not appear in the textbook.)*

|  |  |  |  |
| --- | --- | --- | --- |
| Genome  | Genome size | Number of genes | Number of proteins coded for |
| α-proteobacteria | **1,000−1,500 kb** | 7,000 | **≈700** |
| *Rickettsia prowazekii*  | **1,111 kb** | 7,000 | **684** |
| *A. thaliana* mitochondrion | 370 kb | **57** | 31 |
| *H. sapiens* mitochondrion | 16 kb | 37 | 13 |

2. Have students write a **minute paper** explaining why the Tim complex uses an electrochemical potential for protein translocation, while the Tom complex does not. (LO 12.1.2 Compare and contrast the characteristics of the two mitochondrial membranes and how their characteristics relate to their roles.

 *Answer: The outer membrane, where the Tom complex resides, cannot generate an electrochemical potential.*

3. Ask students in **think-pair-share** groups to answer the question: “Why is the voltage component used to transport ADP/ATP across the mitochondrial inner membrane, but the pH gradient used to transport pyruvate (which has a net charge of −1)?” (LO 12.1.2 Compare and contrast the characteristics of the two mitochondrial membranes and how their characteristics relate to their roles.)

 *Answer: ADP and ATP are both charged (−3 and −4, respectively), so a one-for- one swap results in a net loss of membrane potential, which must be replaced by pumping (electrically charged) protons. However, swapping pyruvate (−1) with a hydroxyl (−1) is electrically neutral but reduces the pH gradient.*

**Clicker Questions**

1. Respiration takes place in the mitochondrion.

a. True

b. False

(LO 12.1.1 Illustrate the functional organization of mitochondria)

2. The majority of the genes in the human mitochondrial genome code for

a. tRNAs and rRNAs needed for translation.

b. proteins needed for mitochondrial electron transport.

c. proteins needed for export of ATP from the matrix.

d. proteins of the citric acid cycle.

(LO 12.1.1 Illustrate the functional organization of mitochondria)

3. Where are the genes for the human mitochondrial RNA polymerase?

a. In the nuclear genome

b. In the mitochondrial genome

(LO 12.1.1 Illustrate the functional organization of mitochondria)

4. The electrical component and the chemical component of the energy stored by the mitochondrial inner membrane are both established by the movement of

a. electrons through the membrane.

b. ATP across the membrane.

c. pyruvate across the membrane.

d. protons across the membrane.

(LO 12.1.2 Compare and contrast the characteristics of the two mitochondrial membranes and how their characteristics relate to their roles)

5. The electrochemical gradient across the mitochondrial inner membrane captures energy that ultimately came from

a. mitochondrial electron transport.

b. photosynthesis.

c. glycolysis.

d. the sun.

(LO 12.1.2 Compare and contrast the characteristics of the two mitochondrial membranes and how their characteristics relate to their roles)

***Answers: 1: b; 2: a, 3: a; 4: d; 5: d***

**Essay/Discussion Question**

What are porins?

*Answer: Porins are transmembrane proteins that form large pores. They are found in chloroplast and mitochondrial outer membranes. They allow the passage of molecules smaller than 1,000 daltons, and thus the space between the inner and outer membranes is equivalent to the cytosol in its concentration of ions and small molecules.*

*Learning Objective 12.1.1:* Illustrate the functional organization of mitochondria.

# **12.2 Oxidative Catabolism of Glucose and Fatty Acids**

**Learning Objectives**

Students should be able to:

12.2.1 Summarize the reactions through which glucose is catabolized during glycolysis and the citric acid cycle and how they contribute to ATP production.

12.2.2 Describe the breakdown of fatty acids and how they contribute to ATP production.

12.2.3 Compare the relative yields in ATP production between glucose and fatty acid catabolism.

**Media Resources**

Animation 12.1 Glycolysis

Animation 12.2: The Citric Acid Cycle

Video: ATP Synthase in Action
Key Experiment: The Chemiosmotic Theory

**Active Learning Exercises**

1. In **small discussion groups,** have students consider how the waste products of cellular respiration, CO2 and H2O, relate to fire extinguishers. (LO 12.2.1 Summarize the reactions through which glucose is catabolized during glycolysis and the citric acid cycle and how they contribute to ATP production.)

Answer: After most of the energy (in the form of high-energy electrons) from the glucose molecules has been extracted to make ATP via substrate level or chemiosmosis, all the atoms and the electrons that carried that energy still remain. The carbon atoms are lost as CO2, and the electrons end up on oxygen to form water. Because the energy was stored in the ATP, CO2 and H2O are low-energy molecules that are good at extinguishing fires.

1. Instruct students to prepare a **sequence map** that lays out the steps in the breakdown of lipids. Then choose a student to share his or her map with the class.

(LO 12.2.2 Describe the breakdown of fatty acids and how they contribute to ATP production.)

Answer: See Figure 12.10.

**Clicker Questions**

1. Glucose brings carbon atoms and *electrons* to glycolysis and the citric acid cycle (CAC). The carbon atoms are lost in \_\_\_\_\_\_\_, whereas the electrons end up \_\_\_\_\_\_\_.

a. the CAC as CO2; in water

b. glycolysis; as NAD+

c. the electron transport chain; in ATP

d. the CAC as CO2; in ATP

LO 12.2.1 Summarize the reactions through which glucose is catabolized during glycolysis and the citric acid cycle and how they contribute to ATP production.

2. Glucose brings carbon atoms and *energy* to glycolysis and the citric acid cycle. The carbon atoms are lost in \_\_\_\_\_\_\_, whereas the energy ends up \_\_\_\_\_\_\_.

a. the CAC as CO2; in water

b. glycolysis; as NAD+

c. the electron transport chain; in ATP

d. the CAC as CO2; in ATP

LO 12.2.1 Summarize the reactions through which glucose is catabolized during glycolysis and the citric acid cycle and how they contribute to ATP production.

3. Why are lipids, and not polysaccharides, the main form of energy storage in animals?

a. Lipids are more easily digested than polysaccharides.

b. Lipids have substantially more energy stored as starch.

c. Lipids are hydrophobic, while polysaccharides are hydrophilic.

d. Animals lack the metabolic pathways to synthesize polysaccharides.

(Have students discuss their answer choices. Then initiate a discussion with this explanation: Animals are mobile and, as such, need to minimize weight. Lipids pack more energy per gram than polysaccharides and they do not bind water. Animals store fat. Plants, on the other hand, are sessile. They “don’t mind” if their energy storage form also binds a lot of water weight because they are stuck in one place. Plants store starch (and all the water that is bound to it). The one instance in which this is not true for plants is seeds, which often store oils (peanut oil, soybean oil, corn oil, canola oil, and many others are all extracted from seeds) and may need to be transported long distances. This also relates to why marathon runners gain a few pounds and feel bloated when they carbo load. The added glycogen binds water, which is heavy.)

LO 12.2.2 Describe the breakdown of fatty acids and how they contribute to ATP production.

**Answers: 1: a; 2: d; 3: b, c.**

**Essay/Discussion Question**

Dihydroxyacetone is interconvertible with glyceraldehyde-3-phosphate. If this were not the case, what would be the net ATP yield from glycolysis?

*Answer: Although both dihydroxyacetone and glyceraldehyde-3-phosphate are products of glycolytic metabolism of glucose, only glyceraldehyde-3-phosphate is metabolized further in glycolysis. In total, for each glucose molecule metabolized through glycolysis, one molecule of dihydroxyacetone and one molecule of glyceraldehyde-3-phosphate are formed. The dihydroxyacetone is metabolized further through glycolysis only if converted to glyceraldehyde-3-phosphate. If this conversion did not occur, only the two molecules of ATP generated directly from the further glycolytic metabolism of glyceraldehyde-3-phosphate would be formed. If only two molecules of ATP were generated from glycolysis, then the net formation of ATP from glucose would be zero, as two molecules of ATP are consumed in the first portion of glycolysis. It is only because dihydroxyacetone is converted that four molecules of ATP are generated in the second half of glycolysis to give a net formation of two molecules of ATP per glucose.*

*Learning Objective 12.2.1:* Summarize the reactions through which glucose is catabolized during glycolysis and the citric acid cycle and how they contribute to ATP production.

# **12.3 Oxidative Phosphorylation**

**Learning Objectives**

Students should be able to:

12.3.1 Compare the mechanisms of ATP formation during glycolysis and oxidative phosphorylation.

12.3.2 Explain chemiosmotic coupling.

12.3.3 Describe how the transfer of electrons from NADH and FADH2 to O2 yields an electrochemical gradient.

12.3.4 Illustrate how a proton gradient across the inner mitochondrial membrane is used to produce ATP.

**Active Learning Exercises**

1. Have students write a **minute paper** in which they compare the oxidation of glucose during respiration to the oxidation of glucose in a burning marshmallow. What happens to the energy that is released? *(*LO 12.3.3 Describe how the transfer of electrons from NADH and FADH2 to O2 yields an electrochemical gradient.? )

*Answer: In both processes, the high-energy electrons in the carbon−carbon bonds of the glucose are transferred from the glucose molecule to molecular oxygen. In the case of cellular respiration, much of the energy is captured and stored in ATP. In the case of the burning marshmallow, the energy is converted to light and heat and lost to the environment.*

2. Have students write a **minute paper** explaining why mitochondrial ATP synthesis is called “oxidative phosphorylation.” (LO 12.3.1 Compare the mechanisms of ATP formation during glycolysis and oxidative phosphorylation.)

Answer: To produce ATP, an inorganic phosphate group is added to ADP (ADP + Pi 🡪 ATP). The energy to drive that reaction is derived from the oxidation of glucose and fatty acids. Hence “oxidative phosphorylation”.

3. Have students form **small discussion groups** and contemplate the following two questions.

1. In chemiosmotic coupling, what is coupled to what?
2. An antibiotic called gramicidin D forms pores in biological membranes . Why would a scientist call a gramicidin D an “uncoupler”? (LO 12.3.2 Explain chemiosmotic coupling.)

Answers: a. The energy in the transmembrane pH gradient is coupled to the synthesis of ATP. b. By poking holes in the inner mitochondrial membrane protons leak back to the matrix without passing through the ATP synthase. The transmembrane pH gradient is collapsed, but no ATP is formed. Hence, energy in the transmembrane pH gradient is uncoupled from the synthesis of ATP

**Clicker Questions**

1. The ATP made via substrate level phosphorylation is different from the ATP made by chemiosmosis.

a. True: The mechanisms of synthesis are fundamentally different.

b. False: ATP is the same molecule regardless of how or where it is made.

LO 12.3.1 Compare the mechanisms of ATP formation during glycolysis and oxidative phosphorylation.

2. Electrons are used to make ATP as they move through the electron transport chain, and they never make it to the end of the chain.

a. True: Electrons are nothing more than packets of energy. When they give up their energy, they cease to exist.

b. False: The electrons are used to reduce oxygen to the level of water. They have lost much of their energy, but they still exist as discrete electrons.

LO 12.3.3 Describe how the transfer of electrons from NADH and FADH2 to O2 yields an electrochemical gradient.

3. Because chemiosmotic ATP synthesis relies on the flow of electrons through an electron transport chain, chemiosmosis is considered to be a redox reaction.

a. True: Any reaction that relies on the transfer of electrons from one molecule to the next is, by definition, a redox reaction.

b. False: The ETC is a series of redox reactions, but the ATP synthase is powered directly by the flow of protons across the membrane.

LO 12.4.3 Illustrate how a proton gradient across the inner mitochondrial membrane is used to produce ATP.

4. In the electron transport chain, negatively charged electrons (e-) and positively charged protons (H+) start on the \_\_\_\_\_\_\_ side of the membrane and end up on the \_\_\_\_\_\_\_ side. This is called \_\_\_\_\_\_\_.

a. matrix (both); cytoplasmic (both); chemiosmosis

b. matrix (e-); cytoplasmic (H+); an electron transport

c. matrix (H+); cytoplasmic (e-); oxidative phosphorylation

d. matrix (both); matrix (e-) and cytoplasmic (H+); an electrochemical gradient

LO 12.3.3 Describe how the transfer of electrons from NADH and FADH2 to O2 yields an electrochemical gradient.

**Answers: 1: b; 2: b; 3: b; 4: d**

**Essay/Discussion Questions**

1. To harvest energy in the absence of oxygen, a cell breaks down glucose at a steady and rapid rate. If oxygen becomes available, the rate of glucose breakdown will decrease and be maintained at a much lower rate than in the absence of oxygen. Explain why this occurs.

 *Answer: In the absence of oxygen, the cell ferments glucose to lactate, using the glycolysis pathway to generate ATP. In the presence of oxygen, the cell switches to oxidative phosphorylation, which generates ATP much more efficiently than glycolysis. Therefore, less glucose is needed to supply ATP at the same rate.*

 *LO 12.3.1:* Compare the mechanisms of ATP formation during glycolysis and oxidative phosphorylation.

2. At low pH, the chemical 2,4-dinitrophenol (DNP) is neutral and can diffuse freely across membranes, including those of mitochondria. At high pH, it gives off a proton, becomes negatively charged, and can no longer diffuse across membranes. What effect would DNP have on ATP production by mitochondria?

 *Answer: DNP would have the effect of dissipating the proton gradient across the inner membrane. It would be neutral on the outside and thus pass freely into the mitochondrial lumen, where it would encounter a high pH environment and release its proton. Thus the overall effect would be proton transport into the mitochondrial matrix. This would result in a halt in ATP production via oxidative phosphorylation, which depends on the proton gradient, and would drastically decrease the overall ATP production of the cell.*

 *LO 12.3.2:* Explain chemiosmotic coupling.

3. Traditionally, it was assumed that ATP synthesis in mitochondria would be via high-energy phosphate group transfer intermediates, but none have ever been found. How does the chemiosmotic theory explain their absence?

 *Answer: According to the chemiosmotic theory, the movement of protons down an electrochemical gradient across the inner mitochondrial membrane drives the formation of ATP. This occurs because the proton movement causes electrically driven shape changes in the F0 subunit of the ATP synthase. The F0 and F1 subunits are coupled. The F1 subunit of the motorlike ATP synthase catalyzes the synthesis of ATP from ADP and Pi. The mechanism is entirely different from that of a high-energy phosphate intermediate.*

 *LO 12.3.2:* Explain chemiosmotic coupling.

4. ATP synthase is composed of two complex proteins, F0 and F1. What is the function of each protein complex, and where is each found in mitochondria?

 *Answer: F0 is a proton channel, and the F1 complex is an ATP synthase. F0 is found in the mitochondrial inner membrane, and F1 is associated with F0 on the matrix face of the inner mitochondrial membrane.*

 *LO 12.3.1:* Compare the mechanisms of ATP formation during glycolysis and oxidative phosphorylation.

# **12.4 Mitochondrial Assembly and Maintenance**

**Learning Objectives**

Students should be able to:

12.4.1 Describe mitochondrial genomes and their contribution to the protein composition of mitochondria.

12.4.2 Summarize how proteins and lipids are imported into mitochondria.

12.4.3 Explain the role of the proton gradient in the transport of proteins and metabolites across the mitochondrial membrane.

**Media Resources**

Video: Mitochondrial Networks
Video: Mitochondrial Dynamics
Molecular Medicine: Mitochondrial Replacement Therapy
Data Analysis Problem: Electron Microscopic Analysis of Mitochondrial DNA
Data Analysis Problem: Staining of Mitochondria with a Fluorescent Dye
Data Analysis Problem: Mitochondrial Protein Import

**Active Learning Exercises**

1. Have students form **small discussion groups** and discuss how mitochondrial replacement therapy works. (LO 12.4.1 Describe mitochondrial genomes and their contribution to the protein composition of mitochondria.)

*Answer: The name is a bit of a misnomer. The mitochondria are not replaced. The chromosomes from an egg with healthy mitochondria are removed and replaced with the chromosomes from an egg with dysfunctional mitochondria.*

2. Mitochondria arose endosymbiotically and therefore have their own genome. However, that genome only codes for <1% of the mitochondrial proteins. The others are encoded in the nucleus and synthesized on cytoplasmic ribosomes. Have students use their devices to research and write a **minute paper** answering the following question: “If the original endosymbiont had a full genome, where did 99% of its genes go?” (LO 12.4.1 Describe mitochondrial genomes and their contribution to the protein composition of mitochondria.)

*Answer: They moved via horizontal gene transfer into the host nucleus. You may wish to also have students research and discuss horizontal gene transfer*.

3. Have students form **small discussion groups** to compare and contrast the Tom and Tim protein translocons. You may also have them compare the Tom/Tic system to the Toc/Tic system found in chloroplasts (section 12.5)

*Answer: See text for details, section 12.4.*

**Clicker Questions**

1. What is the main difference between the Tom and Tim complexes?

a. The Tom complex is located in the inner mitochondrial membrane; the Tim in the outer.

b. The Tom complex is made of lipids while the Tim complex contains proteins.

c. The Tom complex generates its own electrochemical gradient.

d. The Tom complex relies on passive diffusion for transport while the Tim complex used and electrochemical gradient.

LO 12.4.2 Summarize how proteins and lipids are imported into mitochondria.

2. Phospholipid transfer proteins are able to move hydrophobic lipid molecules through the aqueous space of the cytoplasm by

a. adding hydrophilic amino acids to the lipids.

b. changing the charge on the lipid from positive to neutral.

c. burying the lipid in a hydrophobic pocket inside the protein.

d. removing the lipid portion of the molecule.

LO 12.4.2 Summarize how proteins and lipids are imported into mitochondria.

3. A molecule with a net charge of +1 would be transported across the inner mitochondrial membrane by using the \_\_\_\_\_\_\_ of the electrochemical gradient.

a. voltage component

b. pH component

c. both components

d. neither component

LO 12.4.2 Summarize how proteins and lipids are imported into mitochondria.

***Answers: 1: d; 2: c; 3: a.***

**Essay/Discussion Questions**

1. Mitochondrial mRNAs have short poly-A sequences at their 3′ end. Poly-A tails are generally considered to be a feature of eukaryotic, not bacterial, mRNA. How can this observation be reconciled with an endosymbiotic origin for mitochondria?

 *Answer: The mitochondria of today are not the mitochondria originally introduced via endosymbiosis. Present-day mitochondria have evolved from their original status of newly introduced endosymbionts. The genome of present-day mitochondria exhibits a mix of bacterial and eukaryotic traits, which can explain this eukaryotic-like trait of mitochondrial mRNA. The presumption is that the development of poly-A tails originated after the endosymbiotic introduction of mitochondria into eukaryotic cells.*

 *LO 12.4.1:* Describe mitochondrial genomes and their contribution to the protein composition of mitochondria.

2. At low pH, the chemical 2,4-dinitrophenol (DNP) is neutral and can diffuse freely across membranes, including those of mitochondria. At high pH, it gives off a proton, becomes negatively charged, and can no longer diffuse across membranes. What effect would DNP have on the proton gradient between the mitochondrial intermembrane space and matrix?

 *Answer: DNP would dissipate the proton gradient across the inner membrane. DNP in the intermembrane space would be neutral in the presence of the high concentration of protons and thus pass freely into the mitochondrial lumen, where it would encounter a high pH environment and release its proton. This would neutralize the proton gradient and drastically decrease the overall ATP production of the cell.*

 *LO 12.4.3:* Explain the role of the proton gradient in transport or proteins and metabolites across the mitochondrial membrane.

12.5 Chloroplasts and Other Plastids

**Learning Objectives**

You should be able to:

12.5.1 Compare the structural and functional organization of chloroplasts with mitochondria as well as their respective genomes.

12.5.2 Explain how energy from sunlight is harvested by chlorophyll and used to drive production of ATP and NADPH via the light reactions in chloroplasts.

12.5.3 Summarize how the Calvin cycle reactions produce glucose.

12.5.4 Summarize the mechanisms of protein import into chloroplasts.

12.5.5 Describe the roles of other plastids.

**Media Resources**

Animation 12.3: The Light Reactions

Animation 12.4: The Calvin Cycle
Animation 12.5: From Proplastic to Chloroplast

Video: Chloroplasts in *Elodea*

**Active Learning Exercises**

1. Have students **sketch** a mitochondrion and a chloroplast, labeling all components and indicating which membrane is responsible for energy transduction in each organelle. (LO 12.5.1 Compare the structural and functional organization of chloroplasts with mitochondria as well as their respective genomes.)

 *Answer: See Figures 12.3, 12.25, and 12.26.*

2. Have students form **small discussion groups** and determine why a presequence would *not* work in moving chloroplast proteins through the Toc/Tic complex. (LO 12.5.4 Summarize the mechanisms of protein import into chloroplasts.)

*Answer: The presequence on mitochondrial proteins is composed of positively charged amino acids. This allows the electric potential across the mitochondrial inner membrane to drive translocation of the positively charged presequence. The inner membrane of the chloroplast envelope does not establish a membrane voltage.*

3. Have students use their smart devices to research plastids, then write a **minute paper** on the structure and function of any one of the plastid types (chloroplast is excluded). (LO 12.5.5 Describe the roles of other plastids)

*Answer: (Any plastid type can be chosen.)*

4. Have students form small groups and discuss what is incorrect about the following statement: Energy from sunlight is absorbed by chlorophylls, exciting electrons to a higher energy state. These high-energy electrons are then transferred from the chlorophyll molecules through a series of membrane carriers, coupled to the synthesis of ATP and the reduction of NADP+ to NADPH. (LO 12.5.2 Explain how energy from sunlight is harvested by chlorophyll and used to drive production of ATP and NADPH via the light reactions in chloroplasts.)

Answer: The excited electrons of the antenna chlorophyll do not leave the individual chlorophyll molecules and are not transferred through the electron transfer chain. Energy is transferred from antenna chlorophyll to antenna chlorophyll via resonance transfer (see Figure 12.28). Ultimately, an electron is extracted from water and moved through the electron transport chain (see Figure 12.29).

5. A major difference between ATP synthesis in mitochondria and ATP synthesis in chloroplasts is that the mitochondrial ATP is exported to the cytoplasm, but the chloroplast ATP molecules never leave the stroma. Have students form **small groups** to discuss this question: How does the energy in photosynthetic ATP exit the chloroplast? (LO 12.5.3 Summarize how the Calvin cycle reactions produce glucose.)

*Answer: The high-energy terminal phosphate on ATP is transferred to glyceraldehyde-3-phosphate (G3P). The G3P then leaves the chloroplast as a phosphorylated (and reduced, high-energy) molecule. Note: G3P exits via a G3P/PO4 antiporter. If all the chloroplast did was export G3P, it would very quickly run out of the PO4 groups needed for chemiosmosis.*

6. Have students form **think-pair-share** groups to consider the relative life spans of the products of the light-dependent reaction and the light-independent reactions of photosynthesis. How does that relate to petroleum and coal? (LO 12.5.2 Explain how energy from sunlight is harvested by chlorophyll and used to drive production of ATP and NADPH via the light reactions in chloroplasts. LO 12.5.3 Summarize how the Calvin cycle reactions produce glucose.)

Answer: The ATP and NADPH produced by the light-dependent reactions have life spans of less than a millionth of a second. However, the life span of reduced carbon is indefinite. The energy in fossil fuels was stored by the light-independent reactions of photosynthesis some 100−500 million years ago.

**Clicker Questions**

1. The mitochondrion has \_\_\_\_\_\_\_ membrane system(s) and \_\_\_\_\_\_\_ energy- transducing membrane(s) whereas the chloroplast has \_\_\_\_\_\_\_ membrane system(s) and \_\_\_\_\_\_\_ energy-transducing system(s).

a. 1; 2; 1; 3

b. 2; 1; 3; 1

c. 3; 1; 2; 1

d. 4; 3; 2; 1

LO 12.5.1 Compare the structural and functional organization of chloroplasts with mitochondria as well as their respective genomes

2. Ribulose bisphosphate carboxylase/oxygenase has two subunits: large (LSU) and small (SSU). The holoenzyme is composed of eight subunits of each. Hence, it is a 16-mer. The LSU is synthesized in the stroma and the SSU is synthesized in the cytosol and imported to the stroma. In order to efficiently synthesize a functional 16-mer holoenzyme, there must be

a. an effective means of signaling between the chloroplast genome and the nuclear genome.

b. a significant amount of LSU protein imported into the chloroplast.

c. chaperones to correctly assemble the 16 subunits into an active enzyme.

d. export of the LSU mRNA from the chloroplast for translation on cytoplasmic ribosome

LO 12.5.4 Summarize the mechanisms of protein import into chloroplasts.

3. For a protein synthesized in the cytosol to be imported to the thylakoid lumen, it must have

a. a presequence.

b. a signal sequence.

c. a presequence and a signal sequence.

d. two signal sequences.

LO 12.5.4 Summarize the mechanisms of protein import into chloroplasts.

4. All plastids begin as proplastids with their mature form determined by environmental signals and cell differentiation.

a. True

b. False

LO 12.5.5 Describe the roles of other plastids.

5. Chlorophyll is a unique molecule in the way that energy migrates through the photosystem antenna.

a. True: The energy migrates as resonance transfer from one chlorophyll molecule to the next, not as a redox reaction.

b. False: The energy migrates as electrons moving from one chlorophyll molecule to the next, a form of the very common oxidation/reduction.

LO 12.5.2 Explain how energy from sunlight is harvested by chlorophyll and used to drive production of ATP and NADPH via the light reactions in chloroplasts.

6. Cyclic electron flow around photosystem I generates

a. NADPH but not ATP.

b. ATP and NADPH.

c. a proton gradient and NADPH.

d. G3P and ATP.

LO 12.5.2 Explain how energy from sunlight is harvested by chlorophyll and used to drive production of ATP and NADPH via the light reactions in chloroplasts.

7. The substrates for the light-dependent reactions are

a. photons, H2O, and NADP+.

b. CO2 and H2O.

c. H2O and photons.

d. protons and electrons.

LO 12.5.2 Explain how energy from sunlight is harvested by chlorophyll and used to drive production of ATP and NADPH via the light reactions in chloroplasts.

8. The substrates for the light-independent reactions are

a. H2O, CO2, and NADPH.

b. CO2, NADPH, and ATP.

c. NADPH, G3P, and PSII.

d. glucose and O2.

LO 12.5.3 Summarize how the Calvin cycle reactions produce glucose.

9. Consider a chloroplast in the light. If the pH of the lumen is ~5.0, what would you expect the pH of the stroma to be?

a. ~3.0.

b. ~5.0.

c. ~7.0.

d. ~8.0.

LO 12.5.2 Explain how energy from sunlight is harvested by chlorophyll and used to drive production of ATP and NADPH via the light reactions in chloroplasts.

10. The primary end product of photosynthesis is glucose.

a. True: glucose is used to make starch, which is exported from the chloroplast and stored in the cytoplasm before it is excreted as waste.

b. False: The primary end product is G3P, which is exported to the cytoplasm and used as the basis for every carbon-based molecule in the plant (and ultimately humans).

LO 12.5.3 Summarize how the Calvin cycle reactions produce glucose.

***Answers: 1: b; 2: a , c; 3: d; 4: a; 5: a; 6: c; 7: a; 8: b; 9: d; 10: b***

**Essay/Discussion Questions**

1. Explain the role of endosymbiosis in the evolution of mitochondria and chloroplasts.

 *Answer: Because of the prokaryotic nature of the organelle and the striking similarity between the genomes of mitochondria and some bacteria (most notably Rickettsia prowazekii), it has been hypothesized that mitochondria evolved from an endocytic event in which a bacterium was endocytosed by a eukaryotic cell. The eukaryotic cell would have provided the bacterium with protection from the outside world, and the eukaryotic cell would have benefited from the bacterium’s oxidative phosphorylation system for energy production. The evolution of chloroplasts is hypothesized to have occurred in much the same way but at a somewhat later date.*

 *LO 12.5.1:* Compare the structural and functional organization of chloroplasts with mitochondria as well as their respective genomes.

1. Suppose you are visiting a local greenhouse and the greenhouse manager excitedly points out “the cool, white corn plant” nestled among the green ones. How large is the white plant likely to be? Explain your answer.

*Answer: The plant is very small. This is likely a mutant plant that lacks chlorophyll. Therefore, it cannot perform photosynthesis and is doomed to starve to death. Such albino mutants, if left to themselves, rarely grow to more than a few centimeters in height.
LO 12.5.2:* Explain how energy from sunlight is harvested by chlorophyll and used to drive production of ATP and NADPH via the light reactions in chloroplasts.

3. Although none of the reactions in the Calvin cycle directly requires light to produce carbohydrates from CO2 and H2O, the cycle ceases in the dark. What is the reason?

 *Answer: The Calvin cycle uses the ATP and NADPH synthesized in the light reactions to drive the synthesis of carbohydrates from CO2 and H2O, and thus depends on light for its continuation. Though the Calvin cycle can occur in the so-called “dark reaction,” its duration of operation is very limited.*
*LO 12.5.3:* Summarize how the Calvin cycle reactions produce glucose.

4. Explain the functions of mitochondrial matrix processing peptidase (MPP) and chloroplast stromal processing peptidase (SPP).

 *Answer: Peptidases cleave peptide bonds in polypeptides and proteins. In both mitochondrial and chloroplast transport, there are N-terminal amino acid sequences that target polypeptides and proteins to the Tom and Toc complexes in the mitochondria and chloroplast outer membranes and then to the Tim and Tic complexes of the inner membranes. In mitochondria, the targets are the 15–55 amino acid N-terminal presequences, and in chloroplasts, the targets are the 30–100 amino acid N-terminal transit peptides. Once in the inner membrane, MMP cleaves the presequence in mitochondria, and SPP cleaves the transit peptide in chloroplasts.*

 *LO 12.5.1:* Compare the structural and functional organization of chloroplasts with mitochondria as well as their respective genomes.

5. Consider two unlabeled centrifuge tubes, each with a pellet at the bottom. One tube contains a pellet of mitochondria and the other a pellet of chloroplasts. How could the pellets be identified?

 *Answer: Chloroplasts are full of chlorophyll (green) and would produce a green pellet; mitochondria are colorless and would, if pure, produce a white pellet.*

 *LO 12.5.1:* Compare the structural and functional organization of chloroplasts with mitochondria as well as their respective genomes.

6. Presumably, the original chloroplast genome coded for many more proteins than it does today. Some of these genes must have been transferred to the nucleus, with the resulting protein product needing to be imported into chloroplasts. Other genes must have originated in the nucleus and were then adapted for import into chloroplasts. What were the likely protein adaptations that must have occurred for protein import into chloroplasts, and how might one determine whether the protein originated from a chloroplast or a nuclear-coded gene?

 *Answer: In general, a nuclearly coded and cytosolically synthesized protein must have an N-terminal transit peptide that can be recognized by the chloroplast guidance complex. If it does not, the polypeptide will not be guided toward the translocon located in the outer chloroplast membrane. This sequence would need to be added by some kind of gene fusion. Machinery for recognition and translocation would need to be adapted in the outer chloroplast membrane. Homology comparisons could be done to determine whether the protein is more similar to a bacterial or a eukaryotic protein. If it is more similar to a bacterial protein, it presumably originated in the chloroplast, regardless of where the coding sequence resides today.*

 *LO 12.5.4:* Summarize the mechanisms of protein import into chloroplasts.

7. All plant plastids contain the same genome as chloroplasts. However, chromoplasts, amyloplasts, and elaioplasts are clearly different from one another. What mechanism might explain the differences between these plastids that all have the same internal genes?

 *Answer: Plastid development is under the coordinate control of genes within the plastid and the nuclear genomes. Amyloplasts and elaioplasts are forms of leucoplasts. Leucoplasts are found in nonphotosynthetic tissues. Nonphotosynthetic tissues (e.g., roots) often have little, if any, exposure to sunlight, so sunlight may be a cue for chloroplast gene activation. However, in the case of chromoplasts in flowers, sunlight as a cue for gene expression does not provide an obvious explanation. Therefore, there must also be other environmental cues regulating chloroplast gene expression.*

 *LO 12.5.5:* Describe the roles of other plastids.

12.6 Peroxisomes

**Learning Objectives**

You should be able to:

12.6.1 Summarize the roles of peroxisomes in animal and plant cells.

12.6.2 Describe the pathways responsible for peroxisome biogenesis.

**Media Resources**

Molecular Medicine: Peroxisome Biogenesis Disorders

**Active Learning Exercises**

1. Have students prepare a **spider map** for peroxisomes. Place “Peroxisomal Functions” in the middle and the following terms as legs: fatty acid oxidation, catabolism of very-long-chain and branched fatty acids, lipid biosynthesis, synthesis of bile acids, synthesis of plasmalogens, mobilizing stored fatty acids, metabolize Calvin cycle products. Include (where possible) the location. (LO 12.6.1 Summarize the roles of peroxisomes in animal and plant cells.)

*Answer: Functions with locations - fatty acid oxidation (plants, animals and yeasts), catabolism of very-long-chain and branched fatty acids (mammals), lipid biosynthesis (animals), synthesis of bile acids (liver), synthesis of plasmalogens (liver), mobilizing stored fatty acids (seeds), metabolize Calvin cycle products (plants).*

2. Have students form **small discussion groups** and consider the unique aspects of peroxisome biogenesis. (LO 12.6.2 Describe the pathways responsible for peroxisome biogenesis.)

*Answer: Two pre-peroxisomes bud off of the ER, each containing distinct components of a transmembrane protein import complex. New peroxisomes are “empty,” and their internal proteins must be synthesized on cytosolic ribosomes and imported. Peroxisomes may arise de novo or by division of existing peroxisomes*

**Clicker Questions**

1. In plants, one would expect to find peroxisomes in the

a. seed.

b. stem.

c. root.

d. leaf.

LO 12.6.1 Summarize the roles of peroxisomes in animal and plant cells

2. Peroxisome biogenesis requires the fusion of two distinct ER-derived vesicles, V1 and V2, because

a. both V1 and V2 vesicles contain a portion of the importomer.

b. both V1 and V2 vesicles contain a unique set of proteins imported from the cytoplasm.

c. the V1 and V2 vesicles are derived from separate regions of the ER.

d. the V1 and V2 vesicles must be trafficked from the *trans* Golgi network.

LO 12.6.2 Describe the pathways responsible for peroxisome biogenesis

3. Peroxisomal proteins are of a prokaryotic origin

a. True.

b. False.

LO 12.6.1 Summarize the roles of peroxisomes in animal and plant cells

4. In \_\_\_\_\_\_\_, fatty acids are oxidized in both peroxisomes and mitochondria, but in \_\_\_\_\_\_\_ and \_\_\_\_\_\_\_, fatty acid oxidation is restricted to peroxisomes.

a. bacteria; humans; other animals

b. animal cells; yeasts; plants

c. microbes; yeasts; parasites

d. single-cell organisms; fungi; free-living aerobes

LO 12.6.1 Summarize the roles of peroxisomes in animal and plant cells

5. Plant and animal peroxisomes contain the enzyme catalase. Catalase catalyzes the decomposition of

a. plasmalogens.

b. peroxins.

c. fatty acids.

d. hydrogen peroxide.

LO 12.6.1 Summarize the roles of peroxisomes in animal and plant cells

***Answers: 1: a, d; 2: a; 3: a; 4: b; 5: d.***

**Essay/Discussion Question**

Zellweger syndrome is caused by defects in genes coding for peroxisomal protein import. Why are defects in such genes more likely to be lethal than a defect in a gene encoding a single enzyme present in the peroxisomal lumen?

*Answer: A defect in import machinery affects the import of several proteins into the lumen of the peroxisome. A mutation in a single peroxisomal enzyme affects only that enzyme. Therefore, the import machinery mutation would have a greater effect and probability of being lethal.*

*LO 12.6.2:* Describe the pathways responsible for peroxisome biogenesis.