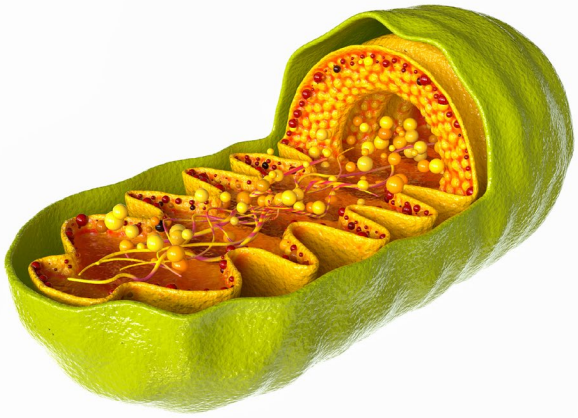
NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Mitochondrial Mysteries:

The Origins of Organelles



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Part I – The Diagnosis

As a freshman in college, Ivy was really enjoying being challenged in classes and making friends also interested in science. She was daydreaming about what magical power she would most enjoy as she sat in the hospital room waiting for her diagnosis. A month earlier, she had gone to her physician to ask if there was any medical basis for her increased clumsiness and weakness in her eyes and eyelids. After what seems like weeks of tests, the doctor had called her in for a face-to-face meeting. She had a gut feeling that today wasn't going to be a good one, but was also hopeful that she might just be blowing things out of proportion or might finally have a treatment plan if she did have a disease.

Just then, there was a knock on the door and Dr. Alvarez entered with a small smile. "Hello Ivy. How are you feeling today?"

"About the same," Ivy stated.

"Well, I think we've figured out what has been causing your symptoms. The genetic testing confirms that you have a large deletion in your mitochondrial DNA, which results in a disease called Kearns-Sayre syndrome. The mitochondria are the part of your cell that converts the food you eat into energy for your cells. The deleted DNA was essentially the blueprint for steps in this process. Without that blueprint, your mitochondria, or energy factories as I like to call them, cannot efficiently make energy."

Ivy was stunned. She had just learned about mitochondria as the "powerhouse" of the cell, but had no clue that these organelles had their own DNA. "I'm just learning about the mitochondria in my introductory biology class. I didn't know they had DNA. Did the genetic testing show how much DNA is missing? Can you be more specific about the overall consequences of this deletion?"

"The results here say there is a 4,997-nucleotide deletion. This causes a loss of 12 proteins used in the energy production pathway within mitochondria. You have the most common deletion that occurs for this disease, but we still know very little about it." Dr. Alvarez paused and sighed. "There are also no known treatments yet, which means we just try to treat the symptoms."

Questions

1. List any information you know about the mitochondria.

2. From the information in the story and your response to Question 1, develop a hypothesis to describe how deletions in the mitochondrial DNA may change the functioning of the mitochondria.

Part II – An Unexpected Visitor

After Ivy was finished meeting with Dr. Alvarez, she didn't feel like going back to her dorm room where her roommates were waiting to hear from her. She decided to process her diagnosis at the local coffee shop. Curling up in an oversized chair with a warm cookie and peppermint hot cocoa with whipped cream always made her feel better. Sitting alone would give her time to think. How could something so important go so wrong in her cells? How did cells get or make the mitochondria anyway? Obviously, cells could survive without mitochondria if they had to, so why had mitochondria evolved in the first place?

Just then, Ali, a fellow student in her biology class who made it a point to sit near her each class, walked over to strike up a conversation. "Hi Ivy! What's up?"

Trying to avoid conversation, Ivy simply shrugged and mumbled, "Not much."

Ali gave her a big smile and said "I've noticed that you take good notes in bio, would you want to study together… over dinner?"

Ivy really didn't need this right now, although she did think he was smart and funny. She decided to ask him about the mitochondria to change the topic. "Speaking of bio, what did you think of the eukaryotic cell structure class? I enjoyed learning about the functions of the different organelles but would like to know more about how they became part of the cell in the first place."

Questions

3. Hypothesize a possible origin of organelles.

4. What kinds of evidence would lead you to support or reject the hypotheses you came up with in Question 3? (Remember, a hypothesis should be testable.)

Part III – Information Overload

Ali sat down with a smile on his face. "This is exactly why I want to become your study partner. Do you have time now? I'm happy to figure this out with you, since my classes are done for the day. Can I buy you a tea and a cookie?" Ali's enthusiasm was actually starting to make Ivy feel a bit better and distract her from her recent diagnosis.

"Tea sounds great," Ivy remarked. "I'm particularly interested in how the mitochondria was first formed or made, since it's the 'powerhouse' of the cell, which seems important."

"You know, I've never really thought about how eukaryotic cells got their organelles. It seems like an important piece of information," Ali commented while deep in thought. "Why don't we consult the omniscient world wide web and see what we can find out? Let's try to come up with a list of clues."

Here is the list of information they compiled:

- Mitochondria have their own DNA. This DNA is circular, encodes ribosomes and tRNAs, and is similar to DNA found in rickettisae, which are small bacteria that grow inside eukaryotic cells.
- Mitochondria use pyruvate, an end product of glycolysis, and oxygen to generate a large amount of ATP.
- Mitochondria have two membranes (an outer and inner membrane) composed of phospholipid bilayers.
- The outermost mitochondrial membrane has porin proteins throughout it to import molecules.
- Mitochondria divide on their own through binary fission, not when the cell divides.
- Protein synthesis in the mitochondria uses ribosomes that are different from the ribosomes in the cytoplasm. Mitochondrial ribosomes resemble those found in prokaryotes.
- Antibiotics used to kill bacteria can also harm mitochondria.
- Most of the proteins needed for the mitochondria to produce ATP are encoded in the DNA found in the cell's nucleus. They are translated in the cytoplasm and then shipped into the mitochondria.

Questions

5. Use the table below to compare and contrast the mitochondrial characteristics Ivy and Ali put together with prokaryotic cells and organelles within the eukaryotic cell.

Characteristics of mitochondria	*Features shared with bacteria*	*Features shared with other eukaryotic organelles*

6. Using the information in your table, revise the hypothesis you originally proposed for Question 4. If you don't update your original hypothesis, then describe pieces of evidence in the table that would support your hypothesis.

Part IV – Mitochondrial Relatives

"Well that's weird," Ivy said, thinking out loud. "Mitochondria look like little bacteria and even have some of their own genes! It seems like mitochondria were once bacteria that infected another cell and then they just stayed together. I'm going to look into why this might have occurred. It doesn't make sense to say that mitochondria are pathogens, since the cell is not harmed. It actually benefits from the association."

Ali cut her off. "*But*, could they have once been a pathogen? Or, what if they weren't bacteria to begin with? Listen to what I found. There are some small, simple eukaryotes called protists that do not have mitochondria like the rest of eukaryotic cells. Instead, they have a mitochondria-like organelle called a mitosome that does not produce ATP, but aids in the formation of iron-sulfur clusters that are incorporated into proteins. Some other protists have a mitochondria-like organelle called a hydrogenosome, which does produce ATP and hydrogen gas, but does not use oxygen. If that's the case, then which came first? I think that the eukaryotic cell may already have had other organelles, possibly mitosomes or hydrogenosomes, that evolved into mitochondria."

Ivy wasn't convinced. "Or did a bacterium infect a cell, which later evolved into mitochondria, mitosomes, or hydrogenosomes?"

Questions

7. Based on the information above and other information you've learned in class, select the hypothesis you think is most likely:
   a. Mitochondria evolved from an engulfed bacterial cell that lost its complex, free-living lifestyle.
   b. Mitochondria evolved energy-producing capabilities from simpler organelles that gained new functions.

   Write a short argument (three to four sentences) in support of your chosen hypothesis.

Part V – Connecting the Dots

Finding support for her argument, Ivy said, "Here is some neat information about a beautiful slug. I think it may help us answer these questions, because the slug actually steals and uses an organelle from the cells of its food. Maybe the mitochondria started as a bacterium that was captured and held hostage by a host cell. Wow, I never thought I'd say slugs were pretty … ." She read the following excerpt from an HHMI BioInteractive module entitled "Slug Power" (https://www.hhmi.org/biointeractive/slug-power).

> *The sacoglossan sea slug* Elysia crispata *can be found sunbathing on Caribbean reefs. The slug feeds on green algae but can survive for more than a month without eating. This is because sea slugs store chloroplasts, organelles in the cells of plants and algae that capture energy from sunlight and convert it to chemical energy by photosynthesis, as they ingest different species of green algae. The chloroplasts are stored in the slug's digestive epithelium and remain active for up to 3–4 months, providing nutrients from photosynthesis, as well as camouflage by making the slug green in color. "Kleptoplasty," or "stolen plastids," is the term for the slugs' remarkable ability. Some marine protists including foraminifera, dinoflagellates, and ciliates are capable of kleptoplasty, but sea slugs are the only animals to exhibit kleptoplasty. They represent a powerful model system for studying the evolution of photosynthesis in eukaryotes through multiple endosymbiotic events.*

Figure 1. Elysia crispata, commonly known as the "lettuce sea slug." *Image credit:* © John Anderson | Dreamstime.com, ID 94096510.

Ali took the notepad and read through the article. "So, these animals can absorb chloroplasts for their own use. Maybe there was something that caused the ancestor of the eukaryotic cell to absorb these bacteria and gain their abilities."

Ivy then had a thought. "I remember Dr. Smith telling us that the early Earth had a different atmosphere than what we have today. There was a point in the Earth's history when the oceans were mostly anoxic (lacking oxygen), and life first evolved in the oceans. So maybe during the time when oxygen was increasing in the atmosphere and oceans, an aerobic bacterium, maybe a rickettisae-like bacterium, was engulfed by an anaerobic host cell that was incapable of using oxygen itself. The host could then benefit from the increasing amounts of oxygen by capturing the extra energy made by the aerobic bacterium. If the bacterium was held hostage, then the host would always have a source of energy!"

Ali smiled and nodded. "I think you're really onto something here. This could be an example of a symbiosis: a long-term physical relationship between two different species. There could have been a benefit for both partners. The host cell receives a constant flow of energy and the aerobic bacterium has a protected environment where it doesn't have to fight others for space and nutrients. The bacteria aren't held hostage, they're a willing participant. Different organisms came together to survive and conquer the changing environment, just like we came together and conquered this question! I knew we'd be a great match… I mean a great study group."

Questions

8. What Ali just described is the endosymbiotic theory. "Endo-" means "inside" and signifies that one partner is living within another partner. Draw a flow chart describing the symbiotic adventure that led to the establishment of mitochondria (i.e., the endosymbiotic theory).

9. People hypothesize that over evolutionary time, the aerobic bacterium inside the host cell slowly lost genes necessary for it to live outside of the host. This caused the association to become essential for the bacteria's survival and reduced it to an organelle because so much of its basic functioning requires the host cell's DNA. With this new information, once again select the hypothesis you think is most likely:
   a. Mitochondria evolved from an engulfed bacterial cell that lost its complex, free-living lifestyle.
   b. Mitochondria evolved energy-producing capabilities from simpler organelles that gained new functions.

   Provide evidence to support your hypothesis. Include at least four different lines of evidence.

10. It's your turn! Chloroplasts are an important organelle in many organisms that share some structural characteristics with mitochondria.
    a. Perform your own research and provide evidence to support that the origin of chloroplasts follows the endosymbiotic theory as well. Create a list like the one in Part III of the case study below.

    b. Draw a well-labeled schematic/flow-chart of the events that resulted in the plant cells having chloroplasts. Refer to your textbook for help (use the index in your textbook to find the pages about the origin of chloroplasts to complete this question).

Wrap Up

Think about the changing climate or environment. If you could acquire any trait that would help you survive what would it be?

Watch the following video to help you review what you have learned:

- *How We Think Complex Cells Evolved.* Running time: 5:41 min. Produced by Adam Jacobson, animation by Camilla Gunborg Pedersen, TED-Ed, 2015. <https://youtu.be/9i7kAt97XYU>

Mitochondrial Mysteries: Cellular Respiration

Part I – Exam Preparation

The following week, Ivy agreed to meet Ali on Sunday in the library to study for their upcoming exam on photosynthesis and cellular respiration.

"Photosynthesis and cellular respiration are inverse processes," Ali pondered while looking at the equations on the whiteboard in their study room. "In photosynthesis, light energy and carbon dioxide produce glucose and oxygen. Then in cellular respiration, glucose is broken down to make carbon dioxide and energy in the form of ATP, and the process requires oxygen."

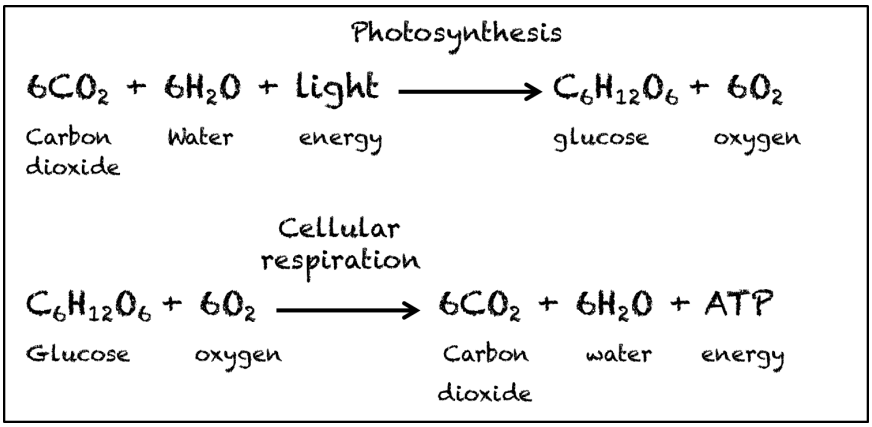


Figure 2. Equations for photosynthesis and cellular respiration.

"That's a good start, but cellular respiration isn't as simple as you put it. I think Dr. Smith is going to ask us much more about cellular respiration than this equation describes." Ivy paused, starting to wonder why she agreed to this. "There are a lot of processes that work together to make enough ATP for our bodies to function. Didn't you read the section in the book about this? I know you were in class and I saw you taking notes. What do you remember from that day?"

She notices when I'm in class… This thought made Ali grin. He'd better step it up so she'd keep meeting with him. "Hold on, let me take out my notebook and see what I've got for this lecture."

Questions

1. Using Ali's class notes (see next page), complete Table 1.

   Table 1. Number of each molecule type produced per 1 glucose molecule.

NADH	*$FADH_2$*	*ATP*

2. Draw a flow chart for the order of processes involved in getting energy from glucose. Indicate the inputs and outputs of each process.
   a. Put a circle around the processes that reduce electron carriers
   b. Put a square around the process that oxidizes the electron carriers.
   c. Place a star next to the processes that produces the most energy for the cell.

Cellular respiration November 11

4 processes

NADH and $FADH_2$ electron carriers

1. Glycolysis: glucose -> 2 pyruvate, 2 ATP, and 2 NADH. Occurs in cytoplasm. No O_2.
2. Pyruvate processing: 2 pyruvates -> CO_2, 2 acetyl CoA, and 2 NADH
3. Citric acid cycle: 2 acetyl CoA -> CO_2, 2 ATP, 6 NADH, and 2 $FADH_2$. Is a cycle.

ETC make proton gradient; OP makes ATP

4. Electron transport chain (ETC) and Oxidative Phosphorylation (OP)
   - NADH and $FADH_2$ are oxidized and their electrons go through the electron transport chain
   - Oxygen is required for this process
   - Energy released from redox rxn actively transports proteins across inner membrane – creates proton gradient H^+ H^+ H^+ H^+ H^+ H^+ H^+ H^+ H^+ H^+ H^+ H^+
   - Proton gradient established
   - ATP synthase (an enzyme, aka protein complex), makes ~25 ATP using proton gradient to drive the energy requiring process

- ❑ BEFORE STUDYING WITH IVY:
  - ❑ Draw out where processes occur in the eukaryotic cell (think mitochondria)
  - ❑ Think build-up of electron carriers, then energy production ramps up.
  - ❑ Know when oxygen is important

Part II – Where the Magic Happens

After finding the page in his notebook for this day, Ali regretted not looking at it sooner. "Ivy, I wrote down that we need to know where each of the four processes in the cell occur, but I didn't remember this until now."

"Can I see your notes?" Ivy asked. "I seem to have written down that oxidative phosphorylation is the payoff phase, but I don't know what's happening in that step. Can you figure that out, and I'll work on locations? I seem to have missed the point about knowing where each step happens, because I don't see it anywhere in my notes. I've been really interested in the mitochondria lately, so I do know something about their structure. Mitochondria have a typical **outer membrane** and then their **inner membrane** is large and folds in on itself a lot. These folds fill in the inside of the organelle with sac-like structures called **cristae**. The space between the inner and outer membranes is known as the **intermembrane space**. The area encapsulated by the inner membrane, where the cytoplasm is found inside a bacterium, is known as the **mitochondrial matrix**.

Questions

3. Using the information from the questions you answered in the previous section (Part I), explain why Ivy is calling oxidative phosphorylation the "payoff phase."

4. Critically read Ivy's description of mitochondrial structure and draw and label a mitochondrion in the space below. Include all of the bolded words from Ivy's description.

Part III – Putting It All Together

After 15 minutes of reading through the textbook, Ali and Ivy came back together to debrief.

"Here's what I read about the payoff phase," Ali began. "It appears to me that while some ATP is made during glycolysis and the citric acid cycle, the majority of the ATP energy is produced during oxidative phosphorylation. So, oxidative phosphorylation is the payoff phase because that's when most of the energy is actually being produced. The previous steps are really just setting up the cell and making the right molecules for this to happen. All of the electron carriers that are reduced during the first three processes are oxidized in the electron transport chain, which creates a **proton gradient**. This proton gradient then provides the large amount of energy needed to drive the production of ATP by ATP synthase. I guess it takes some effort to make energy. But what the heck is this proton gradient and where does it happen? What did you find out about the mitochondria?"

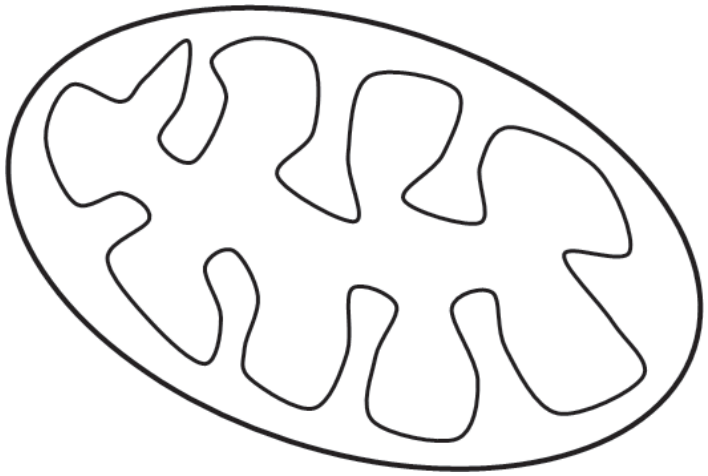
"Dividing to conquer these questions is making our study session quite efficient. We may be done in time for dinner," said Ivy with a smile. "Let me start from the beginning. I'm thinking about this as if our mitochondria are factories, producing ATP according to a defined plan. First, glucose enters the cell and glycolysis occurs in the cytoplasm. Then pyruvate, the product of glycolysis, is transported into the mitochondria through those porin proteins in their membranes. Pyruvate processing and the citric acid cycle occur in the mitochondrial matrix. Here's the tricky part: the electron transport chain involves a set of protein complexes that are located in the cristae of the mitochondrial inner membrane. Those protein complexes accept the electrons donated by NADH and $FADH_2$. This movement of electrons pumps protons (hydrogen ions, H^+) into the intermembrane space to create the proton gradient."

Ali jumped in, "So these protons are being actively put into the intermembrane space by the electron transport chain, meaning there are more protons in the intermembrane space than in the mitochondrial matrix. This seems like pushing water uphill – it's bound to want to come down."

"That's exactly how I thought of it! Now, oxidative phosphorylation is like the doors on a dam being opened so the turbines can create electricity from the flow of water back down the hill. There is another protein complex embedded in the inner membrane known as ATP synthase. This protein functions as the turbine. It has an area where protons can flow from the intermembrane space back into the mitochondrial matrix, and that flow of protons creates enough energy to catalyze ATP synthesis."

Questions

5. Using the drawing of a mitochondrion below, indicate where each of the four processes of cellular respiration occurs. Remember that the mitochondrion is surrounded by the cytoplasm of the cell! Your labels should include the parts of the cell and organelle, the names of the processes, and the molecules that are being shuttled.



6. What is a "proton gradient"? Explain why this is important for the production of ATP.

7. In your opinion, what are the three most important molecules needed for the production of ATP? Explain your reasoning.

8. Additional thought question: use the information in this part and previous information learned in the class to explain why aerobic organisms typically have a faster growth rate than anaerobic organisms.

Part IV – The Dinner

After studying, Ali and Ivy went out to a local sushi joint to relax and get away from campus for a bit. "I'm really glad you agreed to go to dinner with me! It's nice to spend time with someone who is as much of a science nerd as myself." Ali couldn't hold back his excitement, which was making him a little more awkward than he wished. He was always so cool and collected while studying.

"Can I ask you something?" said Ivy suddenly. "Have you noticed anything weird about my eyes?"

"Only that they are the color of Nutella," Ali replied.

"Well, I've started having trouble moving them around and it's becoming progressively harder to keep them open, even though I try to get enough rest. That day you found me in the coffee shop, I had just learned that I have a disease called Kearns-Sayre syndrome. It's caused by a deletion in my mitochondrial DNA. That's why I've been so obsessed with learning about this organelle."

This was a complete shock to Ali. He had thought she looked a little quirky, but had assumed that was just the way she was. "Oh my gosh, Ivy. I'm really sorry to hear that." He paused, wondering if it would be rude to ask more, but his curiosity got the better of him. "What is Kearns-Sayre syndrome? I've never heard of it before."

Fortunately, Ivy didn't seem to mind the question. "Well, do you remember when I said I'm thinking of mitochondria like ATP factories? I think of DNA as the instructions for how to make all of the machinery that the factory needs. While most of those instructions are encoded in the nucleus, a few are still encoded by mitochondrial DNA. Because of the deletion, my mitochondria don't have the blueprints for all of the proteins they need to efficiently make ATP."

"And the end result of too little ATP in your cells is trouble moving your eyes?"

"That's one of the main symptoms, yes. My doctor told me that eye muscles need a lot of energy from mitochondria, which is why they're most affected. But Kearns-Sayre syndrome is a rare disease. Scientists still don't completely understand how the deletion is linked to the specific effects." Ivy's frown grew deeper. "There's also no cure. I can manage the symptoms, but I'll always have trouble with my eyes."

Her voice had gone quiet, and Ali could tell she was feeling self-conscious. "Clearly it doesn't stop you from being a brilliant student," he said, grinning. "And hey, maybe we can both go into medicine and develop a cure someday, now that we're on our way to being experts on the mitochondria!"

Ivy laughed, feeling relieved. She had been worried he would just get up and leave. "Let's not get ahead of ourselves! I just wanted to get that off my chest. Why don't we eat our sushi and focus on the bio exam tomorrow? The rest can wait for another day."

Questions

9. Take five minutes and write down everything you now know about the mitochondria.

10. Compare the answer you gave for the first question in this case study (i.e., list any information you know about the mitochondria) and your response to Question 9 above. Describe three of the most interesting or exciting things you learned from this story.

Wrap Up

Watch the following videos to help you review what you have learned:

- *Cellular Respiration.* Running time: 2:47 min. Produced by RicochetScience, 2016. <https://youtu.be/eBl3U-T5Nvk>
- *Cellular Respiration and the Mighty Mitochondria.* Running time: 7:48 min. Produced by Amoeba Sisters, 2014. <https://youtu.be/4Eo7JtRA7lg>