Cells within cells: An extraordinary claim with extraordinary evidence

Did you know there are more bacteria living in your intestines than there are cells in your entire body? That might be a disturbing thought, but without gut microbes you would have trouble digesting many grains, fruits, and vegetables—and you'd have more allergies and a weaker immune system, not to mention the infections you might get from harmful bacteria if real estate in your intestines weren't already occupied by friendly species.

Figure 1. Lynn Margulis in 2005.

Biologists now think you have an even closer—and more ancient—relationship with bacteria than you might think. They not only live on and in you, but you actually carry the descendents of ancient bacteria inside every cell in your body. You aren't just a house for bacteria; in a very real sense, you *are* bacteria.

How did scientists come to accept this surprising idea? In the 1960s, a young microbiologist named Lynn Margulis (Fig. 1) revived an old hypothesis. Based on a fresh look at evidence from the fields of cell biology, biochemistry, and paleontology, she proposed that several fundamental transitions in evolution occurred, not through competition and speciation, but through cooperation, when distinct cell lineages joined together to become a single organism (Fig. 2). To her colleagues, the idea seemed crazy—like suggesting that aliens built the pyramids—but Margulis defended her work despite this initial resistance. She inspired scientists in far flung fields of biology to test her hypothesis in the lab. As the evidence piled up in the decades following her first paper, even some of her strongest critics had to concede that she'd been right.

You may have already learned about some of her ideas as "facts" in your biology textbook, but you probably haven't heard about how controversial they were when they were first proposed. Let's take a closer look at this story of an extraordinary claim—and the extraordinary evidence that supports it.

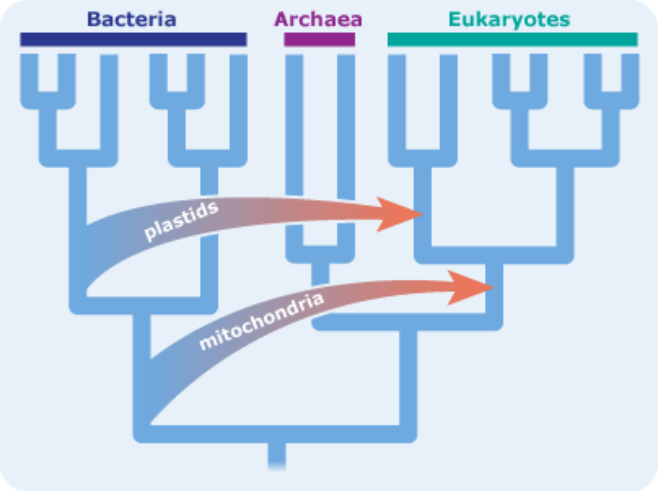


Figure 2. Margulis revived the hypothesis that certain cellular organelles (e.g., plastids and mitochondria) are descended from free-living organisms.

This case study highlights these aspects of the nature of science:

- Science can test hypotheses about events that happened long ago.
- Scientific ideas are tested with multiple lines of evidence.
- Science is a community endeavor that benefits from a diverse and broad range of perspectives, practices, and technologies.
- Scientific ideas evolve with new evidence; however, well supported scientific ideas are not tenuous.
- Through a system of checks and balances, the process of science can overcome individual biases.
- Evidence is the most important arbiter of which scientific ideas are accepted.

A world under the microscope

When Lynn Margulis enrolled at the University of Chicago in 1953, she had planned on becoming a writer. It wasn't until she took a required science class that she developed a passion for biology. In this course, students read

Lynn Margulis photo by Javier Pedreira (CC BY-SA 2.5)

the original works of great scientists instead of a textbook. Gregor Mendel's classic pea plant experiments captivated Margulis. It was the beginning of a life-long fascination with genetics and heredity.

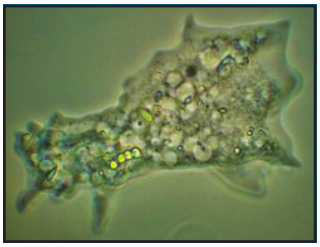


Figure 3. Margulis observed amoebae (like this one) splitting in two.

She decided to study these topics for her masters degree at the University of Wisconsin. It was there that she first watched amoebae under the microscope, observing the way they engulfed their food and reproduced by dividing in two (Fig. 3). First, the amoeba changes its shape, pulling its blob-like form into an almost perfect sphere. Then the nucleus, the structure that carries the cell's genetic material, splits in half. After that, the whole cell starts to split, pinching into two separate cells, each with a nucleus and all the other cell structures it needs to live as an adult amoeba.

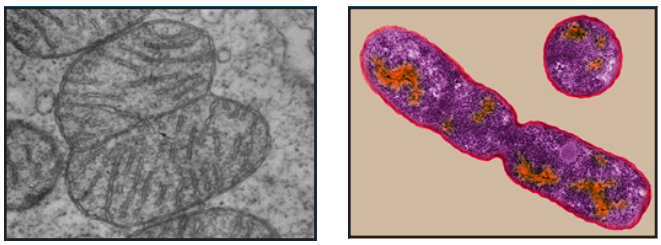


Figure 4. Within a cell's cytoplasm, mitochondria (left) reproduce much like free-living bacteria (right) do. Bacterium image © Dennis Kunkel Microscopy, Inc. (www.denniskunkel.com)

Around the same time, Margulis began to notice the strangely independent behavior of mitochondria, the structures within cells that provide energy by breaking down food molecules. Even though mitochondria are just parts of the cell, they seemed to reproduce the same way that whole amoebae did—by splitting in two (Fig. 4)! Because mitochondria were about the same size and shape as some kinds of bacteria, and since bacteria also reproduce by dividing in two, Margulis couldn't help thinking how much this cell structure, or organelle, behaved like an independent bacterium.

Back to the future

Margulis' observations weren't new to science; many researchers before her[1] had peered through a microscope and noticed the striking similarities between mitochondria and bacteria. Margulis learned from one of her professors that some of those observers, back in the 1880s, had come up with a hypothesis about why mitochondria and bacteria looked so much alike. This was the first time Margulis heard about the "crazy" hypothesis that would shape her career and revolutionize scientists' understanding of how complex cells evolved.

WHAT ARE PROKARYOTES AND EUKARYOTES?

Prokaryotic cells (Fig. 5) are relatively simple. They are small, and their DNA is circular and floats freely inside the cells. All bacteria are prokaryotic cells.

Eukaryotic cells (Fig. 5) are more complex. They are larger, and their DNA is arranged in linear chromosomes and kept inside a nucleus. Eukaryotic cells have some organelles that prokaryotic cells don't have—like mitochondria. All plants, fungi, and animals (including humans!), as well as many single-celled creatures like amoebae, are made up of eukaryotic cells.

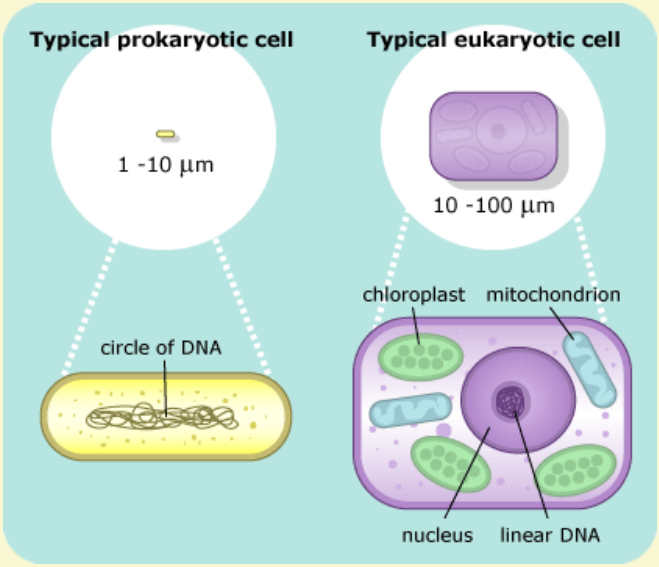


Figure 5.

[1]Notably, Konstantin Mereshkovsky in 1905.

Amoeba photo © www.micrographia.com; dividing mitochondrion image © Rockefeller University Press, 1970; originally published in the *Journal of Cell Biology* 47:373-383.

What was this crazy idea? Margulis' professor explained that over the last eighty years, a number of scientists had proposed that eukaryotic cells evolved when one bacterium (a prokaryote) engulfed another and the two began living together. Over many generations, and through many smaller changes, the engulfed cell evolved into an organelle, like the mitochondrion. According to this idea, mitochondria look and act so much like bacteria because they once *were* bacteria!

This ecological relationship is called endosymbiosis. "Endo" is Greek for "within" and "symbiosis" is Greek for "living together"—so endosymbiosis means one organism living inside another. In Margulis' day, scientists knew that many organisms have endosymbionts—like termites, which depend on microorganisms in their guts to digest wood (Fig. 6)—but nobody thought that this relationship could evolve to be so close that the two would become a single organism.

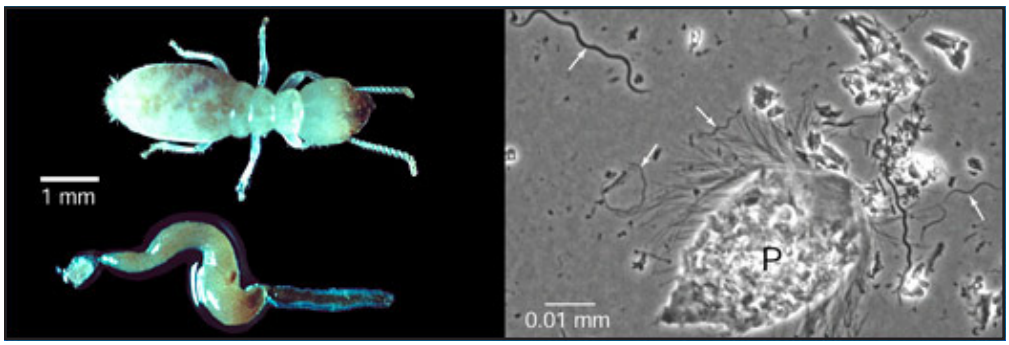


Figure 6. At left, a wood-eating termite is pictured next to a gut removed from a another individual. When contents removed from the hindgut are examined under a microscope (right), many symbionts are revealed, including protozoans (labeled P) and a number of spiral and wavy-shaped bacteria (indicated by arrows).

Each of the times that the endosymbiotic hypothesis was proposed, most of the scientific community thought it sounded too far-fetched. Two different organisms coming together to form a single one? Ridiculous! It would never have worked!

To Margulis, the idea didn't sound crazy, but as a graduate student, she didn't have much time to mull it over either. She was busy thinking about genetics and working on her Ph.D. research at UC Berkeley. As we'll see, however, her research and her observations of another organelle, the chloroplast, would lead her back to this strange idea.

An old idea gets a fresh look

Margulis' research followed in the footsteps of one of the scientists who had inspired her to become a biologist in the first place. With his peas, Gregor Mendel (Fig. 7) had shown that genetics was predictable; if you know what genes parents have, you can predict what genes their offspring are likely to have. Later research explained why: genes are located on DNA and DNA follows strict rules when it is copied and passed to offspring. However, at the time, scientists were discovering more and more cases of inheritance that broke Mendel's laws. How could this be? Margulis decided to try to find out.

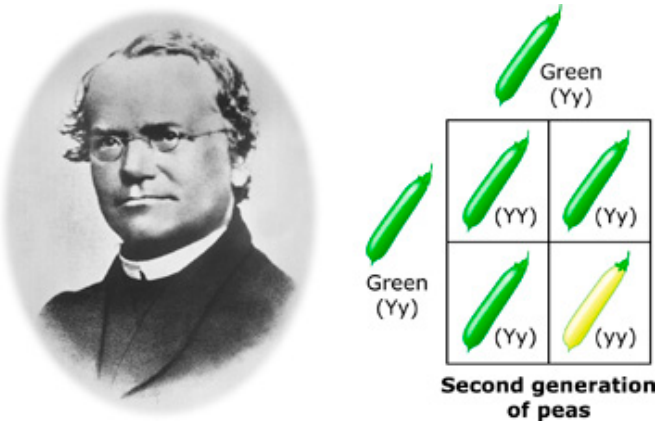


Figure 7. Gregor Mendel (left) showed that if you know the genotypes of the parents in a cross, you can predict the ratios of different offspring genotypes (right).

She, along with many other scientists, suspected that cells might have DNA *outside* of the nucleus and that this DNA might not follow the same rules of inheritance that nuclear DNA does. Margulis' first research was on *Euglena*, a single-celled eukaryote. She found that it had DNA inside its chloroplasts, not just in its nucleus. What was the DNA doing there? Was it responsible for any of the traits that seemed to break Mendel's laws?

Termite and termite gut microbes photos courtesy of John Breznak, Michigan State University; Gregor Mendel photo in the public domain

While considering these questions, Margulis remembered the endosymbiotic hypothesis. She knew that chloroplasts (Fig. 8) reproduce by splitting in two, as bacteria do and as the mitochondria that she had observed earlier do. And now she was sure that chloroplasts also had their own DNA. Suddenly, she was captivated by a new question: If chloroplasts contain their own DNA and reproduce by splitting in two, could it be that these organelles really were once free-living bacteria??

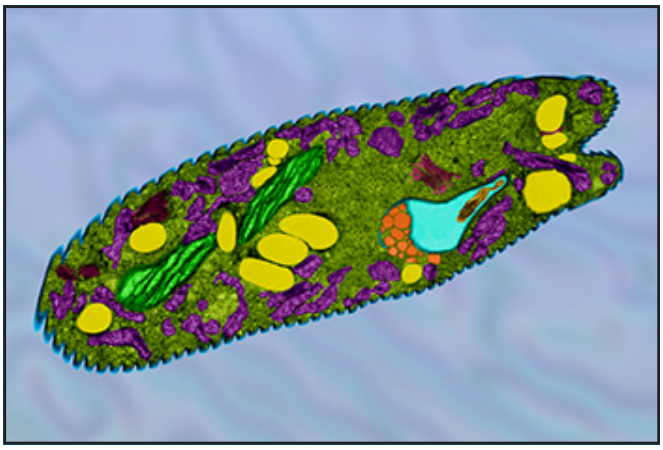


Figure 8. The green organelles in this *Euglena* are chloroplasts. The purple bodies are mitochondria. Image © Dennis Kunkel Microscopy, Inc. (www.denniskunkel.com)

Margulis began to explore the idea in earnest. She didn't do any new research beyond her initial investigations of chloroplasts' DNA, but she did read about other scientists' research to find the most up-to-date evidence relevant to her hypothesis. She found that many scientists had made observations that would make perfect sense if eukaryotic cells had evolved via endosymbiosis.

Before we examine the evidence she presented, let's look at her new, expanded version of the older hypothesis.

How many became one

The story Margulis told begins with the dawn of life on Earth. Three and a half billion years ago, nothing but bacteria lived on our hot, barren planet, and there was no oxygen in the atmosphere. Around three billion years ago, some of these bacteria evolved the ability to use energy from the sun to create food for themselves through photosynthesis. The waste product of this process was oxygen, and these bacteria produced so much of it that it dramatically changed the atmosphere.

OXYGEN: A DOUBLE-EDGED SWORD

We usually think of oxygen as essential to life, and for those that have evolved to use it, it is. But part of what makes oxygen so critical to most life also makes it dangerous. Oxygen can generate free radicals—atoms or molecules with a spare electron that makes them extremely reactive. Many of those reactions are detrimental, causing mutations and other forms of damage to living cells. For organisms that have not evolved the ability to prevent and repair this damage, oxygen can be toxic.

The oxygen poisoned many bacteria, but others evolved the ability to use it. Over many generations, some of these bacteria became dependent on oxygen to break down their food. Margulis proposed that these bacteria experienced several episodes of endosymbiosis (Fig. 9):

- First, some amoeba-like bacteria ingested some of the bacteria that could use oxygen to break down food. Eventually, they evolved to live together, with the oxygen-users permanently installed inside the amoeba-like bacteria. With

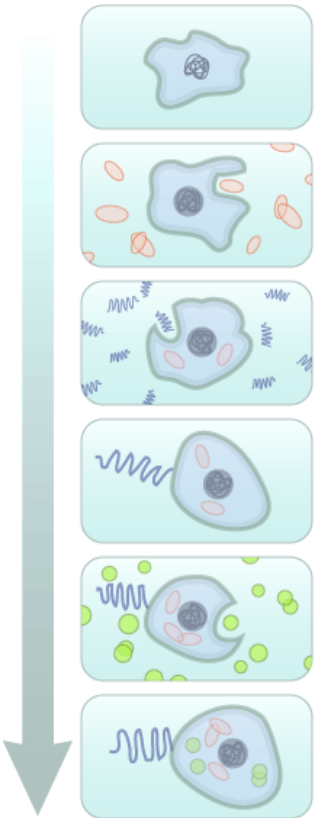


Figure 9. Margulis proposed that a bacterial lineage experienced repeated episodes of endosymbiosis, eventually leading to modern eukaryotes.

their new oxygen-using residents, the amoeba-like bacteria thrived in the oxygen-filled environment. These organisms were the ancestors of all eukaryotes, and the bacteria they ingested evolved into mitochondria.

- Next, one of these early eukaryotes ingested another sort of bacterium—a long, spiral-shaped type. Eventually, they too evolved to live together permanently, with the spiral-shaped bacteria living alongside the mitochondria in the host cell. These organisms were the ancestors of all animal cells, and the spiral bacteria they had ingested evolved into a number of important structures, like cilia and flagella, which help animal cells move around.
- Finally, some of those early animal cells ingested even more bacteria—the kind that had evolved the ability to photosynthesize—and these too evolved to live together permanently. These cells were the earliest ancestors of plants, and those photosynthetic bacteria within them evolved into structures called plastids—for example, the chloroplast—which allow plant cells to perform photosynthesis.

If Margulis was right, endosymbiosis had happened many times (Fig. 10) and played a major role in the evolution of life on Earth!

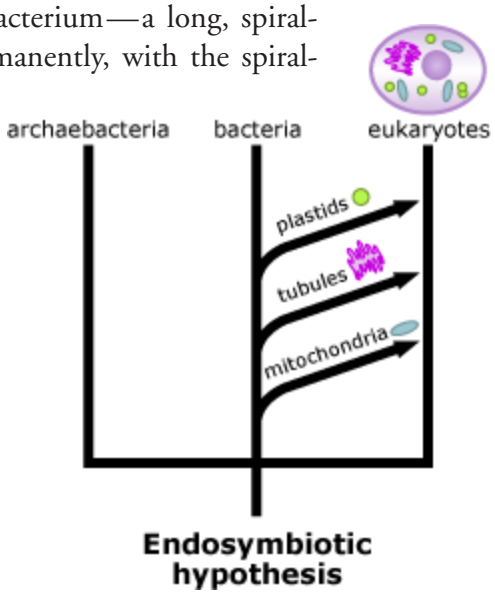


Figure 10.

Obstacles to acceptance

Margulis knew that other scientists had proposed similar hypotheses about endosymbiosis in the past and were ridiculed for it. Why hadn't the idea ever gained acceptance? In science, ideas can be rejected for many different reasons—and most of them applied in the case of *this* hypothesis:

1. Lack of evidence

Scientists strive to scrutinize the evidence for everything, even things that seem obvious. This means that to be accepted, a scientific idea must be more than just plausible; it must be tested and supported repeatedly with multiple lines of evidence. Earlier scientists had tried to test the endosymbiotic hypothesis, but they didn't have the technology that they needed to design a truly fair test of the idea—so there was simply no strong evidence for the idea (Fig. 11). Sure, mitochondria *look* a lot like bacteria, but that wasn't enough to convince scientists that they had once actually *been* bacteria.

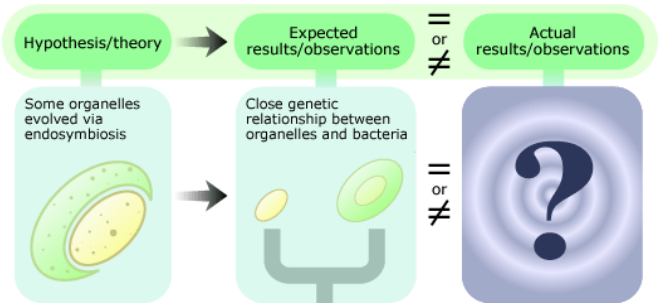


Figure 11. Some scientists didn't get behind the endosymbiotic hypothesis because key evidence that could have supported or contradicted it could not be obtained with the technology of the day.

2. Inconsistency with an accepted theory

Many scientists were skeptical of the endosymbiotic hypothesis because it didn't seem to fit into the theory of evolution as it was understood then (Fig. 12). Between 1900 and 1950, biologists made many key discoveries in the field of genetics by focusing on small, random changes in DNA—mutations—that occur when a cell reproduces. These genetic "mistakes" were clearly an important mechanism of evolution, and many biologists thought that *all* evolution occurred as a result of the accumulation of many small mutations over time. However, the new hypothesis proposed big evolutionary advances through symbiosis—not slow and steady change through tiny mutations. The endosymbiotic hypothesis seemed, at first, to be a poor fit with what scientists of the day understood about how evolution works.

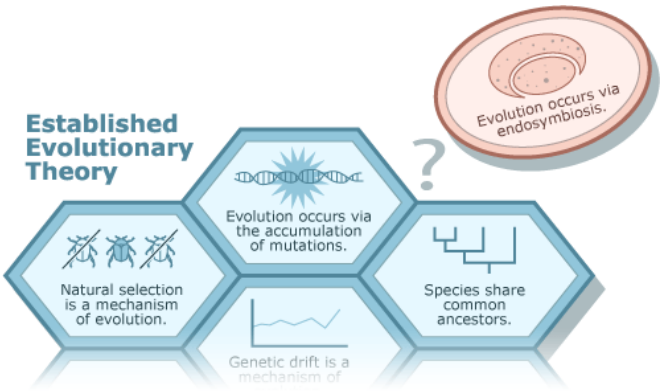


Figure 12. Some scientists didn't accept the endosymbiotic hypothesis because it didn't easily fit into the theory of evolution as it was then understood.

3. Parsimony

Scientists are more likely to accept simpler, or more parsimonious, ideas over more complex ones, all other things being equal. And accepting the new idea would have made evolutionary theory more complex. Instead of proposing one main mechanism (the accumulation of small mutations over time), the theory would have had to incorporate symbiosis as an additional mechanism of evolutionary change. Scientists didn't see why they should look for a new way to explain evolutionary change when the old way had so much supporting evidence and seemed to explain most of what they had observed. Extra evidence was needed to convince them that evolutionary theory had to make room for an additional mechanism of change.

4. Biases

Scientists strive to work objectively, but they are still human and vulnerable to biases just like everyone else. In this case, scientists had two big biases that tainted their reaction to the endosymbiotic hypothesis. First, ever since Darwin, evolution had been about competition between organisms fighting it out for territory, mates, and food. But the endosymbiotic hypothesis focused on *cooperation* (Fig. 13). Evolutionary theory didn't say that cooperation *couldn't* happen, but scientists just weren't used to the idea that evolution could occur as the result of two organisms working together.

Figure 13. Scientists were used to the idea that competition (left, represented by head-butting bighorn sheep) led to evolution, but weren't as comfortable with the idea of cooperation (right, represented by a clownfish seeking protection amongst the tentacles of an anemone) leading to evolution.

Second, most of the scientists doing research in evolution at the time worked with relatively large animals—fruit flies, birds, and mice—not microorganisms like Margulis' amoebae and bacteria. Scientists who worked with microorganisms knew that one organism living inside another was commonplace, but those working on large animals had seen few examples of endosymbiosis. Today, we know that endosymbiosis is common even in complex, multicellular animals (like the algae that live in giant clams and perform photosynthesis), but at the time, scientists working on large animals assumed it was exceedingly rare. These scientists had trouble accepting the hypothesis because they weren't familiar with endosymbiosis from the animals they studied.

Obviously, scientists are not always won over by new ideas right away. This kind of resistance can make science progress slowly, but it also works to ensure that every new idea is thoroughly tested before gaining acceptance. In her first publication on the hypothesis, Margulis did her best to explain all the tests of the hypothesis that had already been done and which were still waiting to be performed …

Bighorn sheep photo courtesy of the USGS; clownfish photo by Jenny Huang (CC BY 2.0)

Marshalling the evidence

At the time Margulis proposed her new version of the endosymbiotic hypothesis, the dominant view in the scientific community was that mitochondria and similar structures had evolved in a step-by-step manner from other parts of the cell (Fig. 14). So how did Margulis make a case for her idea? All scientific arguments work in the same way. You imagine what you would expect or predict to observe in a particular situation if the hypothesis were true, and then you see if that expectation (or prediction) matches reality. If it does—and if no other hypothesis generates the same expectation—the idea is supported; if not, it is contradicted. Most scientific hypotheses and theories generate many different expectations, all concerning different lines of evidence that might or might not support the idea.

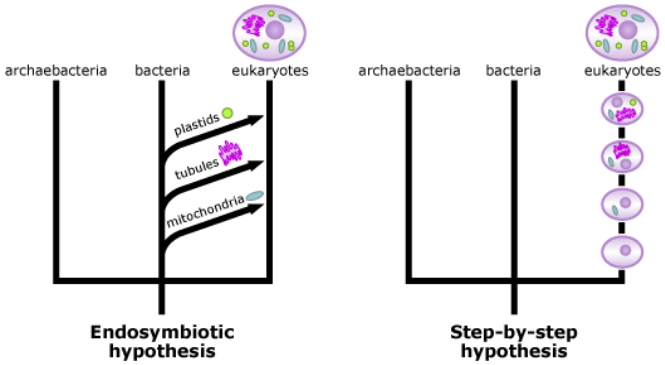


Figure 14.

Let's see what unique expectations the endosymbiotic hypothesis generated that the step-by-step hypothesis did not. Though Margulis' hypothesis dealt with mitochondria, tubular structures, and plastids (e.g., the chloroplast), we'll start by focusing on just the mitochondria.

If mitochondria evolved from an independent bacterium engulfed by another, then we'd expect that:

1. **Mitochondria reproduce themselves and are passed down from parent to offspring**

Free-living bacteria reproduce themselves; they are not built by another organism. So if mitochondria are really the descendents of free-living bacteria, we'd expect them to reproduce themselves and be passed to an individual's descendents—not be constructed anew from other parts of the host cell with each new generation. Margulis herself had seen the way that mitochondria made more of themselves by dividing in half (Fig. 15). And other scientists had published observations of these new mitochondria being divided between daughter cells when the host cell split. There was no doubt that mitochondria fulfilled the first expectation. So far, so good!

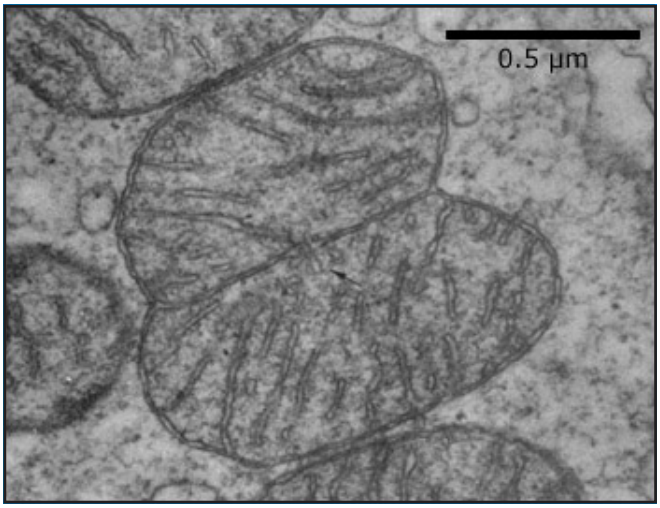


Figure 15. A mitochondrion in a cell from a butterfly prepares to divide.

2. **Mitochondria have their own genetic material**

All organisms have genetic material, so if mitochondria had once lived on their own as bacteria, they should have their own DNA. Just as Margulis had gone looking for DNA in Euglena chloroplasts, other scientists had been looking for DNA in mitochondria—and had found it! Mitochondria met Margulis' second expectation.

3. **Mitochondrial DNA codes for its own traits**

If mitochondrial DNA is really the DNA of what was once a distinct bacterium, we'd expect it to code for specific traits that the original bacterium had (e.g., using oxygen to break down food)—traits that the DNA in the nucleus doesn't code for. But how can you tell if a trait comes from DNA in mitochondria or DNA in the nucleus? Margulis came up with two tests:

Test #1: The easiest method would be to remove the mitochondria, then check and see if the trait (e.g., production of a certain protein for breaking down food) still exists in the cell. Unfortunately, most cells die when

their mitochondria are removed, and that makes it tough to say whether any traits are missing or not! For mitochondria at least, this test was inconclusive.

Test #2: The second test relied on the way traits are inherited. Mendel was able to predict what traits offspring would have because, in most cases, offspring get half their genetic material from Mom and half from Dad. But it turns out that in many multicellular organisms, mitochondria get passed down from just one parent—usually from the mother (Fig. 16). This is because mitochondria are generally inherited just from the egg, not from the sperm. That means that if specific traits (e.g., using oxygen to break down food) are carried by the mitochondrial DNA (and not by the nuclear DNA), those traits should have unusual maternal patterns of inheritance. Other scientists had already discovered such traits! Kearns-Sayre syndrome was investigated long after Margulis proposed her hypothesis, but it provides a good example of this sort of trait. Kearns-Sayre syndrome is a rare, human genetic disorder caused by a decreased ability of cells to get energy out of food. When scientists studied the inheritance pattern of this disease, they found that it was only passed down from the mother—just as we'd expect if the gene that causes this disorder was located on mitochondrial DNA. Evidence gathered later also supported the idea that the gene for the syndrome was on mitochondrial DNA. Margulis knew that such maternally inherited traits did exist. Furthermore, these are traits that seem related to the mitochondrion's job in the cell. This was strong evidence supporting the idea that some traits are only encoded in mitochondrial DNA. Mitochondria passed this test too!

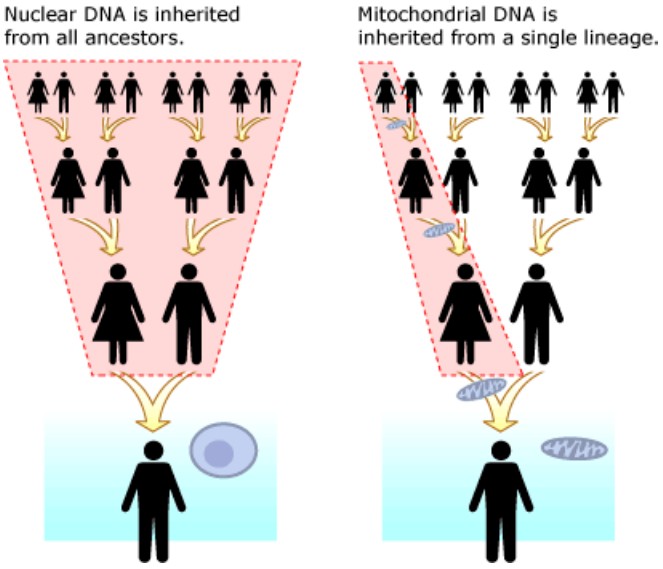


Figure 16. Unlike nuclear DNA (left), mitochondrial DNA is only inherited from the maternal lineage (right)—a quirk which allowed Margulis to determine whether mitochondrial DNA encoded unique traits.

4. **Mitochondria have bacterial relatives; mitochondria are more closely related to free-living bacteria than they are to the cell they reside in**

If mitochondria evolved from free-living bacteria, mitochondria should have long-lost bacterial cousins. But how could scientists figure out who those cousins might be? Complex traits, like a long DNA sequence, a complicated organ, or an intricate biochemical process are often good indicators of evolutionary relationships. If two organisms have the same complex trait, it's much more likely that they inherited it from the same ancestor than that the same complex trait just happened to evolve twice in two separate lineages.

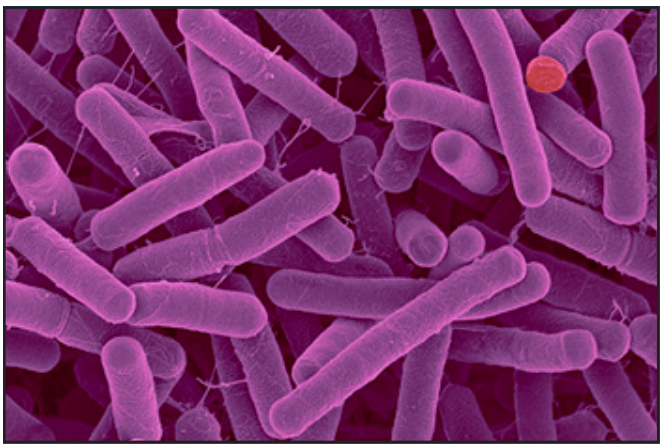


Figure 17. *Bacillus atrophaeus*, an aerobic, rod-shaped bacterium. Image © Dennis Kunkel Microscopy, Inc. (www.denniskunkel.com).

Margulis didn't have to look very hard to find a whole group of bacteria that fit the bill. The aerobic bacteria (Fig. 17) shared an essential complex trait with mitochondria—the ability to use oxygen to break down food molecules. Mitochondria and these free-living bacteria even use the same biochemical steps in the process! Aerobic bacteria were a perfect candidate for mitochondria's bacterial cousins.

Examining the alternative

All of the observations described previously make most sense if mitochondria evolved from free-living bacteria. But if the alternative—that mitochondria originated step by step inside the cell (Fig. 18)—is true, then there's no reason to expect mitochondria to be passed on to offspring, to have DNA that codes for unique traits, and to have close bacterial relatives. To give the alternative a fair hearing, Margulis tried to imagine what expectations it generated—to see if it had any evidence supporting it. She reasoned that …

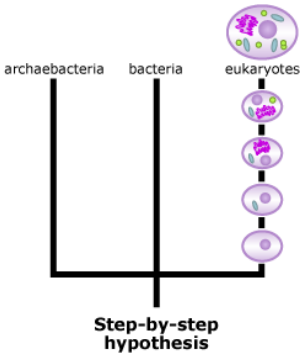


Figure 18.

If mitochondria evolved step-by-step inside the cell, then we'd expect that:

There are organisms that preserve early stages of mitochondrial evolution—that contain "proto" mitochondria

Biologists know of many examples of transitional forms, living or extinct organisms that have "intermediate" structures that help us understand how major changes in the history of life happened. It seemed reasonable to suppose that if mitochondria evolved from another structure in the cell, we might be able to find some organism with "transitional" mitochondria—early evolutionary forms of mitochondria. However, try as they might, no scientist had (or has yet today) observed anything like this. All the cells known to science either contained full-blown mitochondria or none at all. This makes perfect sense if mitochondria evolved by endosymbiosis, but not if they evolved from another part of the cell.

Based on the available evidence, the accepted hypothesis did not look very compelling, and Margulis' hypothesis looked reasonable, but there still wasn't any smoking gun. Figure 19 summarizes all the lines of evidence discussed so far.

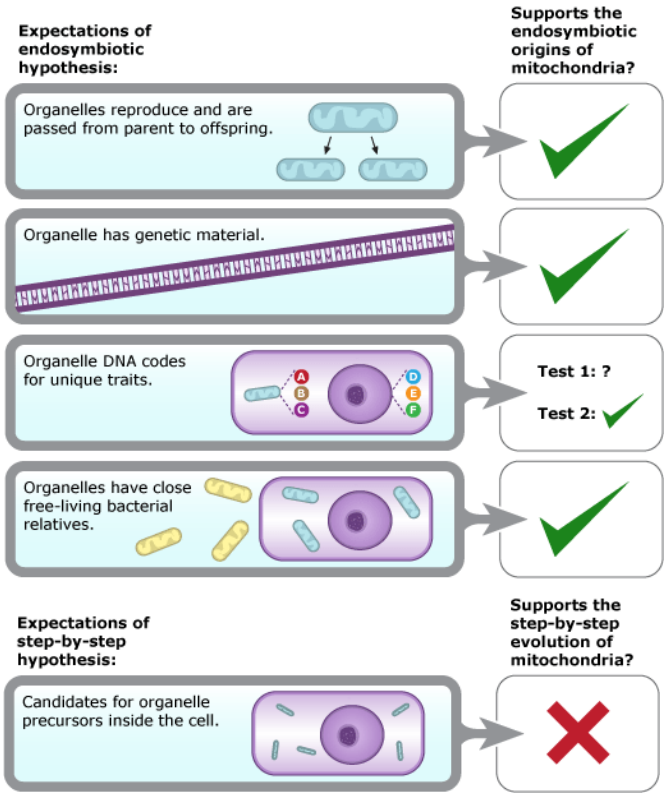


Figure 19.

The other organelles

We've taken an in-depth look at the lines of evidence Margulis developed to test her hypothesis, using mitochondria as an example. But, of course, Margulis had proposed that it wasn't just mitochondria that had evolved from endosymbionts; she thought that plastids and tubule organelles had evolved from endosymbionts too. In the same article where she reported all the evidence she'd gathered about mitochondria, she also explained the evidence relevant to these other organelles (Fig. 20). Plastids, at least, did even better than mitochondria. You can see on the chart on the next page that every test result supported the idea that plastids evolved via endosymbiosis.

The tubule organelles, however, didn't fare as well. They are passed to offspring in some cases and there didn't seem to be any organisms that contained precursors to these structures, but most of the tests were inconclusive or simply hadn't been done yet. Margulis still thought they had evolved via endosymbiosis, but the evidence supporting this view wasn't very strong.

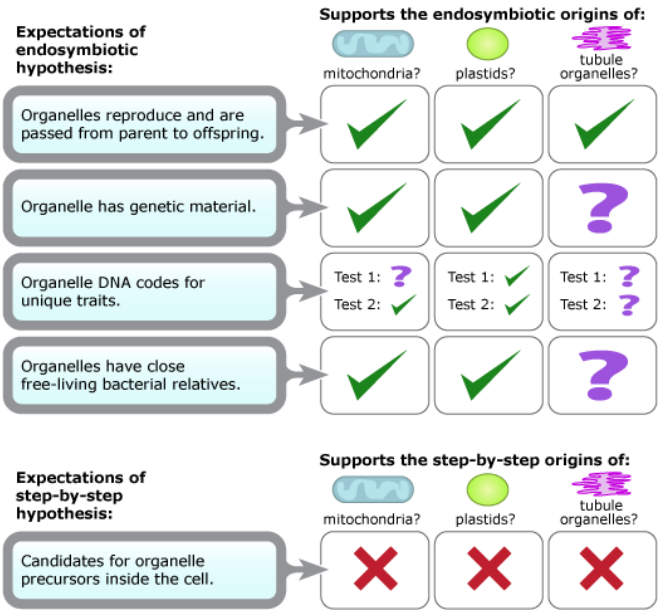
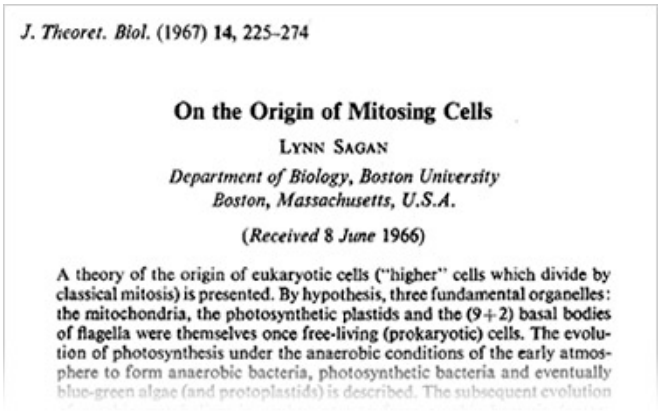


Figure 20.

The community reacts

Margulis' article laid out her complete hypothesis—when each endosymbiotic event had happened historically—and all the lines of evidence relevant to all the different types of organelles. She sent out her article to more than a dozen scientific journals, but they all rejected it—not because they thought it was bad science, but because it didn't fit neatly into any of the single subject areas that these journals usually covered. Margulis' paper discussed fossils, geology, genetics, biochemistry, and a whole zoo of organisms spread across the tree of life. Finally, the *Journal of Theoretical Biology*, which covers a range of disciplines, accepted it. The article was published in 1967 under the name Lynn Sagan, since Margulis was married to Carl Sagan at the time (Fig. 21).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On the Origin of Mitosing Cells

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(*Received* 8 *June* 1966)

A theory of the origin of eukaryotic cells ("higher" cells which divide by classical mitosis) is presented. By hypothesis, three fundamental organelles: the mitochondria, the photosynthetic plastids and the (9+2) basal bodies of flagella were themselves once free-living (prokaryotic) cells. The evolution of photosynthesis under the anaerobic conditions of the early atmosphere to form anaerobic bacteria, photosynthetic bacteria and eventually blue-green algae (and protoplastids) is described. The subsequent evolution

Figure 21. Margulis' article on the endosymbiotic hypothesis.

So was Margulis' argument enough to convince her colleagues? Yes—and no! Her paper sparked a lot of interest right away and it even won an award for best faculty publication of the year at Boston University where Margulis taught. Many of Margulis' colleagues working in genetics and microbiology needed little convincing to accept the idea. Some other biologists were swayed by the many lines of evidence she'd assembled. But many researchers in other fields seemed downright disturbed by the idea that vital cell structures like mitochondria could have evolved through endosymbiosis. Some critics argued that they could think up plausible scenarios in which the eukaryotic cell evolved in slow gradual steps and still met the expectations generated by the endosymbiotic hypothesis.

For example, one of the relevant lines of evidence involved the shape of the DNA in mitochondria. Mitochondrial DNA forms a circle (Fig. 22), just like bacterial DNA does. Nuclear DNA, on the other hand, is bundled up in linear strands. Some scientists reasoned that this indicated that mitochondria were more closely related to bacteria than to the cells they were inside of and viewed this as evidence supporting Margulis' hypothesis. Critics, however, interpreted the evidence differently. Based on the knowledge that circular DNA was the first kind of DNA that existed, they thought that bacterial DNA and mitochondrial DNA had both evolved from this early circular DNA, but along separate paths. In other words, they thought that bacterial DNA and mitochondrial DNA were similarly shaped, not because they were closely related, but because neither had ever evolved away from DNA's original shape (Fig. 23).

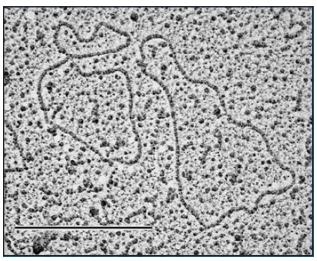

Figure 22. Electron microscope image of circular mitochondrial DNA in *Diplonema papillatum*, a marine flagellate. Scale bar is 0.5 µm.

Maybe that doesn't sound like a very likely explanation compared to Margulis' hypothesis, but in some ways, science can be like a courtroom—the burden of proof is often on the person who makes the new claim. It was up to Margulis to convince skeptics that she was right.

Margulis was savvy enough to recognize that convincing other scientists to think seriously about her hypothesis was going to take more than one paper. She had to overcome the scientific community's resistance to ideas that were unfamiliar to them and that didn't fit neatly into evolutionary theory as it existed then. To that end, she expanded her arguments into a full length book that, after an initial rejection by a publisher, was finally published in 1970. The book format allowed her to reach a wider interdisciplinary audience, expand on her arguments, and offer counterarguments to some of her critics.

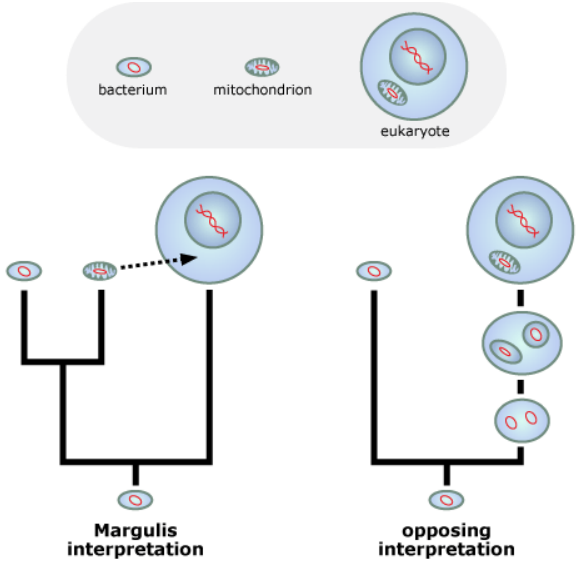

Figure 23. Two possible interpretations of the observation that mitochondria and bacteria both have circular DNA, while eukaryotic nuclear DNA is arranged in linear strands.

Though the book encouraged many scientists to take the endosymbiotic hypothesis seriously, it didn't convince them. In science, evidence is king. Ideas live and die by the evidence that supports or refutes them—and most scientists simply wanted stronger evidence before accepting the new idea.

The smoking gun: Support from a new technology

For the next ten years or so, the controversy raged. While new data and new arguments were brought in on both sides, none of the emerging research settled the matter to everyone's satisfaction. Though we often think

Circular mitochondrial DNA image reproduced with permission from the American Society for Microbiology, from Marande, W., J. Lukes, and G. Burger. 2005. Unique mitochondrial genome structure in diplonemids, the sister group of kinetoplastids. *Eukaryotic Cell* 4(6):1137-1146, fig. 5. DOI:10.1128/EC.4.6.1137-1146.2005

of scientists as neutral, logical people, there was a lot of passion on both sides. Margulis continued to champion her hypothesis, and was called tenacious and audacious by her supporters. Her detractors called her the same things, but some said it with a whole lot less admiration!

Throughout the 1970s, while some scientists were debating Margulis' hypothesis, others were at work on a new technology that would eventually settle the matter: DNA sequencing—techniques that would allow us to read the chemical code that makes up our genes. DNA sequencing is one of the most powerful tools in biology. Because closely related species have similar genes, DNA sequencing can help us figure out how species are related to one another—and this was just what scientists needed to know to evaluate an important line of evidence on Margulis' checklist: whether mitochondria, plastids, and tubule structures have close bacterial relatives (Fig. 24).

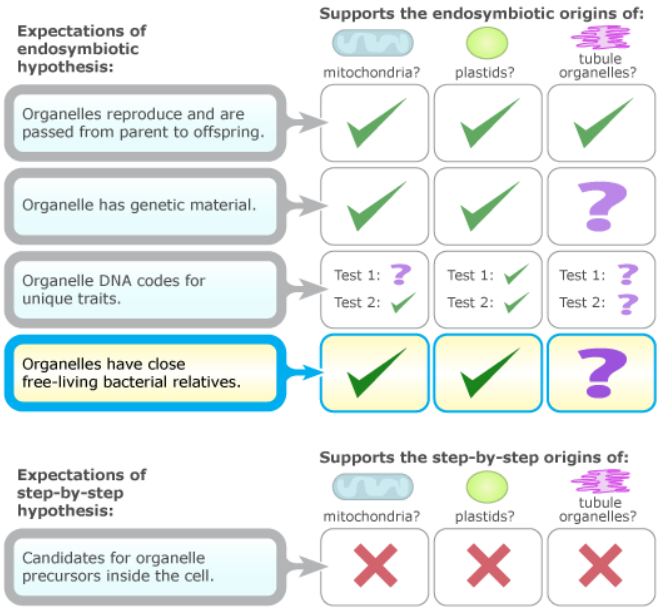

Figure 24.

Michael Gray and W. Ford Doolittle (Fig. 25) were interested in applying the new sequencing technologies to the debate about endosymbiosis. They wanted to know if the DNA inside plastids was more closely related to bacterial DNA or to the DNA inside the nucleus of the cell. If plastids evolved via endosymbiosis, we'd expect their DNA to have a similar sequence to that of free-living bacteria. On the other hand, if plastids evolved step-by-step inside the eukaryotic cell, we'd expect their DNA to be more like DNA in the nucleus.

By 1982, the results were in. In a paper that year, Doolittle and Gray summed up their results, as well as those of others: plastid DNA was much more similar to the DNA of free-living, photosynthesizing bacteria than it was to the DNA of the host cell. There was little doubt now: these organelles almost certainly evolved from endosymbionts.

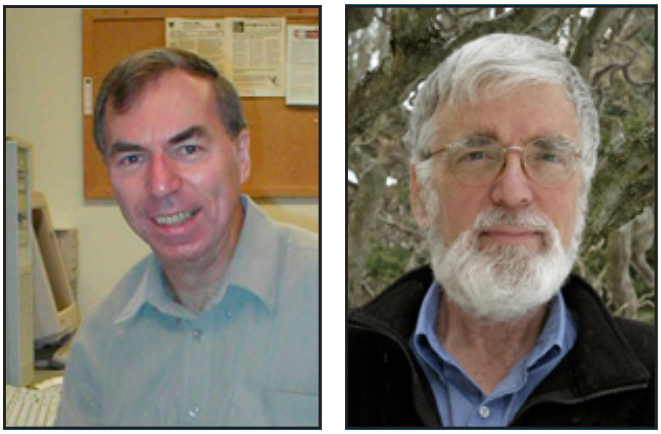

Figure 25. Michael Gray (left) and W. Ford Doolittle (right), both of Dalhousie University, Halifax, Nova Scotia.

Scientists still weren't certain about mitochondria, but just one year later they had genetic sequences from mitochondrial DNA too—and that DNA turned out to be remarkably similar to the DNA of free-living oxygen-using bacteria. This convinced most scientists that mitochondria had also evolved endosymbiotically from bacteria. Sixteen long years after Margulis had first published her ideas, the evidence was too powerful to ignore. Most scientists accepted her ideas about the importance of endosymbiosis. Evolutionary theory would have to make room for a new mechanism: lineages don't just split via speciation; they can also merge together via endosymbiosis to form a brand new lineage.

The debate about tubule organelles rages on

But wait! What about the tubule organelles, such as cilia and flagella (Fig. 26), which Margulis was convinced had also evolved via endosymbiosis? The evolutionary story of these organelles turned out to be a lot more difficult to figure out, and the debate about their origins continues today.

Gray photo courtesy of Michael Gray; Doolittle photo courtesy of W. Ford Doolittle

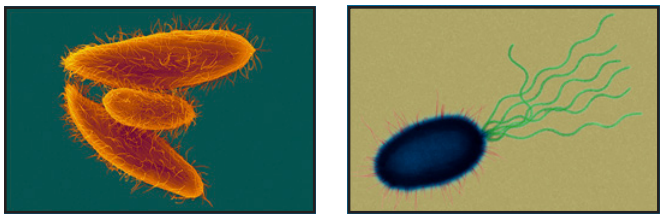

Figure 26. *Tetrahymena* (left), a freshwater protozoan with rows of fine cilia, and *Pseudomonas* spp. (right), a bacterium with several flagella. Images © Dennis Kunkel Microscopy, Inc. (www.denniskunkel.com).

DNA was found inside mitochondria and plastids pretty early on in our story, but the same wasn't true for the tubule organelles. Because they are big and sticky, stray DNA from the nucleus and from the environment tends to cling to them. These cellular dust bunnies always seemed to wind up contaminated with other DNA—and this makes it difficult for scientists to figure out for sure if they have DNA of their own!

Joan Argetsinger (Fig. 27), the first scientist to test the idea that there is DNA inside tubule organelles, published her results in 1965. She found DNA, but couldn't determine whether it was from the organelle itself or from contamination. For decades, the debate went back and forth. Many claimed to find evidence of genetic material, either DNA or RNA (another information-carrying molecule in the cells)—but it was always shown to be a result of failed methods, mistakes, or contamination.

Figure 27. Joan Argetsinger in 1963 when she was working on tubule organelles.

Despite the lack of evidence, Margulis remains convinced that we'll eventually find evidence to support the idea that tubule organelles, like plastids and mitochondria, are the result of endosymbiosis. In 2006, she published a revised version of her hypothesis that fits with what we'd observed up to that point—namely, that it's really hard to find genetic material belonging to tubule organelles. According to the new version of the hypothesis, tubule organelles evolved via endosymbiosis but have less genetic material (which would make it more difficult to find) because they were the very first endosymbionts to be swallowed up, and they've had all this time to lose more genetic material than the other organelles. However, many scientists were not any more convinced by her updated hypothesis regarding the tubule organelles than they were by the original.

In 2008, it looked like her hypothesis about tubule organelles might get some support. A team of scientists found convincing evidence that a certain type of tubule organelle does have its own genetic material—RNA! Would this bit of data convince the scientific community that Margulis had been right all along about an endosymbiotic origin for tubule organelles?

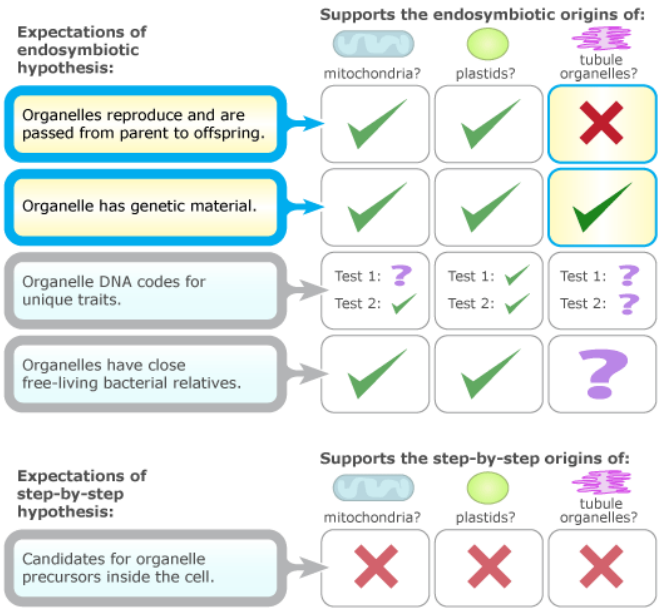

Figure 28.

No. The excitement was short-lived. Only a year later, another group of scientists showed that if you remove tubule organelles from a eukaryotic cell, *they can grow back*. According to Margulis' original criteria, self-replication and getting passed on to daughter cells was an important expectation generated by the endosymbiosis hypothesis. If a cell can grow an organelle from scratch, it likely means that that the organelle doesn't copy itself and get passed on to daughter cells. This discovery argued against the idea that these organelles evolved via endosymbiosis (Fig. 28). Based on all the available evidence, most biologists reject the hypothesis that tubule organelles descended from endosymbionts.

Argetsinger photo courtesy of Joan Argetsinger Steitz, photo by Joseph Gall

Updating evolutionary theory

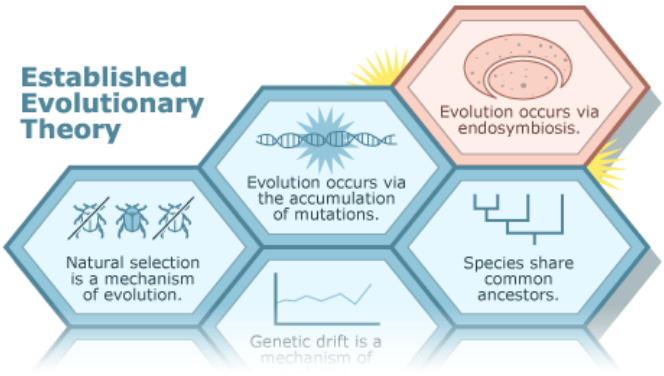

Figure 29. The idea of endosymbiosis became an established part of evolutionary theory.

Margulis may have been wrong about tubule organelles resulting from endosymbiosis—many biologists think that this part of her hypothesis is not supported by the evidence—but her ideas have still made one of the most tremendous contributions to evolutionary biology of the last hundred years. She didn't overthrow any of the core ideas of evolution, but she did force some of them to move over and make room for modifications (Fig. 29)! Margulis established that genetic mutations are not the only source of new traits in life and that competition is not the only strategy that living things can employ to get ahead in the evolutionary game. Through symbiosis, distantly related organisms can cooperate to form an entity more fit than the individual species involved, and over time, that relationship can grow so intimate that what was once two or three distinct species becomes one. Today, biologists accept the idea that this sort of endosymbiosis is common.

In the end, powerful evidence is what gave Margulis' hypothesis validity, but part of the battle was won with persistence and time. Evidence is always the most important factor in the acceptance of scientific ideas, but science doesn't happen in a vacuum, and many other factors can affect the pace of its progress. New ideas, or ones that seem to stray far from accepted theory, face greater obstacles to acceptance than more familiar ideas. This can make scientific knowledge slow to change—but that may not be a bad thing. This kind of skepticism ensures that new hypotheses are tested rigorously, with multiple lines of independent evidence, before the scientific community gets behind them. However, it's important to note that all ideas in science must be testable so that evidence regarding their validity can be gathered—even if that process takes decades.

Figure 30. Lynn Margulis at a symposium celebrating Darwin in 2009.

Lynn Margulis' story of discovery shows us how scientific ideas change over time. What began as a fringe hypothesis that couldn't be tested with the tools of the 19th century was revived and expanded by Margulis when the appropriate technology for testing began to be developed. With it, she convinced the scientific community that the evidence was strong enough to take a strange idea about endosymbiosis seriously. Through the efforts of many scientists working in a range of fields, the idea was tested until the toughest critics had to agree that, at least for mitochondria and plastids, the idea was correct.

Margulis' tenacious work on this hypothesis changed the way scientists understand how evolution works and inspired a whole new world of questions: Did any cellular features besides mitochondria and plastids evolve via endosymbiosis? How did an endosymbiont's DNA wind up in the host cell's nucleus? How often does this DNA transfer occur? Is the transferred DNA usually beneficial, harmful, or neutral? How has this process affected genome evolution? As scientists in many different fields seek answers to such questions, we are slowly building a better understanding of the key roles that endosymbiosis and cooperation have played in the evolution of life on Earth.

Want to learn more? Check out these references

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