The Origin of Eukaryotic Cells*

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The origin of life on Earth is a question of great interest. The origin of eukaryotic cells is yet another question that has boggled evolutionary biologists, naturalists, cell biologists, and molecular biologists alike for quite a while. Although the question is far from settled, evidence suggests that some of the organelles in the present-day eukaryotic cells have arisen from symbiosis events. This article examines morphological and some molecular evidence for the endosymbiotic origin of mitochondria and plastids in eukaryotes. Many of these arguments are based on a classic paper by naturalist and microbiologist Lynn Margulis and follow up work from other scientists. She described eukaryotes as multigenome systems, where all biochemical reactions are encoded in the DNA of either the nucleus or the subcellular organelles of symbiotic origin.

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Symbiosis is a living arrangement of individuals of two different species in an association that can be either beneficial or unfavourable. Symbiogenesis or endosymbiosis theory explains the origin of specific organelles in the present-day eukaryotic cells. The theory posits that chloroplast and mitochondria arose from the engulfment of specific prokaryotic cells by another prokaryotic cell during the evolution of life on Earth. The currently held view of endosymbiotic theory also takes into account specific geochemical and atmospheric conditions on Earth.

The endosymbiotic theory for the origin of eukaryotic cells has had many proponents in the last century and a half. Andreas Schimper, a German botanist, was the first to propose the endosymbiotic origin of chloroplasts in his 1883 paper 'On the development of chlorophyll grains and color bodies'. The term

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symbiogenesis was coined by the Russian botanist Konstantin Mereschkowski in his 1905 paper 'The nature and origins of chromatophores in the plant kingdom' and elaborated upon in a 1910 paper, 'The theory of two plasms as the basis of symbiogenesis: A new study of the origins of organisms'. He worked extensively on lichens, a composite organism representing a symbiotic relationship between fungi and algae or cyanobacteria. Boris Kozo-Polyansky, another Russian botanist, developed the symbiogenesis theory further in his 1924 paper 'The new principle of biology: An essay on the theory of symbiogenesis'. He proposed that symbiosis drives the evolution of new traits that are subject to natural selection, thereby integrating symbiogenesis with Darwinian evolution.

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Lynn Margulis was an American naturalist and microbiologist who expanded on the endosymbiont theory of evolution of eukaryotes by carefully organizing and illustrating the evidence for the endosymbiotic origin of mitochondria and chloroplasts [1]. As laid out here, she crafted this tale with a series of compelling pieces of evidence combined with logical predictions that have withstood the test of time over the past half-century. In addition to anatomy and cell biology, Lynn also supported her theory with evidence from geochemistry and molecular biology.

Evolution of Photosynthesis and Respiration in Prokaryotes in Reducing Atmosphere on Earth

The terrestrial atmosphere on the Earth was a reducing one 4.5 to 2.7 billion years ago. It is believed that some of the biomolecules came about non-enzymatically in the primordial soup in the reducing environment on Earth in this period. Indeed, a variety of organic molecules such as ATP and amino acids can be synthesized much more easily under reducing conditions than in oxidizing conditions in the laboratory. This led to the idea that life arose under reducing conditions of the Earth's atmosphere [2]. Some of the events described here are numbered for the sake of clarity, though not done by Lynn Margulis herself.

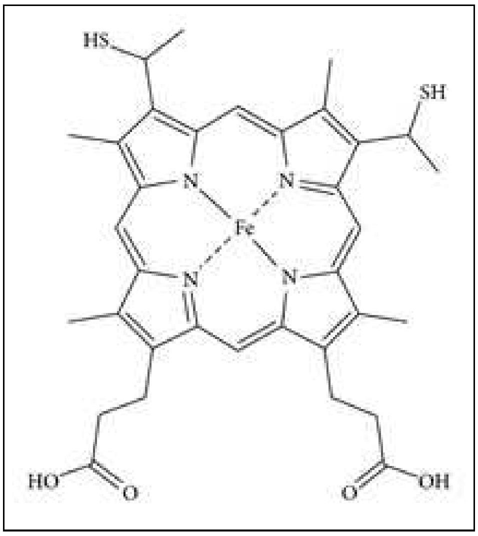

Figure 1. Protoporphyrin IX, a precursor for heme, cytochrome c and chlorophyll.

Event 1: Prokaryotes with nucleic acid genomes abounded in the reducing environments. Photodissociation of water vapor in the upper atmosphere resulted in the production of free hydrogen and molecular oxygen, the latter threatening the nucleic acid genome of free-living self-replicating organisms. Chance emergence of organisms with the ability to produce chelating porphyrins[1] (*Figure* 1) that protected the organism from naturally produced oxidizing agents such as oxygen would have led to their selection. Some of these cells then may have evolved ways to produce ATP using solar energy absorbed by the porphyrins. This could have been via a molecule like the present-day chlorophyll. Using energy released from ATP and the free hydrogen (present in the Earth's atmosphere at that time), CO_2 was reduced to make cell material such as sugars. Such organisms would have been the primitive phototrophs[2].

Event 2: Heterotrophs[3] relied on fermentation of sugars for ATP production. Some of the cells with porphyrins developed more efficient oxidation of carbohydrates. This included the evolution of cytochrome-mediated production of ATP via electron transport systems leading to the emergence of anaerobic respirers that released either nitrate or hydrogen sulfide.

[1] Porphyrins are heterocyclic macrocycle organic compounds composed of 4 pyrroles interconnected at the center. Porphyrins and related molecules are essential components of redox-active proteins such as cytochromes and chlorophyll.

[2] Autotrophs are organisms capable of making their own food. They store chemical energy in carbohydrates by fixing carbon. Phototrophs are autotrophs which use the energy of light to fix carbon.

[3] Heterotrophs are organisms lacking the ability to synthesize their own food. They consume autotrophs or other heterotrophs.

Event 3: Primitive phototrophs continued to use hydrogen from photodissociated water to reduce CO_2 into carbohydrate, leading to the production of gaseous oxygen as a byproduct of photosynthesis. In places where gaseous oxygen became abundant, anaerobic heterotrophs evolved the final and aerobic step of respiration by donating hydrogen to oxygen, thereby eliminating CO_2 and water.

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Lynn presented geochemical evidence for the transition of the Earth's atmosphere from reducing to oxidizing by analyzing the oxidation of uranium in sediments. Specifically, oxidized rocks as old as 2.5 billion years old have been found, suggesting that oxygen must have been abundantly present at the time. Microfossil structures of the blue-green algae, known more correctly as cyanobacteria, presumably the first phototrophs, have been found in rocks dating 2.1 billion years ago [3]. Oxidized limonite beds as old as 2.5 billion years have been found, suggesting that photosynthesis ending in the release of gaseous oxygen must have evolved before that time.

Event 4: Some autotrophic microbes with the ability to use porphyrin for anaerobic photosynthesis in light also appear to have developed the ability to use the same porphyrins for aerobic respiration in the absence of light. This led to the evolution of prokaryotic algae with both photosynthetic and respiratory mechanisms for ATP production. These ancestors to blue-green algae were many times more efficient in the production of cellular material (growth) and energy and must have increased rapidly in abundance.

Event 5: Prokaryotic algae and aerobic microbes continued to eliminate gaseous oxygen and reactive oxygen species and accelerated the transition of the Earth to an oxidizing atmosphere. This must have happened sometime before the emergence of oxidized rocks on Earth (4.5 and 2.1 billion years ago).

The Evolution of Eukaryotic Cells from Prokaryotic Cells

Geological evidence suggests that oxygen was present in the atmosphere of Earth as early as 2.7 billion years ago and became relatively abundant 1.2 billion years ago. This would have caused the loss of all life forms lacking the ability to protect themselves from oxidation. This would also have put a stop to the production of abiogenic (non-cellular) organic molecules. Heterotrophs would have been forced to consume autotrophs with photosynthetic or chemoautotrophic ability. Although prokaryote fossils have been found in rocks as old as 3.1 billion years, the first eukaryotic alga (phototroph) appears only in the rocks dated 0.4 billion years or younger. The lack of a missing link between the prokaryotic cyanobacteria and eukaryotic algae, both phototrophs, in the fossil evidence, led Lynn to argue for the theory of endosymbiotic origin of plastids responsible for photosynthesis.

Event 6: The oxidizing environment would have put enormous pressure on anaerobic microbes. Ingestion of an aerobic microbe (protomitochondrion) by an anaerobic heterotroph could have led to a symbiotic relationship. The selective advantage provided by the endosymbiont could have then caused the relationship to become obligate (*Figure* 2). This led to the emergence of the first aerobic amoeboid organism. Such cells continued to perform anaerobic oxidation of glucose to pyruvate in the cytoplasm (Embden–Meyerhof pathway) while further oxidation occurred in the symbiotic mitochondrion using molecular oxygen, via the Krebs cycle. In this cycle, hydrogen atoms from organic acids combine with FAD and cytochrome, resulting in the generation of ATP and elimination of water.

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Event 7: Greater amounts of energy available after the incorporation of mitochondria would have led to larger heterotrophic cells with amoeboid movements. Engulfment of one microbe by another may also have resulted in a eukaryotic phospholipid membrane and the formation of nucleus and endoplasmic reticulum.

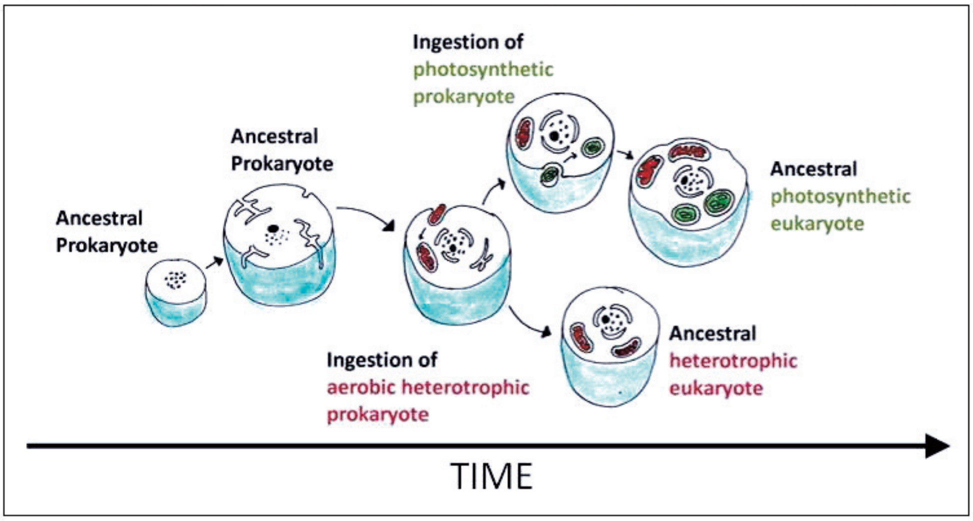

Figure 2. The endosymbiont theory for the origin of eukaryotic cells. A heterotrophic prokaryote ingested an aerobic heterotroph (protomitochondrion). Subsequent ingestion of a photosynthetic prokaryote led to the emergence of ancestral photosynthetic eukaryote.

Evolution of Eukaryotic Plants

Event 8: Lynn Margulis argued, and logically so, that plant cells must have come from a heterotroph that acquired a photosynthetic prokaryotic endosymbiont. It is unlikely that plant cells evolved an oxygen eliminating capability first and later packaged them into membrane-bound plastids. Lower eukaryotic algae have a large diversity in cellular structures. This implies that these evolved by the ingestion of different photosynthetic prokaryotes (protoplastids) by heterotrophic eukaryotes at various time points during the evolution of eukaryotes (*Figure* 2).

General Features of an Endosymbiont

What should be the criteria to call an organelle an endosymbiont? Lynn Margulis posited that a symbiont must have the following properties as a free-living organism prior to ingestion by a host cell:

1. Presence of genetic material in the form of self-replicating DNA
2. Presence of messenger RNA complementary to the DNA

3. Presence of a functioning protein synthesis machinery

4. A source of ATP and other nucleotides

5. A source of small molecules from which to make proteins and nucleic acids, and

6. A cell membrane synthesizing system.

Upon entry into a host cell, a symbiont may lose none to all of its features except the ability to self-replicate its DNA and synthesize mRNA from that DNA. It is also very likely that after long association, the redundancy in the functionality between host and symbiont genomes will be selected against. The symbiont is likely to relegate all dispensable metabolic functions to the host genome, and the relationship will progressively become more obligate.

For a symbiotic relationship to work, the host cells must ensure a mechanism to faithfully distribute the endosymbiont organelles to both daughter cells during cell division. Any mutation which will ensure reasonably equal distribution will be selected. All eukaryotic cells have mechanisms to ensure that the daughter cells receive one or more mitochondria, fulfilling this criterion. Some photosynthetic cells can lose their chloroplast in a process called bleaching but then require special conditions to survive. If a eukaryotic cellular organelle is indeed an endosymbiont, there should be no organism containing intermediate intracellular stages of the organelles. For example, one does not find plant cells with free porphyrins capable of photosynthesis. Plant cells always have them encased in membrane-bound plastids. Indeed, the entire series of the metabolic capability of the organelle, necessary for photosynthesis, is acquired together as a unit.

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If a symbiont is lost, all the metabolic functions encoded in the symbiont genome should be lost from the host altogether. These functions can only be reacquired altogether by the reingestion of the free-living symbiont. Endosymbionts have their own genes and may not obey the laws of Mendelian inheritance. For example, in humans, the zygote receives the mitochondrial genome uniparentally and only from the mother. Do mitochondria fulfill all the requirements for organelles originating in symbionts? Lynn argued that there are no organisms that have features of eukaryotes but lack mitochondria. This also suggests that this symbiosis is so ancient, it has become obligate. In addition, we have no examples of nucleated or plastid-containing cells where mitochondrial enzymes are unpackaged or where only a few enzymes are present, and the rest are missing. Lynn further argued that for a cell to evolve a mitochondrion *de novo*, it would have to acquire 30000 base pairs (assuming each mitochondrion needs 100 different enzymes of 100 amino acids each), making it an improbable event.

Lynn further argued that the evolution of plastids by evolution of the protein-coding capacity of the nuclear genome in an aerobic cell resulting in a phytoflagellate is also highly improbable. This evolution would require several thousands of specific mutations in the genome to encode for the function enabling photosynthesis. Further, the chloroplast DNA has been found in different photosynthetic eukaryotes [4]. As early as 1965, a satellite band of DNA was recovered from photosynthetic cells. The band was missing from bleached cells, and such cells completely lacked the potential for chloroplast formation. Moreover, mRNA complementary to chloroplast DNA was found in the organelle. Blue-green algae may be considered a free-living prokaryote counterpart of plastids. Like mitochondria, neither fossil evidence nor present-day examples of intermediate photosynthesis-capable organisms exist. Chloroplast DNA also follows the example of non-Mendelian cytoplasmic heredity.

As early as 1965, a satellite band of DNA was recovered from photosynthetic cells. The band was missing from bleached cells, and such cells completely lacked the potential for chloroplast formation. Moreover, mRNA complementary to chloroplast DNA was found in the organelle.

Fossil or present-day organisms containing chloroplasts, but lacking mitochondria have not been found, suggesting that mitochondria became obligate endosymbionts before the ingestion of a protoplastid by an aerobic organism (*Figure* 2).

Both mitochondria and chloroplasts are also closer in size to present-day free-living prokaryotes. In her 1967 paper, Lynn posited that all the eukaryotes must contain at least 3 specific types of DNA: nuclear, mitochondrial, and (9 + 2) homologue (precursor to cilia and of centrosomes responsible for motility and cell division via mitosis respectively). Moreover, an additional DNA type corresponding to chloroplasts must be found in eukaryotic photosynthesizing organisms. This prediction of Lynn is true except for the presence of DNA corresponding to (9 + 2) homologue. Lynn suggested that the search for (9 + 2) endosymbiont DNA has evaded detection because it has little metabolic function, and thus DNA is very small. Lynn's description of eukaryotes as multi genome systems, except for 9 + 2 homologue, has been validated further by genomics. Woese and Fox [5] analyzed the ribosomal RNA sequence of eukaryotes, eubacteria, and archaea and showed that they are three distinct lineages. 16S rRNA of archaea were a lineage as far from eubacteria as they were from eukaryotic cytoplasmic 18S rRNA. 16S rRNA of eubacteria formed a distinct third lineage. Later analysis revealed that ribosomal RNA of mitochondria is similar to ribosomal RNA from eubacteria.

Additional evidence for support of endosymbiotic origin of mitochondria and chloroplast:

- Mitochondria and plastids contain circular DNA similar to the DNA of prokaryotes, while the nuclear DNA of eukaryotes is organized into many linear chromosomes.

- New mitochondria and plastids arise by binary fission, the form of cell division used by extant prokaryotes, while nuclear DNA of eukaryotes is segregated via mitosis.

- The genome of mitochondria and Proteobacteria called Rickettsial bacteria are related suggesting that *Rickettsia* may be their free-living counterpart.

- Genomes of plastids and cyanobacteria are related.

- The ribosomes of mitochondria and plastid are similar to those of bacteria (70S) and different from the cytosolic ribosomes of eukaryotes (80S).

- Proteins encoded by mitochondrial or plastid genome begin with N-formyl methionine, as is the case in bacteria. Proteins encoded

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by nuclear genes in eukaryotes, on the other hand, begin with methionine.

Candidatus Prometheoarchaeum syntrophicum, an archaeon from deep-sea sediment, is a complex anaerobic prokaryote with the complexity of a eukaryote [6]. In the laboratory, this Lokiarcheon can be grown together with a methanogenic bacterium and with sulfate-reducing bacterium in a symbiotic relationship. Addition of sugar, electron acceptor, or other building blocks to this Archeon alone does not increase the cell yield suggesting that the symbiotic relationship is essential for the host. The Lokiarcheon-proteobacterium symbiosis provides an example of an event preceding the evolution of eukaryotic cell from a prokaryotic anaerobe. Although the endosymbiotic origin of mitochondria and chloroplast is believed to be true in light of several lines of evidence presented above, several questions remain unanswered. Mitochondria and chloroplast have genomes far smaller than the genome of free-living counterparts, proteobacteria, and cyanobacteria respectively. This suggests that the symbiosis event is ancient, and the endosymbiont has transferred a large fraction of its metabolic capacity to the nuclear genome, perhaps for better cellular economy and integration. However, the mechanisms of gene transfer to the nucleus are not fully known. Nevertheless, the success of endosymbiont theory in explaining the emergence of aerobic respiration and photosynthesis keeps the search and research on for origins for other cellular organelles.

Although the endosymbiotic origin of mitochondria and chloroplast is believed to be true in light of several lines of evidence presented above, several questions remain unanswered.

Suggested Reading

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