Endosymbiotic Theory

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Key Points

- A brief history of the endosymbiotic theory
- The endosymbiotic theory for the mitochondrial origin
- The ubiquity of mitochondria and origins of cellular complexity
- The endosymbiotic theory for the plastid origin
- Secondary endosymbiosis
- Endosymbiotic gene transfer and its evolutionary consequences
- Limits and challenges of the endosymbiotic theory

Glossary

Aerobic organisms Aerobic organisms live in oxic environments. Obligate aerobes are strictly dependent on oxygen and need it to grow. Aerobes usually use oxygen as the terminal electron acceptor in energy metabolism.

Alveolates The alveolates are a group of unicellular eukaryotes, including dinoflagellates, apicomplexans, and ciliates.

Amoebozoa The Amoebozoa are one eukaryotic supergroup containing ameboid microorganisms.

Anaerobic organisms Anaerobic organisms do not require oxygen for growth. For obligate anaerobes oxygen is harmful, because various enzymes of anaerobes are readily inactivated by oxygen. Facultative anaerobes can live with or without oxygen.

Apicomplexa Apicomplexa are a large group of obligate parasitic, unicellular eukaryotes belonging to the supergroup Chromalveolata.

Archaeplastida The Archaeplastida are one eukaryotic supergroup containing glaucophyta, rhodophyta, and chlorophyta (with land plants). Archeaplastida possess primary plastids.

Autotrophy Autotrophic organisms are able to produce organic compounds (food/energy-source) from inorganic ones using light or chemical reactions.

Chlorarachniophytes Chlorarachniophytes are ameboid eukaryotes belonging to the rhizaria. They possess secondary (green) plastids with four membranes and a nucleomorph.

Chlorophytes The lineage of Archaeplastida having green primary plastids.

Endoplasmic reticulum A network of membranous tubules and sacs located within eukaryotic cytosol, primarily responsible for protein and lipid synthesis assembly of membrane bound intracellular compartments such as endoplasmic reticulum, golgi and lysosomes, located within eukaryotic cytosol.

Endosymbiosis One cell living in stable symbiosis within another.

Facultative anaerobic organisms Facultative anaerobes are able to grow with or without oxygen.

Fermentation Enzymatic conversion of organic compounds (sugars) into acids, gases, or alcohol.

Functional redundancy (through endosymbiosis) The retention of divergent but homologous gene copies donated both by host and endosymbiont at organelle origin for the same functions (e.g., ribosomal proteins of chloroplasts, mitochondria, and the cytosol).

Glaucophytes A group of unicellular algae with plastids that still possess a rudimentary peptidoglycan wall and that together with the rhodophytes and the chlorophytes comprise the eukaryotic supergroup Archaeplastida.

Heterocyst Differentiated cells of some cyanobacteria that are specialized for nitrogen fixation.

Heterotrophy Heterotrophic organisms use reduced organic substances as their source of carbon.

Homologs Proteins in different species that are similar in sequence due to evolutionary descent from a common ancestor

Hydrogenosome Hydrogenosomes are anaerobic mitochondria that have a double membrane and synthesize ATP via hydrogen-producing fermentations. They lack cytochromes, a membrane-associated electron transport chain, and (except in rare cases) a genome. Hydrogenosomes arose several times independently in evolution and can be found in trichomonads, anaerobic ciliates, and some fungi.

Invention cost In the context of evolutionary biology, the cost (chiefly energetic, but also survival and reproductive cost) incurred by the trial and error until a functional unit under selection (e.g. protein, a protein complex or a process) evolves (i.e. a functional DNA sequence is available on the genome for vertical or horizontal inheritance).

Last Common Ancestor A theoretical concept referring to the most recent common ancestor shared by all members of a group at any taxonomic level (e.g., species, genus, family), from which they have descended.

Maintenance cost In the context of biology, the cost (chiefly energetics) of replicating, expressing and recycling or degrading a unit (e.g. a protein or protein complex) that is already genome encoded and inherited (vertically or horizontally).

Meiotic cell cycle A specialized cell division, observed in sexually reproducing eukaryotes, in which the chromosome numbers are reduced by half during the production of reproductive cells.

Methanogen Methanogens are archaea that produce methane as a metabolic byproduct of core energy metabolism.

Mitosome Mitosomes are organelles of mitochondrial origin that do not produce ATP. They have retained components of FeS cluster assembly or sulfate activation.

Natural selection pressure Environmental factors that differentially influence the survival and reproductive success of a unit under selection (e.g. an amino acid, a protein, an organism or an ecosystem).

Nucleomorph Found only in some groups of eukaryotic algae whose plastids stem from secondary endosymbiosis, the nucleomorph is the highly reduced nucleus of the eukaryotic endosymbiont, it is located inside the periplastidal compartment. The nucleomorph is lost in most algae with secondary plastids but still can be found in chlorarachniophytes and cryptophytes.

Nucleosome A fundamental unit of eukaryotic chromatin, composed of a segment of DNA wrapped around a core of histone proteins.

Opisthokonts Another name for the group consisting of animals and fungi.

Outer membrane vesicles (OMVs) Vesicles that bud out from the outer membranes of prokaryotes (bacteria and archaea) into their surrounding

Periplastidal compartment The periplastidal compartment can be found in plastids of secondary origin within the chlorarachniophytes and cryptophytes. It corresponds to the cytosol of the eukaryotic endosymbiont.

Phagocytosis Phagocytosis is the engulfment of cells or particles by living cells.

Primary plastids Designates plastids that stem from a symbiotic association of a cyanobacterium with a eukaryotic host.

Proteobacteria Proteobacteria are a major group of bacteria. They are Gram-negative.

Pseudogene Pseudogenes are DNA segments resulting from multiple mutations, which look like genes, but are dysfunctional.

Rhodophytes Red algae, a group of Archaeplastida.

Ribosome Ribosomes are cellular particles composed of proteins and rRNA, where proteins are synthesized. They can be found in the cytosol, in mitochondria, and in plastids. In algae with a nucleomorph, a fourth set of ribosomes occurs in the periplastidal compartment.

SAR An eukaryotic group of organisms including stramenopiles (heterokonts), alveolates, and rhizaria.

SCH An eukaryotic group including stramenopiles, cryptophytes, and hacrobia.
Secondary plastids Designates plastids that stem from secondary endosymbioses in which the product of the primary endosymbiosis (a green- or a red-algae) came to reside into a heterotrophic, eukaryotic host.
Symbiosis Living together. When symbiosis involves benefit for both partners, it is mutualism.
Syntrophy "Eating together," designates a kind of metabolic association in which one cell is dependent upon a metabolic endproduct of another. The metabolic endproduct is often molecular hydrogen.

Abstract

Endosymbiotic theory designates a class of hypotheses that view various organelles in eukaryotic cells as descendants of endosymbionts, whereby the term endosymbiont designates a microbial cell that has come to live stably inside another microbial cell (a host). In its oldest and most familiar versions, endosymbiotic theory posits that mitochondria and plastids were once free-living bacteria. Mitochondria (the powerhouses of eukaryotic cells) stem from free-living proteobacteria. Plastids (the chlorophyll-containing solar panels of plant cells) stem from cyanobacteria. This article explains the basic principles of endosymbiotic theory, including the concept of secondary endosymbiosis, in which the plastids of some algal groups arose through an endosymbiosis of a eukaryotic alga within a eukaryotic host.

Introduction: A Brief History

Endosymbiotic theory designates a class of hypotheses that view various organelles in eukaryotic cells as descendants of endosymbionts: cells that came to live inside another cell (a host). In its oldest and most familiar versions, endosymbiotic theory posits that mitochondria and plastids were once free-living cells: mitochondria (the powerhouses of eukaryotic cells) stemming from free-living proteobacteria and plastids (the chlorophyll-containing solar panels of plant cells) stemming from cyanobacteria. The Russian botanist Constantin Mereschkowsky is generally credited with the first formulation of endosymbiotic theory. He described plastids as reduced cyanobacteria that entered into a symbiosis with a heterotrophic host, which itself originated via a symbiosis between a heterotrophic host cell and a smaller endosymbiont that, in his view, gave rise to the nucleus (Mereschkowsky, 1905, translation in Martin and Kowallik, 1999). Mereschkowsky's reasoning was remarkably modern with regard to the origin of plastids. He did not consider that mitochondria might also be of endosymbiotic origin (Mereschkowsky, 1910, translation in Kowallik and Martin, 2021). That idea probably traces back to the French biologist Paul Portier (1918), who developed ideas about the relationship between bacteria and mitochondria. But Portier proposed that mitochondria could be cultured outside their host cells, and this precipitated considerable criticisms from peers (Archibald, 2014). The American biologist Ivan Wallin developed endosymbiotic theory further for mitochondria (Wallin, 1927). He was convinced that mitochondria are descendants of endosymbiotic bacteria, but he did not expound upon the ancestry of the host that acquired them (Wallin, 1927). Like Portier, he thought that the cultivation of mitochondria outside their host should be possible. Though initially quite popular in the early 1900s, endosymbiotic theories endured scathing criticism in a leading college textbook of the day (Wilson, 1928), whereupon they fell into disrepute for decades.

Endosymbiotic theory was revived in 1967 when Lynn Sagan (later named Margulis) argued that chloroplasts and mitochondria had descended from separate endosymbionts. Sagan envisaged as a host for the origin of mitochondria a heterotrophic anaerobic prokaryote, in whose cytoplasm an aerobic prokaryotic microbe had taken up residence. The resulting heterotrophic protozoan later engulfed a cyanobacterium, resulting in the origin of plastids (Sagan, 1967). However, germane to all of Margulis's versions of endosymbiotic theory, from 1967 onward, is the notion that the eukaryotic flagellum arose from a symbiotic spirochete (Margulis, 1970; Margulis et al., 2006)—a view that never received reproducible experimental support and that remained outside the mainstream of developments surrounding endosymbiotic theory. Since Margulis's revival of the idea, more than 30 different versions of endosymbiotic theory, with varying degrees of detail, and with different areas of focus, have been put forward (reviewed in Martin et al., 2015). Some versions introduce new ways to imagine the origin of mitochondria and chloroplasts, other versions suggest endosymbiotic origins for other cell compartments like peroxisomes or the nucleus, or aim to account for the origin of various eukaryotic traits. In the main, however, endosymbiotic theory is about the origin of chloroplasts and mitochondria.

Mitochondria

Theory

In spirit, different endosymbiotic theories of mitochondrial origin have converged onto a symbiosis between a bacterium (the mitochondrial ancestor, the endosymbiont) and an archaeon (the host cell), as a starting point. In different formulations of endosymbiotic theory, the genomic and cellular nature of the host and endosymbiont take various forms.

The Nature of the Endosymbiont and the Host

Most models for the origin of mitochondria posit that the mitochondrial endosymbiont was an aerobic bacterium, if they take a stance on its physiology at all. But various anaerobic forms of mitochondria like hydrogenosomes also occur among eukaryotes (Müller et al., 2012) and these also need to be accounted for under endosymbiotic theory. One variant of endosymbiotic theory, called the hydrogen hypothesis, directly accounts for these anaerobic mitochondria. It posits a symbiotic association of an anaerobic, strictly hydrogen-dependent and autotrophic archaebacterium as the host with a facultatively anaerobic, heterotrophic bacterium as the endosymbiont, with specialization and differential loss leading to aerobic and anaerobic forms of mitochondria (Martin and Müller, 1998).

It is a particularly curious aspect of endosymbiotic theory that ideas about the bacterial ancestry of mitochondria developed historically long before concepts about the host for the origin of mitochondria appeared. Ideas about the nature of the mitochondrial host came as a necessary afterthought in the wake of the more pressing debate about whether endosymbiosis for organelle origins was a good idea or not. Early models for the origin of mitochondria have a primitive mitochondrion-lacking (amitochondriate) microbe as the hosts of an aerobic bacterium (De Duve, 1969). In the early 1970s and well into the 1990s it was customary to view the mitochondrial host as a mitochondrion-lacking eukaryote—a cell that had mastered the evolutionary transition from being a prokaryote to one that had a nucleus, a cell cycle, and all the other myriad attributes that separate eukaryotes from prokaryotes (for a long list of such attributes see Cavalier-Smith, 2002). Following the discovery of archaebacteria (archaea), an archaeon was often viewed as the host that acquired the mitochondrion (Van Valen and Maiorana, 1980; Doolittle, 1980).

The model of Vellai and Vida (1999) operates with a prokaryotic host, as does the sulfur cycling theory of Searcy (1992). López-García and Moreira (2006) proposed a three-partner endosymbiosis between a fermenting, heterotrophic, hydrogen-producing ancestral myxobacterium (delta-proteobacterium) that serves as the host, a strictly anaerobic, methanogenic archaeon that becomes the nucleus, and an alpha-proteobacterium that was then surrounded by the syntrophic couple and became the mitochondrial ancestor. The model presented by Martijn and Ettema (2013), like that put forward by Yutin et al. (2009), suggests a phagocytosing archaeal host, which engulfed an alpha-proteobacterium.

All the while it should have been evident that the host for the origin of mitochondria was related to archaea (or was an archaeon outright), because the eukaryotic cytosol harbors archaeal ribosomes (Esser et al., 2004). Improvements in phylogenetic methods have gradually brought forth a picture in which the host for the origin of mitochondria branched within the archaea (Cox et al., 2008; Williams et al., 2013; Spang et al., 2015), not as a sister to the archaea, as the older rRNA tree of life implied (Pace, 2006). Some views, based in phylogenetic analyses, point to newly characterized clades of archaea (Imachi et al., 2020) as relatives of the host. Together, data suggest that the mitochondrion was acquired by an archaeon (a prokaryote), as some of the endosymbiotic models had suggested.

The endosymbiotic origin of mitochondria in an archaeal host is illustrated in **Fig. 1**.

Contributions of the Endosymbiont

The initial contribution of endosymbiont (then symbiont) most likely pertained to a metabolic exchange, the exact details of which vary across models, for example, in form of hydrogen exchange or sulfur recycling (reviewed in Martin et al., 2015). The contributions of the bacterial endosymbiont were instrumental in transforming the prokaryotic host into the last eukaryotic common ancestor (LECA). Eukaryotic genomes are chimera of bacterial (endosymbiont) and archaeal genomes (Rivera and Lake, 2004). A vast majority of these bacterial genes pertain to carbon and lipid metabolism. Metabolism hence remains an important contribution of endosymbiont from the starting point of syntrophy. The differences between archaeal lipids (isoprene ethers) and eukaryotic lipids (fatty acid esters) are also traced to the endosymbiont and likely to its outer membrane vesicles (Gould et al., 2016).

Contributions of the Host

The archaeal host contributed most of the information processing and, predominantly, the protein synthesis machinery. Eukaryotes harbor massive genomes in their compact nucleosomes that also allow a finer gene expression control, interconnected to the physiology of the cell and environment. Archaeal contributions were the histone fold proteins, combined with a possibility of multiple replication start sites, which allowed eukaryotes to store, replicate and express uniquely large genomes (Brunk and Martin, 2019).

The Nature of FECA and LECA

It is now widely accepted that the LECA possessed most of the defining intracellular structures of eukaryotes such as the nucleus, mitochondria and the endomembrane system. However, today some models for the origin of mitochondria entail the assumption that the host that acquired the mitochondrial symbiont was already a eukaryote, others operate on the premise that the host was a prokaryote and that the origin of eukaryote cell complexity came later (reviewed in Martin et al., 2015), respectively sketching a cell biologically complex and simple first eukaryotic common ancestor (FECA). Homologs of some proteins responsible for eukaryotic complexity, encoded on the genomes of the asgrad (Zaremba-Niedzwiedzka et al., 2017) appear to support the complex archaeal host and a complex FECA. However, they remain at odds with microscopic evidence of a bona fide prokaryotic asgard archaea engaged in a metabolic syntropy (Imachi et al., 2020). Present data tend to favor the view that the host was a prokaryote,

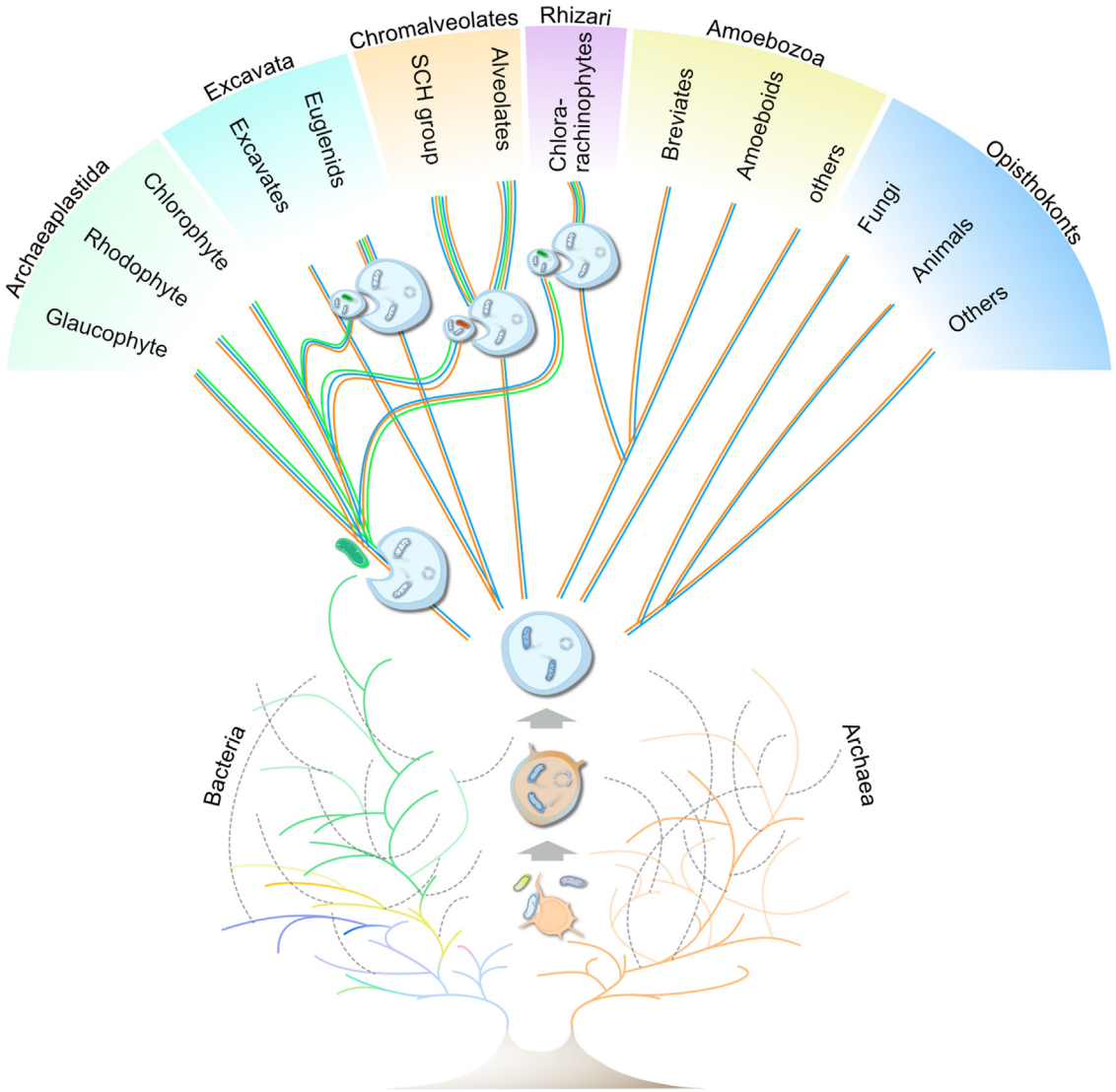

Fig. 1 A schematic representation of the origin of the three domains of life and their relationships: The bacteria are shown in blue while the archaea are shown in orange. Each colored line serves to show the number of genomes involved in shaping that lineage while also indicating phylogenetic relationships between the various groups. Life arose from alkaline hydrothermal vents as the two bacterial kingdoms the bacteria and the archaea. Prokaryotic evolution is rampant with Lateral Gene Transfers (LGT)—shown in dotted gray lines between the various groups—that shape the various prokaryotic lineages. A symbiotic association of an archaea and bacteria gave rise to eukaryotes where genome evolution is dominated by endosymbiotic events rather than LGT. Evolution of eukaryotes from prokaryotes was characterized by increase in genome size, cell size and complexity as well as a shift in membrane lipid composition (depicted as a change in color from archaea to eukaryotes). The taxonomic groups shown correspond to those recognized by Adl et al. (2005). The term SCH-group is introduced here to designate Stramenopiles, Cryptomoads, and Haptophytes, whose plastids appear to share a single common origin (Zimorski et al., 2014; Gould et al., 2015).

specifically an asgard archaeon in most current views (Lane and Martin, 2010; Williams et al., 2013; Bolte et al., 2015; Raymann et al., 2015; Spang et al., 2015) and the intracellular complexity evolved between FECA and LECA.

The Origins of the Endomembrane System

Under the view of a simple FECA, the endomembrane system, a unique and unifying eukaryotic feature, evolved between FECA and LECA along with most of the defining eukaryotic traits. The defining eukaryotic feature, the nucleus, can indeed be considered as part of the endomembrane system as it is an extension of the endoplasmic reticulum (Anderson and Hetzer, 2008), and a theory has it that the membrane bound vesicles secreted by the mitochondrial endosymbiont likely forged endoplasmic reticulum as well as nucleus and other eukaryotic compartments (Gould et al., 2016; McBride 2018; Raval et al., 2022). Hence, the origin of the

eukaryotic endomembrane system at large is best explained endogenously, postdating the mitochondrial endosymbiosis inside a morphologically simple FECA.

This is in contrast to the view that the eukaryotic endomembrane system originated in archaea, predating mitochondria, a view based in homology of a dozen archaeal proteins with the proteins of eukaryotic endomembrane system (Zaremba-Niedzwiedzka et al., 2017) and a notion of an already complex FECA. Such an exogenous origin, in the absence of a microscopic evidence of an endomembrane system in archaea, remains less likely as compared to the endogenous origin of the endomembrane system as the mitochondrial endosymbiosis being a key explanatory factor which is also ubiquitous among eukaryotes.

The Ubiquity of Mitochondria and Origins of Cellular Complexity

All eukaryotes, including the ones that once appeared to lack mitochondria, actually have or had mitochondria after all, albeit sometimes in highly reduced forms (Tovar et al., 1999, 2003; Williams et al., 2002). This places the origin of mitochondria at the very base of eukaryote evolution (Embley and Martin, 2006), a unifying feature of eukaryotes, not unlike nucleus. As a consequence, the origin of eukaryotic-specific traits might have come in the wake of mitochondrial symbiosis in contrast to the view, summarized succinctly by Doolittle (1998), that the mitochondrial host became eukaryotic more or less by point mutation, and eukaryotes that were then known to lack mitochondria were most simply seen as descendants of that host.

In line with the view that the endosymbiosis preceded key eukaryotic traits, the nucleus could have arisen in the wake of mitochondrial endosymbiosis, the proliferation of (rapidly self spliced) group II introns and their transformation into (slowly spliced) spliceosomal introns may have caused the need for a nuclear membrane to separate splicing from translation (Martin and Koonin, 2006).

Also, in agreement with the view that eukaryote complexity emerged in the wake of the mitochondrial endosymbiosis is the comparatively recent recognition that the many evolutionary inventions that separate eukaryotes from prokaryotes did not come for free, they came at an energetic price. The configuration of bioenergetic membranes that mitochondria conferred upon the ancestor of the eukaryotic lineage allowed it to do the evolutionary inventing required to forge the eukaryotic lineage (Lane and Martin, 2010). Such bioenergetic considerations would readily explain why mitochondria are ubiquitous among eukaryotes (they were required for eukaryote origin) and why no prokaryote on its own ever made the leap to eukaryote-like complexity: without a mitochondrial endosymbiont, it lacked the energy per gene to do so (Lane and Martin, 2010; Lane, 2014). Eukaryotes without mitochondria often live as symbionts or parasites within other eukaryotic cells (Karnkowsa et al., 2016) and depend on their host's mitochondria to maintain the cost of complexity, the invention cost having already been paid during eukaryogenesis (Raval et al., 2022).

The last ingredients required for the emergence of complexity, as with many biological phenomena, are natural selection pressures. A presence of an endosymbiont in a prokaryotic (archaeal) cell presented a series of cell biological challenges, solutions to which likely culminated into a complex cell biology that we label as "eukaryotic" (Raval et al., 2022). A particularly difficult challenge was the transition from prokaryotic cell division to cell division with a presence of an endosymbiont, especially once replication was decoupled from the membrane upon the emergence of nucleus. This likely led to a decoupling of chromosome division from cell division, leading to a syncytial (multinucleated) LECA, where the initial complete cell cycle arrest was rescued by the emergence of meiotic cell cycle (Garg and Martin, 2016). The selection pressures that brought forth most key aspects of eukaryotic complex cell biology can be traced to the mitochondrial endosymbiont, and can be furthermore understood as responses to selective pressures that emerge in response to the presence of a mitochondrial endosymbiont in an archaeal host (Raval et al., 2022). Together, lipids, energy and selection pressure from the mitochondrial ancestor forged a complex eukaryotic cell. Complex (eukaryotic) cells that never hosted a mitochondrial endosymbiont have not been observed so far. Under endosymbiotic models, which stipulate a simple prokaryotic host, the evolutionary origin of eukaryotic cells was accompanied by invention of thousands of proteins families, a process energetically facilitated by the mitochondrial endosymbiont, but also induced by endosymbiosis, because many of the major cytological differences that distinguish prokaryotes from eukaryotes can be understood as evolutionary responses to the conversion of the mitochondrial endosymbiont into a bioenergetic organelle (Raval et al., 2022). Thus, the ubiquity of mitochondria among eukaryotes, including among anaerobic eukaryotes (Müller et al., 2012), is perhaps best seen as evidence that endosymbiosis really was crucial to eukaryotic origin, not just in terms of making eukaryotes more efficient at what they do, but bringing them into existence in the first place.

Plastids

The Nature and Contributions of the Endosymbiont and the Host

All models for the origin of chloroplasts propose that the host was already a eukaryote. The nature of the symbiotic association between host and plastid symbiont varies across models. Today, plastids are involved in photosynthesis, carbon fixation, amino acid biosynthesis, lipid and cofactor biosynthesis as well as nitrogen metabolism. This gives rise to several hypotheses about the physiological context for the establishment of the plastids.

In Mereschkowsky's version of endosymbiotic theory, the production of carbohydrates for the host was the key contribution by the cyanobacterial endosymbiont right from the start (Mereschkowsky, 1905). Another reason for the establishment of the symbiosis could have been the low concentration of oxygen in the air at the time of endosymbiosis, such that the oxygen produced by the

symbiont subversed the host's mitochondrial respiration (Martin and Müller, 1998). However, if we look at modern symbioses involving cyanobacteria, they mostly involve cyanobacterial nitrogen fixation (Kneip et al., 2007; Ran et al., 2010). Today's plastids do not fix nitrogen. Possibly they have lost this attribute (and the associated genes) as a consequence of the evolution of the nitrogen cycle (there is more nitrate in the environment today because of environmental O_2). This aspect of endosymbiotic theory for plastid origin (nitrogen fixation) is not directly supported by data, but is congruent with recent analyses that today's filamentous, heterocyst-forming and nitrogen fixating cyanobacteria (sections IV and V) are most similar, from the genomic perspective, to the plastid ancestor (Deusch et al., 2008; Dagan et al., 2013).

In some literature, a proposal for the origin of plastids involving chlamydia continues to surface. It posits that a chlamydial endosymbiont was involved as a kind of metabolic helper in the origin of plastids (Ball et al., 2013), a kind of preplastidial infection that the cyanobacterial symbiosis somehow cured. That suggestion, though published in prominent journals, is problematic, because the observations upon which it is founded (phylogenetic trees) are subject to simpler alternative interpretations that do not require the participation of any symbionts other than a cyanobacterium at the origin of plastids (Deschamps, 2014; Zimorski et al., 2014; Domman et al., 2015). Moreover, the chlamydia story has the problem that if one infers the existence of a symbiont from a few gene trees (which is how the chlamydia symbiosis narrative operates), then for every unexpected branch that we observe in phylogenetic trees we would have to infer a new endosymbiont, and that kind of reasoning in endosymbiotic theory simply does not work (Ku et al., 2015). The origin of plastids is sketched in **Fig. 1**A member of the rhizaria, the ameba *Paulinella chromatophora*, harbors within its cytoplasm cyanobacteria of the genus *Synechococcus* (Marin et al., 2005; Yoon et al., 2006). These endosymbiotic cyanobacteria have been known for over 100 years and are called chromatophores (Mereschkowsky, 1905; Melkonian and Mollenhauer, 2005). Their genome is reduced compared to their closest free-living relatives, but is larger than typical plastid genomes (Nowack et al., 2008). Some authors refer to this classical cyanobacterial endosymbiont as a "photosynthetic organelle" (Marin et al., 2005; Nowack and Grossman, 2012).

The Chronology and Ecology of Mitochondrial and Plastid Origins

The timing in Earth history of mitochondrial and plastid origin cannot be pinpointed, but plausible ranges are often cited that stem from fossil evidence. The oldest eukaryotic microfossils are about 1.8 billion years old (Knoll, 2014). Because mitochondria arose only once in eukaryote evolution (Lane and Martin, 2010), this age can also be seen as a minimum age for mitochondria. The origin of plastids has been estimated at about 1.5 billion years ago (Parfrey et al., 2011), the minimum age for plastids is 1.2 billion years ago because a fossil red alga called *Bangiomorpha* (Butterfield, 2000) is found in rocks of that age.

The ecological context of mitochondrial origin remains discussed. However, there is a little doubt that the eukaryotic life originated prior to the current levels of oxygen were established (Betts et al., 2018; Porter, 2020; Mills et al., 2022) and niches allowing anaerobic syntrophies amongst prokaryotes therefore remains a parsimonious starting point for eukaryogenesis, as formulated by the initial eukaryogenesis theories (Martin and Müller, 1998; Moreira and Lopez-Garcia, 1998) and the versions more than two decades later (Sousa et al., 2016; Lopez-Garcia and Moreira, 2020; Spang et al., 2019). Archaeal and bacterial syntrophies involving H_2 exchange and methanogenesis are prominent in anoxic niches, where is makes the most physiological and thermodynamical sense (Schönheit et al., 2016; Stams and Plugge 2009). The archaeal clade phylogenetically closest to the archaeal host of eukaryogenesis also shows evidence of anaerobic syntrophy (Imachi et al., 2020), particularly, in the direction explicitly predicted by one of the models for mitochondrial (eukaryotic) origins—the hydrogen hypothesis (Martin and Müller, 1998; Sousa et al., 2016). Anaerobic syntrophy with H_2 producing mitochondrial ancestor also explains reduced, hydrogen producing, mitochondria that is hydrogenosomes as well as isolated presence (retention) of anaerobic metabolism among eukaryotes. Therefore, chronologically, physiologically, thermodynamically and ecologically, an autotrophic host in anaerobic syntrophy with the mitochondrial ancestor in an anoxic niche, explains the beginning of mitochondrial and eukaryotic origins most parsimoniously (Schönheit et al., 2016; Sousa et al., 2016; Martin et al., 2017; Mills et al., 2022).

Secondary Endosymbiosis

Mitochondria and chloroplasts can take on a diversity of form and function across different eukaryotic groups. But they have one attribute in common: chloroplasts and mitochondria are always surrounded by two membranes. This is a telling character that betrays their endosymbiotic origin. The two membranes surrounding chloroplasts and mitochondria correspond, in terms of homologies, to the plasma membrane and lipopolysaccharide layer of the Gram-negative bacteria from which the organelles arose (Lister et al., 2005; Schleiff and Becker, 2011). Yet there are photosynthetic eukaryotes whose plastids are surrounded by three or even four membranes (Gould et al., 2008; Stoebe and Maier, 2002; Van Dooren et al., 2001), such organelles are often called "complex plastids." The plastids of euglenids and dinoflagellates are surrounded by three membranes. The plastids of the chlorarachniophytes, the cryptophytes, the diatoms, the brown algae, and relatives are surrounded by four membranes.

How did these algae obtain their plastids? Under endosymbiotic theory, these additional membranes are explained as a result of secondary symbiosis. That is, the plastids of these algal groups stem from secondary endosymbiosis: symbioses between a eukaryotic host and a eukaryotic alga. In contrast to the single endosymbiotic origin of primary plastids from cyanobacteria, these secondary symbioses have occurred at least three times independently in evolution: once in the euglenid lineage, once in the chlorarachniophyte lineage, and (at least) once in the "chromalveolate" lineage (for a discussion of what chromalveolates are, see Zimorski et al.

(2014) and Gould et al. (2015)). The euglenid and chlorarachniophyte lineages acquired their plastids from green algae, the chromalveolate lineages acquired their plastids from a red alga (or red algae).

The uncertainty about the number of secondary endosymbiotic events in the red algae has to do with the conflicting data from molecular phylogenies (gene trees). The gene trees that would address the question of how many secondary endosymbiosis took place among the chromalveolates conflict with one another, giving rise to many suggestions for independent origins of the red secondary plastids. Considerations relating to protein import into red secondary plastids argue for a single secondary endosymbiotic event at the origin of this group (Zimorski et al., 2014; Gould et al., 2015). The additional two membranes surrounding red secondary plastids are most easily interpreted as the inner and outer leaves of the endoplasmic reticulum (ER) of the host that acquired the red algal endosymbiont (Zimorski et al., 2014; Gould et al., 2015). The workings of secondary endosymbiosis in algal evolution are shown in **Fig. 1**.

The number and nature of secondary hosts involving the origin of red secondary plastids remain unclear—some red secondary plastids have been suggested to be of tertiary or even quaternary endosymbiotic origin (Stiller et al., 2014). Lineages with secondary red plastids include the nucleomorph-bearing cryptophytes, the haptophytes, the diatoms (stramenopiles), some dinoflagellates, the chromerids, and the perkensids and some apicomplexans (McFadden, 2014), which secondary lost their photosynthetic ability—all lineages of the SCH-group and alveolates. Within these lineages the dinoflagellates are the only organisms with three-membrane bound plastids. Considerations relating to protein import suggest that it was the second outermost membrane (corresponding to the host's distal ER leaf) that was lost in the dinoflagellates (Zimorski et al., 2014; Gould et al., 2015), all other secondary plastids derived from red algae are surrounded by four membranes. In the context of membrane homologies in endosymbiotic theory, there have been several suggestions that the nucleus was once an endosymbiont (reviewed in Martin, 2005). However, such theories often state that the nucleus is surrounded by two membranes (or a double membrane), which is incorrect: the nucleus is surrounded by one folded membrane that is contiguous with the ER (Martin, 1999).

Endosymbiotic Gene Transfer

One of the important aspects of endosymbiosis is that it can, and does, lead to gene transfer from organelles to the nucleus (Martin et al., 1998; Martin and Herrmann, 1998; Timmis et al., 2004). Ninety years ago, even Wallin sensed that somehow the process of endosymbiosis should be connected to a transfer of genetic material from the organelle to the host. He wrote: "It appears logical, however, that under certain circumstances, [.] bacterial organisms may develop an absolute symbiosis with a higher organism and in some way or another impress a new character on the factors of heredity. The simplest and most readily conceivable mechanism by which the alteration takes place would be the addition of new genes to the chromosomes from the bacterial symbiont" (Wallin, 1925, p. 144). That is a fairly modern formulation of a process that is now called endosymbiotic gene transfer (Martin et al., 1993). About 15%–18% of the genes in a higher plant's nuclear genome come from the cyanobacterial antecedent of plastids (Martin et al., 2002; Deusch et al., 2008), and in eukaryotes that lack plastids, such as yeast, the vast majority of genes having prokaryotic homologs come from bacteria, not archaea (Esser et al., 2004; Cotton and McInerney, 2010; Thiergart et al., 2012). The simplest interpretation is that these bacterial genes in nonphotosynthetic eukaryotic lineages come from the mitochondrial ancestor (Pisani et al., 2007; McInerney et al., 2014). Three major consequences of endosymbiotic gene transfer are endosymbiont genome reduction, genome retention and protein import.

Genome Reduction

The process of endosymbiotic gene transfer entails the integration of bulk chunks of organellar chromosomes, or in some cases even a whole organelle genome spanning more than 100 kb (Huang et al., 2005). The evidence that this has happened can be seen at the computer by comparing organelle genomes to nuclear genomes (Hazkani-Covo and Covo, 2008) and in laboratory experiments where organelles are transformed with constructs that only become active in the nucleus (Huang et al., 2003, 2004). The mechanism of DNA insertion entails nonhomologous end joining and most eukaryotic genomes are replete with such recent organelle insertions (Hazkani-Covo and Covo, 2008). One might wonder how organelle DNA gets to the nucleus in the first place so that it can recombine. The most likely mechanism is simply stress induced organelle lysis, and there is some evidence for this in plants (Lane, 2011; Wang et al., 2012). Importantly, organelle lysis means that there has to be more than one organelle copy in the cell, one to lyse and one for progeny, and this is the crux of the "limited window" hypothesis (Barbrook et al., 2006).

There is another important aspect to gene transfer to the nucleus. Both at the origin of mitochondria and at the origin of plastids, host, and symbiont possessed a large number of genes for homologous functions. Such genes would include ribosome biogenesis, amino acid biosynthesis, nucleotide biosynthesis, core carbon and energy metabolism, cofactor biosynthesis, and the like. Chloroplasts and mitochondria have both retained their own ribosomes, for example, and divergent members of homologous gene families for ribosomal proteins as one example, but other examples have been well studied, including core carbohydrate metabolism. This phenomenon is called "functional redundancy through endosymbiosis" (Martin and Schnarrenberger, 1997). It generates highly divergent copies of genes homologous to prokaryotes even though they reside on eukaryotic chromosomes.

Genome Retention

Potential benefits of relinquishing genes to the nucleus are manifold, including recombination and an escape from Muller's ratchet. Nevertheless, organelles retain dozens, sometimes hundreds of genes, that encode for the defining functions of bioenergetic organelles: components of their bioenergetic electron transport chains (and components of the ribosome needed to synthesize them). Colocation of gene and gene product for redox regulation of gene expression (CoRR) theory suggest that genes are retained in the close proximity to the electron transport chains to execute regulation in response to redox signals specifically from a given organelle (Allen 2015; Allen and Martin, 2016). An alternative suggests protein sorting constraints on highly hydrophobic membrane proteins stipulates their retention (von Heijne, 1986), yet very few organelle encoded proteins are hydrophobic. The CoRR hypothesis readily accounts for the observation that chloroplasts and mitochondria have undergone massively convergent evolution to retain components of bioenergetic membranes and ribosomal proteins (Maier et al. 2013).

Protein Import

The origin of organellar protein import machineries played an important role in the evolution of mitochondria (Dolezal et al., 2006) and plastids (Schleiff and Becker, 2011), because it allowed the genetic integration of host and endosymbiont while allowing the endosymbiont to maintain its biochemical identity. In the early phases of organelle evolution, before the invention of the protein import apparatus that allowed plastids and mitochondria to import proteins from the cytosol, the transferred genes either became pseudogenes or became expressed as cytosolic proteins. In this way, endosymbionts can easily transfer whole pathways from the organelle to the cytosol. The transfer of whole pathways from the cytosol to an organelle is also possible, but the mechanisms are different (Martin, 2010).

With the advent of organelle protein import, however, transferred genes had the opportunity to obtain the necessary expression and targeting signals to be targeted back to the organelle from which the nuclear gene was acquired. This process has resulted in an expansion of the eukaryotic nuclear gene repertoire and in reductive genome evolution in the organelle. While it has long been known that the genes retained most tenaciously by plastids and mitochondria encode for proteins involved in the electron transport chain of the bioenergetic organelle or for the ribosome required for their synthesis (Allen, 2003, 2015), only recently was it recognized that even within the ribosome, the same core of proteins has been retained independently by plastids and mitochondria, probably owing to constraints imposed by the process of ribosome assembly (Maier et al., 2013).

The Endosymbiotic Theory: Limitations and Challenges

Endosymbiotic theories were built around photosynthesis, as a mechanism to account for the presence of plastids in plants (Mereschkowsky 1905). Endosymbiotic theories for the origin of mitochondria are faced with the twofold problem of explaining both the origin of the organelle (simple) and the difference between prokaryotic and eukaryotic cell organization. Endosymbiotic theories for mitochondrial origin do not focus on the mechanism by which the bacterium entered the host. Phagocytosis is often called upon to solve this "entry problem." As such, older theories of mitochondrial origin posit the existence of phygocytosing prokaryotes capable of engulfing the mitochondrial endosymbiont (Margulis et al., 2006). However, phagocytosis is engulfing of prokaryotes as food particles via a dynamic plasma membrane (freed from the electron transport) and subsequent intra-cellular digestion that releases the energy contained within the food particle. Prokaryotes synthesize ATP at their plasma membrane, a vital function that precludes use of the plasma membrane in ATP synthesis and concomitant phagocytosis-like processes (Martin et al., 2017). Eukaryotes have specialized bioenergetic membranes that harbor electron transport chains in the mitochondrial inner membrane. This allowed the plasma membrane of the archaeal host to relinquish its bioenergetic function and participate in membrane flux. This reasoning, in the absence of observed phagocytotic lifestyle in prokaryotes, makes phagocytosis before mitochondria implausible, and solutions to the entry problem remains debated, although there are a number of prokaryotic cells that harbor prokaryotic endosymbionts (Martin et al., 2017).

Any free-living eukaryote that has no sign of having ever hosted mitochondria would challenge the endosymbiotic theory of mitochondrial (and eukaryote) origin. Evidence in support of endosymbiotic theory have historically been indirect. However, experimental setups are emerging that can model mitochondrial and plastid origin using synthetic endosymbiosis (Mehta et al., 2018; Cournoyer et al., 2022; Gäbelein et al., 2022).

Evidence of eukaryotic like intra-cellular complexity in prokaryotes is lacking so far. As a consequence, the existence of a mitochondrial endosymbiont is strictly (always) correlated to the existence of eukaryotic cellular complexity. Under autogenous (non-endosymbiotic) theories for eukaryotic origin, the correlation between the presence of mitochondria and the presence of eukaryotic traits is the result of pure coincidence (Cavalier-Smith, 1975; Schavemaker and Munoz-Gomez, 2022). Under endosymbiotic theory, there is a causal and mechanistic connection between the origin of mitochondria and the origin of eukaryotic cell complexity (Martin and Müller, 1998; Lane and Martin, 2010; Gould et al. 2016) and the driving force behind that symbiosis was physiological, with morphogenesis emerging as a consequence.

Conclusion

Endosymbiotic theory explains why some organelles of eukaryotic cells are so similar to prokaryotic cells. It is a fairly powerful theory in that it can explain a number of disparate observations within a single unifying framework. Mutation theory, population genetics and selection can explain many aspects of evolutionary divergence among cells, but they cannot explain how mitochondria, chloroplasts, and complex plastids arose; for those major events in evolutionary cell biology, endosymbiotic theory is the only explanatory tool available. It works quite well, but it works best when used sparingly and in close conjunction with neighboring disciplines like microbial physiology and genetics.

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Multimedia

Origin of eukaryotes (2021): Developed by William F. Martin, Heinrich Heine University Düsseldorf (Germany): https://www.youtube.com/watch?v=oW3DT18jxqI