

Title: The Endosymbiotic Theory Revisited: the Origins and Evolution of Mitochondria in Living Eukaryotes

Introduction-

One of the features that distinguish a eukaryotic cell from a prokaryotic cell, besides the presence of a “true nucleus” encasing its DNA, is the presence of interior organelles such as the mitochondria. The theory is that there was a time when the ancestors of modern eukaryotes did not have mitochondria but later obtained them from an outside source, which had found its way into the host cell. This is known as the serial endosymbiotic theory (SET), which was proposed by Lynn Margulis in the 1970s and in later reports (Margulis, 1996). The endosymbiosis is usually described as the endocytosis or phagocytosis of a bacterium, some type of aerobic alpha- proteobacterium, into the host cell, which resulted in the eventual formation of mitochondria (e.g., Martin and Müller, 1998; Lang et al., 1999). The theory of endosymbiosis itself has been well supported by fairly recent genetic data since Margulis first seriously proposed it a few decades ago. For instance, there is an ample amount of organelle genome sequencing data to suggest that mitochondria (and plastids) are not only descended from a bacterial ancestor but likely arose from a single ancestral source (Gupta, 2000; Lang et al, 1999).

However, there are some competing hypotheses about how the process of endosymbiosis actually occurred and about what specific types of early cells were involved in the process. Three of these will be discussed in this paper: the Archezoa hypothesis, the hydrogen hypothesis, and the syntrophic hypothesis (the modern version of classic fusion theory).

Each hypothesis presents a different model for the order of the origin of the endosymbiosis event as well as the species of host and symbiont that were involved. The order of origin for the endosymbiosis in particular is very important to our understanding of the evolution of modern eukaryotes. If the endosymbiosis happened later, perhaps after the early eukaryote had already developed true eukaryote properties (i.e. a proto-nuclear envelope) and perhaps even a cytoskeleton for phagocytosis of the symbiont (Cavalier-Smith, 2002) then maybe the endosymbiosis wasn't really a major factor in eukaryogenesis. If the endosymbiosis happened very early, say when the host was still an Archeon (Martin and Müller, 1998; Lang et al, 1999) or some other prokaryote, then the endosymbiosis of the alpha proteobacterium could have had a much more pivotal role in eukaryogenesis. Likewise, in order for the model to be plausible, there needs to be phylogenetic data, as will be shown later, for the origin of the eukaryote features (nucleus, mitochondria) according to which species were involved. This would be the genetic relationship of the species from the context of the three main *taxa* of the phylogenetic tree: Bacteria, Archaea, and Eucaryota.

The Archezoa and Syntrophic hypotheses suggest that there was a point when true, primitive eukaryotes (those possessing a nuclear envelope) existed during the early evolution of cells but started off as premitochondriate. Consequentially, eukaryotes acquired mitochondria from the alpha- proteobacterium symbiont. The problem with these models is that the early ancestral premitochondriate eukaryote is used as a theoretical model and not supported by direct evidence. This paper will argue that the more evolutionarily plausible model is the hydrogen hypothesis, which suggests that the endosymbiont was acquired first and then only after the endosymbiosis did the cell

develop the mitochondria, nuclear envelope, and other genetic and organelle features of true eukaryotes (Martin and Müller, 1998). The first part of this paper will examine the evidence supporting each of the three hypotheses. The second part of the paper will put each of the hypotheses in the more general context of phylogeny and the evolutionary significance of the symbiosis. Finally, this paper will argue for the hydrogen hypothesis in terms of phylogeny and evolutionary significance, namely that it is best supported by the phylogenetic data and proposes the most plausible explanation for the symbiosis of host and proteobacterium.

The Archezoa Hypothesis

This model suggests that there was an amitochondriate (no mitochondria) eukaryote and that this eukaryote was a direct descendent of a prokaryote from the domain *archaea* as opposed to a descent of the domain *bacteria*. Proponents of this model note the vast differences between the domains, namely the RNA, protein, metabolic differences (Margulis, 1996; Doolittle, 1999), and that the genes of the modern eukaryote nucleus have more in common with archaea than with bacteria. The study of the small-sub-unit rRNAs (SSU rRNAs) of the archaeal lineage shows a striking similarity to eukaryotes when it comes to the replication, transcription, and translation systems (Doolittle, 1999). The eukaryote lineage is not strictly composed of archaeal genes, however, since the SSU rRNA study of species from the eukaryote lineage shows that the genes for enzymes involved in cytosolic metabolism (and also fatty acid metabolism in *Archaeoglobus fulgidis*) are of bacterial origin or homologs to bacterial genes (Doolittle, 1999). The cause of this bacterial presence is attributed to lateral gene transfer (LGT), a process by which genetic material is exchanged between organisms, even those of different species,

without the need for reproductive activity. In this case, the LGT is occurring between the archeal and bacterial domains. The most common occurrences are between housekeeping and catabolic genes (Doolittle, 1999). One of the proposed mechanisms of this lateral gene transfer is that the archaeon would eat (ingest) bacteria and then acquire small amounts of genetic material in a process referred to as “ratcheting” (Doolittle, 1998). Consequently, this small amount of genetic material would be laterally transferred and more ingestion will result in more small lateral transfers. Supposedly, those transfers had replaced the archaeal genes, which are already very similar to eukaryotes, with the bacterial genes that provide the functioning properties of proto-eukaryotes (Doolittle, 1998). Some of those would include the prerequisite genes for some functions of a mitochondrion.

All of this data implies that the genetic material for the nuclear envelope, cytoskeleton, and other important eukaryote features were already made present in this proto-eukaryote. So it came about without the need for bacterial genes from a bacterial endosymbiosis. As a result, many of the eukaryote features would be developed autogenously (no outside source necessary) and there would be a true eukaryote without mitochondria. Consequently, the mitochondria were introduced to the amitochondriate eukaryote through phagocytosis (the necessary cytoskeletal structures are present) by the alpha- proteobacterial endosymbiont only after the other structures were formed. These two events: the autogenous origin of the nuclear envelope and other structure and the inclusion of a bacterial symbiont required a ‘merging’ of genetic material. Cavalier-Smith suggests that this merge was successful because the eukaryotic properties that were now functioning in the amitochondriate eukaryote (i.e phagotrophy, endomembrane system

including peroxisomes, cytoskeleton, nucleus, mitosis and sex) were overlapping and synergistic with the genetic material from the endosymbiont (Cavalier-Smith, 2000).

The Syntrophic Hypothesis

This model is similar to the Archezoa hypothesis in that it supposes an amitochondriate stage in eukaryote evolution and an initially archaeal host. However, the syntrophic model describes a considerably different mechanism with regards to the formation of the primitive amitochondriate eukaryote. Whereas the Archezoa model describes the eukaryote structures developing autogenously and with genetic additions by LGT, the syntrophic model suggests that not one but two proteobacteria (eubacteria) endosymbionts were involved. The first symbiosis is considered to be between a methanogenic archaea and a delta- proteobacterium (an ancestral sulphate-reducing myxobacteria) (López-García, 1999). Unlike the endosymbiosis in the Archezoa model, the cytoskeleton does not exist yet; the cell is still an Archaeon. So instead, the two prokaryotes are described as coming together by syntrophy (López-García, 1999). Syntrophy is when a host cell is selected by large surface area and corresponding high surface contact. As a result, the cells either exchange substantial genetic material or they fuse together. This concept of endosymbiosis by syntrophy is taken from the classic fusion model that preceded this one because of its ability to describe the mosaic distribution of many genes (Gupta, 2000).

This cell fusion could have eventually resulted in the amitochondriate eukaryote if the fusion would exchange enough homologous material. To account for this, the phylogenic study done by López-García demonstrates that the genes for signaling pathways in

Myxobacteria are homologous with the genes in eukaryote signaling pathways. Likewise, methanogenic archaeal lipids, lipid-synthesis pathways, DNA-related enzymes, histones, and nucleosomes are described as both homologous to eukaryotes and very similar in actual function (López-García, 1999). Now that there is much homologous genetic material, the archaeal host can evolve into the amitochondrial eukaryote. Following thereafter is the phagocytosis of the second endosymbiont, an alpha- proteobacterial methanotroph, which results in the formation of the mitochondria.

Supposedly, the reason why the cell fusion would have occurred in the first place is that there is a mutually advantageous, symbiotic relationship between host and symbiont. This can even be said of both of the proteobacterial symbionts that find their way to the host methanogenic archaeon. The Myxobacteria produces hydrogen and carbon dioxide by fermentation and the methanogen benefits by consuming them. Likewise, the Myxobacteria get to speed up its metabolic rate since the host archaeal methanogen cell provides it with a hydrogen sink (López-García, 1999). This symbiosis is extended even further when the alpha- proteobacterial methanotroph is phagocytosed. The amitochondriate methanogenic eukaryote provides methane as food for the methanotroph symbiont and the methanotroph releases carbon dioxide like the Myxobacterium (López-García, 1999). As a result, the methanogenic eukaryote consumes the carbon dioxide, speeding up its methanogenesis and feeding the methanotroph symbiont in a great cycle of beneficence. Now that this second endosymbiont is part of the relationship, there can be yet another genetic exchange and the formation of mitochondria.

The Hydrogen Hypothesis

This model is fundamentally different from either the archezoa or the syntrophic models in that it does not suppose an intermediate amitochondriate eukaryote. However, it still involves an archaeal host and proteobacterial symbiont while it proposes an explanation for the symbiosis and cell fusion that is, in many ways, very similar to the one proposed in the syntrophy hypothesis that came shortly after this one was formulated. The syntrophy (cell fusion) was also used in the hydrogen hypothesis and is the same as the kind used in the López-García syntrophy. One can even consider the syntrophy hypothesis to be an extension of this older hydrogen hypothesis, though a questionable one as will be shown later in this paper.

However, the hydrogen hypothesis differs in key ways from the López-García syntrophy model: there is only one endosymbiosis of a proteobacterium and that relationship is between an alpha proteobacterium and an obligatory (strict) anaerobic autotrophic methanogenic archaeon that required hydrogen and carbon dioxide from the environment (Martin and Müller, 1998). The alpha- proteobacterium is fermentative (producing hydrogen and carbon dioxide) just like the delta- proteobacterium in the newer syntrophy model. Also, this endosymbiosis happened while the host cell was still an archaeon. Consequently, the symbiont gave rise to the nuclear envelope, mitochondria, and other features of hypothetical early eukaryotes all at once.

Whereas as the other models of symbiosis explain the evolution of archaeons into eukaryotes by means of lateral gene transfer from temporary contacts (Doolittle, 1999) or by gene 'ratcheting' from consumed food pieces (Doolittle, 1998), the hydrogen hypothesis suggests that the endosymbiosis itself was the cause. In that way, the formation of mitochondria and the origin of eukaryotes were synonymous. In order to

explain how the endosymbiosis caused all of this to happen, Martin and Müller propose an explanation based almost entirely from the evolutionary benefits acquired by the cells involved.

The autotrophic archaeon receives hydrogen and carbon dioxide from the alpha-proteobacterium and the proteobacterium received methane in turn. This is very similar to the syntrophic hypothesis. However, the relationship is taken one step further: the archaeon starts to become reliant on the proteobacterium symbiont for its supply of hydrogen and carbon dioxide, thus having to rely less on the environmental supply (Martin and Müller, 1998). Eventually, the anaerobic archaeon would be so reliant that it would eventually undergo the syntrophy with the proteobacterium and start using the importing membrane transport systems and carbohydrate metabolism of the proteobacterium. The final stage of the evolutionary model is an aerobic heterotrophic (doesn't produce own food) eukaryote that has become reliant on the primitive mitochondria provided by the endosymbiont. Supposedly, the reason why it would do away with its hydrogen consuming, methanogenic needs is that the mitochondria provide more efficient metabolism for the cell (Martin and Müller, 1998). As a result, the model explains the evolution of eukaryotes without such a reliance on gene ratcheting and LGT as explanations.

The origin of the nucleus and cytoskeleton-before or after the endosymbiosis

All three hypotheses are in agreement that the mitochondrion originated from a proteobacterial endosymbiont at some point in the history of eukaryotes (Margulis, 1996; Martin and Müller, 1998; López-García, 1999; Cavalier-Smith, 2002; Lang,

1999). However, as stated before, one cannot determine the role of the endosymbiosis in eukaryogenesis without considering the origins of the nucleus and other key structures such as the cytoskeleton. There are two suggested routes for the origin of the nucleus and other cytoskeleton: 1) They evolved autogenously (from inside the archezoan cell) and then the mitochondrion evolved after the endosymbiont was phagocytosed by the host 2) The genes for the nucleus and cytoskeleton were introduced by an endosymbiont.

Each of the three hypotheses above suggests different origins for the nucleus and cytoskeleton. The archezoan model suggests an autogenously derived nucleus and cytoskeleton. First, the necessary genes for the development of the cytoskeleton would arrive by gene ratcheting (Doolittle, 1998) or lateral gene transfer (Doolittle, 1999). Second, the cytoskeleton would form in the ancestral prokaryote so that it could arrange a homologous early nuclear envelope and the pore complexes of the nucleus. However, this would require a prokaryote, in this case an Archaeon, to possess a cytoskeleton. The problem is that there is no known prokaryote that possesses a cytoskeleton (Martin, 1999; Doolittle, 1999).

The model for an autogenous origin of eukaryote structures prior to the endosymbiosis

The Archezoa model attempts to resolve this problem by appealing to LGT and gene ratcheting. Both mechanisms would occur very frequently. The ancestral archeon would come into contact with a great many other prokaryotes and have many opportunities to acquire a few genes here and there from LGT. Likewise, the cell would frequently have to feed on other prokaryotes (bacteria) and, thus, have even more

opportunities to acquire the genes of other species through gene ratcheting. Every once in a while a gene of bacterial origin would end up expressing the functions of a nuclear gene or those required for a given cytoskeleton function. If this doesn't happen, evolution would still have many more opportunities to do this again (Doolittle, 1998). This model looks plausible in terms of gene selection. After all, a cell that can eventually phagocytose useful bacteria would sometimes have advantages over the others and there are, as stated before, metabolic genes of bacterial origin that can be found in archaeons.

The bacterial genes found in archeal cells have cytosolic functions and most bacterial genes are found in mitochondria. However, the problem is that the genes for the tubulin-and-actin based cytoskeletons found in eukaryotes are simply not found in bacterial cells. There are homologues but those are very distantly related (Doolittle, 1999). Therefore, bacterial genes acquired through LGT or ratcheting would not likely lead to the autogenous formation of the cytoskeleton and nucleus.

The model for an endosymbiotic origins of the nucleus and cytoskeleton

The hydrogen hypothesis and the syntrophy hypothesis each present some phylogenetic data to explain a possible endosymbiotic origin of the nucleus, cytoskeleton, and other important eukaryote components (Martin and Müller, 1998; López-García, 1999). They suggest that the early eukaryote could have acquired the genes for the eukaryote cytoskeleton and nucleus functions by means of a close symbiosis between the host and endosymbiont and from the endosymbiont itself. The syntrophy model proposes a close relationship between the methanogenic archaeon and sulfur-reducing delta-

proteobacterium that caused the two prokaryotes to co-evolve and become dependent on each other (López-García, 1999). In the process, there was a transfer and replacement of the genes that were non-informational (non-DNA processing). The result of this, supposedly, would be genes of a chimeric origin (López-García, 1999; Doolittle, 1999) that could produce the nucleus and the cytoskeleton that would be needed to phagocytose the second endosymbiont.

The hydrogen hypothesis describes a similar co-evolution that involves the selective transfer of metabolic genes rather than non-informational genes (Martin and Müller, 1998). This occurred by means of the metabolic dependence between the archaeon host and the mitochondrion that has arisen from the endosymbiont. Phylogenetic data concerning species of ciliate protists indicate a resemblance between mitochondria and hydrogenosomes (a hydrogen producing organelle) and that hydrogenosomes might have evolved from mitochondria (Lang, 1999). The hydrogenosome possesses some genes found in the nucleus that are thought to be of eubacterial origin (Martin and Müller, 1998). As a result, these evolved mitochondria could bring about other cytological structures. Neither the syntrophy nor the hydrogen hypotheses propose an actual mechanism for the generation of the cytoskeleton and nucleus but neither does the archezoa hypothesis.

The selective pressures for the endosymbiosis with regards to archeal/ proto-eukaryote metabolism

The Archezoa model proposes an explanation for the selective advantages of the endosymbiosis. The alpha-proteobacterium receives physical protection from the archaeon host at the loss of its own reproductive autonomy through reduction evolution (Doolittle, 1998). The reduction evolution results in consequent augmentation of ATP production in the endosymbiont. In turn, the archaeon host receives the benefit of nutrients (and subsequent ATP) that it would not be able to use without the help of the proteobacterial endosymbiont. This amounts to a one-way symbiotic exchange of nutrients. It's not very clear that the benefit of protection received by the endosymbiont would be beneficial enough to merit losing both reproductive autonomy and some of its nutrition. The endosymbiont in this model is considered to be an ancestor of an alpha-proteobacterium of the order *Rickettsia*. A study of the SSU rRNA of *Rickettsia* suggests a very close phylogenetic relationship between this species and mitochondria (Emelyanov, 2001). However, *Rickettsia* is known as an obligate intracellular parasite that depends on the cytosol of living cells to grow and reproduce. The primary evolutionary benefit of having that physical protection from the host is so that it can reproduce. Therefore, the acquired protection may not be beneficial enough for the endosymbiont.

The hydrogen model and the syntrophy model present two-way metabolic exchanges that may have arisen from selective pressures for the endosymbiosis (Martin and Müller, 1998; López-García, 1999). The syntrophy model looks to microbial ecology where it would appear that the most widespread symbiosis (in this case, syntrophy) is between sulphate-reducing bacteria and methanogenic archaea (López-García, 1999). The hydrogen hypothesis is based on a similar abundance of bacterial symbiotic relationships

among contemporary species. In this case, anaerobic archaeons and hydrogen producing alpha-proteobacteria (Martin and Müller, 1998). Both models involve strong symbiotic relationships between bacteria that are living today. However, there is evidence from the fossil record to suggest that the environment of the early Earth would have had a great abundance in sulfate and hydrogen producing bacteria and much less free oxygen in the atmosphere (López-García, 1999). Therefore, the hydrogen and syntrophy hypotheses present mutually beneficial symbiotic relationships between host and endosymbiont that very likely could have occurred on the early Earth.

The missing proto-eukaryote

Although the syntrophy and hydrogen models each present plausible mechanisms for symbiosis, there is one significant way that they disagree. The syntrophy model suggests that there was one point when an amitochondriate eukaryote existed and it was an ancestor of modern eukaryotes. It developed a cytoskeleton and nucleus by means of the co-evolution of the myxobacteria and the methanogenic archaeon host but did not produce a mitochondrion until a second endosymbiont was phagocytosed. The problem is that phylogenetic studies of modern amitochondriate eukaryotes indicate that they at one time in their evolutionary history possessed a mitochondrion and subsequently lost the organelle (Martin, 1999). This does not rule out the possibility that amitochondriate eukaryotes may have existed but there is no known phylogenetic explanation to date. The hydrogen hypothesis description of a simultaneous evolution of eukaryote mitochondrion, cytoskeleton, and nucleus is best supported.

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