
Final Papers

On the origin of eukaryotic cells; the evolution of a theory

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The origin of eukaryotic cells has been a topic of discussion for many years. The split from prokaryotes to eukaryotes has been of major importance to understanding how complex organisms have been able to develop. To understand what caused this split, one has to look at different aspects of the development of the cell. The formations of certain organelles such as the nucleus and the mitochondria have been fundamental in the evolution of eukaryotes. Furthermore, the evolutionary relationship between Eukaryota and the other two domains can tell us more about the way eukaryotes have evolved from prokaryotes. Central to the improvement of these ideas, is the advancement of technology. Major breakthroughs in how we think have usually followed the advancement of the technology. Due to these advancements, new discoveries have recently been made and we might be facing another major development in how we think about eukaryotic origins.

One of the biggest questions in human history is the question of origin. Where did life come from? To answer this question, one must look at the most basic life forms we know of; cells. There are two main types of cells. Prokaryotes are often considered more basic life forms, while eukaryotes are usually, but not always, more complex. A few features lead to this distinction. Eukaryotes contain a nucleus, which separates the DNA from the rest of the cell, and multiple organelles with different functions. From providing energy to the cell (mitochondria, chloroplasts), to transporting proteins from one place to the other (Golgi apparatus) and many other functions (fig. 1). One of the reasons they have been able to develop this complexity is the phagocytosis of another unicell (Lane & Martin, 2010).

Prokaryotes, on the other hand, are almost always single celled, with the exception of some cyanobacteria (Flores & Herrero, 2010). They do not have a nucleus, but instead usually contain circular DNA also called a plasmid, which resides freely within the cytoplasm (fig. 1).

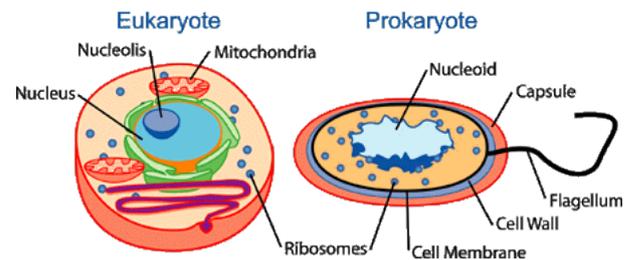


Figure 1 - Taken from *The worlds of David Darling* (<http://www.daviddarling.info/>)

A typical example of the eukaryotic and prokaryotic cells. You can see a few organelles in the eukaryote, such as the nucleus and mitochondria are absent in the prokaryote. Many other organelles are also usually present in eukaryotic cells, but are not depicted here.

Importance

With the better understanding of the origin of eukaryotes, we gain insights in how cells have developed and evolved over time. These insights can be of tremendous importance in other fields of science. For instance, to produce our own synthetic cells (Deshpande, Caspi, Meijering & Dekker, 2016).

Not only can insight into the origin of eukaryotes be of importance to other fields, it is also a merit in its own right. Long have we been searching for the truth behind our own, human origin. This origin can be

traced back to the origin of our basic cells, namely the eukaryotes.

Origin of organelles

One of the differences between prokaryotes and eukaryotes are the organelles. When talking about eukaryotic origin, it is important to look at where these come from. One of these organelles, the mitochondrion, has been thoroughly investigated. It is an organelle which provides the cell with energy. It is generally accepted that an event called endosymbiosis took place (Sagan, 1967). This means that the mitochondria originated from a free living bacteria that got phagocytized by another cell.

Another important organelle is the nucleus. It contains the genetic information of the cell, the DNA. The nucleus has allowed for more advanced gene control, due to the ability of actively transporting translational factors in and out of the nucleus. Prokaryotes do not have a nucleus, to shield their genetic code from being translated. This means that eukaryotes have another level of control over gene expression.

While the theory of endosymbiosis is widely accepted and supported by a great deal of evidence, the hypotheses for the formation of the nucleus are not that common ground. Many different hypotheses have been proposed and they have been heavily discussed (Pennisi, 2004).

Three domains

Beyond the ways to distinguish prokaryotes from eukaryotes, there is a widely accepted way to arrange different types of organisms into groups. A commonly accepted model, used to this day, was introduced by Woese et al. in 1990. They dismissed the, at that time, current model and suggested a new one. The model that had been used to that day was of the five kingdoms. The Animalia, Plantae, Fungi, Protista, and Monera. Woese et al. (1990) commented on the fact that the old division of organisms has been frequently challenged, but never truly altered. They describe in their paper that there is no molecular evidence to support that model and instead propose a model that has been used up to this day. They found that there were three main groups that differentiate from each other, in terms of their genetic makeup. He divided them in so called domains and named them the Bacteria, Archaea and Eukarya. Archaea and Bacteria are both prokaryotes, while the Eukarya consists of eukaryotes (fig. 2).

They also suggested that there has been a single common ancestor, from which these three domains have ultimately originated. First, at a certain point in

history, there was a split between the Bacteria and a common ancestor of the Archaea and Eukaryota. At a later point, these two groups also split off from each other. This is now known as the Tree of Life and is used worldwide as the phylogenetic model for the origin of life (Woese et al., 1990).

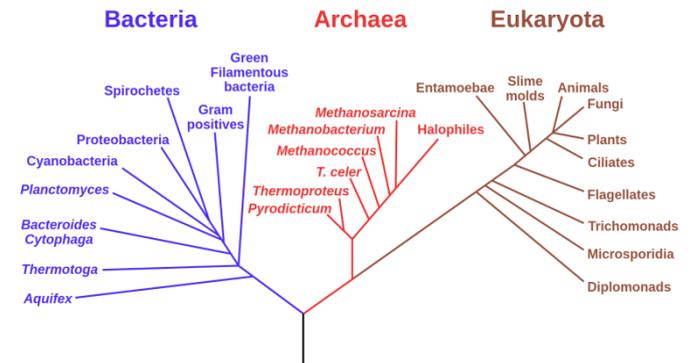


Figure 2 - An adaptation of the tree of life, first proposed by Woese et al. (1990)

The "Tree of Life". Rooted at the bottom is the Last Universal Common Ancestor (LUCA). Each node shows a group of organisms and the lines represent their evolutionary relationships between them based on RNA analysis. The three domains of living organisms are shown. Bacteria split off from a common ancestor of Archaea and Eukaryota. This common ancestor then split into these two domains.

Eocyte hypothesis

In 1984, a group of bacteria was found to closely relate to eukaryotes. This group was coined the eocytes (Lake, Henderson, Oakes & Clark, 1984). In this study, they analyzed the ribosomal subunits of these eocytes compared to that the Eukaryota, archaeobacteria and eubacteria (now known as the Bacteria and Archaea, respectively). They came to the conclusion that although the group consists of sulphur-dependent bacteria, they are more closely related to the Eukaryota than either the Bacteria or the Archaea in their ribosomal subunit structure. Lake et al. (1984) proposed the 'eocytic tree' (fig. 3) as opposed to an earlier version of the 'archaeobacterial tree' proposed by Woese et al.

In the end, the model of Woese et al. (1990) was preferred over the model proposed by Lake et al. (1984). However, in the 21st century, new phylogenetic data reheated the discussion, and birthed an alternative to the three domain model, called the eocyte hypothesis.

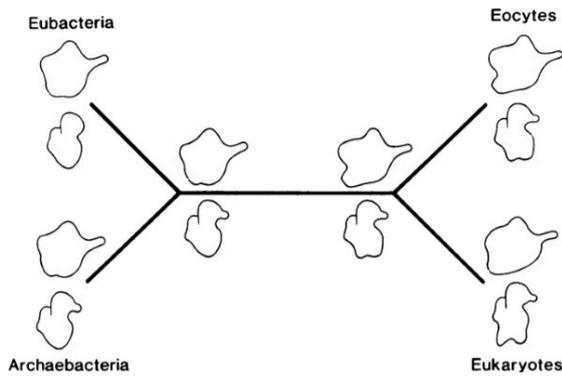


Figure 3 - Taken from Lake et al. (1984)

The unrooted Eocytic Tree as proposed by Lake et al. in 1984. It is based off the structures of ribosomal subunits. These structures show that eukaryotes are more closely related to the eocytes than the archaeobacteria and eubacteria (Archaea and Bacteria, respectively).

Advancement of technology

New discoveries and the advancement of technology have been crucial to finding the answer to many questions, including the question of eukaryotic origin. One can think of many hypotheses considering the question of origin, but without actual evidence to back it up, it will lead to nothing more but speculation. When you look back, there is a clear correlation between the way we think about this question, and the development of new technologies. Already the example given of Woese et al. (1990), who opposed an idea that was mainly based off of visual evidence (animals and plants look and move extremely different), and countered it with a new idea that was backed by modern technology; the sampling and understanding of the genetic code.

During this thesis, I will try to highlight the different ideas concerning the origin of eukaryotes, look at how technology helped achieve this goal and finally discuss and analyze these ideas. The way we think has been changed numerous times and we might reach another breakthrough in the coming decade.

Endosymbiosis

Endosymbiosis literally translates to “together living”. The name for this theory is a literally description of what it entails. It is believed that at one point in history, an anaerobic host fused with an aerobic prokaryote. Instead of digesting, the

prokaryote kept its function, providing the host cell with energy produced by aerobic oxidation. They coexisted together, and when the host cell divided, the endosymbiont divided too. At that point, they were endosymbiotic, or; together living.

What led to this theory? The advancement of technology was the leading reason for the development of this theory. The theory was properly formed by Lynn Margulis in the sixties. The use of electron microscopy allowed her to look at cell organelles in detail, and subsequently discover striking similarities between mitochondria and bacteria. Furthermore, there was DNA found inside the chloroplasts (Sagan, Benshaul, Epstein & Schiff, 1965). At the time, it was not possible yet to actually sequence genes, which would be discovered several years later (Jou, Ysebaert, Fiers & Haegeman, 1972).

With the next step in technology available; the sequencing of DNA, the theory of Margulis got further support from the research of Schwarz and Dayhoff (1978). They found that the DNA of the mitochondria was close to that of aerobic bacteria, supporting that they are descendant of free living organisms that got engulfed by another cell.

More evidence for this theory is the fact that mitochondria carry their own DNA, called mitochondrial DNA (mtDNA).

Further evidence comes from looking at the mtDNA of several organisms. The mtDNA that resembles a bacteria the most (Lang et al., 1997) (mtDNA of *Reclinomonas americana*) is often compared to the bacterial genome that resembles a mitochondria (*Rickettsia prowazekii*) (Andersson et al., 1998). Evidence from this study shows that the mitochondrial genome is probably monophyletic (Gray, Burger, & Lang, 1999), implying there has been a single event of endosymbiosis of mitochondria. A second event of endosymbiosis occurred, where a eukaryotic host cell, already containing mitochondria, engulfed a cyanobacterium, subsequently giving rise to photosynthetic autotrophs. This event is also thought to be singular and monophyletic (Matsuzaki et al. 2004).

These two events are called primary endosymbiosis and are thought to be rare occurrences. These events of primary endosymbiosis have had a great impact on evolution, causing the divergence of prokaryotes and eukaryotes, and also the divergence of plants and algae within the eukaryotes.

There have been several events of secondary endosymbiosis, after the initial primary endosymbiosis. Secondary endosymbiosis is when the

result of primary endosymbiosis (a eukaryotic cell combined with mitochondria/plastids) is engulfed by another cell. These events gave rise to a diverse group of algae and other eukaryotes.

Lynn Margulis

One of the pioneers in research on eukaryotic origin, is Lynn Margulis. She is the one that pushed the theory of endosymbiosis even though the idea of endosymbiosis has been around since around 1905, proposed by Konstantin Mereschkowski (translation of 1905 article; Martin & Kowallik, 1999). Mereschkowski was often ridiculed for his idea (Sapp, Carrapiço, & Zolotonosov, 2002). In hindsight, it is easy to say that it was wrong to ridicule him, since he was right in the end, but in reality it was hard to provide actual evidence for the theory. How would they know the inner environment of a bacterium or eukaryote without the use of an electron microscope? This novel instrument was developed in 1933 and available for general use in the fifties.

It was not until Lynn Margulis (then still married; Lynn Sagan) revisited the idea and kept pushing it (Sagan, 1967). She had access to electron microscopes, which allowed her to see the inner environment of a cell in detail and observed several things that made her think. She saw cell membranes and wondered how organelles in a cell could develop something like that. A logical explanation would be that if a unicell which already possesses a membrane, phagocytizes another unicell, which also has a cell membrane, effectively giving rise to a membrane within a membrane. Due to the power of magnification that is available with electron microscopes, she could observe these things and draw conclusions. Without this ability to observe membranes, or other organelles, this closely, it would've been hard to convince others of this idea.

Evidence

A decade later, more tangible support for Sagan's theory was found. Schwarz & Dayhoff (1987) analyzed the amino acid and nucleotide sequences of two proteins (ferredoxins, c-type cytochromes) and of 5S ribosomal RNA. These molecules were taken from blue-green algae, bacteria and eukaryotes. From this data, they made an evolutionary tree, which resembles the evolutionary relationships between the different types of organisms, based on the sequences of those molecules.

Schwarz & Dayhoff (1987) were aware of two different hypotheses concerning the origins of eukaryotic organelles. They state there are two different schools of thought at that time, the

organelles either arose by compartmentalization of DNA within the cytoplasm of an evolving protoeukaryote, or the hypothesis that they arose due to endosymbiosis.

For endosymbiosis to be true, they argued that the sequence of acquired proteins and RNA of chloroplasts and mitochondria, should be close to that of free living bacteria, since they were once free living bacteria themselves. They conclude that this is indeed the case. Mitochondria of eukaryotic organisms appear to be closely related to aerobic bacteria. This genetic evidence reinforces the theory of endosymbiosis.

Origin of nuclei

A major difference between prokaryotes and eukaryotes is the nucleus. In eukaryotes, the genetic material of the cell is contained within this membrane bound organelle, while prokaryotes have their genetic code freely in the cytoplasm. The nucleus is impenetrable for most large molecules, but contains nuclear pores that can transport certain molecules in and out. This allows eukaryotic cells a new level of control over gene expression. Transcription of the genetic code is done within the nucleus, but the translation is done outside of the nucleus. This means there needs to be transport from the nucleus, which can be controlled, allowing for another level of control over gene expression, which prokaryotes lack. It can be argued that this factor of more control over gene expression, also leads to more complexity in cells.

It is clear that the nucleus is an important factor in the development of eukaryotic cells, but how did it originate? There are multiple hypotheses concerning this question (Pennisi, 2004).

Syntrophy Hypothesis

Much like the theory of endosymbiosis, a hypothesis for the formation of a nucleus closely follows the same ideas. López-García & Moreira (2006) suggest that the nucleus originated from the phagocytosis of a methanogenic archaea by a fermenting myxobacterium. Also, the proto-mitochondrion is acquired at the same time. These 3 organisms symbiotically evolved. The archaea bacteria loses its membrane, and the nuclear envelope is formed, while the host cell (myxobacterium) transfers its genetic information to the archaea and subsequently loses its own 'self'.

Inside-out hypothesis

Another hypothesis also looks at endosymbiosis as origin for the nucleus, but from a different angle. Baum & Baum (2014) suggest that instead of having a

host cell which engulfs a prokaryote homologous to what is now the nucleus, such as described before, instead the prokaryote homologous to the nucleus was the host cell. They describe an event where such a prokaryote extruded membrane-bound 'blebs' around its own cell wall. These 'blebs' would then be used to facilitate material exchange between this prokaryote and another organism; the ancestral mitochondrion. This would be called ectosymbiosis, as opposed to endosymbiosis described up until now. In time, the blebs would form around the proto-mitochondrion. The gaps left behind would become what are now known as the endoplasmic reticulum, and due to slowly fusing of the blebs, a cell membrane would be formed.

Viral origin

Some researchers proposed that the nucleus was created by a viral infection of a bacterial cell (Villarreal & DeFilippis, 2000; Bell, 2001; Takemura, 2001). A virus either infected the host cell or got phagocytized. Instead of killing the host cell or becoming digested, the virus would have survived and slowly co-existed with the host cell, much like the endosymbiosis theory for mitochondria, chloroplasts, etc. In turn, the virus would become what we now know as the nucleus.

One of the arguments for this hypothesis is that several important eukaryotic proteins (DNA polymerases) are closely related to that of some viruses, but not at all to any bacteria (Villarreal & DeFilippis, 2000; Takemura, 2001). These proteins are of great importance during DNA replication. This close resemblance suggests that viral infection has played a major role in the development of a nucleus.

Bell (2001) compares some features of eukaryotes to that of some viruses, bacteria and archaea. He found that some of these features, like the capping of mRNA transcripts and the transport of mRNA, occur in both eukaryotes and some viruses, but not in the bacteria or archaea.

Molecular co-evolution

Cavalier-Smith (2010) suggests an entirely different view. Most of the discussed hypotheses have some form of external factor for the development of a nucleus. Based on extensive research, Cavalier-Smith (2010) proposes that the nucleus evolved from within the cell. He states that the formation of the nucleus has been a co-evolution, together with many eukaryotic traits. In his own words; "According to the coevolutionary theory of the eukaryote cell, cytoskeleton, endomembranes, peroxisomes, cilia and genetic systems are part of a unified whole".

Evolutionary Relationships

When talking about origin of the eukaryotic cell, it is not only important to look at the cellular changes in the cell, like the event of endosymbiosis and the acquiring of a nucleus. It is also important to look at the evolutionary relationship of the eukaryote with the other domains. The idea proposed by Woese et al. in 1990 still holds ground to this day. His phylogenetic tree contains at the base the last universal common ancestor (LUCA). This is the organism that allegedly, all life originates from. From there, the Bacteria split from the last common ancestor of Eukarya and Archaea, which then also split from each other.

There are, however, contradicting hypotheses which will be discussed in the following paragraphs.

Early eukaryotes

While endosymbiosis is a theory supported by much evidence and widely accepted, not all aspects of the origin of eukaryotes are widely accepted. As discussed earlier, the origin of the nucleus has many different hypotheses (Pennisi, 2014). Apart from that, there is also still discussion about the origin of the first eukaryotic host cell. For a time, it was thought that an amitochondriate primitive eukaryote (a cell containing a nucleus, but not mitochondria) was the original host, underwent endosymbiosis. The implications of this are that there has been a eukaryotic cell without mitochondria, but with a nucleus. The evidence for this theory was mainly that there have been found amitochondriate eukaryotes, which are early diverging from other eukaryotes (Gray, Burger & Lang, 1999). It was thought these eukaryotes, commonly grouped as the Archezoa (Cavalier-Smith, 1989), branched off from the other eukaryotes, forming a lineage that contains a nucleus, but did not undergo endosymbiosis, so never acquired mitochondria.

With more advancement in DNA technology, this theory was found to be unsupported. The amitochondriate group did in fact split off after endosymbiosis took place, but their original endosymbiont didn't evolve to mitochondria as we know it. Instead, these cells contain what are known as 'hydrogenosomes'. These hydrogenosomes are similar in mitochondria that they have a double membrane which produces ATP, but do not contain DNA. However, it was found that they do contain proteins with specific RNA signatures, corresponding to mitochondria and their related proteobacteria. This heavily implies hydrogenosomes share a common ancestor with mitochondria, meaning this amitochondriate group split off after endosymbiosis

took place (Bui, Bradley & Johnson, 1996). This suggests that mitochondria and the nucleus have originated around the same time, or in very close succession. This is supported by some hypotheses concerning the origin of the nucleus (López-García & Moreira, 2006; Baum & Baum, 2014; Cavalier-Smith, 2010).

It is therefore unlikely that cells originated a nucleus long before they acquired mitochondria. If there was an intermediate eukaryote with nucleus and mitochondria, all intermediate forms have gone extinct, although no evidence has (yet) been found for such an intermediate. An interesting view on this subject comes from Fuerst (2006). He proposes that the Last Universal Common Ancestor of all life, the LUCA, might have been a eukaryote-like cell. His idea stems from the fact that he found structures that closely resemble nuclear membranes in prokaryotes. This could imply that the LUCA already contained a nucleus, but it degraded in the prokaryotes, while it remained in the group that we now know as eukaryotes.

However, this hypothesis does not have a lot of support. Cavalier-Smith (2010) claimed it is “a complete red herring as they are totally irrelevant - crude analogies at best”. He argues that “there has been no serial sectioning analysis to show that they actually define a distinct cellular compartment separating nucleoid and cytoplasm”.

Eocyte hypothesis

Opposed to the three domain system generally used up until now, the eocyte hypotheses proposes another model. It suggests that instead of three domains, there are actually two domains (fig. 4). In the eocyte hypothesis, the eukaryotes are not a domain, but rather a branch of the Archaea (Gribaldo, Poole, Daubin, Forterre, & Brochier-Armanet, 2010; Guy & Ettema, 2011; Williams, Foster, Cox, & Embley, 2013; Spang et al., 2015).

This idea stems from the discovery of the so called eocytes (Lake et al. 1984). This group of organisms is prokaryotic, but very close related to the eukaryotes. Lake et al. (1984) wanted to introduce them as a new kingdom, but this never caught on in the mainstream ideology. In the 21st century, new phylogenetic data could be accumulated, due to the ability to sequence genomes of single cells (Guy & Ettema, 2011).

Guy & Ettema (2011) state that there is a so called superphylum within the tree of the Archaea. They call this group the TACK superphylum, named after the different phyla within the group. The Thaumarchaeota, Aigarchaeota, Crenarchaeota

(formerly known as the eocytes) and Korarchaeota. They propose, based on their phylogenetic analyses, that the ancestral eukaryote did not split off from the Archaea as suggested by Woese et al. (1990), but rather emerged from within the TACK group of the Archaea.

This argument is supported by a recent finding. The Lokiarchaeota is a proposed archaeal phylum, which was found in deep sea marine sediments. This phylum has a close phylogenetic relationship to the eukaryotes, and they are said to be monophyletic (Spang et al., 2015).

However, according to Gribaldo et al. (2010), it is hard to ascertain which of the two presented models hold truth. They analyzed seven different studies and have found that four of them support the 2D model and three support the 3D model. Intriguing is the fact that even though the results are very dissimilar, the datasets used largely overlap. Gribaldo et al. argue that the use of different phylogenetic methodologies is key here. They think that the difference in accuracy is what leads to the discrepancy in results.

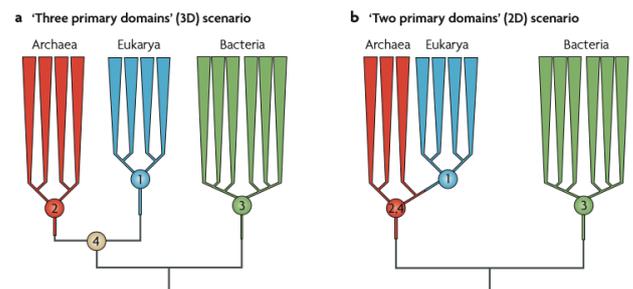


Figure 4 – Taken from Gribaldo et al. (2010)

The two different models. a) the three domain, or 3D, model as originally proposed by Woese et al. (1990). b) the two domain model proposes that instead of being split off from the Archaea at 4, eukaryotes are instead a group within the Archaea itself

Ring of life

Another view on the evolutionary place of eukaryotic origin is quite contrary to most models presented here. A so called ring of life hypothesis, opposes the standard tree of life model. Instead of eukaryotes being the direct descendant of either a common ancestor with Archaea, or the descendant of the Archaea, this theory proposes that eukaryotes are a chimeric organism, formed from two different prokaryotes and formed a new type of organism; the eukaryote (Rivera & Lake, 2004).

Akanni et al. (2015) argue that the tree of life model has long been debated and that horizontal gene

transfer (HGT) plays a major role. In a tree-like model, horizontal gene transfer is not really considered. A node is descendant from a node before it, implying there is no genetic transfer between organisms from other nodes on the tree.

Other recent articles seem to support this view, as a review of McNerney, Pisani and O'Connell (2015) concludes that "it is clear that eukaryotes cannot be correctly defined as 'derived' Archaeobacteria, or as 'derived' Eubacteria".

Discussion

As we have seen, there are many different hypotheses on several aspects of the origin of the eukaryotic cell. Some ideas are more commonly accepted than others, like the theory of endosymbiosis (Sagan, 1976) or the tree of life model (Woese et al., 1990). Others are less common ground, mainly due to conflicting views (Gribaldo et al., 2010; Pennisi, 2014)

The theory of endosymbiosis mainly came to be due to breakthroughs in technological advancement. The idea has been around for a long time (Martin & Kowallik, 1999), but there was no hard evidence to support it. The electron microscope, which had only been available commercially for a few years when Lynn Margulis used it to look at organelles, has caused a major shift in the way people thought. Evidence kept piling and it was looking more and more like a theory, instead of a mere hypothesis.

A more recent development is the regained attention for the eocyte theory. First discovered in 1994 (Lake et al., 1994), new data fanned the flame around the idea that eukaryotes are not a sister group of Archaea, but rather originated from within the Archaea. Again, technology has played a key role in this trend. The ability to isolate DNA from a single cell on site has been paramount for this development. Spang et al. (2015) found the proposed Lokiarchaeota phylum due to this technology.

However, as Gribaldo et al. (2010) stated, there is still an unresolved debate around this matter. It is not yet possible to realistically conclude whether the eukaryotes have originated from within the Archaea or as a sister group of them.

On the other side of the story, Cavalier-Smith (2010) is quite certain he holds the key to a part of the debate, namely the origin of the nucleus. He states that "Symbiogenetic theories, especially, have been a 40-year distraction from the core problems of how a bacterium was transformed into a eukaryote".

Truly, the work of Cavalier-Smith (2010) seems to be the most thought out and comprehensive idea on the origin of the nucleus. He argues that other theories seem incomprehensive or unexplained. For example, the theory provided by López-García & Moreira (2006). He states that the theory has too many assumptions, making it needlessly complex, and thus probably wrong. López-García & Moreira (2006) provided a view based on an evolutionary reasoning standpoint. However, they state that the archaeal bacterium, which would be engulfed and eventually become what we now know as a nucleus, would have to lose its membrane. On top of that, the host cell would have to lose its membrane and part of its genome. These assumptions indeed make their hypothesis needlessly complex.

Other hypotheses concerning the matter of nuclear origin, namely that of viral origin and the inside-out hypothesis, still remain questionable since there seems to be a lack of data on these subjects. Baum and Baum (2014) stated that they were only aware of one other author discussing the idea of the nuclear envelope resembling boundaries of an ancestral cell.

Conclusion

Technology has and will be an important factor in science. Major breakthroughs in ideas on the origin of eukaryotic cells have followed the advancement of technology closely. Many hypotheses surround the origin of the nucleus, all with their own supportive data. Arguments can be made for and against these theories. Possibly, with a new advancement in technology, more certainty can be gained regarding this subject. However, we might be facing another such breakthrough, with the eocyte hypothesis gaining more ground over the three domain system. The 2D model vs 3D model debate has been unresolved for now, but with new data coming in every day, like the discovery of the Lokiarchaeota, which are thought to be monophyletic with eukaryotes, there might be a shift in how we perceive the eukaryotic, and thus our own origin.

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