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Meiosis: Its Origin According to the Viral Eukaryogenesis Theory

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1. Introduction

The core meiotic machinery is largely universal in eukaryotes indicating that a substantial proportion of the meiotic machinery had already evolved in early eukaryotes [1]. Since there appear to be no close homologues to meiosis and the sexual cycle in either the bacterial or archaeal domains, it seems reasonable to propose that the origin of meiosis appears to be associated with the early origin of the eukaryotes themselves. Given the complexity and the level of integration of the intricate processes required to effectively maintain meiosis and a sexual cycle, it has been difficult to envisage how the full meiotic and sexual cycles could appear in the eukaryotic domain, without clear homologues in the prokaryotic domains of life. This is a particular challenge if all life descends directly from a Last Universal Common Ancestor (the LUCA hypothesis) and life evolves only according to the classical neo-Darwinian principles of incremental changes. It is not surprising then that the origin of sex, (and by extension the origin of meiosis), has been described as the queen of evolutionary problems [2].

If we investigate the characteristic features of the eukaryotic domain, we find that meiosis is only one of a wide array of features that have such a wide distribution within the eukaryotes that they appear to have been present and almost fully developed in the ancestor of all living eukaryotes [3]. These features include the nucleus, endomembrane systems, linear chromosomes with telomeres, mitochondria, peroxisomes, the cell division apparatus, mitosis, nuclear pores, mRNA capping, introns, the spliceosomal apparatus and the nuclear pore proteins [3]. Given this range of complex inter-related characters apparently present at the origin of the eukaryotes, the earliest common eukaryotic ancestor was clearly very different in design compared to any cells of the bacterial or archaeal domains. In addition, it has been clear for some time that the eukaryotic genome is an ancient chimera, a blend between bacterial metabolic genes and archaeal information processing genes [4]. Together, these observations

make the evolutionary relationship between the eukaryotes and the prokaryotes very challenging to elucidate since there is apparently an insurmountable chasm between eukaryotic and prokaryotic cells.

If we accept the paradigm of a 'Last Universal Common Ancestor' (e.g. LUCA), the apparently abrupt emergence of the eukaryotes in their modern form without transitional organisms is a major challenge to the classic Darwinian view of evolution. In particular, if we restrict our understanding of evolution to that of the classical neo-Darwinian school of evolution, every small evolutionary step leading to the origin of the first eukaryote should have conferred a selective advantage on the cell, thus the evolution of the complex traits observed in the transition from prokaryotes to eukaryotes (or visa versa) should have proceeded via small steps, each of which provided a selective advantage during the transition in cellular design. However, the transition in design between prokaryotic and eukaryotic design involves changes so profound, no convincing series of plausible small changes, each with its own selective advantage has been proposed that would begin to allow for this transition. In addition, no such transitional forms have been recognised either as fossils or survived as 'missing links'. Simply put, there are no credible evolutionarily intermediates between the prokaryotic and eukaryotic domains. That is, there are no organisms found with a eukaryote-like nucleus without linear chromosomes, or a mitochondrion without an endoplasmic reticulum, or a meiotic replication cycle without a cytoskeleton, that would indicate a stepwise evolutionary acquisition of critical eukaryotic features. Rather, all eukaryotes that we can observe today appear to descend from a common ancestor that already possessed a complex suite of multiple characteristics and none are descended from any earlier intermediates without many of the critical characteristic eukaryotic features [3].

One possible solution to this evolutionary impasse lies in the symbiogenic mode of evolution. A major role for symbiogenesis in evolution was postulated over 100 years ago when it was proposed that chloroplasts were descended from free living photosynthetic organisms [5]. However, for reasons that are difficult to decipher [6], the theory was scorned by the scientific community. By the early 1960's the theory drew attention in some textbooks only as a 'bad penny that has been in circulation far too long' [7]. It took a re-launch of the theory by scientists such as Lynn Margulis in the late 1960's and 1970's [8] to resurrect symbiogenesis as a mainstream scientific topic and combat the resistance to the concept. Despite the scientific community's dogmatic reluctance to embrace the symbiogenic model for evolution, it is now the accepted paradigm for the evolution of key eukaryotic features such as both the chloroplasts and mitochondria [9].

The symbiogenic mode of evolution can be interpreted as a specialised form of neo-Darwinian evolution, since it involves the gradual evolution of two organisms that obtain some mutual advantage from their relationship (mutualism) such that the evolutionary trajectories of each organism are related to each other. In the symbiogenic mode of evolution, each of the organisms are subject to classical neo-Darwinian selection where small infinitesimal changes in their individual genetic codes allow for closer and closer integration with the other member so that each organism evolves to adapt to the presence of the other. In some cases the intimate relationship between the two organisms effectively creates a third organism that is unlike

either ancestor alone. A classic example is that of lichens, which are composed of a photobiont (cyanobacteria or alga) and a fungal host that have co-evolved to such an extent they are classified as "lichen" species, despite the fact that each lichen consists of at least two separate organisms that can relatively easily enter into and leave the symbiotic relationship [10]. Lichens demonstrate how a symbiogenic mode of evolution can allow for complex changes in organismal design to appear whilst adhering strictly to the neo-Darwinian mode of gradualistic evolution for each of the organisms involved.

A case of symbiogenesis directly relevant to the origin of the eukaryotes is the case of the evolution of the chloroplast. As mentioned above, the idea that the eukaryotic chloroplast is symbiotic in origin is an old one [5] and despite being dogmatically resisted by the scientific community for over 50 years it is currently supported by so many lines of evidence that it is a widely considered incontrovertible [11]. Critically the presence of symbiogenic chloroplasts provides a clue as to the timing of the eukaryotic divergences and thus addresses one of the critical issues of the origin of the eukaryotes. In the case of chloroplasts, it is clear that they originally derive from a prokaryotic cyanobacteria of domain bacteria [12]. Since the chloroplast could not have originated prior to the origin of the cyanobacteria, it is clear that the eukaryotic plant lineage as a whole could not have evolved prior to the evolution and differentiation of the cyanobacteria from other bacterial groups. Therefore the first eukaryotic plant cell (presumably an alga) could not have evolved before photosynthesis had evolved in the bacterial domain, and the cyanobacteria had diverged from the other bacteria both photosynthetic and non photosynthetic. With regards to the timing of the origin of the ancestor of all modern eukaryotes, chloroplasts are not one of the universal eukaryotic features, as they are only found in the photosynthetic lineages, and thus the symbiogenic origin of the chloroplast does not inform us about the origin of the eukaryotes themselves since it seems likely that plant cell ancestors were eukaryotic before they entered into the symbiosis with the cyanobacteria. However the endosymbiotic origin of the chloroplasts is an important example since it clearly shows that symbiogenesis can and has made a major contribution to the evolution of at least some complex features of modern eukaryotes.

If we examine mitochondria rather than the chloroplasts, we find that they are also derived from a symbiogenic origin [9], but unlike the chloroplasts, mitochondria are one of the unique universal eukaryotic features that appeared prior to the origin of the last universal ancestor of the eukaryotes alive today [3]. Thus, like the transition from circular to linear chromosomes, or the acquisition of a nucleus, or the invention of mRNA capping, the origin of the mitochondria is associated with the origin of the last eukaryotic common ancestor. This is highly informative since if the mitochondrion is descended from a bacterial endosymbiont, and the last universal ancestor of the eukaryotes possessed a mitochondrion, then the last universal ancestor of the living eukaryotes could only have originated after the origin of the bacterial domain. According to this logic, the existence of mitochondria in the last eukaryotic common ancestor implies not only that the eukaryotes emerged after the bacterial domain had originated, but also that the eukaryotes evolved in a world that already possessed bacteria. Phylogenetic analysis of the mitochondria shows that they are not just descended from a generic ancestral bacterium, but rather they descend from an alpha-proteobacteria probably

related to the Ricksettsiales [13], and thus the last ancestor of all modern eukaryotes must have evolved after the bacterial domain had already differentiated into a wide range of bacterial phyla, including the extensive proteobacterial divisions.

If the mitochondria shares a common ancestor specifically with alpha-proteobacteria, then the ancestor of all modern eukaryotes must have emerged from an environment in which the bacteria had already evolved into their modern groups, and therefore presumably their modern forms. This is consistent with other lines of evidence such as the fossil record which suggests that the first traces of eukaryotic cells post dates that of recognisable cyanobacterial fossils by over a billion years [7].

If the bacteria were already essentially modern in design before the evolution of the last eukaryotic common ancestor, the question arises as to what other life forms were present. With regards to the archaea, given that fossil evidence suggests that methanogenesis arose greater than 2.8 billion years ago [14], and methanogenesis is an exclusively archaeal process, it would appear that the archaea were also present prior to the origin of the eukaryotes. This is confirmed by the observation that the ancestral eukaryotic genome is a chimera of bacterial and archaeal genes [4] which indicates that prokaryotes of the archaeal domain were also present at the origin of the eukaryotes. Significantly, recent research confidently indicates that the genome of the last common ancestor of all extant archaea apparently was at least as large and complex as that of typical modern organisms in this domain of cellular life. [15] The evidence thus suggests that both bacteria and archaea were highly evolved and already differentiated into major groups when the eukaryotes evolved and therefore the eukaryotes evolved in a 'prokaryotic world' which contained at least bacteria and archaea.

It also seems likely that viruses, phages, and plasmids were also present in this 'prokaryotic world' as there is evidence that the last common universal ancestor of cellular organisms was infected by a number of different viruses. [16] Further support for the proposal that complex DNA viruses predated the origin of the eukaryotes comes from analysis of some of the most complex known viruses, the NCLDV viruses. Phylogenetic studies on the NCLDV viruses have shown that this class of DNA viruses was present before or at the origin of the last universal eukaryotic ancestor. [17], [18], [19] In addition, phylogenetic analysis of key nuclear/NCLDV signature genes such as mRNA capping enzymes indicate that they branch from the phylogenetic branch leading to the earliest eukaryotes. [20] Further support for the existence of phage/viruses at or close to the origin of the eukaryotes comes from molecular analysis of mitochondria where the enzyme used in transcription of mitochondrial genomes are related to T7 phage RNA polymerases rather than typical bacterial RNA polymerases. [21] The phage version is found in the majority of the groups of eukaryotes, with the only currently known exception being the RNA polymerase in the protist *Reclinomonas*. [21] Since the phage version is almost ubiquitous in eukaryotic mitochondria, it suggests that the T7 bacteriophage were present close to the ancestor of all living eukaryotes.

In conclusion, several lines of evidence can be used to argue with some degree of confidence that the ancestor of all modern eukaryotes descends from an ancestor that appeared relatively 'late' in the evolution of life on earth, and that it lived in a 'prokaryotic world' in which bacteria, archaea and complex DNA viruses had already evolved into recognisably modern forms.

Furthermore, the symbiogenic origin of the mitochondria and the chloroplasts in algal/plant lineages indicates that symbiogensis was a critical evolutionary process active in the origin and early evolution of the eukaryotes. It is in this prokaryotic world in which the Viral Eukaryogenesis theory for the origin of the eukaryotes, meiosis, and the sexual cycle is set.

2. The Viral Eukaryogenesis theory : A prokaryotic community evolves into the eukaryotic cell

At its simplest level, the Viral Eukaryogenesis theory proposes that the eukaryotic cell is descended from a prokaryotic world community consisting of three phylogenetically unrelated organisms. These three organisms were, an archaeal ancestor of the eukaryotic cytoplasm, an alpha-proteobacterial ancestor of the mitochondria and a viral ancestor of the nucleus. [20] Although it is now widely accepted that the eukaryotic mitochondria is descended from an endosymbiotic bacterium, it is the idea that the eukaryotic nucleus could be a viral endosymbiont that is usually considered to be a radical or 'far-fetched' aspect of the Viral Eukaryogenesis theory. It would appear to be particularly radical due to what I believe to be prejudices and outdated paradigms about viruses. For example, viruses were long defined by their small size and inability to replicate outside of a cell, and thus the viruses failed to even meet the definition of life, and were more like inanimate poisons that could be crystallised and stored as an inert substance. Even the name virus is derived from the Latin word for poison which implies both that viruses are entirely destructive of life, and that they are more like a liquid chemical poison than a living organism. In the original version of the Viral Eukaryogenesis theory, [20] it was proposed that eukaryotic nucleus was descended from a pox-like virus (which at the time was one of the most complex viruses known), a scourge of humanity, and not exactly a wholesome image for a relative, no matter how distant.

Over the last decade, however, the established viral paradigm has gradually been modified by the discovery of complex NCLDV viruses such as the Mimivirus, Mamavirus, Megavirus and CroV viruses. These viruses are so large and complex that they exceed the complexity of some living prokaryotes, and some authors have even proposed that the Mimiviruses and its kin are a fourth domain or supergroup of life, rivalling the bacteria, archaea and eukaryotes. [17, 18] The Mimivirus was the first of these giant viruses to be discovered, and its discovery has led to the realisation that viruses can be far more complex than previously thought. [22] Indeed, the Mimivirus was unlikely to have been found using standard methods, because the old viral paradigm included a criteria that they could pass through a Chamberland filter that would filter out bacteria sized particles like the Mimivirus. Sequencing of the Mimvirus and other members of the giant viruses has revealed that not only are the viruses large, they are genetically complex, possessing many features that were previously considered to be restricted to cellular organisms. [22] Amongst the > 1000 genes present in the giant viruses are many genes involved in translation, transcription, and genetic information processing. [22]

One of the objections to the idea that a virus could be the ancestor of the eukaryotic nucleus is that the viruses are non-living and therefore not really a valid candidate to be an endosymbiotic

ancestor of the eukaryotic nucleus. However, if one makes a comparison between the modern Mimivirus and the modern bacterium *Rickettsia belli* it can be seen that in terms of size, complexity and habitat there are many similarities between these two organisms despite one being a virus, and the other being a bacterium related to the eukaryotic mitochondrion. In terms of size, these two organisms are fairly similar, and in fact the Mimivirus was named for the fact that it was a 'Mimicking microbe'. In terms of complexity, the organisms are also similar, with both possessing genomes in the range of 1200-1500 protein coding genes, [22, 23] both organisms are membrane bound, and both possess genes involved in translation, transcription and genetic information processing. Most strikingly however, is that both organisms are obligate internal parasites, and both are known to be capable of replicating in the same host. That is, the Mimivirus was originally discovered infecting *Acanthamoeba polyphaga*, [22] a host in which *Rickettsia bellii* can also replicate. [23] Significantly in terms of the Viral Eukaryogenesis theory, phylogenetic analysis of the genes of both these organisms show that they descend from ancestors that existed before the eukaryotes arose. [9, 17, 18, 19, 20] If it can be accepted that an alpha-proteobacterial ancestor of Rickettsia could have established a permanent endosymbiosis within the lineage leading to the origin of the eukaryotes and thus evolved into the mitochondria, there seems no scientific reason why an ancestor of an equally complex obligate internal parasite like the Mimivirus could not have similarly established a permanent endosymbiosis within the lineage leading to the origin of the eukaryotes and thus evolved into the nucleus. Of course, in the Viral Eukaryogenesis theory, it is proposed that one critical difference between the endosymbionts was that during the evolution of the eukaryotes, the alpha-proteobacterial genome was dramatically reduced in complexity as it evolved into the mitochondria whilst the viral genome increased in complexity as it evolved into the nucleus. Presumably this difference in evolutionary trajectory occurred due to the different functions of the organisms within the community as they evolved specialised functions within the eukaryotic cell; the mitochondria as a centre for active metabolism, and the nucleus as a centre for transcription and replication of genetic material.

One consequence of the proposed derivation of the eukaryotes from three members of the 'proto-eukaryotic' community, is that the modern eukaryotic genome should be a palimpsest of genes derived from the three phylogenetic sources. In this way the VE theory provides a basis for understanding the observation that the eukaryotic nucleus is a chimera of genes derived from bacterial and archaeal sources [4] and explains why the information processing genes of eukaryotes are generally of an archaeal nature, whereas genes of more general metabolic nature are of a bacterial origin. As explained in the VE theory, the tripartite consortium initially contained three separate but interacting genomes. The archaeal host genome possessed genes characteristic of those in archaeal organisms, and thus the ribosomes, translation apparatus, DNA metabolism genes, cell replication genes and other general metabolic genes of the cytoplasm would have originally been derived from archaeal origins. In contrast, the viral genome possessed genes characteristic of those of the NCLDV viral lineage, and thus the genes for virus/nuclear replication, assembly, membrane folding, DNA replication, DNA maintenance, and mRNA capping would have originally been derived from viral origins. These viral genes would also be genes that evolved to interact with the archaeal information processing apparatus since the virus was dependent on host archaeal machinery

for translation and replication. Finally, the third genome, the bacterial genome, possessed genes characteristic of those in bacterial organisms, and thus the future mitochondrial ribosomes, translation apparatus and many metabolic genes would have originally been derived from bacterial origins. It is proposed that the different functions and thus evolutionary trajectories of the genomes of the three members of the consortium influenced their location in modern eukaryotes. In the case of the original alpha-protoebacterial endosymbiont, despite being separated from the future viral/nuclear genome by the symbiont membrane, the vast majority of genes of the original endosymbiont were either re-located to the viral/nuclear genome or lost, with only a few genes remaining in the modern mitochondrial genome and, in the even more reduced hydrogenosomes, the endosymbiont genome as a separate entity completely disappeared. In the case of the host archaeal genome, it is proposed that its 'information processing' genes, where needed, were re-located to the future viral/nuclear genome, and the archaeal genome as a separate entity, like that of the hydrogenosome genome, completely disappeared. As a result of these processes, the eukaryotes ended up with two independently replicating genomes, the much reduced mitochondrial genome, and the greatly expanded viral/nuclear genome representing a chimera of genes derived from the original virus, the archaeal host and the a range of genes from bacterial endosymbiots including the ancestor of the mitochondria.

As shown in Figure 1, it is proposed that the unique genetic architecture of the eukaryotes arose because the eukaryotic cell is the descendent of a symbiogenic prokaryotic world community rather than a single ancestral cell. It is proposed that the community became integrated to such an extent that, like the lichens, we see the components of the community as a single organism, which we currently recognise as the eukaryotic cell. Thus the unique design of the eukaryotic cell arose because of the integration of the three lineages into a single unit. [20] It is further proposed that the co-ordination of their replication cycles led to the evolution of mitosis, meiosis and the unique eukaryotic cell cycle. [24]

A key aspect of Viral Eukaryogenesis theory that differentiates it from many other models is that it proposes that the eukaryotes did not result from a single chance 'event' such as a chance fusion between cells, but rather that the three symbiogenic lineages that were involved in the emergence of the eukaryotic cell were evolutionarily linked together and the eukaryotic cell we see today resulted from a process that over time increasingly linked each of the organisms together. That is, the archaeal lineage is linked to the evolution of the bacterial lineage because of a mutualistic symbiotic relationship between the two organisms, and the virus and the archaea are evolutionarily linked together in a symbiotic host/parasite relationship. As a result the three lineages were found together in the same ecological community, and they shared a linked evolutionary process that lead to the evolution of the eukaryotic cell. In this community, each member of the consortium evolved according to classical neo-Darwinian models, however as they became more and more integrated, they eventually became unrecognisable as separate organisms, but rather were integrated into a single permanent consortium that we call the eukaryotic cell.
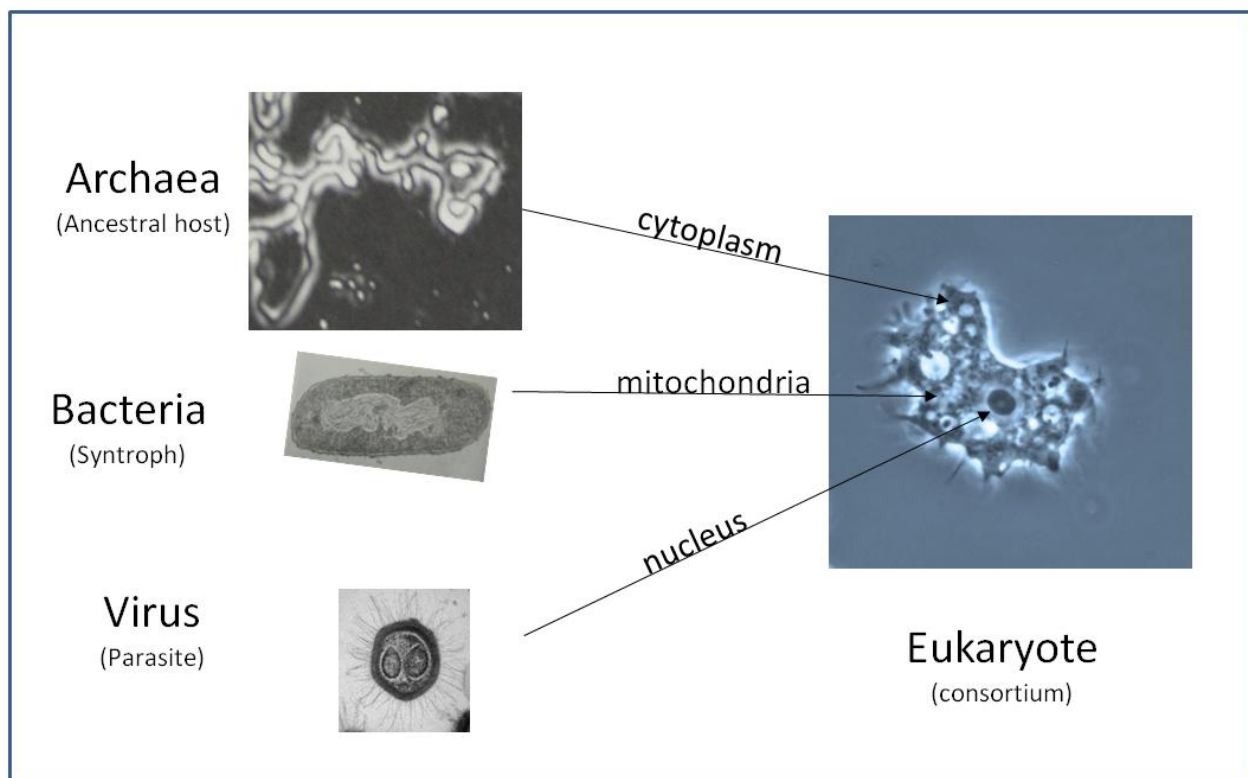
Figure 1. The eukaryotic cell evolves from a prokaryotic world community/consortium. In the Viral Eukaryogenesis theory, the eukaryotic cell is descended from a consortium of three originally independent prokaryotic world organisms. The eukaryotic cytoplasm is descended from an archaeal cell that did not possess a cell wall and was subject to infection by a variety of agents including a range of bacteria and viruses. The eukaryotic mitochondrion is descended from an alpha-proteobacteria that was originally in a syntrophic relationship with the archaeal host, and eventually invaded the host cytoplasm and established an permanent endosymbiotic presence in the host cell. The eukaryotic nucleus is descended from a NCLDV virus that was originally in a parasitic relationship with the archaeal host, and eventually established a permanent lysogenic presence within the host cytoplasm. The three organisms of the consortium eventually shared a common evolutionary trajectory and eventually evolved into the eukaryotic cell. In the process of permanently establishing the 'eukaryotic cell', the viral ancestor of the nucleus took over the roles of DNA replication and transcription by means of its capping/cap binding proteins that directed preferential translation of the viral transcripts into proteins. As a result, the virus became the genetic information processing centre of the cell and gradually acquired genes from the other organisms. The alpha proteobacterial ancestor of the mitochondria transferred the majority of its genes to the virus/nucleus in the process of evolving into the mitochondria, and in the case of hydrogenosomes, all of its genes. The host archaeal cytoplasm maintained its role in translation of mRNA transcripts and eventually transferred all of its 'eukaryotic' genes to the nucleus.

3. An archaeal ancestor of the eukaryotic cytoplasm

An archaea is proposed as the ancestor of the eukaryotic cytoplasm in the Viral Eukaryogenesis theory primarily because it is clear that the eukaryotic ribosomes are more closely related to those of the archaea than the bacteria. [4] More specifically, an archaea without a cell wall is proposed because it can be argued that the eukaryotic ancestor did not possess a cell wall since many modern eukaryotes such as animals do not possess cell walls, and those that do, possess a range of cell wall materials (eg mannoproteins in yeast, cellulose in plants, etc) that were most likely evolved independently of each other. [20] There have been at least two archaea

described that do not possess a cell wall, *Thermoplasma* and *Methanoplasma elizabethii*. It has been proposed several times that *Thermoplasma* may have been related to the ancestry of the eukaryotes. [25, 26] However, *Methanoplasma elizabethii* is proposed in the Viral Eukaryogenesis theory as an archetype for the ancestor of the eukaryotic cytoplasm. *M. elizabethii* is a modern member of a syntrophic consortium consisting of bacteria and archaea that metabolise fatty acids into methane. [27] Although, '*M. elizabethii*', is not expected to be particularly closely related to the ancestor of the eukaryotic cytoplasm, it was chosen because it is illustrative of the evolutionary forces that could link the evolution of an archaeal ancestor of the cytoplasm with a bacterial ancestor of the mitochondrion. In the modern consortium in which *M. elizabethii* is found, the bacteria break down fatty acids and produce hydrogen and carbon dioxide as waste products, which are used by the archaeon as a source of raw materials for methanogenesis. [27] This type of syntrophic relationship provides an evolutionary and ecological linkage between the two species. In the Viral Eukaryogenesis theory it is proposed that a syntrophic relationship between an archaeon and a bacterium ensured the long-term co-evolution of the two species since they existed in a mutually advantageous relationship. Although not fully elucidated, the recent discovery of anaerobic methane oxidation consortia has demonstrated that syntrophy between methane oxidising archaea (ANME) and bacteria occurs on a global scale and is responsible for major geochemical carbon cycles in the earth's biosphere. [28] Critically the ANME type syntrophy is independent of the presence of oxygen and these types of syntrophy could have been abundant prior to the time that the first eukaryotes appeared.

4. A bacterial ancestor of the mitochondrion

An alpha-proteobacteria is chosen in the Viral Eukaryogenesis theory to be the ancestor of the mitochondria since it is clear from phylogenetic and structural studies that the mitochondria are descended from an alpha-proteobacterium. [29] For example, the genes of the electron transport chain in the mitochondria are clearly homologous and specifically related to those of the alpha-proteobacteria. [13] Thus in the Viral Eukaryogenesis theory, the ancestor of the mitochondria was an alpha-proteobacteria that existed in a syntrophic relationship with the archaeal ancestor of the eukaryotic cytoplasm.

5. A viral ancestor of the eukaryotic nucleus

The viral ancestor of the nucleus is proposed to have been an ancient member of the NCLDV viruses that infected the archaeal ancestor of the eukaryotic cytoplasm. [30] In the initial presentation of the theory, [20] a pox-like virus was chosen as a symbiotic ancestor of the nucleus because they, like the eukaryotic nucleus, possess large double stranded DNA genomes. [20] In addition to the large ds DNA genome, the poxviruses, possess several other features that are common to the eukaryotic nucleus. [20] They possess tandem DNA repeats at their telomeres, they encode DNA polymerases that are homologous to eukaryotic poly-

merases, they replicate in their host cell cytoplasm, they are membrane bound, and critically, they encode their own apparatus required to cap and polyadenylate mRNA prior to extrusion into the host cytoplasm, [20] a process that is otherwise only known to be performed by the eukaryotic nucleus and a range of other viruses.

In the years since the original Viral Eukaryogenesis hypothesis was published, it was found that the pox-like viruses are actually members of a larger grouping of viruses, the NCLDV viruses. [31] Included amongst the NCLDV group of viruses are the relatively newly described giant viruses, the Mimivirus, Mamavirus, Megavirus and CroV viruses. These large viruses have genomes larger than the smallest bacterial genomes. [22] Furthermore, phylogenomic analysis of the NCLDV viruses appears to show that they are ancient, emerging from the archaeal branch shortly before the eukaryotes originated. [17, 18, 19] Phylogenetic analysis of several specific genes, including the mRNA capping enzymes and DNA polymerases, place the genes of the NCLDV viruses at the base of the eukaryotic radiation, suggesting that they differentiated shortly before the eukaryotes evolved, and that they contain genes that are phylogenetically related to those that are used by the eukaryotic nucleus, [20, 32] Since mRNA capping is not observed in either bacteria or archaea, but is observed in NCLDV viruses it is argued that mRNA capping is a viral invention which ensured preferential translation of viral mRNA, and this feature has been passed on to its nuclear descendent. Significantly, NCLDV viruses such as the Mimivirus also possess their own highly divergent cap binding protein EIF4E which appears to be endogenous to the virus, and not obtained from the *Acanthamoeba* host. [33, 34] It therefore appears that the Mimivirus, like the eukaryotic nucleus, has its own DNA directed RNA polymerase, its own mRNA capping enzyme, and its own cap binding protein (eIF4E) to ensure translation of the viral transcripts by the host ribosomes. If the Viral Eukaryogenesis theory is valid these three sets of genes were present in the ancient NCLDV ancestor and allowed the virus to take over the translational apparatus in the host archaeon by directing the host ribosomes to recognise the viral mRNA which was differentiated from the typical uncapped prokaryotic host mRNA. In the process of taking over the transcription/ translational regime, the virus re-organised the prokaryotic transcription/translation regime into the typical eukaryotic one where mRNA is capped prior to extrusion into the cytoplasm where the cap binding protein directs translation of the capped mRNA.

In addition to the discovery of the Mimivirus and related giant viruses, recent research into the *Acanthamoeba*, the host of the Mimivirus, illustrates the kind of 'community of organisms' proposed in the Viral Eukaryogenesis theory. In this case, it has been shown that *Acanthamoeba* is not just the host for the Mimivirus, it is also the host of many bacteria and viruses and where it acts as a kind of melting pot for the horizontal transfer of genes between bacteria and viruses. [35] Thus the *Acanthamoeba* cytoplasm can be the host of a variety of bacteria, as well as a variety of complex NCLDV viruses including the Mimivirus and Marseillesvirus, and that these 'invaders' can and do exchange genes with each other. It is proposed in the Viral Eukaryogenesis theory that the *Acanthamoeba* is a modern organism that represents a direct descendent of the kind of community that evolved into the eukaryotic cell. In terms of the Viral Eukaryogenesis theory, this community can be considered to be an 'ecosystem' of sorts containing a variety of independently derived 'organisms', some of which, like the mitochon-

dria and nucleus are permanently linked to the single host, whereas others like the bacteria and Mimivirus are transient members of the ecosystem and capable of being transferred between communities by infecting new hosts.

5.1. The origin of meiosis and the eukaryotic cell cycle according to the Viral Eukaryogenesis theory

According to the Viral Eukaryogenesis theory, the three lineages that make up the modern eukaryotic cell originally replicated independently of each other, and due to natural selection, they eventually evolved to have the co-ordinated replication cycles seen today. [24, 36] In the process of evolution, it is proposed that the mechanisms of viral and cellular replication were exapted into the eukaryotic cell cycle.

In the case of the mitochondria, it is relatively simple to envisage a situation whereby the alpha-proteobacterial endosymbiont evolved its replication cycle to ensure that there were always several endosymbionts per cell, and thus it was highly likely that any daughter cells would posses at least one copy of the mitochondria. In the case of the viral lysogen there were more options available since viruses have evolved several complex mechanisms by which they are either maintained within a host (lysogeny) or transfer themselves to new hosts (infection). It is proposed that the mitotic cycle of the eukaryotes evolved from the mechanisms by which the virus established a permanent lysogenic presence in the host cell, [24] and that the meiotic cycle and sex arose from the mechanisms by which the virus transferred itself to new hosts.

5.2. Mitosis

Observations of modern viruses demonstrate that viruses can be maintained indefinitely within a host lineage either by integrating into the host genome like lambda or replicating as a stable low copy plasmid like lysogen in the host cytoplasm like P1. [37] Where a virus integrates into the host chromosome, its replication and stability are ensured by the host genome's mechanisms of replication and transfer to daughter cells. However, where a virus replicates as a plasmid-like lysogen, the virus must provide mechanisms to ensure its own replication and stable transfer to all daughter cells. In the case of the P1 phage, it has been found that the virus has a complex mechanism to ensure that the viral genome is replicated and maintained at a low copy number. [38] It is thought that keeping a low copy number is crucial to ensure that the metabolic burden of maintaining the virus is limited, so that the host is not at a competitive disadvantage when compared with hosts that have not been infected. [39] In addition to the mechanism to ensure a low copy number, the P1 phage has evolved at least two mechanisms to ensure that it is stably propagated to the daughter cells. [38] Firstly, the virus has evolved efficient partitioning mechanisms to ensure that a copy of the virus is mechanically segregated to each daughter cell before the daughter cells are divided. Secondly, the P1 phage has evolved a toxin/antitoxin mechanism to ensure that if the segregation fails to ensure both cells receive a copy of the virus, the cell not receiving the virus will die. This is achieved by having a long-lived toxin and a short-lived antitoxin that is continually produced by the viral genome [38] and is suggestive of the kind of mechanisms that viruses evolve to ensure that the viral lysogen is maintained indefinitely.

Studies at the molecular level on a variety of lysogens, such as other viruses (eg N15) and large conjugative plasmids such as F and R1 has shown that these low copy number lysogens have independently evolved similar, but non homologous mechanisms to ensure low copy number/ partitioning occurs as required. [40] In the systems studied at a molecular level, it has been found that copy number control is achieved by the possession of centromeric regions that perform several crucial roles. After the genome has been replicated there are two copies of the chromosome, both of which are 'handcuffed' together at the centromere region, which has the function of preventing further rounds of replication. [40] Subsequently when the cell is dividing, the centromere performs a second function, whereby it binds the proteins that are required to segregate the copies of the daughter chromosomes to daughter cells. [40] Once the segregation proteins are bound to the centromeres, the chromosomes are segregated via filament polymerisation to either pole of the cell. In the case of the R1 plasmid, these segregation proteins are closely related to the actin genes, [40] in other cases such as some of the large linear *Bacillus* plasmids they are be related to tubulin [41] and in the case of N15 phage and the F plasmid other classes of proteins are used. By segregating the chromosomes to either pole of the cell, the division of the host cell at the equator ensures both daughters obtain a copy of the chromosome, and the centromere is freed from the 'handcuffing' proteins, and thus free to enter into another round of replication. By this mechanism the copy number is kept low, and the viral chromosome is segregated efficiently to the daughter cells.

Several of the 'themes' of the replication of the large lysogens such as P1, R1, F and N15 are mirrored by the mechanisms controlling the replication of the eukaryotic chromosomes during mitosis and are thus consistent with the Viral Eukaryogenesis theory. [25] For example, the term centromere, representing a region of the chromosome that binds the two copies of the genome together, and representing the region to which the segregation proteins will bind and segregate the chromosomes to either pole of the cell is equally applicable to eukaryotic mitosis as it is to the segregation of lysogenic viruses and plasmids. The mechanism by which the chromosomes are segregated to either pole of the cell, i.e. via filament polymerisation, is also equally applicable to mitosis as it is to plasmid segregation and, amongst the range of proteins used by the lysogenic viruses and plasmids, there are tubulin-like proteins that are related to the tubulin used by eukaryotic cells to segregate chromosomes to either pole. [41] The evolution of mitosis in the eukaryotes is thus consistent with the Viral Eukaryogenesis theory in which the eukaryotic nucleus descends from a complex DNA virus that established a permanent lysogenic presence in its host. It is thus argued that the mechanisms by which the eukaryotic nucleus and chromosomes replicate and are segregated to either pole of the cell is a direct result of the descent of the eukaryotic nucleus from a large DNA virus [23] and arose directly from the mechanisms by which the ancestor of the eukaryotic nucleus maintained itself as a single copy lysogen in the host of the archaeon.

5.3. Meiosis and sex

In the Viral Eukaryogenesis theory, it is proposed that three general viral characteristics were exapted by the community to allow the evolution of meiosis and the sexual cycle. These three features were viral immunity, viral incompatibility, and viral infection, which when combined

with the existing 'mitotic' copy number control mechanisms resulted in the evolution of sex and meiosis.

One of the viral features proposed to have been exapted from viruses in the evolution of meiosis and the sexual cycle is viral immunity functions. In the case of viral immunity, it has been observed that viruses often possess a mechanism to prevent multiple infection of a host by the same virus. For example, infection of *E. coli* by T4 bacteriophage immediately prevents further infection by other T4 viruses. [42] Similarly, the N15 virus (which lysogenises its host as a linear cytoplasmic prophage) possesses immunity functions that prevent lysogenised cells from being further infected by the N15 virus. [43] Conjugative plasmids, which share many features with lysogenic viruses, have also evolved immunity mechanisms which prevent donor cells transferring the plasmid to lysogenised recipient cells and these immunity mechanisms are well understood. [44]

Another critical viral feature exapted in the evolution of the meiosis and the sexual cycle is the viral/lysogen feature of incompatibility. Incompatibility can occur when a host is infected by two lysogens that utilise common copy number control mechanisms. This can occur because immunity functions do not in general prevent infection of the host by a different lysogen, and thus a host can potentially be infected by two lysogens at the same time. When multiple infections of a host by different lysogens occur, it is found that the lysogens are either compatible with each other, in which case they can replicate indefinitely in the same host cell, or incompatible with each other, in which case they will be segregated into separate host cell lines. [45] It has been found that compatibility or incompatibility of lysogens is dependent upon their mechanisms for replication and segregation. If they use non-homologous mechanisms, they are compatible, whereas if they use homologous mechanisms, they are usually incompatible with each other and thus cannot be maintained in the same lineage. [45] It is thought that the interaction between copy number control mechanisms, and maintenance of related but not identical lysogens would lead to incompatibility if a host was infected by two related lysogens which shared a copy number control mechanism.

Another critical feature exapted from viruses in the evolution of meiosis and the sexual cycle is proposed to have been the mechanisms by which the virus could horizontally infect new hosts. A critical feature of viruses (and plasmid lysogens) is that they can be transferred horizontally between hosts. In the case of viruses, the completion of the lytic cycle produces multiple virions that escape the cell and have many features that allow them to attach to and infect new hosts. In the case of lysogenic viruses, these viruses will enter the new host, and either establish a lysogenic cycle or enter a lytic cycle to produce more infectious virions. In contrast to viruses, conjugative plasmids do not have an extracellular stage to allow transfer from host to host within a population. Instead, these plasmids have evolved conjugation mechanisms that allow them to be transferred from an infected cell into an uninfected host cell. [44] In the case of the F plasmid, conjugation involves over 40 genes, including genes for pilus formation, surface exclusion, mating pair stabilisation, regulation and DNA mobilisation, [44] indicating that these processes are highly evolved and strongly selected for.

It is proposed in the Viral Eukaryogenesis theory that sex (syngamy) is exapted from the mechanisms by which the virus spread to new hosts. In this case, the virus either was produced

as a free virion that used membrane fusion to enter new hosts, or the virus caused the host to conjugate with hosts that did not contain the virus. As shown in Figure 2 the virus/host would normally replicate via the lysogenic 'mitosis' like cycle in the hosts. However, under specific conditions, the host would produce new infectious viruses that could infect a new potential host that had not yet been infected by the virus. Once the new host was infected, the virus would enter into either the lysogenic (mitotic) cycle or enter into the lytic cycle to produce more infectious virions.
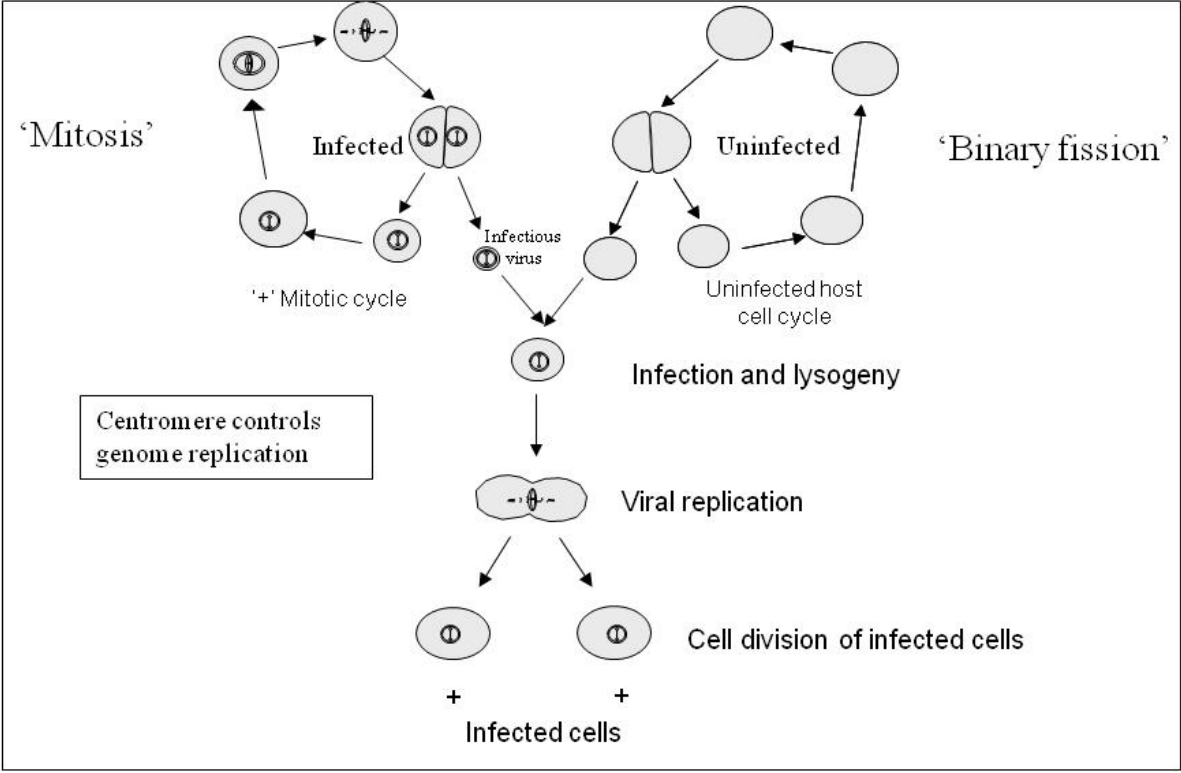
Figure 2. The lysogenic and infectious cycle of the viral ancestor of the nucleus. As shown, the archaeal ancestor of the eukaryotic cytoplasm initially replicated via a typical archaeal/prokaryotic binary fission style of replication (top right). However, when infected with the proposed viral ancestor of the nucleus, the virus maintained itself in the host using a mitosis-like process in which the copy number control of the virus was regulated through the use of 'centromere' sequences as is commonly observed in modern viruses and plasmids (top left). The virus could also enter into an 'infectious' cycle whereby an infectious virion would be produced that could infect new hosts that had not already been infected by the lysogen.

It is proposed in the Viral Eukaryogenesis theory that the eukaryotic sexual cycle evolved when two closely related but incompatible viral lysogens (designated 'a' and 'alpha') evolved from the original viral ancestor of the nucleus (Figure 3). When a cell infected by lineage 'a' encountered a host infected by lineage 'alpha', neither cell would recognise the other as being infected. As a result a virus could be transferred to a new host that already possessed a closely related lysogenic virus. Critically the two homologous but slightly different viruses would utilise the same mechanisms for copy number control. Once infected by the second related virus, the host would contain two viruses, and these viruses would be incompatible since they

shared a common mechanism for copy number control. When the lysogens replicated, four viral chromosomes would be produced, and since they possessed homologous centromeric regions, the four chromosomes would be bound together by the 'handcuffing' proteins at their homologous centromeres. Once the four viral chromosomes were handcuffed together, the cells would be committed to go through two divisions before the centromeres were no longer handcuffed together. Only once the chromosomes were no longer 'handcuffed' together would the origins of replication be exposed, and the chromosomes be capable of entering into another round of replication.
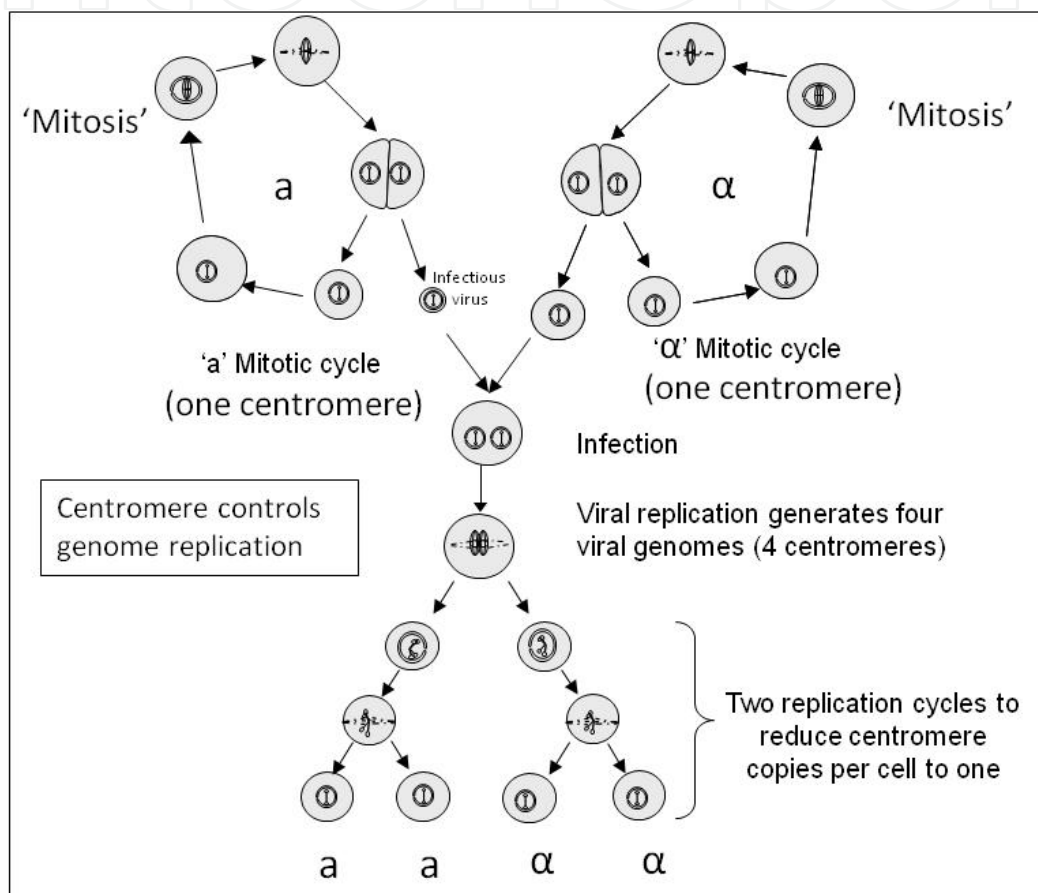
Figure 3. The eukaryotic sexual cycle arose when the lysogenic ancestor of the nucleus diverged into two closely homologous but not identical viruses utilising the same copy number control mechanisms. As shown two related viruses, designated 'a' and 'alpha' evolved that could maintain themselves in the archaeal host via the mitosis like lysogenic replication cycle. In this mitosis like cycle the copy number of the virus was maintained at unity by the use of centromeric regions. Since the two viruses were closely related to each other, they shared homologous centromeric regions, however the viruses were sufficiently different such that neither virus recognised the other virus as 'self' and thus a single host could be infected by both viruses. When an 'a' virion encountered a host infected by the 'alpha' virus, the 'a' virus would infect the 'alpha' host since it failed to recognise that it was infected already. As a result, both viruses would replicate, generating four homologous viral chromosomes that shared the same copy number control mechanisms. The four viral chromosomes would be then be 'handcuffed' together preventing further replication. The viral segregation mechanisms would ensure that the viral pairs would be segregated to either pole of the cell during replication. After a single round of replication there would still be two viral chromosomes attached to each other per cell and thus no further viral replication could proceed. As a result, the cell would have to enter a second replication cycle before the individual viral chromosomes could be segregated as a single copy into the host cells.

It is therefore proposed that the origin of sex and meiosis occurred when a complex DNA virus entered a new host via membrane fusion processes to produce a host containing two highly homologous, but not identical lysogenic viruses (Figure 3). Due to the incompatibility of the viruses, the next replication cycle of the cell would be a meiosis like cycle, using the same molecular machinery as the mitosis-like cycle. Thus the cell infected with the two homologous viral chromosomes would have to enter into a replication cycle in which the two viral chromosomes replicated once to produce four chromosomes, but the host cell would have to replicate twice, producing four daughter cells before the copy number control mechanism of the virus would allow the viruses to replicate again (Figure 3). The proposed cycle, based on the 'infectious strategy' of the lysogenic virus mirrors the eukaryotic sexual cycle, provides a mechanistic explanation for the reductive divisions observed in meiosis, and provides an explanation for why mitosis and meiosis share common themes and molecular machinery, yet result in quite different outcomes in evolutionary terms. [24] It also suggests that since mitosis and meiosis evolved at the same time, meiosis did not evolve from mitosis, and that the ancestral eukaryotic state possessed both mitosis and the sexual cycle.

6. Discussion

"The purpose of a scientific theory is to unite apparently disparate observations into a coherent set of generalizations with predictive power..... Historical theories, which necessarily treat complex irreversible events, can never be directly tested. However they certainly can lead to predictions. Even if this theory should eventually be proved wrong it has the real advantage of generating a large number of unique experimentally verifiable hypotheses. (Lynn Margulis, 1975)."8

Like the endosymbiotic theory for the origin of the mitochondria and the chloroplasts, the Viral Eukaryogenesis theory is an historical theory which treats complex irreversible events that can never be directly tested. Like the endosymbiotic theory for the origin of the chloroplasts and the mitochondria, it will be its ability to coherently unite apparently disparate observations, and make predictions that can be tested experimentally that will determine whether it is ultimately accepted.

If the radical concept of a viral ancestor of the nucleus can be entertained, then as shown in this chapter, the theory does unite a wide variety of disparate observations into a coherent set of generalisations. It can coherently explain many of the differences in genetic design between the eukaryotes and the prokaryotic bacteria and the archaea. For example, the origin of the nuclear membrane, the change from circular chromosomes to linear chromosomes, the invention of telomeres, the invention of mRNA capping, the invention of cap directed translation, the separation of transcription from translation, the invention of nuclear pores, the invention of karyopherins, are all a simple consequence of the evolution of the nucleus from a complex DNA virus that already possessed these features. That is, if it is accepted that a complex DNA virus like the Mimivirus evolved into the nucleus, there are no biologically

implausible steps required in the evolution of the eukaryotic nucleus since the Mimivirus is already membrane bound, possesses a linear chromosome with telomeres, caps its own mRNA, encodes its own cap binding protein (eIF4E), does not translate mRNA within its own virion, and exports its own capped mRNA into the host cytoplasm for translation. Thus the origin of the nucleus from a complex DNA virus has the potential to explain in a coherent fashion many of the dramatic changes in genetic architecture in the transition from prokaryotic to eukaryotic design. It also leads to testable predictions about the phylogenetic relationship between the eukaryotes, prokaryotes and NCLDV viruses. Although many of these testable predictions have not yet been tested, testing of some, such as the phylogenetics of the mRNA capping genes, have supported the theory. [20]

In addition to providing an explanation for the radical change in genetic design from the prokaryotes to the eukaryotes, the theory is one of the few theories for the origin of the eukaryotes comprehensive enough to provide a plausible explanation for the origin of the unique cell cycle of the eukaryotes. It predicts that the origin of mitosis, meiosis and the sexual cycle are all consequences of the very origin of the eukaryotic cell, and its viral derived nucleus. That is, because in the Viral Eukaryogenesis theory, the eukaryotic cell is actually a consortium of three phylogenetically independent members of a consortium, the eukaryotic cell cycle was by necessity quite different from any replication cycle of either cellular or viral organisms. Rather, the eukaryotic cell cycle that we see today is a synthesis of the replication cycles of three previously independent organisms, the nucleus, the cytoplasm and the mitochondria. Simply put, in the Viral Eukaryogenesis theory, mitosis is the mechanism by which the virus maintained itself as a single copy lysogen in the archaeal host, sex is the mechanism by which the virus transferred itself to a new host/ or the host was infected by new viruses, and meiosis is a natural consequence of the copy number control mechanisms being incompatible when two highly homologous viruses using the same copy number control mechanism infected the same host.

If the eukaryotes did evolve as proposed in the VE theory, a critical question to be asked is what made this new consortium uniquely capable of evolving beyond the comparatively simple 'prokaryotic world' of phages, bacteria and archaea and into the much more complex 'eukaryotic world' of complex multicellular organisms, and how did that evolution relate to the new modes of replication such as mitosis, sex and meiosis? It is the opinion of this author that both the new cellular design, where the 'cell' was uniquely compartmentalised into functionally specialised 'organelles' such as the mitochondria and nucleus, and the new modes of replication (sex and meiosis) were both critically important in the evolution of complexity in the eukaryotic lineages. In the case of the new cellular design it is proposed that the 'proto-eukaryotic' community eventually evolved into an amoeba-like eukaryotic ancestor that invented an unprecedented predatory life cycle that eventually lead to an evolutionary arms race in which the more complex and larger predators and prey had greater fitness. Critically however, the invention of sex and meiosis introduced a new mode of evolution, one that allowed the newly evolved eukaryotes to escape the limitations that Muller's ratchet imposes on prokaryotic evolution. In particular, the evolution of the 'meiosis' like cycle induced by infection with a second closely related viral lysogen allowed both repair of detrimental mutations within any one lysogen via recombination, and the spread of new advantageous mutations/genes that had evolved to increase the fitness of the viral genome, thus providing

an immediate advantage to the consortium over its prokaryotic rivals. It is interesting to note here that when high mutation loads are deliberately introduced into viruses (including NCLDV virus such as poxviruses), the co-infection of a single host with multiple mutated virons allows the regeneration of functional viruses through a recombination process termed multiplicity reactivation (MR). [46, 47] This suggests that multiple infections of a single host by related viruses as proposed in the VE theory can side step the problem of accumulating mutational load seen with Muller's ratchet. As a result, the new meiosis like mode of replication allowed the integrity of the genetic information of the consortium to maintained at a higher level than seen in the prokaryotic world and this, combined with the new unique cellular organisation of the eukaryotes may have facilitated the evolution of complexity in the eukaryotic lineages.

The Viral Eukaryogenesis theory paints a radical new picture of the origin of the eukaryotes that would have been impossible to present even a few years ago. Prior to the 1970's symbiogenesis was scorned by the scientific community, despite the fact that today the evidence appears incontrovertible that the chloroplasts and mitochondria are symbiogenic in origin. Even the fact that there are three domains of life was only established with some difficulty by Woese in the 1970's, and the diversity of the Archaeal domain in particular has been expanded over the last 10 years or so to include phylogenetically new groups such as the Thaumarchaeota and Korarchaeota. The 21st century discovery of the hitherto unexpected giant viruses such as the Mimivirus have also shown that our knowledge of the natural biological world is still incomplete and most likely there will be more surprises in the future to challenge our accepted biological paradigms. Only time will tell whether ongoing scientific discoveries will prove or disprove the Viral Eukaryogenesis theory, but since we do not have an established paradigm that explains the origin of mitosis, sex, meiosis and the unique cellular architecture of the eukaryotes, it is perhaps time to entertain radical new theories for the origin of the eukaryotes, especially if they can be experimentally tested.

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