Evolving complexity: a biography of the Last Eukaryotic Common Ancestor

Where did we come from? Colloquially, this is a question that has perplexed and intrigued us as humans from the dawn of our existence. Before the discovery of cells, many including the Italian embryologist Marcello Malpighi believed all embryos to have existed since the world was created[1]. Even earlier was the Greek Mythological view that it was the God of Fire, Prometheus, who first carved man from mud. Taking a modern scientific perspective, the question rather becomes: how did eukaryotic cells originate? Without this transition from single-celled bacteria and archaea to infinitely intricate eukaryotic cells, such organisms as the fire chaser beetle to sea foam would not exist - indeed we would not either – for it formed the lynchpin to which all subsequent diversification revolved. What is presented in this essay is a possible account for the Last Eukaryotic Common Ancestor, plucked with cautious consideration from a plethora of hypotheses for eukaryogenesis, for the truth, obfuscated by nature, is yet to be truly demystified.

Though these cells may be "little more than fancy froth on the surface of bacterial life,"[2] the sheer diversity and complexity of eukaryotes is undisputable. To understand the origin of such "fancy froth", we must first distinguish the characteristics which make them so complex. Unlike archaea and bacteria, the eukaryotic genetic material is compartmentalized by a nucleus to limit chemical damage to DNA and to act as a physical barrier to prevent integration of foreign DNA[3]. Research conducted by Katsumi Chiyomaru and Kazuhiro Takemoto from the Kyushu Institute of Technology confirmed an increased genome complexity in eukaryotes, with the haploid gene number for eukaryotes being 11038 (in median) greater than prokaryotes. They also found that the power per gene was greater for eukaryotes, 0.14fW in median compared to 0.0027fW in median for prokaryotes[4]. This leads to the belief of mitochondria being characteristically eukaryotic, acting as the 'power-house' to provide for the more energy-demanding metabolic processes. In addition, eukaryotes are unique for their possession of SNARE proteins which help fuse biological membranes efficiently and controllably[5], for example during vesicle fusion at a synapse, and account for the highly pertinent and unique eukaryotic feature of membrane-bound organelles - sectioned and specialized compartments within the cell[6]. Identification of some of these characteristics forming within prokaryotic cells can therefore lead to an understanding of eukaryogenesis.

Crucial to understanding the evolving complexity of LECA is an appreciation for the creativity of nature and an openness to bypass traditional scientific ways of thinking. In the case of evolution, one may conjure ideas of the 'tree of life' but, as Richard Dawkins writes, "history is usually a random, messy affair"[2] and eukaryogenesis cannot simply be explained through the branching out of a universal common ancestor. More allegiant to the truth is the 'ring of life' hypothesis[7] proposed by Lake and Rivera which takes into account that it is also the fusion of genomes and involvement of horizontal gene transfer that resulted in *Eukaryota*. Figure 1 shows a revised version of Lake and Rivera's ring of life.

Under this model, it is understood that one prokaryote (the *Eocyta* or eocytes, part of archaea) acted as a the central genome, involved more heavily in informational processes[2] and this host cell fused with bacteria (alpha-proteobacteria which formed mitochondria) whose genes are operational[7]. This begs the following questions: what was the nature of this eocyte? How was fusion of these prokaryotes achieved? And was this process a gradual one or rather a "big-bang"[2]?

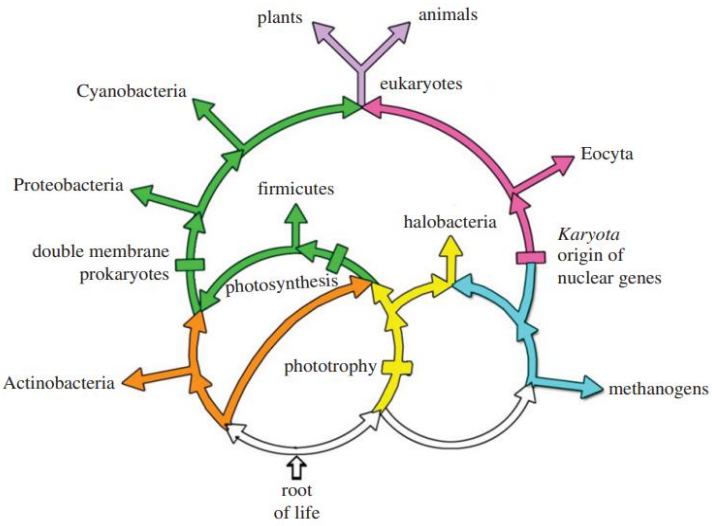

Fig. 1 Ring of Life (from ref. 7)

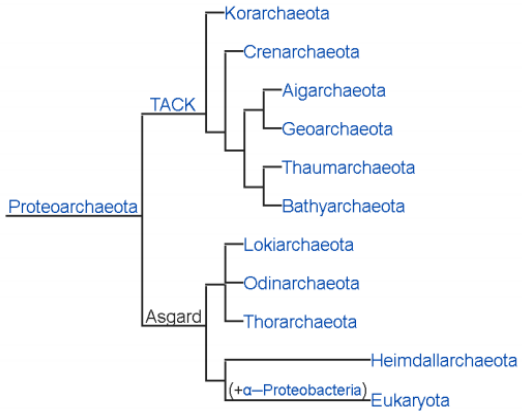

Fig. 2 Phylogenetic Tree for Proteoarchaeota (from ref. 9)

The eocytes are a subset of Archaea which have been proposed to contain the proto-eukaryotic cell. Research into this subset clarified a group known as the Asgard group as "the closest prokaryotic relatives of eukaryotes"[8]. This group includes *Lokiarchaeota*, *Thorarchaeota*, *Odinarchaeota* and *Heimdallarchaeota*[9] – the latter of which is now thought to be the most closely related to eukaryotes as shown in Figure 2. This conclusion was drawn due to phylogenomic analyses and analysis of genome content showing Heimdall to code for multiple genes previously thought of as specifically eukaryotic. These associated proteins include ESCRT complex proteins, actin homologues and GTPases[10]. Further to this, analyses conducted by the University of Lausanne[6] found that, though there is scepticism as to whether Archaea possess SNARE proteins, the Heimdall genome was shown to contain SNARE-like factors. This suggests that the diverse set of eukaryotic SNAREs evolved from an "archaeal precursor"[6], likely the Heimdall.

With this established, what is it about the nature of Asgard which led to the formation of eukaryotes? The answer is seemingly simple: blebs. These are extracellular protrusions[11], typical of archaea, produced to increase the surface area and therefore capability of interaction with its external environment, including other prokaryotes. Pertinent to this is the first picture taken of Loki (within the Asgard group along with Heimdall) in 2020 and proves the presence of extracellular protrusions[12].

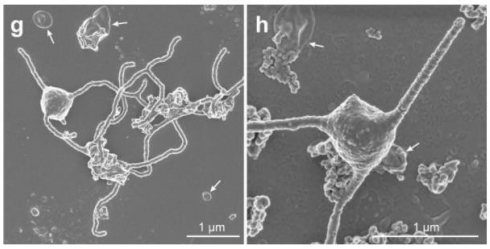

Fig. 3 Extracellular Protrusions in First Picture of Loki (from ref. 12)

David and Buzz Baum claim these blebs to be pivotal in the 'inside-out' model they propose for the formation of a nucleus and gradual symbiosis of mitochondria[11] and indeed eukaryogenesis as a whole. Like the 'ring of life' hypothesis, this 'inside-out' model does not follow conventional lines of thought which tend towards an 'outside-in' theory where an archaeal cell simply swallowed other prokaryotic cells, and through an endosymbiotic relationship, they, as if by miracle, became one. What ignites this as a commonly held view is how there has been little to no intermediary cell between prokaryotes and eukaryotes found. However, amongst other reasons, what seems unconvincing about this theory is how it presumes Archaea to be capable of engulfment processes homologous with phagocytosis when little to no evidence of this has been supplied. Rather, the 'inside-out' model suggests that an archaeal cell lived in a biofilm with bacteria and through its protrusions, turning into larger blebs, and through benefitting from a mutualistic relationship, they gave rise to the eukaryotic cell. Figure 4 depicts this process.

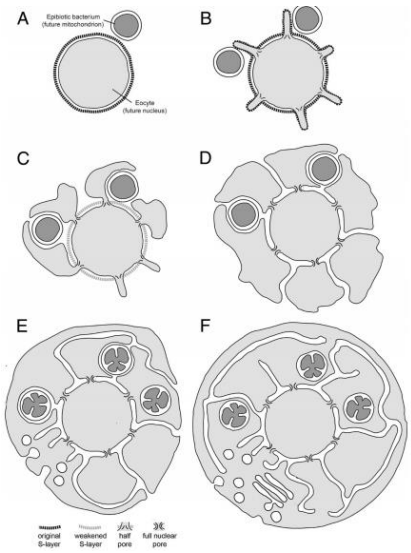

Fig. 4 The 'inside-out' Model of Eukaryogenesis (from ref. 11)

The first question in need of answering is how the roughly two metres of DNA molecules inside each eukaryotic cell was compartmentalized into a single nucleus[1]. Baum and Baum proposed that the original eocyte cell became the nucleus and the protrusions which expanded to become 'blebs' became the cytoplasmic exterior. This happened through the gradual separation of metabolic processes of DNA replication, transcription and ribosome assembly in the nucleoplasm, from the rest of the cytoplasmic compartments; Baum and Baum put forward that this would have been beneficial for preventing "exposure of the genome to the dangerous by-products of metabolism (for example, reactive oxygen species generated in mitochondria)"[11].

However, the process of synthesizing membrane-bound DNA has been a major point of controversy. An alternative method is proposed by Masaharu Takemura[13] who, after analysing a group of viruses, known as poxviruses, identified its DNA polymerase to be similar to that of eukaryotes. Further investigation lead him to believe that a subgroup of virus, known scientifically as Nucleucytoplasmic Large DNA Viruses or NCLDVs, either became the nucleus or acted as a manual to which proto-eukaryotic cells could then learn to make their own membrane to protect chromosomes. Acting to refute this theory is the fact that viruses are known for taking genes from their hosts and so it becomes difficult to distinguish whether viruses gave eukaryotes the similarities seen or whether they were traits stolen by viruses after eukaryotes were created. Other hypothesis tend towards the formation of a nucleus simply being a thermodynamic process of coagulation due to high quantities of DNA[3], this being a non-adaptive process. This process seems more difficult to rebut and perhaps worked in connection with the inside-out model explanation.

The next complexity is the symbiosis of mitochondria, or rather, alphaproteobacteria, the involvement of which can be seen in Figure 2. Baum and Baum[11] suggest that alphaproteobacteria shared a mutualistic relationship with the archaean host cell through ectosymbiosis – a mutually beneficial relationship whereby one lives on the surface of the host. What materials were in need of exchange between these two prokaryotes? This, too, has been a topic of debate but possibilities include hydrogen, sulphur, hydrogen sulphide, organic acids and ATP[11]. Then proposed is that, over a long amount of time, the protrusions turned to blebs in order to increase the surface area and therefore contact with its external environment – particularly to increase the exchanges between itself and the alphaproteobacteria. The gradual enclosing of the cell seen in Figure 4 then had the further benefit of preventing external pathogens from entering. Also crucial is the gradual loss of the S layer on the archaeal cell surface membrane. This is a protein surface layer which acts as protective coats, molecule and ion traps as well as maintains cell shape and controls what molecules enter and leave the cell[14]. Baum and Baum suggest that this layer is lost at contact points between alphaproteobacteria and the archaea to increase exchange.

The endoplasmic reticulum can then be understood as the spaces between extracellular blebs which makes sense due to the suspected relationship between mitochondria and ER. Soyeon Lee and Kyung-Tai Min[15] report regulatory communication between these organelles via a mitochondrial-associated ER membrane to "provide another level of regulations in energy production, lipid process, Ca2+ buffering and apoptosis."[15]

The formation of the continuous plasma membrane – the final step in the creation of LECA – follows naturally to be explained. The inside-out model hypothesizes that it is due to proteins found in the endoplasmic reticulum known as cell-cell fusogens. These proteins stimulate the fusion of bleb membranes around the exterior of the cell. The difficulty in ensuring a continuous membrane however is particular tubes of the evolving cell which connect the ER to the cell's external environment which must be removed. The solution: Dynamin. This protein, involved in membrane fission[16], assembles at the cytoplasmic part of the plasma membrane and internal ER network. Severing the connections between the ER and plasma membrane, it allows the ER to simply be spaces unattached to the membrane within the cell[11].

Will it be the 'outside-in', 'inside-out' or another hypothesis entirely that future advancements unveil as the truth? The image taken of Loki only last year[12] serves as a paradigm of what may come and there are certain hopes that scientists may one day culture and analyse a *Heimdallarachaeota* cell. After conferring with Professor Buzz Baum himself, he assures that though modern Heimdall cells may differ from the original cell which evolved to create eukaryotes, it would certainly give ground-breaking insight as to the ancestral history of complex life and bring us one step closer, or rather 1.8 billion steps backwards through time, to answering the pivotal question of where indeed did we come from.

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