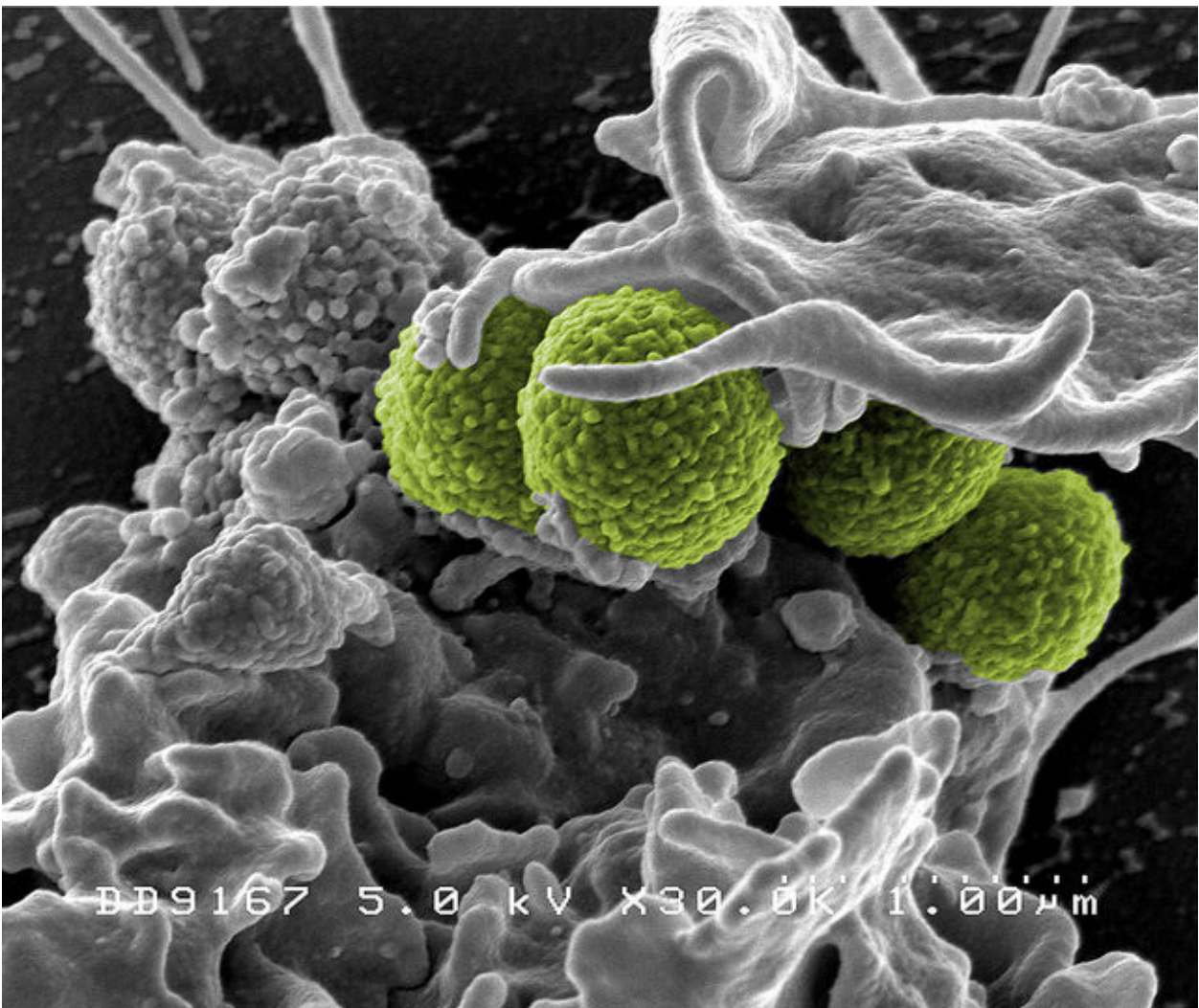# Asgard Archaea bring us closer to the truth about the tree of life

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(Timmer, 2017) Asgard Archaea

Abstract

Since the discovery of the first Asgard Archaea, researchers have been extensively describing their properties and genomic resemblances to eukaryotic cells. Asgard Archaea were found to mostly be obligate anaerobes, with varied ecologies and metabolic properties from one species to another. Most are thought to be able to degrade H_2 and organic compounds for energy, as well as produce H_2 as a possible byproduct. It was found that Asgard Archaea had genes that encoded for properties earlier only ascribed to Eukarya, called Eukaryotic Signature Proteins (ESPs). After the isolation of the first Asgard Archaea in Japan, a model called Entangle-Engulf-Endogenize (E3) was described by Imachi (2020). Here, considering the syntrophic relationship of the Asgard archaea with an O_2-scavenging partner and a sulfate-reducing deltaproteobacterium, indications of a first archaeal host cell that evolved into an eukaryotic cell were made. The E3 model, as well as the ESPs found in Asgard members' genome, both support the two-domain tree of life where Eukaryotes are part of the Archaea domain.

Introduction

In 2015, a group of evolutionary microbiologists at Uppsala University in Sweden described the DNA sequence of Lokiarchaeota. This was the first of 6 archaeal phyla to later be ascribed to the Asgard superphylum, all named after Gods of ancient Norse mythology. (Spang et al., 2015). Even though they were archaeal organisms for sure, all phyla of Asgard harbored various special traits never seen before in other archaeal species. Asgard members held genes that encoded for a broad range of eukaryotic processes, including membrane-related processes and cell shape dynamics (Zaremba-Niedzwiedska et al., 2017; Mcleod et al., 2019). These genes were previously found specific to Eukarya, the discovery of these genes in an Archaea species, therefore, gave a new perspective on the origin of the eukaryotic cell (eukaryogenesis) (Zaremba-Niedzwiedzka et al., 2017). Furthermore, in 2020 a research group in Japan succeeded to isolate an Asgard archaeon for the first time (Imachi et al., 2020), giving new insights into the origin of the eukaryotic cell (eukaryogenesis). This organism, as well as the earlier discovered Asgard members, all showed to survive on syntrophic relations with symbiotic partners (Lopez-Garcia et al., 2020). Researchers came up with models for eukaryogenesis starting from an archaeal host cell that likely was not much different from the Asgard members (Spang et al., 2019; Imachi et al., 2020). Now, there are still two sides to the story that shaped the beginning of evolution. The first story being the Woese theory, where Eukarya were a separate domain of life. The second being the Eocyte theory, where Eukarya are part of the Archaea domain of life.

This essay aims to describe the Asgard superphylum in terms of lifestyle, metabolism, and genetic (eukaryotic) properties. Furthermore, the implications of the discovery and isolation of

the Asgard superphylum have on the phylogenetic tree of life and the evolution theory will be discussed.

Properties of members of the Asgard superphylum

Since the discovery of Lokiarchaota in 2015, and the implication that the Asgard superphylum plays a big role in the model tree of the evolution of life, much effort has been made to understand the properties of Asgard members. The distribution of said species is widespread and their properties differ from one another. With metagenomic sequences from samples acquired around the world, classification has been implemented to map a phylogenetic tree of the Asgard family (Zaremba-Niedzwiedska et al., 2017). Four groups have been described in this tree: Lokiarchaeota, Thorarchaeota, Odinarchaeota, and Heimdallarchaeota. Later on, also Gerdarchaeota and Helarchaeota were discovered and were added to the Asgard Superphylum (Seitz et al., 2019; Cai et al., 2020)

Archaeal properties

More than 40 years ago, due to their differences from Eukarya and Bacteria, Archaea were first described as a primary division of the tree of life (Woese et al., 1977). For example, the peptidoglycan usually found in cell walls of Bacteria was lacking in Archaea, and their membranes were made from totally different lipids than both Bacteria and Eukarya because they comprised of the glycerol-1-phosphate ether lipids. Lastly, their way of transcription greatly resembled that of eukaryotes, using an archaeal DNA-dependent RNA polymerase. These findings resulted in the conclusion that Archaea species were unique and should be taxed independently from Bacteria and Eukarya (Spang & Ettema., 2017). Since culturing of Archaea was still difficult at the time, researchers established a method for sampling small-subunit ribosomal RNA sequences directly from soil or sediment. Due to this new technique discovery of new archaeal strains skyrocketed (Pace., 2009). Further cultivation-independent metagenomics and single-cell genomics analysis have enabled researchers to predict properties like the type of carbon fixation, generation of energy, and protective mechanisms (Sangwan et al., 2016). Later with the use of full-length microbial 16S and 18s rRNA gene sequencing researchers were capable of assembling genomes even more precisely. With these techniques at hand, after the discovery of the first Asgard member, the Lokiarchaeota, very quickly other members were discovered and described.

Asgard ecology

Most Metagenome-assembled genomes (MAGs) of Asgard Archaea were found in anoxic sediment environments around the world and appeared to inhabit diverse environments (Cai et al., 2020). Until now samples were found most frequently in sediments and soil environments, mostly marine, estuary, or river sediments (Bulzu et al., 2019). Lokiarchaeota were mostly found in sulfate-methane transition zones and were supposedly linked to the methane oxidation and sulfate reduction processes there. Examples of places where Asgard samples were collected are hydrothermal vent sediments in the Arctic Sea, marine water in the Red Sea, microbial mats in Australia, mangrove sediments in Southeast Asia, and Hot spring sediments in North America (Mcleod et al., 2019).

Carbon metabolism

With metagenomic data assembly, where the individual genomes from complex metagenomes are reconstructed, multiple studies predicted the metabolic pathways of Asgard phyla (Williams et al., 2020). It was predicted that all Asgard Archaea can degrade amino acids and have restricted biosynthetic capacities, indicating that amino acid degradation and partner dependence may be a common feature across Asgard members (Imachi et al., 2020). They either use hydrogen under autotrophic growth or produce hydrogen under heterotrophic growth, consuming small organic compounds such as peptides or other short-chain hydrocarbons (Spang et al., 2019; Huang et al., 2019; Manoharan et al., 2019)(fig1). Most members of the Asgard superphylum are thought to be facultative anaerobes, except for 2 strains from the Heimdallarchaeota group that showed signs of an aerobic lifestyle, since they harbored the complete cytochrome c oxidase (Imachi et al 2020).

All Asgard members were capable of degrading organic compounds and H_2 for energy. For degradation of H_2, most Asgard Archaea held a great number of the genes for the Wood-Ljungdahl-Pathway (WLP), which allows for the reduction of carbon dioxide to acetyl-Coenzyme A, which is then used to support autotrophic carbon fixation and energy conservation (Spang et al., 2019). Spang (2019) also identified putative formate dehydrogenases in Thorarchaota and Lokiarcheota, which indicated that Asgard Archaea possibly also supported their growth with formate as an energy and/or carbon source. All members of the Asgard superphylum hold genes for the 3b NiFe-hydrogenase, which is a group 3 NADP-coupled metalloenzyme that catalyzes the reversible oxidation of hydrogen gas (Spang et al., 2019). This being said, MCleod (2019) suggested a mixotrophic lifestyle for the entire superphylum, where the Asgard members use both autotrophic and heterotrophic pathways to obtain energy and carbon fixation. Later an exception was found; Gerdarchaota, can only function as a strictly anaerobic autotroph, but it also holds enzymes involved in the WL Pathway (WLP) (Lu et al., 2021; Cai et al., 2020). Interestingly, in a paper by Seitz (2019) another new phylum called Helarchaeota was introduced as a thermophilic fermentative

heterotroph and was found to be the only phylum able to metabolize short-chain alkanes anaerobically.
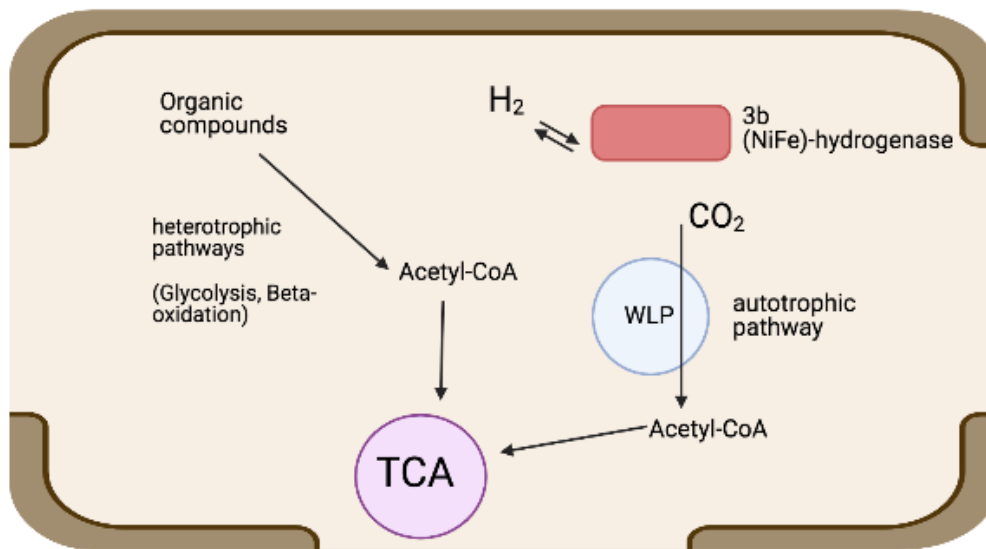
Common metabolic features Asgard Archaea

Fig.1: Schematic representation of common metabolic features in Asgard Archaea: Lokiarchaeota, Heimdallarchaeota, Thorarchaeota, and Odinarchaeota. All use multiple heterotrophic pathways to degrade organic compounds such as amino acids to gain acetyl-CoA. Also, all Asgard Archaea hold all or most genes (in the case of Heimdallarchaota and Odinarchaeota) for the Wood-Ljungdahl Pathway (WLP), an autotrophic pathway that uses CO_2 to obtain Acetyl-CoA. Acetyl CoA goes into the Tricarboxylic Acid cycle (TCA cycle) to gain high-energy electrons and ATP. 3b (NiFe)-hydrogenase functions as a metalloenzyme that catalyzes the bidirectional oxidation of H_2.

Phototrophic properties, nitrate and sulfate cycling & copper and arsenite resistance

Thorarchaeota, Lokiarchaeota, and Heimdallarchaeota harbor ribulose bisphosphate carboxylase (RuBisCo), which is the main gene in the phototrophic carbon fixing Calvin-Benson-Bassham pathway. However, phylogenetic analysis revealed that Asgard archaeal RuBisCo is not used for the photosynthetic carbon fixation pathway, and is affiliated with type Ⅲ archaeal RuBisCo, an enzyme that acts in a three-step pathway for conversion of AMP to glycerate-3-phosphate, which is a metabolic intermediate in both glycolysis and the Calvin cycle (McLeod et al ., 2019; Sato et al., 2007). Although, Photosynthesis was later reintroduced as an energy capturing mechanism in the Asgard Superphylum. Many organisms use rhodopsin pigments to capture or sense sunlight, and heliorhodopsin was found to be distantly related to the type 1 proton-pumping rhodopsin and show photo cycles that are longer

than one second, which is suggestive of light-sensory activity. Thorarchaota and Heimdallarchaeota both harbor genes for this enzyme, leading to the belief that many years ago Archaea were likely located in places where they could capture sunlight (Mcleod., 2019; Pushkarev et al., 2018).

Asgard members possess genes for both nitrate reductase and nitrite reductase, suggesting that Asgard Archaea play a role in nitrogen transformation. Also, all Asgard Archaea encode for sulfate adenylyltransferase and phosphoadenosine phosphosulfate reductase, suggesting the ability for sulfur cycling. Furthermore, all Asgard members were predicted to reduce arsenite and copper since they harbor copper efflux systems and arsenite transporters (Mcleod et al., 2019).

Isolation of Asgard

A research group in Japan was the first to succeed in isolating an Asgard species and called it the *Candidatus Prometheoarchaeum syntropicum* (Imachi et al., 2020)*. This was an important step in the research on the metabolism of the Asgard Archaea since earlier assumptions on metabolic functions were based on metabolic data assembly (Lopez-Garcia et al., 2020). They engineered a methane-fed continuous flow bioreactor system for more than 2000 days to enrich the organism they obtained from samples from anaerobic marine methane-seep sediments and obtained the Asgard Archaea members Loki-, Heimdall- and Odinarchaeota (Imachi et al., 2020). In the end, they isolated the Loki strain MK-D1, which showed a doubling time of 14-25 days. The entire process of enrichment and isolation took them over a decade. Stable growth was obtained by supplementation of all 20 amino acids and powdered milk, and a triculture with 2 other organisms: a *Methanogenium* archaeon and a *Halodesulfovibrio* bacterium (Imachi et al., 2020)*. Further research using microscopy showed that the MK-D1 strain looked like a small coccus and formed aggregates surrounded by extracellular polymeric substances and that they did not contain organelles. Furthermore, the cells produced membrane vesicles, blebs, and membrane protrusions. Their membranes consisted of typical archaeal ether-type lipids and the organism lacked genes for ester-type lipid synthesis (Imachi et al., 2020).

Isolated Asgard metabolism

The obtained MK-D1 strained could catabolize 10 amino acids and peptides through syntrophic growth with the *Halodesulfovibrio* bacterium and the *Methanogenium* archaeon through interspecies hydrogen and formate transfer (Imachi et al., 2020). This was determined by the measured depletion of amino acids, and the fact that the addition of high concentrations of formate and hydrogen suppressed the growth of the MK-D1 strain. (Imachi et al., 2020). Furthermore, they found genes for one NiFe hydrogenase and formate dehydrogenase, that mediate reductive H_2 and formate generation, respectively, as well as genes for the degradation of the 10 amino acids. The identified amino-acid-catabolizing pathway recovered energy through degradation of a 2-oxoacid intermediate, and MK-D1 showed to be able to degrade it hydrolytically or oxidatively to yield acyl-CoA that can further be degraded to ATP. In the

hydrolytic path, formate is produced and passed through to the methanogenic Archaea partner. And in the oxidative path, 2-oxoacid is oxidized to obtain CO_2, which is again reduced to H_2 and formate respectively.

By isolating the MK-D1 strain, for the first time, a closed genome of the Asgard superphylum was obtained that showed a relationship between MK-D1 and Eukarya. 80 Eukaryotic Signature Proteins (ESPs) were localized in the genome of MK-D1 that were also found in related Archaea, next to RNA-based evidence that these genes were expressed in the organism.

Eukaryogenesis

Since the discovery of the Asgard Superphylum, the discussions about the origin of the first eukaryotic cells began to rise again. The Asgard species all had ESPs encoded in their genome, which were genes that indicated a relationship between the Asgard Archaea and the first eukaryotic cell. Furthermore, the Asgard members found in samples, but also the isolated MK-D1 strain showed optimal growth when in syntrophy with metabolic partners. Suggesting that through this syntrophy, endogenization of partners led to the special features we see in the successful eukaryotic cell nowadays. This all goes against the classical model of the tree of life, where life was separated into three branches, and Eukarya did not evolve from an archaeal host cell. In the second part of this essay these ESPs and symbiotic relationships, as well as proposed models for eukaryogenesis and the tree of life will be described.

Eukaryotic like Proteins

Eukaryotes have been found to descend from a common ancestor (the Last Eukaryote Common Ancestor, or LECA). All organisms that are characterized as descendants of this ancestor and therefore bear the name eukaryote have similar characteristics; these characteristics include the mitochondrion, nuclear envelope, nuclear pores, and an endomembrane system, among many others (Koonin, 2010), and are found specific to eukaryotes, meaning that they developed over the years in a separate lineage from Archaea and Bacteria (Fournier & Poole, 2018). The main reason why scientists hypothesized that eukaryogenesis started from an Archaeal host cell, was the detection of Eukaryotic Signal Proteins (ESPs) in the genome of the Lokiarchaeota (Spang et al., 2015; Zaremba-Niedzwiedska et al., 2017). Later, with the discovery of Thorarchaeota, Odinarchaeota, and Heimdallarchaeota, More ESPs were found, suggesting that ESPs are widespread across the Asgard superphylum (Mcleod et al., 2019). These ESPs included components that in eukaryotic organisms were needed for various regulatory processes including cytoskeleton remodeling, endosomal sorting, eukaryotic-like ubiquitination, nucleocytoplasmic transport, and vesicular trafficking (Zaremba-Niedzwiedska et al., 2017). However, most of these eukaryotic systems encoded in the Asgard superphylum are incomplete and functionally uncharacterized (Mcleod et al., 2019). Meaning, these ESP are predicted to have similar functions in the Asgard phyla as they have in Eukarya, but this is not proven yet.

Also lately, it has been found that ESPs are not eukaryote-specific at all, since they have been found in multiple other organisms such as the TACK and Asgard lineages of Archaea and across eukaryotes (Fournier & Poole, 2018). This raises the question if ESPs are a good indication for a species to be related to eukaryotes, or that there are simply not enough discovered species that harbor these ESP to know if they are widespread over all sorts of lineages. Through the broader discovery of ESPs a pattern was also discovered, where ESPs shared by TACK and Asgard lineages are mostly informational and ribosomal proteins that are associated with highly conserved cellular functions (Fournier & Poole, 2018). Whereas ESPs found in only Asgard lineages are related to cytoskeleton remodeling and vesicle trafficking, which are characteristic of eukaryotes (Spang et al., 2018). This however does not make Asgard species under the definition of Eukaryotes, since there is no evidence that Asgard species have a nucleus or other organelles or systems associated with eukaryotes (Fournier & Poole, 2018). It merely suggests that Eukarya were not a separate branch from Bacteria and Archaea, but that they rather descend from an archaeal host cell as stated before.

The ESPs discovered by Zaremba-Niedzweidska (2017) were cytoskeletal components, endosomal sorting complexes required for transport (ESCRT), and a wide variety of small GTPases. They based their analysis on genetic markers and employed phylogenetic approaches with 16S and 23S rRNA, and r-proteins that were only shared between Archaea and Eukarya to find and determine these ESPs. Each Thorarchaeal genome encoded TRAnsport Protein Particle (TRAPP) complexes, which in eukaryotes represent multi-subunit vesicle-tethering factors involved in trafficking activities, such as transport of proteins from the Endoplasmatic Reticulum to the Golgi apparatus (Zaremba-Niedzweidska et al., 2017).
Conserved actin homologs called "Lokiactins", gelsolin- and profilin-domain proteins in all lineages of the Superphylum indicated that Asgard Archaea contain sophisticated cytoskeletal machinery (Zaremba-Niedzweidska et al., 2017). Prolifins are known as regulators of eukaryotic cytoskeleton dynamics, and the Asgards prolifins have shown to interact with eukaryotic actin (Mcleod et al., 2019). Furthermore, the ESCRT and ubiquitin modifier system components that were previously only known to be used in eukaryotes for protein degradation and endosomal sorting, were found in a co-organized gene cluster in the genomes of Heimdallarchaeota and Odinarchaeota (Zaremba-Niedzweidska et al., 2017). The ubiquitin modification system was later found to be expressed in the uncultured archaeon *Candidatus "Caldiarchaeum subterraneum",* indicating that the ubiquitin modification pathway may have eukaryotic-like functions in Archaea (Mcleod et al., 2019).
In the first isolated Asgard Archaea, the Lokiarchaota MK-D1, the genes for actin, gelsolin, ubiquitin, and the ESCRT pathway were all expressed, suggesting that the pathways earlier only ascribed to eukaryotes, could be functional across the entire Asgard superphylum as well (Imachi et al., 2020).

Syntrophy

Since all Asgard members miss strong electron acceptors in their ecosystems, they were predicted to have used the help of metabolic symbioses or syntrophy mediated by hydrogen

and/ or electron transfer between Archaea and Bacteria. It was predicted that all Asgard Archaea were capable of syntrophic degradation of amino acids, and are dependent on symbiotic interactions for both catabolism and anabolism, for example for H_2, formate, and metabolite transfer. High hydrogen inhibits prometheoarchaeum growth, suggesting it requires syntrophic hydrogen sinks (Lopez-Garcia et al., 2019). Later, the first cultured Asgard member showed also to be an organism that was dependent on syntrophy, since it grew in symbiosis with a sulfate-reducing Deltaproteobacterium, a methanogenic archaeon, or both (Imachi et al., 2020).

Models for Eukaryogenesis: Reverse flow model vs E3 model

Based on the metabolic potential of the discovered Asgard species, Spang (2019) proposed a model for the Last Asgard archaeal Common Ancestor (LasCA). Keeping in mind the possibility of horizontal gene transfer from different sources, they saw it likely that LAsCA had the potential to metabolize organic substrates. Furthermore, since genes for the WLP were present in Lokiarchaeota and Thorarchaota, and also partly in the other Asgard species, it was suggested that the LasCA used the WLP pathway as well. The presence of homologs of RuBiSCO in all Asgard species was found unrelated to the carbon fixation cycle and presumably acquired through horizontal gene transfer. Lastly, the presence of the NiFe-hydrogenases in all Asgard members showed no clear signs to be a key subunit of LasCA in phylogenetic essays. Altogether they concluded that LasCA had the potential to grow on H_2 and CO_2, as well as on organic substrates using the WLP, and might even be able to fermentatively produce H_2 with the bidirectional NiFe hydrogenase. Based on these assumptions the reverse flow model was introduced, which suggested an opposite syntrophic interaction than earlier presumed by other studies. These studies suggested that there was a syntropic interaction based on the transfer of H_2 between an H_2-dependent methanogen and an H_2-producing bacterial partner (Martin & Muller., 1998). In the reverse flow model, the eukaryogenetic syntrophy was based on hydrogen transfer. The reverse flow model stated that the archaeal ancestor of eukaryotes used fermentative pathways to produce H_2 (and also acetate or formate), which were syntropically metabolized by the facultative anaerobic Alphaproteobacterium (Lopez-Garcia et al., 2020)

The second model introduced was the E3 model, which stands for Entangle-Engulf-Endogenize and was proposed by Imachi (2020) after the successful isolation of the first Asgard Archaea. This model is based on a dual symbiosis, where the archaeal host cell degrades amino acids and produces H_2, which is then caught by a sulfate-reducing bacterium. Furthermore, toxic O_2 is scavenged by an aerobic organotrophic Alphaproteobacterium. The general idea was that through a merge of the archaeal host cell and the aerobic Alphaproteobacterial endosymbiont the first eukaryotic cell was born (Imachi et al., 2020). In this model, the early Archaea likely needed to be able to live with increasing O_2 levels and as a result, it started to depend on facultative O_2-respiring organisms (Imachi et al., 2020). This partner would remove the toxic oxygen from the archaeal host cell and protect it against O_2-damage (Imachi et al., 2020). Later the archaeal host cell engulfed this organism with extracellular polymeric structures, and through the final step of sharing nutrients and mixing of membranes, the archaeal host cell endogenized its partner. Next to its O_2-scavenging partners the archaeal host also lived in syntrophy with a sulfate-reducing bacterium that made sure H_2 didn't accumulate in the

cytoplasm. The facultative O_2-respiring oxygen was postulated to be the ancestor of the mitochondria. The nucleus and associated membrane system were formed from vesicles produced by the Alphaproteobacterium, where the archaeal membrane phospholipids would have been fully replaced by the bacterial phospholipids from these vesicles (Lopez-Garcia et al., 2020) according to the E3 model, the archaeal host cell already developed a complex cytoskeleton and endomembrane system before the acquisition of the mitochondrial ancestor (Imachi et al., 2020). These findings all strongly suggest that indeed the Asgard Archaea resemble the archaeal host cell of eukaryogenesis at a time where the symbiotic O_2 scavenging partner was not yet evolved to a mitochondrion and the sulfate-reducing partner was not yet engulfed and endogenized. Making the Asgard superphylum an excellent model archaeon for research into eukaryogenesis. However, since the models of eukaryogenesis are based on Archaea that live nowadays, and not back when the process of eukaryogenesis started, it is not sure whether they can truthfully encompass the history of life. It is merely possible to hypothesize such models based on presumptions in genomic analysis.
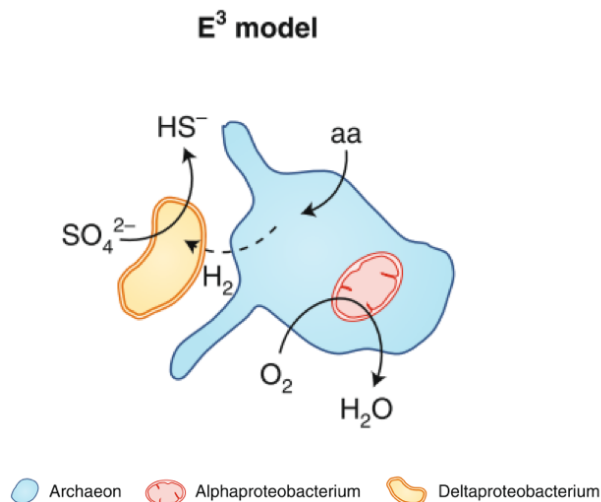

Fig.2: Entangle-Engulf-Endogenize model (E3)(Lopez-Garcia et al., 2019). An Amino acid degrading archaeon working together with an O_2-scavenging Alphaproteobacterium and a Sulfer-reducing Deltaproteobacterium

Indication for evolution and tree of life

The Discovery and Isolation of the Asgard Archaea gave researchers the means to support the Eocyte tree of life, where the first Eukaryotic was categorized in the Archaea phylum, separating all life into 2 main domains; Archaea and Bacteria (Fig.3B). This went against earlier assumptions that stated that life was classified into 3 domains; Archaea, Bacteria, and Eukarya (Fig.3A). After the discovery of Lokiarchaota by Spang (2015) and the other Asgard Archaea by Zaremba-Niedzwiedska (2015), Seitz (2019), and Cai (2020), an opposing group of researchers tried to disprove their hypotheses by criticizing their methods. For example, a study done by Da Cunha (2017) suggested that ESPs found in the genome of Asgard phyla were a result of contamination or homologous recombination from other eukaryotic species in the examined soil. And that removal of a certain elongation factor 2 (EF-2) crushed the evidence of the Eocyte hypothesis. As a reaction to this Spang (2018) disproved this claim by addressing the affiliation of eukaryotes with the Asgard superphylum in a phylogenetic analysis performed on 3 different concatenated datasets, in which none of them included EF-2. Later with the development of long-read genomic sequence analysis, the arguments against the Eocyte theory were disregarded even more, since the chance of mistakes due to homologous recombination declined significantly (Cai et al., 2020). Furthermore, the argument of lipid divide, where 2 different kind of lipids leads to an unstable membrane, and therefore the archaeal ancestor with glycerol-phosphate ether lipids could have never incorporated the eukaryotic glycerol-3-phosphate ester lipids, made the Eocyte hypothesis wobble again. However, Caforio (2017) succeeded in making a stable hybrid heterochiral membrane through lipid engineering of the bacterium *Escherichia coli*, again reinforcing the possibility of eukaryotic descent from an archaeal ancestor. Overall the research on Asgard Archaea by all the studies combined give a strong, plausible version of the origin of life. The metabolism mechanisms discovered, the symbiotic partner relationships, the ESPs, and the anoxic environment of the habitats Asgard members all strongly support the theories surrounding eukaryogenesis that start from an Archaeal Host cell. Thereby rejecting the original Woese theory that states that life originated out of 3 branches. The Isolation of the first Asgard species reinforced the assumptions by showing that in vivo the Asgard worked together in symbioses with a sulfate-reducing bacterium and an O_2 scavenging partner, leading to the E3 theory of eukaryogenesis, which is now a widely accepted model for eukaryogenesis. Starting from the E3 model, it is now possible to further analyze the process of eukaryogenesis and the historic timeline of the evolution eukaryotic cells went through, giving them the properties that lead to the diverse forms of life categorized in the Eukarya domain.
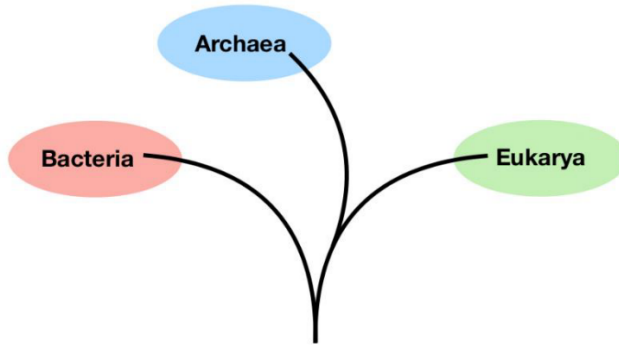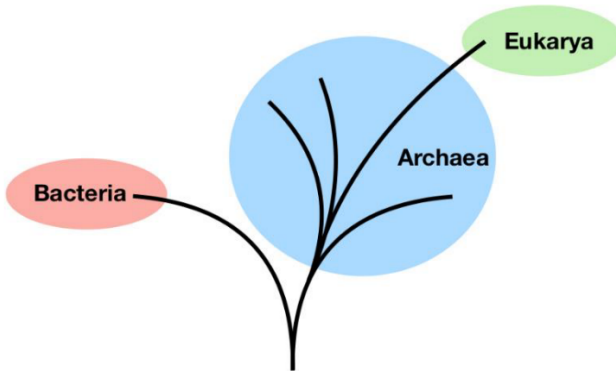
Fig.3: Trees of life. A: The Woese theory supporting the 3 domains of life; Bacteria, Archaea and Eukarya. B: 2 domains of life, supported by the Eocyte theory and the discovery of ESPs and the E3 model (Figure by Mcleod et al., 2019).

Conclusions

This review has highlighted the most important properties of members of the Asgard superphylum in regards to ecology, carbon metabolism, and other metabolic properties. Namely that all Asgard strains were capable of processing organic compounds and H_2 for energy, some were equipped with genes for aerobic respiration and some had genes that indicate special functionalities like nitrogen and sulfur cycling. Furthermore, the Isolation of the first Asgard species was described and put into perspective with already known properties and symbiotic relationships. The Asgard showed to likely live best in an anoxic environment with an O_2-scavenging partner and a sulfate-reducing bacterium. The discovery of ESPs and their possible functions in the Asgard species are described. Asgard showed to harbor genes for cytoskeleton remodeling, endosomal sorting, eukaryotic-like ubiquitination, nucleocytoplasmic transport, and vesicular trafficking. The E3 model described a possible history of the origin of eukaryogenesis and was based on the findings of ESPs and the metabolic and symbiotic processes in Asgard species. It showed a plausible theory for the Eocyte tree of life where the first eukaryotic cell descended from an archaeal host that probably closely resembled the Asgard Archaea of nowadays. More extensive research has to be done to see if all ESPs are expressed in Asgard Archaea and phylogenetic analysis has to be continued to find out the exact timeline of the process leading to the first eukaryotic cell.

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