Cultured Asgard Archaea Shed Light on Eukaryogenesis

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The first cultured Asgard archaeon lives in metabolic symbiosis with hydrogen-scavenging microbes. Its full-genome analysis authenticates the existence of Asgard archaea, previously only known from metagenome-assembled genomes, confirms their closer phylogenetic relatedness to eukaryotes and reinforces the idea that the eukaryotic cell evolved from an integrated archaeal-bacterial syntrophic consortium.

The recent cultivation of a tiny archaeon from the deep-sea by Imachi and co-workers (2020) is shaking the scientific community by providing new clues on the origin of the eukaryotic cell. Why?

The origin of the eukaryotic cell, or eukaryogenesis, long remained impenetrable. This major evolutionary transition led to an extraordinary diversity of unicellular, protist lineages and paved the way to the organized multicellularity observed in plants, fungi and animals. Because of the higher average complexity of eukaryotic cells, historical models of eukaryogenesis generally favored a prokaryote-to-eukaryote transition. However, accepting the crucial role of symbiosis in this process has taken time. Initially scorned, the endosymbiotic origin of mitochondria and chloroplasts popularized by Lynn Margulis was acknowledged since the 1970s. Yet, models proposing that eukaryotes resulted from a symbiosis directly involving bacterial and archaeal ancestors were deemed speculative (Lopez-Garcia et al., 2017). Rather, the prevalent scenario, inspired by Carl R. Woese's proposal of three primary domains of life, invoked an independent proto-eukaryotic lineage sister to archaea that developed most typical eukaryotic traits (complex cytoskeleton, endomembranes, phagocytosis, nucleus, etc.) before it acquired the alphaproteobacterial ancestor of mitochondria. Some reservation followed the realization that eukaryotes predating the mitochondrial symbiosis are not known and the suggestion by some phylogenomic analyses that eukaryotes might descend directly from archaea (Lopez-Garcia et al., 2017; Williams et al., 2019). Nonetheless, close archaeal relatives were not identified and the idea of a symbiotic origin of eukaryotes did not permeate cell biology. The situation radically changed with the improvement of sequencing and bioinformatic methods enabling the reconstruction of individual genomes from complex metagenomes (metagenome-assembled genomes, MAGs). MAGs affiliating to an uncultured lineage previously known from environmental 16S rRNA gene sequences (Deep-Sea Archaeal Group/Marine Benthic Group-B) contained several typical eukaryotic genes not shared by other archaea. Furthermore, phylogenetic analyses placed eukaryotes within this clade (including Loki-, Hela-, Thor-, Odin- and Heimdallarchaeota), which was renamed Asgard archaea in reference to the Norse pantheon (Spang et al., 2015; Williams et al., 2019; Zaremba-Niedzwiedzka et al., 2017). This strongly reinforced the idea of a cooperative, symbiogenetic origin of the eukaryotic cell. Many Asgard eukaryotic-like genes encoded membrane-remodeling proteins, which promoted the hypothesis that eukaryotes evolved from a complex archaeon that developed endomembranes and phagocytosis. Phagocytosis would have been essential for the engulfment of the alphaproteobacterial ancestor of mitochondria (Spang et al., 2015). In addition, MAG-based metabolic predictions suggested that, except for some potentially aerobic Heimdallarchaeota, Asgard archaea are anaerobic, in agreement with their natural anoxic environments (sediments, deep microbial mat layers), using hydrogen under autotrophic growth or producing hydrogen (or reducing equivalents) when growing on small organics such as peptides or other short-chain hydrocarbons (Spang et al., 2019). Based on inferred Asgard ancestral metabolisms and on the typical absence of strong electron acceptors in their natural ecosystems, eukaryotes were proposed to have evolved from a metabolic symbiosis (i.e., syntrophy, or cross-feeding), mediated by hydrogen and/or electron transfer between archaea and bacteria (Spang et al., 2019), rejuvenating similar ideas proposed two decades before (López-García and Moreira, 1999). However, the lack of Asgard archaea in culture prevented validating MAG-inferred metabolic capabilities and even raised skepticism in certain circles about their very existence.

Imachi and coworkers (2020) have now succeeded in culturing one Asgard archaeon, *Candidatus* Prometeoarchaeum syntrophicum MK-D1, from deep-sea sediment. As its name indicates, it lives in syntrophy with either a methanogenic archaeon, a sulfate-reducing deltaproteobacterium, or both (**Figure 1A**). This archaeon grows slowly; enriching it took over a decade. Genome prediction and stable isotope probing showed that Prometeoarchaeum can metabolize ten different amino acids and small peptides through syntrophic growth based on interspecies hydrogen (and/or formate) transfer with hydrogen-scavengers. High hydrogen itself inhibits Prometeoarchaeum growth, supporting the idea that it requires syntrophic hydrogen sinks. This Asgard archaeon displays striking morphology. The cells are very small, ~500 nm diameter on average, and lack organelle-like inclusions or endomembranes. However, they produce membrane vesicles, chains of blebs and extended protrusions, and also form aggregates with exopolymeric substances. Prometeoarchaeum's cell membranes possess, like all known archaea, typical archaeal phospholipids, which are very different from the bacterial-like eukaryotic phospholipids. Its complete genome (4.46 Mbp) contains 80 eukaryotic-like proteins that are also present in other Asgards. Several of them are highly expressed including, among others, cytoskeleton and membrane remodeling proteins (actin, ESCRTIII, small GTP-binding domain proteins). The predicted metabolic potential, shared with other Asgard archaea, confirms the observed metabolism. Although fermentation and more specific metabolisms are inferred for other Asgard archaea, comparative analyses suggest that their last common ancestor was an amino-acid-degrading anaerobe that produced H_2 and fatty acids as by-products, acquired ATP primarily from substrate-level phosphorylation by catabolizing 2-oxoacid intermediates and depended on metabolic partners (Imachi et al., 2020).

Based on these features, Imachi and co-workers (2020) proposed a new symbiogenetic scenario for eukaryogenesis: the entangle-engulf-endogenize (E^3) model (**Figure 1B**). Eukaryotes would have evolved around 2 billion years ago, when atmospheric oxygen started to accumulate, from an initial syntrophy similar to the one observed today between Prometheoarchaeum and its deltaproteobacterial sulfate-reducing partner. However, this hydrogen-scavenging bacterium would have been lost in favor of an aerobic alphaproteobacterium (future mitochondrion) that allowed aerotolerance through detoxifying oxygen. Because Asgard archaea have small cells and might not produce enough energy to carry out phagocytosis, Imachi et al. suggest that the alphaproteobacterium was engulfed by slow entanglement via cell-protrusion trapping. After engulfment, the host would have shared amino acid-derived metabolites (e.g. pyruvate, 2-oxobutyrate) with the endosymbiont that, in return, respired oxygen and provided the host with building blocks it could not synthesize. Two additional steps helped convert this consortium into the eukaryotic cell: the acquisition of an ATP transporter by the oxygen-respiring ATP-generating endosymbiont and the replacement of archaeal membrane lipids by bacterial ones.

The cultivation of *Candidatus* Prometeoarchaeum syntrophicum is important for several reasons. It corroborates the reality of Asgard archaea, putting to rest criticisms suggesting that these archaeal MAGs were artefacts of metagenomic binning or chimeras. On the contrary, beyond the generation of computer files, metagenomic binning has a formidable potential for the discovery of novel prokaryotic lineages. It also validates MAG-derived metabolic predictions and suggests that hydrogen-evolving amino acid degradation and syntrophic dependence on hydrogen-scavenging partners are widespread, likely ancestral traits of the Asgard superphylum (Imachi et al., 2020; Spang et al., 2019). This has implications for the origin of eukaryotes, supporting symbiogenetic models based on hydrogen-mediated syntrophy. However, contrary to previous predictions (Spang et al., 2015), Asgard archaeal cells do not exhibit internal cell complexity or phagocytosis capacity. This opens the possibility of mechanisms other than classical phagocytosis for endosymbiont incorporation. Nonetheless, although the cultivation of Asgard members reinforces a syntrophic origin for eukaryotes, the E^3 model entails

questions and challenges that are also shared by competing eukaryogenetic models that explicitly propose syntrophic interactions, such as the Syntrophy, the Reverse Flow or the Hydrogen hypotheses (Figure 1C-E). The classical Syntrophy and Hydrogen hypotheses were based on hydrogen transfer from bacteria to a methanogenic archaeal host (López-García and Moreira, 1999). Upon the discovery of Asgard archaea, the Reverse Flow model proposed an opposite flow of hydrogen or electrons from an Asgard archaeon to the bacterial ancestor of mitochondria (Spang et al., 2019). More recently, the Syntrophy and Hydrogen hypotheses have been updated to accommodate an Asgard-like archaeon instead of a methanogen. The Hydrogen hypothesis proposes that eukaryotes derive from an autotrophic Asgard archaeon that used hydrogen produced by the alphaproteobacterial ancestor of mitochondria (Sousa et al., 2016). The revised Syntrophy hypothesis proposes a tripartite symbiosis between a sulfate-reducing deltaproteobacterial host scavenged hydrogen from an endosymbiotic Asgard archaeon (future nucleus) and sulfate from a sulfide-oxidizing alphaproteobacterial endosymbiont (future mitochondrion) (López-García and Moreira, 2020). These models differ not only in the number and type of symbiotic partners but also in the timing and mechanisms leading to the evolution of typical eukaryotic traits (e.g. endomembranes and nucleus, mitochondrion) (López-García and Moreira, 2020). Two major questions remain unsolved in the E^3 model. The first, shared with all models proposing an archaeal host, is the archaeal-to-bacterial membrane transition. Indeed, no such transition has ever been observed and, although an engineered *Escherichia coli* strain can incorporate up to 30% archaeal phospholipids in its membrane, higher percentages of phospholipids impair growth and result in aberrant morphologies and asymmetric division (Caforio et al., 2018). This, together with the need to adapt the whole membrane proteome to a very different physicochemical lipid environment, might be important barriers to such lipid transition (López-García and Moreira, 2020). The second big challenge for the E^3, as for some other models, is to explain why (which selective forces) and how (which mechanisms) the eukaryotic nucleus evolved. Although Prometeoarchaeum is a modern archaeon and may not necessarily represent their 2-billion-year old ancestors, a deeper exploration of cell and molecular biology of Asgard archaea and their bacterial symbiotic partners, as well as their metabolic interactions and behavior in their natural microbial ecosystems, should help us realistically reconstruct the evolutionary path to the eukaryotic cell.

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FIGURE LEGEND

Figure 1. Syntrophy and Eukaryogenesis Models

(A) Metabolic symbiosis established between the Asgard archaeon *Candidatus* Prometeoarchaeum syntrophicum MK-D1 (center) and its deltaproteobacterial (left) and methanogenic archaeal (right) symbiotic partners identified in the mixed cultures established by Imachi and coworkers (2020).

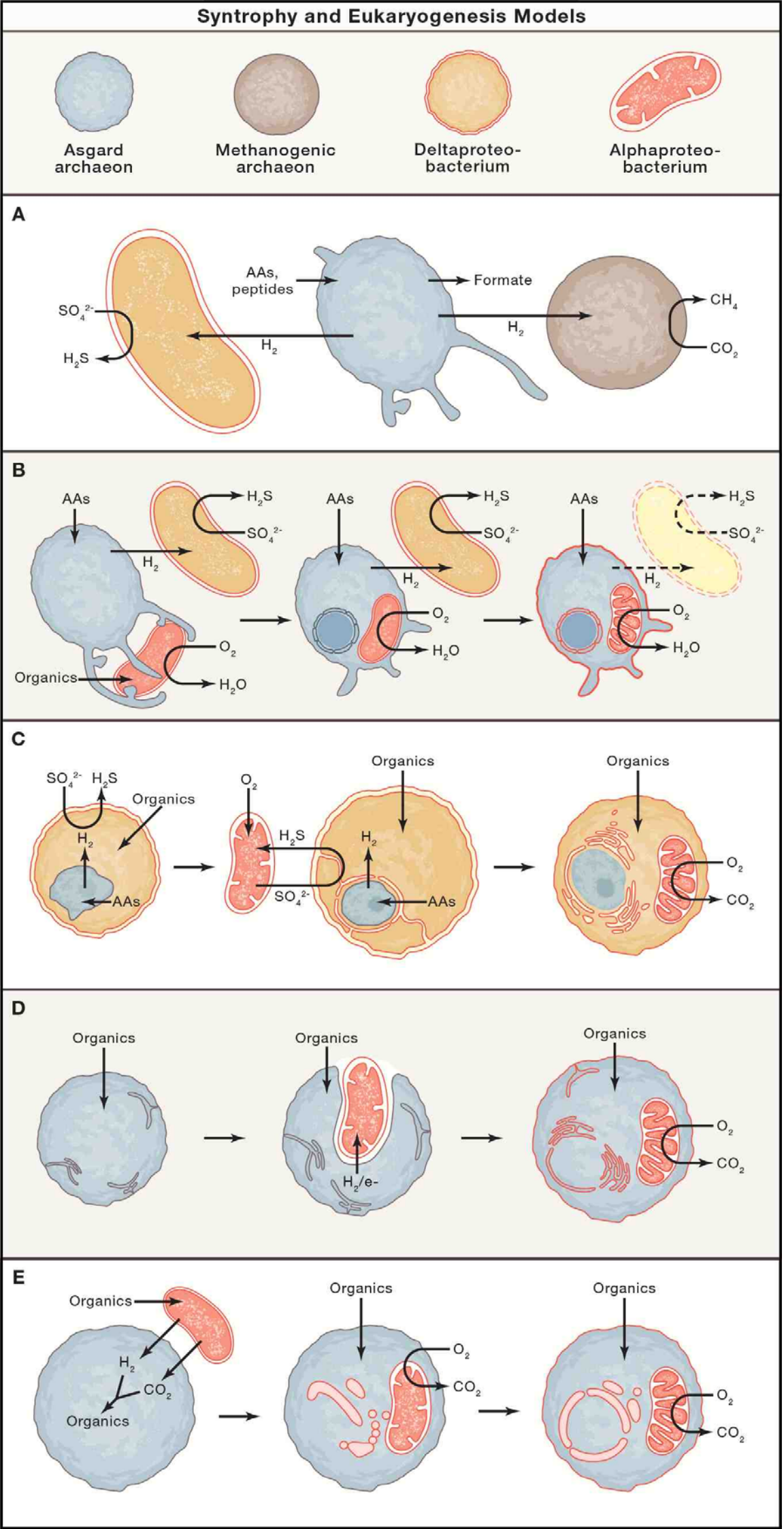
(B) The Entangle-Engulf-Endogenize (E^3) model (Imachi et al., 2020) proposes that eukaryotes evolved from a progressive internalization of an alphaproteobacterium (ancestor of mitochondria) inside a H_2-producing Asgard archaeon initially associated to a sulfate-reducing deltaproteobacterium that would later disappear from the consortium (indicated by discontinuous lines).

(C) In the revised Syntrophy hypothesis (López-García and Moreira, 2020), eukaryotes evolve from the initial incorporation of a H_2-producing Asgard archaeon (future nucleus) within a sulfate-reducing deltaproteobacterial host before the acquisition of the facultatively aerobic, sulfide-oxidizing alphaproteobacterial ancestor of mitochondria.

(D) The Reverse Flow model (Spang et al., 2019) involves a H_2-producing Asgard archaeon with a developed endomembrane system that incorporates by phagocytosis a H_2 (or electron)-consuming alphaproteobacterium (future mitochondrion).

(E) The revised Hydrogen hypothesis (Sousa et al., 2016) proposes the acquisition of a H_2-producing alphaproteobacterium by a hydrogen-dependent autotrophic archaeal host as the triggering event of eukaryogenesis.

With the exception of the Syntrophy hypothesis, all these models require an archaeal (blue) to bacterial (red) membrane phospholipid transition to explain the current eukaryotic membrane biochemistry (López-García and Moreira, 2020). Organics refers to small organic compounds (e.g. peptides, short-chain hydrocarbons, amino acids); AAs, amino acids.


Syntrophy and Eukaryogenesis Models
Asgard archaeon
Methanogenic archaeon
Deltaproteo-bacterium
Alphaproteo-bacterium
A
AAs, peptides
Formate
SO_4^{2-}
H_2S
H_2
CH_4
CO_2
B
AAs
H_2S
SO_4^{2-}
H_2
O_2
H_2O
Organics
C
SO_4^{2-}
H_2S
Organics
H_2
AAs
O_2
CO_2
D
Organics
H_2/e^-
O_2
CO_2
E
Organics
H_2
CO_2
O_2