# Eukaryogenesis, a syntrophy affair

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Eukaryotes evolved from a symbiosis involving Alphaproteobacteria and archaea phylogenetically nested within the Asgard clade. Two recent studies explore the metabolic capabilities of Asgard lineages, supporting refined symbiotic metabolic interactions that might have operated at the dawn of eukaryogenesis.

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Eukaryogenesis, the evolutionary process leading to the origin of the eukaryotic cell, has remained elusive for a long time. The endosymbiotic origin of mitochondria and chloroplasts from Alphaproteobacteria and cyanobacteria, respectively, became established 40 years ago. However, models proposing the symbiotic origin of eukaryotes directly from bacterial and archaeal ancestors, such as Lynn Margulis' serial endosymbiosis theory or the hydrogen and syntrophy hypotheses, were considered the heretical face to the prevailing orthodox view that a proto-eukaryotic lineage sister to archaea had evolved all typical eukaryotic traits (for example, phagocytosis and the nucleus) except for the mitochondrion[1–3]. In recent years, methodological advances and the exploration of microbial diversity in natural environments have allowed for the reconstruction of genomes of uncultured microorganisms from metagenomes, and have brought new light into eukaryogenesis by allowing for the discovery of the Asgard archaea. These deep-branching archaea share more genes with eukaryotes than other archaea, and phylogenomic analyses place eukaryotes within this clade[4,5]. This provides support for the occurrence of only two primary domains, bacteria and archaea, and for the symbiogenetic origin of eukaryotes[6]. According to this view, third, but secondary, domain of life. If the symbiogenetic origin of eukaryotes is now making its way to mainstream science, most existing models fail to propose plausible, detailed evolutionary processes and leave many questions unexplained[2], starting with the specific nature of the symbiotic interaction at the origin of eukaryotes. The most detailed models in this sense were the hydrogen and the syntrophy hypotheses which, 20 years ago, converged in proposing a metabolic interaction based on interspecies hydrogen transfer from a bacterial fermenter to an archaeal methanogen[7] (Fig. 1). In the hydrogen hypothesis, that bacterium was the endosymbiotic ancestor of mitochondria; in the syntrophy model, that bacterium was the host that incorporated an archaeon (future nucleus) and a second endosymbiont (future mitochondrion). At that time, although the occurrence of metabolic symbioses (syntrophies) between methanogenic archaea and Deltaproteobacteria was well known in oxygen-deprived systems, our knowledge about archaeal diversity and metabolism was extremely fragmentary. However, a plethora of archaeal phyla has been discovered since then. Although members of these lineages remain mostly uncultured, we can start unveiling their metabolic potential from genomes assembled from metagenomes of natural communities and proposing more plausible metabolic interactions at the onset of eukaryogenesis.

This is exactly what Spang et al.[8] and Bulzu et al.[9] have done in this issue of *Nature Microbiology* by reconstructing the metabolism of various Asgard archaeal lineages from metagenome-assembled genomes (MAGs), mostly from sediments. Asgard archaea exhibit high metabolic versatility. Loki- and Thorarchaeota likely fix carbon using the Wood–Ljungdahl pathway (WLP) and can derive electrons from diverse organic substrates, including complex carbohydrates, fatty acids, amino acids, peptides and alcohols. Lokiarchaeota also seem to have a reverse tricarboxylic acid cycle[9]. Although they might be able to grow lithoautotrophically on H2, Loki- and Thorarchaeota

likely use it to reduce CO2. They can also use formate as carbon or as an energy source. Loki- and Thorarchaeaota can link the oxidation of organics to the generation of membrane potential and produce hydrogen from fermentation. Thus, depending on environmental conditions, they can either consume or produce hydrogen and, if strong electron acceptors are lacking (as very often in anoxic sediments), they might engage in syntrophic interactions with hydrogen or formate scavengers. The presence of reductive dehalogenases additionally suggests that they can also shuttle electrons to organohalide compounds. However, whereas Lokiarchaeota use WLP in reverse to oxidize organics, Thorarchaeota possess a canonical respiratory chain complex I coupling the respiration of organics to the generation of an electrochemical gradient[8]. The thermophilic Odinarchaeota are able to ferment organics and can couple ferredoxin oxidation to respiratory H+ reduction.

While Loki-, Thor- and Odinarchaeota possess metabolisms typically associated with anoxic environments, Heimdallarchaeota, which so far seem the closest known archaeal lineage to eukaryotes, are somewhat different. They have also flexible metabolism and, like Odinarchaeota, some Heimdallarchaeota can ferment and couple ferredoxin oxidation to a respiratory chain for H+ reduction. Likewise, some can use organohalides as electron acceptors. Others use hydrocarbons as substrate. However, unlike the rest of their known Asgard relatives, some Heimdallarchaeota show a more oxygendependent metabolism, likely oxidizing organics using nitrate or oxygen as terminal electron acceptors. Furthermore, they contain the oxygen-dependent kynurenine pathway for NAD+ biosynthesis that is widespread in eukaryotes, which they likely imported horizontally from bacteria[9]. In addition, Bulzu et al.[9] identify the presence of several rhodopsins in Heimdallarchaeota, including putative type-1 proton pumps, the functionally enigmatic heliorhodopsins and a new family of rhodopsins discovered also in Loki- and Thorarchaeota — the schizorhodopsins. This suggests exposure to light, and therefore a habitat, linked to sediment surface.

These observations lead Bulzu et al.[9] to propose the ‘aerobic protoeukaryote’ model, stating that both symbiotic partners at the origin of eukaryotes, the bacterium and the archaeon, used oxygen for respiration in a microoxic niche without nonetheless advancing any specific metabolic (or other) interaction underlying the eukaryogenetic symbiosis. Inferring a fully aerobic metabolism for the archaeal partner may be premature. Not all Heimdallarchaeota are aerobic — oxygen and nitrate respiration having been imported from bacteria at a relatively later stage that is difficult to date[8,9]. In addition, our knowledge of archaeal diversity remains partial, as reveals the recent identification of a new Asgard lineage, the Helarchaeota, which anaerobically oxidize short-chain hydrocarbons[10]. It is therefore possible that other archaeal lineages even more closely related to eukaryotes exist. Indeed, one should always bear in mind the limits of actualism when trying to infer past metabolisms from modern organisms occupying a few terminal branches in phylogenetic trees. From this perspective, the inference of ancestral metabolism by a relaxed common denominator approach combined with phylogenetic information seems safer. Spang et al.[8] thus conclude that the last Asgard common ancestor thrived in anoxic environments, possessed the WLP, had the ability to use various organics — likely including fatty acids — and lacked terminal reductases for exogenous electron acceptors. It also had the capacity to either produce or consume hydrogen depending on the environmental conditions.

Based on this primeval metabolic potential and on the fact that syntrophic interactions are widespread in anoxic environments[3], Spang et al. propose the ‘reverse flow model’[8], stating that the eukaryogenetic symbiosis involved the transfer of electrons or hydrogen from one Asgard archaeon to the alphaproteobacterial ancestor of mitochondria. Although these authors invoke only one bacterium, they also leave open

the possible involvement of additional symbiotic partners8, as in recent suggestions of serial prokaryotic symbioses predating the mitochondrial acquisition11. Likewise, although Spang et al. favour an archaeon-to-bacterium flow of reducing equivalents, they do not discard the opposite flow, as proposed by the original hydrogen and syntrophy hypotheses, since Asgard archaea can be hydrogenogenic or hydrogenotrophic. The specific involvement of methanogens meant that the hydrogen and syntrophy hypotheses were less supported when Asgard archaea were discovered, since methanogens were only known within Euryarchaeota. The hydrogen hypothesis was updated to suggest that the archaeon might have been a non-methanogenic hydrogendependent autotroph12. However, although Asgard archaeal methanogens are not known, hydrogenotrophic methanogenesis has been discovered in other deeply branching archaeal phyla13, suggesting that methanogenesis and possibly shortalkane-based metabolism14 might have been ancestral in archaea. Whatever the directionality, the chances for eukaryotes to derive from a metabolic symbiosis involving electron or hydrogen transfer, as suggested long ago7, are becoming increasingly high. From this perspective, the reverse flow model adds fresh air to the field of eukaryogenesis. Future studies providing more informed comparative genomic evidence for early archaeal metabolism, as well as archaeal functional characterization and ecological interactions in anoxic and microoxic settings, should help to constrain evolutionary models for the origin of eukaryotes.

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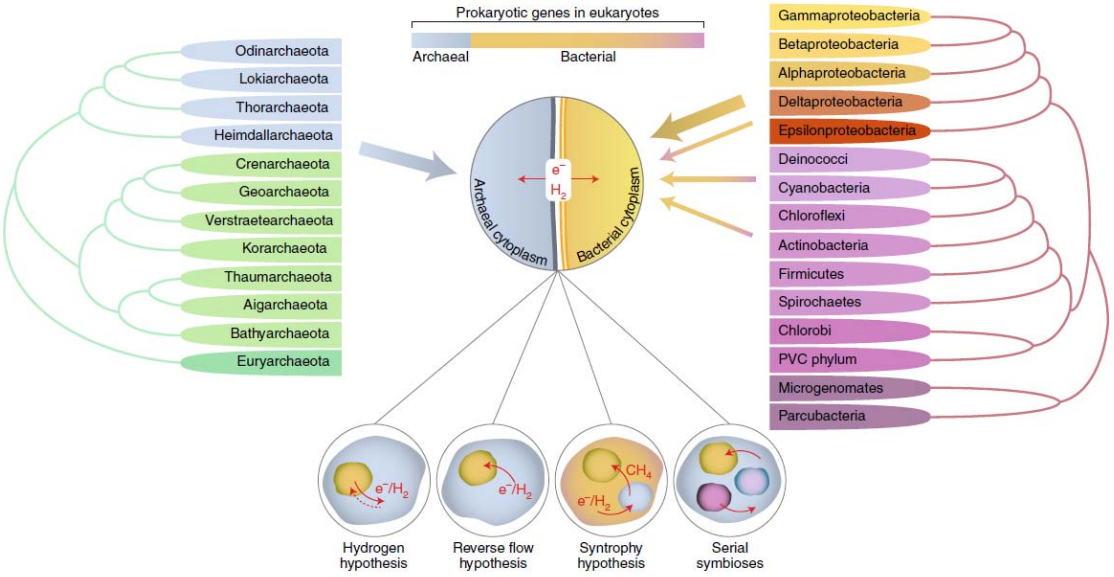


**Fig. 1 |** Metabolic symbiosis at the origin of eukaryotes. Current phylogenomic evidence supports symbiotic models for the origin of the eukaryotic cell. Eukaryotic genomes are mosaics containing a substantial number of genes (~1,000) of archaeal and bacterial ancestry that can now be traced to specific lineages[4,5,11]. This information supports the idea that eukaryotes evolved from a symbiosis between a member of the recently described Asgard archaea more closely related (so far) to the Heimdallarchaeota and, at least, the facultatively aerobic alphaproteobacterium that gave rise to the mitochondrion. Comparative analyses of the Asgard archaeal metabolic potential allow Spang et al.[8] and Bulzu et al.[9] to conclude that Asgard archaea were primarily organoheterotrophic organisms that can produce and consume hydrogen. Some Heimdallarchaeota also gained the capability to use oxygen and nitrate as final electron acceptors by horizontal gene transfer in a later stage. Based on Asgard archaeal metabolic reconstruction and ecological considerations, Spang et al.[8] propose the 'reverse flow model'. This refined symbiogenetic model for the origin of eukaryotes invokes a metabolic symbiosis, or syntrophy, mediated by hydrogen or electron transfer between archaea and bacteria. However, unlike the original hydrogen and syntrophy hypotheses, which proposed interspecies hydrogen transfer from the bacterial to the archaeal symbiont, the 'reverse flow model' involves electron or hydrogen flow from the archaeal to the bacterial symbiont. This eukaryogenetic syntrophy likely established in anoxic or microoxic environments[8,9]. Although the model specifically involves one archaeon and one bacterium, Spang et al.[8] leave open the possibility that symbiotic interactions with other prokaryotes might have intervened, in consonance with recent proposals for serial symbioses[11].