Think again

Energy for two: New archaeal lineages and the origin of mitochondria

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Metagenomics bears upon all aspects of microbiology, including our understanding of mitochondrial and eukaryote origin. Recently, ribosomal protein phylogenies show the eukaryote host lineage – the archaeal lineage that acquired the mitochondrion – to branch within the archaea. Metagenomic studies are now uncovering new archaeal lineages that branch more closely to the host than any cultivated archaea do. But how do they grow? Carbon and energy metabolism as pieced together from metagenome assemblies of these new archaeal lineages, such as the Deep Sea Archaeal Group (including Lokiarchaeota) and Bathyarchaeota, do not match the physiology of any cultivated microbes. Understanding how these new lineages live in their environment is important, and might hold clues about how mitochondria arose and how the eukaryotic lineage got started. Here we look at these exciting new metagenomic studies, what they say about archaeal physiology in modern environments, how they impact views on host-mitochondrion physiological interactions at eukaryote origin.

Keywords:
acetogenesis; Bathyarchaeota; endosymbiosis; eukaryotic origin; Lokiarchaeum; mitochondria

Introduction

The origin of eukaryotes is one of life's most important evolutionary transitions. The more we learn about eukaryote origin, the more the origin of mitochondria appears to have been the decisive step. From the energetic perspective, mitochondria had everything to do with eukaryote origin [1]. Energy metabolism in eukaryotes, whether aerobes or anaerobes, is typically the job of mitochondria [2], programmed cell death is governed by mitochondria [3]. The nucleus apparently arose in the wake of mitochondria [4]. Even the eukaryotic endomembrane system appears to come from mitochondria [5].

But the mitochondrion had to have a host. What do we know about the host that acquired the mitochondrion? Although, the vast majority of genes that the eukaryote ancestor possessed appear to come from mitochondria [6], in modern phylogenetic schemes that link the eukaryotic lineage to prokaryotes, the eukaryotes are depicted as emerging from within the archaea. This is because microbial systematics is done with rRNA [7], or more recently, with ribosomal proteins [8, 9]. Eukaryotes do indeed have archaeal ribosomes in the cytosol, and in contrast to the old tree of life [7], ribosomal proteins now link eukaryotes to specific lineages of archaea that people are discovering in marine sediment [10–12]. Metagenomics seems to be homing in on the host.

And what are metagenomic studies finding? They are finding new archaeal lineages, many incomplete genome assemblies, numerous contigs, and interesting phylogenetic trees. About a year ago, the Lokiarchaeum lineage caused quite a stir [11]. Previously characterized as a member of the Deep Sea Archaeal Group [13, 14], its draft genome assembly was pieced together from sequences in marine sediment. It was reported as a "complex" cell that provides a missing link between prokaryotes and eukaryotes [11], though no one can yet say how "complex" it is or not, because no images of its cells are available.

The ancestor of complex cells (eukaryotes) had mitochondria

A slight detour is in order at the term "complex." When we read the word "complex" in the context of the prokaryote–eukaryote divide, the first thing that comes to mind is eukaryote-type cell complexity, that is, having an endoplasmic reticulum, membrane traffic, a nucleus, and the like. There was a time about 20 years ago, when people thought that

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the most ancient complex cells ("primitive" or "early branching" eukaryotes [18]) never had mitochondria. They were called archezoa [15], and they comprised anaerobic eukaryotic lineages like *Giardia* and *Trichomonas* that were thought to be deep diverging and were thought to lack mitochondria. Accordingly, many 1990's versions of the endosymbiont hypothesis for the origin of mitochondria typically envisaged an anaerobic archaeon that became complex, phagocytotic, and acquired, through phagocytosis, a mitochondrion that in such theories is seen as an undigested food bacterium that imparted oxygen respiration to eukaryotes [16, 17].

It turned out that the archezoa were neither early branching nor did they lack mitochondria. The trees were full of artifacts, the eukaryotic anaerobes were not primitive early branching lineages, rather they branched among 5–6 eukaryotic supergroups, members of which had mitochondria [18, 19]. And they possessed mitochondria after all, but reduced forms of mitochondria called hydrogenosomes and mitosomes [2, 20–23]. Hydrogenosomes are anaerobic mitochondria, that generate H_2 as an end product of fermentative ATP synthesis via substrate level phosphorylation [2], while mitosomes seem to have no function at all in ATP synthesis, having functions in FeS cluster assembly [24] and sulfur metabolism [25] instead.

Even though the archezoa theory failed in every respect in that every prediction it generated turned out to be wrong, the reincarnation of archezoa in the form of the "phagocytosing archaeon theory" [26] or as "archaea capable of primitive phagocytosis" [27] has re-emerged. Rather than resurrecting the archezoa, it seems to us that the field should be considering alternative theories that directly account for the data. The predictions of some of those theories have fared quite well.

Endosymbiosis and eukaryote origin

There are a number of theories out there for eukaryote origin [28–30]. One such alternative – one that is particularly relevant in the context of Lokiarchaea [31] – is the hydrogen hypothesis [32]. It suggested that mitochondria did not arise via phagocytosis (organelle origin through indigestion), but that they arose through anaerobic syntrophy [33, 34] instead: one cell living from molecular hydrogen that is the end product of another organism's fermentative metabolism. The hydrogen hypothesis suggested that the mitochondrion's ancestor was a facultative anaerobe that was able to respire (as perserved in mitochondria), but also able to perform anaerobic H_2-producing fermentations (as preserved in hydrogenosomes), and that the host was H_2-dependent. In that view, H_2 was the selective force that brought the mitochondrial ancestor into physical association with its host, initially in symbiotic association of free-living microbes [33, 34], later as an endosymbiont within the host. Metabolic interactions and gene transfer led to establishment and integration of the mitochondrial ancestor [32, 35] and its conversion to an ATP-producing organelle, which provided the energy required to make the major evolutionary transition from a union of prokaryotes into a eukaryotic cell [1]. Ecological specialization to aerobic and anaerobic niches brought forth aerobes and anaerobes [32] in all eukaryotic supergroups, as well as a few eukaryotes with facultative anaeobic mitochondria as well, for example *Euglena* [2]. Other hypotheses involving syntrophic interactions have been suggested to account for eukaryote origin [36, 37], but they do not readily account for the observation that mitochondria of various eukaryotic lineages still generate H_2 today [2].

How did the mitochondrial ancestor gain entry to the cytosol of its host? The phagotrophic theory maintains that the host ingested the mitochondrial symbiont, but did not eat it all up [16, 17, 26, 27]. From the standpoint of physiology, metabolic interactions, and energetics, the list of problems with the phagotrophic theory is long [1, 5, 30]. In fact, we can think of no data at all that actually speaks in favour of the phagotrophic theory, it is just the way that some people prefer to think about the origin of eukaryotes problem. For example, some authorities have espoused a phagotrophic origin of eukaryotes, regardless of whether eukaryotes are being derived from a phagotrophic cyanobacterium [38], from a phagotrophic proteobacterium [39], or from a phagotrophic sister of archaea [40]. Some biologists clutch to phagotrophy as the unwaivering constant in a world of changing theories and new data on eukaryote origin, shoehorning phagotrophic origins of mitochondria into congruence with whatever phylogenetic data might come along [41]. Nonetheless, in the real world, many fungi harbour bacterial endosymbionts [42–44] even though no fungi are phagotrophic (it is part of the definition of the group). Clearly, the fungi alone demonstrate that phagocytosis is not required for bacteria to become endosymbionts within other cells, though that circumstance will not faze proponents of phagotrophic eukaryogenesis. The reasons why some scientists cling to phagotrophy as their favorite step at eukaryote origin is not our concern in this paper. Our concern is phylogeny and physiology at eukaryote origin.

Some theories have it that the endosymbiont came to reside within a prokaryotic host without requiring the host to have been phagocytotic as the mechanism of entry [30, 32, 36, 37]. The hydrogen hypothesis posits that prokaryotes can come to live within other prokaryotic cells, and indeed, in the real world, symbioses in which prokaryotes live within other prokaryotes are known [45–47]. By contrast, neither phagotrophic archaea nor true archezoa have yet been observed. At face value, the phagocytosing archaeon theories predict that we should find phagocytosing cells that never possessed mitochondria, for which there is no evidence so far. By contrast, the hydrogen hypothesis predicted that all cells that phagocytose should possess or should have possessed a mitochondrion. That prediction has fared quite well. But its predictions went further.

The hydrogen hypothesis clearly predicted interleaving of aerobes and anaerobes in eukaryotic phylogeny, which phylogenetics bore out: anaerobic mitochondria-bearing lineages are found among all currently recognized eukaryotic supergroups [48]. It predicted the presence of mitochondria in the eukaryote common ancestor, which subsequent studies also bore out [2, 49, 50]. Because the hydrogen hypothesis posits that the mitochondrial ancestor was a facultative anaerobe [32], it accounts naturally for the various forms of anaerobic mitochondria that are found across diverse eukaryotic lineages and for the circumstances (i) that eukaryote

anaerobes typically use the same small set of enzymes for anaerobic energy metabolism; and (ii) that anaerobic energy metabolism in eukaryotes is typically linked to mitochondria [2, 49]. Furthermore, it predicted that the host should be an archaeon, branching within the archaea, not as the sisters to archaea, which phylogenies also came to support [8, 9, 11]. Moreover, it generated a very radical and specific prediction that the host lineage was not phagotrophic, but should turn out to be hydrogen-dependent instead. The identification of novel archaeal lineages that branch more closely to the host lineage than any others raised the question of whether they are phagotrophic or hydrogen-dependent.

While Spang et al. [11], like others [27], discussed the possibility that Lokiarchaeon (Loki) might be phagotrophic or on its way to becoming phagotrophic, no one had actually reported investigations of Loki's metabolism. We had a look at its genome from the standpoint of energy metabolism, and we found in Loki's metagenome data considerable evidence that Lokiarchaeon is hydrogen-dependent [31]. That bears out one of the most specific predictions of the hydrogen hypothesis and can be seen as evidence providing support in its favor [32], although it is clear that the search for new archaeal lineages has just begun. At the same time, people have begun to look at some of the key genes initially suggested to support a "complex" lifestyle for Loki: the first results do not bear out expectations that Loki has "complex" cells as it concerns Rab GTPases [51] or proteins involved in membrane traffic [52]. This is because (i) the proteins that Spang et al. [11] interpreted as Loki's Rab GTPases turn out to lack in particular characteristic membrane-anchoring motifs that define eukaryotic Rab GTPases [51]; and because (ii) certain domain fusions that are crucial to proteins associated with eukaryotic membrane traffic are not present in the Loki metagenome [52].

Complexity and the endomembrane system

A new twist has recently come into the issue of eukaryotic cell complexity: it concerns the origin of the eukaryotic endomembrane system. In roughly 50 years of thoughts on the topic, evolutionary cell biologists have come up with two basic ways to derive the eukaryotic endomembrane system: from invaginations of the plasma membrane and through hypothetical cellular fusion processes involving symbiotic interactions that did not entail the origin of mitochondria (reviewed in refs. [28–30]). Recently, a new, simpler, and more natural way to account for the origin of the eukaryotic endomembrane system was proposed that takes the outer membrane vesicles that prokaryotes produce into account [5]. In that model, the basic structure of the eukaryotic endomembrane system stems from outer membrane vesicles secreted by the bacterial ancestor of mitochondria, which accumulated in the cytosol of its archaeal host at mitochondrial origin. Bacterial outer membrane vesicles have been known for decades [53]. In the context of endosymbiotic theory, if they are produced by the mitochondrial endosymbiont in the host's archaeal cytosol, this generates a primitive endoplasmic reticulum function upon which selection can act and from which the nuclear membrane is derived, just as it occurs in the cell cycle of modern eukaryotes [5]. Outward vesicle flux from the mitochondrion to the host's plasma membrane would also account for the chemical transformation of the host's plasma membrane from archaeal lipids to bacterial lipids, including the loss of chemiosmotic energy conservation at the host's plasma membrane and the transition to mitochondrial ATP synthesis in eukaryotes [5]. Clearly, mitochondria and gene transfer were important at eukaryote origin [1, 6].

Metagenomics also bears on the lipid biosynthesis issue at eukaryote origin. A recent phylogenomic analysis proposes that Loki, as well as some uncultivated archaeal MGII/III lineages, might lack the capacity to synthesize typical G1P archaeal lipids, but might be able to synthesize G3P-based lipids, which might reflect a transitional stage in terms of membrane lipid biosynthesis [54]. If so, such lipids should be out there in the environment, an exciting prospect. But as with carbon and energy metabolism (see Patchwork metabolism in metagenomes section) the lack of complete genomes and cultured strains for these new lineages impairs progress: When genes are missing in the metagenome, there is always the nagging question of whether they are really missing in the genome or whether are they are present in the genome but just missing in the metagenomic assembly. Conversely, when surprising genes crop up in a metagenome, the nagging question is then whether they stem from the same genome corresponding to a ribosomal RNA or ribosomal protein tree, or whether they are just common in the environment where the new lineage lives. At present, these questions are not easily answered. So, as exciting as the new archaeal metagenome lineages are, one can not be sure, from the genomic data, about how these microorganisms are making a living in their environment. Once data on complete genomes, growth, cell morphology, and the like become available, they will help to sort out what really belongs to Loki, what is in prokaryotes from the same environment, and what is missing. Whether the organisms behind the new archaeal metagenomics turn out to look like eukaryotes, as some suspect [27], or like normal archaea, as we suspect [31], are suspenseful questions. From our perspective, the endosymbiotic origin of mitochondria appears to have had more far-reaching consequences than most ever envisioned. And as we stated at the outset: from our perspective, at eukaryote origin, mitochondria seem to have been the decisive step [1–6, 19, 30–32, 35].

Patchwork metabolism in metagenomes

Coming back to metabolic interactions: What, exactly, is Loki doing with H_2? Hydrogen dependent archaea typically use H_2 as a source of electrons for their core bioenergetic reactions, for example, sulfur reduction [55] or CO_2 reduction in the case of methanogens [56–58]. We found a complete archaeal version of the Wood–Ljungdahl (WL) pathway (also called the acetyl-CoA pathway) in the Loki genome, a typical pathway of autotrophic CO_2 fixation [57], and the pathways that hydrogenotrophic methanogens use to synthesize methyl groups during methanogenesis, the process by which they generate their ion gradient for ATP synthesis [58, 59]. When we looked

at the Lokiarchaeum genome from a bioenergetic perspective to see if we could find evidence for its core ATP-producing reaction, we could not find the canonical enzymes of methane synthesis or any kind of respiration, sulfur-based, or otherwise [31]. So despite having an archaeal WL pathway, it is not completely clear at present how Loki makes a living in terms of core energy metabolism (ATP synthesis).

But Loki is not alone in that respect, as many other novel lineages of archaea are currently being described from anaerobic environments [10, 12] and those metagenomes are also not clearly revealing which types of energy metabolism are keeping these cells alive. For example, Evans et al. [10] described new archaeal lineages (Bathyarchaeota) from marine sediment that have the archaeal version of the acetyl-CoA pathway, but have no clear evidence of known forms of archaeal energy-conserving metabolism. In very recent work, He et al. [12] reported Bathyarchaeota lineages that appear to be performing a very simple and suspected primitive form of energy metabolism that, in nature, is otherwise only known from bacteria so far: acetogenesis.

While bacterial acetogens and archaeal methanogens both possess the acetyl-CoA pathway, they harbor very different versions, the bacterial one being folate-dependent, the archaeal one being methanopterin-dependent [57]. Importantly, the enzymes of the archaeal and bacterial WL pathways are generally not related at all [60], the acetyl-CoA synthase/carbon monoxide dehydrogenase and its associated methyltransferases being exceptions [60].

The pathway that He et al. [12] propose for the Bathyarchaeota is noteworthy in that it combines two kinds of energy metabolism into one. As one part of this dual energy conservation proposal, they suggest that Bathyarchaeota can live from anaerobic fermentations of amino acids and cell wall components, producing H_2 as an end product of that process. This would not be spectacular in itself, except that – in contrast to bacterial fermenters – archaeal fermenters tend to use environmental sulfur as an electron acceptor in anaerobic fermentations, typically involving an ion pumping membrane protein called Mbx [61–63]. Accordingly, these are not really fermentations in the strict sense, because an environmental electron acceptor ($S \pm 0$) is used, such that their energy metabolism is sometimes called "facilitated fermentation" [64]. Though many archaeal fermenters can use protons as terminal electron acceptors [63], they typically do so via a membrane bound hydrogenase complex called Mbh, which couples electron transport to the generation of a transmembrane ion gradient [63], and subunits of which were reported in three out of the six new bathyarchaeal metagenomic lineages [12]. So if the Bathyarchaeota are generating H_2 from fermentations, they are doing something that appears trivial, and that is very widespread among bacteria, though bacterial fermenters typically use soluble hydrogenases, while archaeal fermenters typically come equipped with Mbx related sulfur reducing complexes [63].

The other part of their proposal has it that Bathyarchaeota can use the H_2 and CO_2 from fermentations to fuel acetogenesis. This is also noteworthy, in two respects. First, archaeal acetogens have not been found in nature so far, though some archaea can grow as acetogens under specific laboratory conditions. For example, a *Methanosarcina* strain can grow acetogenically for a short time when grown on CO [65]. So archaeal acetogenesis in the wild would be something new. The second point of interest is that the metabolic scheme that He et al. [12] present has the cell gaining energy from acetogenesis either with H_2 and CO_2 from the environment, or with H_2 and CO_2 that it generates itself from fermentations. If that is true, then it would be a kind of intracellular syntrophy where one cell lives from its own metabolic end products.

That hardly seems shocking, one might think: Why provide food and energy for the community when one can have it all one's self? But prokaryotes are not generally greedy: they often live and let live, even when the opportunity to "take it all" is there. One example is nitrification, the conversion of ammonia to nitrate with the help of oxygen [66]. Usually the process is divided among different prokaryotes, one group synthesizing ATP by oxidizing ammonia to nitrite and another group synthesizing ATP by oxidizing nitrite to nitrate. Only recently were bacteria characterized that perform both processes within the same cell [66], which might be advantageous in environments where substrates are very limited. Another example is the observation of cross-feeding in laboratory chemostat cultures: a pure strain evolves into two strains, the second living from the waste products of the first [67]. Other examples of cross feeding (syntrophy) are given in Costa et al. [68].

We have noticed [31] that the repertoire of enzymes in central intermediary metabolism that are found in the published Lokiarcheum genome [11] is generally quite similar to that found in the Bathyarchaeota [10, 12]. Might Lokiarchaeum have both a fermentative and an acetogenic type of metabolism? Before enrichment cultures are available that will permit some physiological measurements, it is impossible to say. In fact, the only thing that is absolutely certain at the moment is that nobody knows exactly how these new archaeal lineages are making a living in their anaerobic environments. It might be that they require acetoclastic methanogens in syntrophic association to remove acetate [12]; it might be that they are embedded in more complicated syntrophic interactions entailing sulfate reduction [10]; it might be that they have acetogenic energy metabolism but satisfy their carbon needs heterotrophically. It could be that none of the above is true, or that all of the above are true, but that Lokiarchaeota and Bathyarchaeota are generalists of sorts that can adjust their mode of growth according to environmental conditions.

As fascinating as the new archaeal lineages are, the sobering truth is that there are still no cultured representatives and no closed (complete) genomes for these new groups. Furthermore, the metagenomic data still leave much room for interpretation, because many crucial genes are missing, for example, the rotor stator ATPase in many lineages [10] or missing soluble hydrogenases, which would likely be required for both H_2 production and acetogenic growth, in the case of some of the He et al. [12] Bathyarchaeota lineages. Are they really missing, or are the metagenomes just incomplete?

It is clear that the new lineages have to be anaerobes, because where they live, no oxygen is available. Acetate, formate, CO_2, and H_2 play an important role in

such environments [33, 59, 69]. The search for clues to energy metabolism among these new archaeal groups is guided by what is known only from a few model anaerobes: methanogens [57], acetogens [70], and fermenters [58]. It is also complicated by the circumstance that in anaerobic niches H_2 partial pressures can determine whether an organism gains energy in the CO_2 reducing or CO_2 generating reaction, for example *Thermacetigenium phaeum* [71, 72], which can grow in either direction, depending on H_2 partial pressure. This is why, in reference to the Bathyarchaeota metagenomes in their study, Evans et al. [10] discuss both syntrophic methane oxidation (a metabolism possible only at very low H_2 partial pressures) and acetogenesis, which requires higher H_2. In anaerobic sediments, thermodynamics has the last word on what is possible [73], syntrophic interactions are very common [33] and the thermodynamics of acetogenesis in deep environments are becoming increasingly important [74].

Hydrogen transfer in symbiotic associations

Coming back to mitochondrial origin, the hydrogen hypothesis [32] posits that the symbiotic association that led to the origin of mitochondria entailed anaerobic syntrophy [33] between a H_2-producing endosymbiont and a H_2-dependent host. Other symbiotic models for eukaryote origin also invoke anaerobic syntrophy, albeit in the opposite direction, with the archaeon producing H_2 rather than consuming it [28]. One model involves syntrophic interactions between an anaerobic methane oxidizer, viewed as the ancestor of the nucleus, and a sulfate reducer, which is suggested to have served as the host for a hypothetical endosymbiotic origin of the nucleus [75]. As in the case of H_2 dependent methanogens interacting with H_2-producing bacteria [33, 34], symbiotic interactions between anaerobic methane oxidizers, and sulfate reducers are quite common in anaerobic environments. Recent advances indicate that sulfate-dependent anaerobic methane oxidation does not involve H_2 transfer, though, rather electrons appear to be directly transferred from the membrane of the archaeal methane oxidizer to the sulfate reducers [76, 77]. Those fascinating new findings underscore the ability of microbes to undergo tight metabolic interactions, but as a metabolic model for mitochondrial origin, they do not accommodate the ancient H_2-producing ability of mitochondria [2].

In the case of anaerobic methanotrophic consortia, the archaeal Wood–Ljungdahl pathway, which is reversible [57], is likely running backwards [76, 77]. Might the same be true of Loki and – more to the point of this paper – might the same have been true at the origin of eukaryotes? This is where evolutionary genome analysis can help discriminate between alternatives: eukaryote genomes do not reveal evidence for participation of sulfate reducers at eukaryote origin [6]. Rather the genes that eukaryotes share with prokaryotes only uncover the participation of an archaeon and an alpha-proteobacterium in the eukaryote common ancestor [6], as the hydrogen hypothesis predicts. In addition, the hydrogen hypothesis has fared very well when it comes to accounting for the H_2-producing ability of modern mitochondria, the proteins they share with hydrogenosomes, and the widespread occurrence of anaerobic mitochondria among eukaryotic lineages [2, 31, 49]. One can invoke scenarios that are more complicated than the hydrogen hypothesis and entail more symbiotic partners. But, when it comes to accounting for the origin of mitochondria in a manner that naturally accounts for hydrogenosomes, a H_2-producing endosymbiont in a H_2-dependent host entails the bare minimum of possible partners. As with the origin of the endomembrane system [5], alternative models can get more complicated, but they cannot get simpler. In the realm of theories for eukaryote origin, we view simplicity as a virtue, and physiology as a good guide.

Conclusion

Much of the interest in the new archaeal lineages is phylogenetic. The metagenomic lineages link the host at mitochondrial (and eukaryote) origin to archaea closer than ever before. But they also have implications for the very earliest phases of evolution on Earth, because the metagenomic lineages also now place methanogenesis at the base of the archaea [9], which fits very well indeed with the predictions of those theories for the origin of life that have microbial metabolism arising at serpentinizing (alkaline, H_2-producing) hydrothermal vents [57, 78, 79]. The finding of He et al. [12] that some archaea appear to be acetogenic also fits very well with that view. But the main course of the new metagenomic lineages will be unraveling and pinning down their carbon, and energy metabolism, because only then will we have a better understanding for how they survive in nature. That in turn should enrich our understanding of metabolic interactions at the origin of mitochondria.

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