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Lokiarchaeon is hydrogen dependent

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The nature of the host that acquired the mitochondrion at the eukaryote origin is an important microbial evolutionary issue. Modern phylogenetics indicates that the host was an archaeon. The metagenome sequence of *Candidatus* Lokiarchaeon has identified it as being the closest relative of the host yet known. Here, we report comparative genomic evidence indicating that Lokiarchaeon is hydrogen dependent, as one theory for the eukaryote origin—the hydrogen hypothesis—predicts for the host lineage.

The origin of eukaryotes is a critical evolutionary transition[1]. Recent metagenome data indicate that '*Candidatus* Lokiarchaeon' (Loki) is, by the measure of ribosomal protein phylogeny, the closest known free-living archaeal relative to the host[2] that acquired the mitochondrion. Loki's genome sequence lists several protein families that link archaea with eukaryotes, including ESCRT proteins, ubiquitin domain proteins and eukaryotic-type Ras GTPases, prompting discussion of the possibility that the mitochondrion might have been acquired by an archaeon that possessed primitive phagocytotic capabilities[2,3]. Here, we show that Loki's genome harbours a complete tetrahydromethanopterin (H_4MPT)-dependent Wood–Ljungdahl pathway and enzymes revealing it to be a hydrogen-dependent, strictly anaerobic and very probably autotrophic archaeon, attributes of the host that are specifically predicted by the hydrogen hypothesis for the origin of eukaryotes[4]. In archaea studied to date, ESCRT proteins are involved in cell division[3,5], not phagocytosis, and the presence of an H_4MPT-dependent Wood–Ljungdahl pathway indicates a hydrogen-dependent autotrophic lifestyle. Loki's genome data, although incomplete[2], shed light on the physiology of archaeal lineages thought to be closely related to the host and implicate a role for hydrogen dependence[4] and anaerobic syntrophy[4], rather than phagotrophy[2,3], in acquisition of the mitochondrion at the eukaryote origin.

In search of evidence for the physiology of Loki in its metagenome, we first asked whether it harbours the hallmark pathway of hydrogen-dependent anaerobic autotrophs: the Wood–Ljungdahl (or acetyl-coenzyme A (acetyl-CoA)) pathway[6]. The acetyl-CoA pathway exists in two forms, a tetrahydrofolate (H_4F)-dependent bacterial version and an H_4MPT-dependent archaeal version[6]. Loki possesses a complete methyl synthesis branch of the H_4MPT-dependent acetyl-CoA pathway (Fig. 1). It also possesses an almost complete pathway for the biosynthesis of H_4MPT, the typical archaeal one-carbon carrier and the cofactor that underpins the archaeal acetyl-CoA pathway[6] (Fig. 1). Two subunits of bifunctional carbon monoxide dehydrogenase/acetyl-CoA synthetase (CODH-ACS) in the acetyl-CoA pathway are missing, but the Loki genome is estimated to be only about 90% complete[2].

The H_4MPT-dependent acetyl-CoA pathway is characteristic of hydrogen-dependent archaeal anaerobic autotrophs[6,7]. The Loki genome lacks almost all genes that are specific to methanogenesis and anaerobic methane oxidation. Of the ion-pumping MtrA-H complex, only MtrH (a methyl-H_4MPT-dependent methyltransferase subunit)[8] is present, and methyl-CoM reductases essential for methanogenesis and anaerobic methane oxidation[9] are missing. Autotrophs that fix CO_2 via the acetyl-CoA pathway use pyruvate synthase (pyruvate:ferredoxin oxidoreductase, PFO) to generate pyruvate[6], and Loki possesses an archaeal PFO (Fig. 1).

Further evidence for the anaerobic lifestyle of Loki can be drawn from the presence of several enzymes that perform flavin-based electron bifurcation (Supplementary Table 2), a newly characterized mechanism of energetic coupling that is widespread among anaerobes[7]. Loki's electron bifurcating enzymes include NADH-dependent ferredoxin:NADP oxidoreductase[7] (NfnAB) fused to a coenzyme F_{420} hydrogenase subunit B (FrhB) domain having high similarity with the sulfide dehydrogenase (SudHI/II) from *Pyrococcus furiosus*, the electron transfer flavoprotein Bcd/EtfBC[7] and a complete hydrogen-dependent electron bifurcating MvhADG-HdrABC hydrogenase system including the Hyp NiFe maturation system[7] required for nickel insertion, cyanide ligand synthesis from carbamoyl phosphate, and cyanide transfer to the active site of the MvhA subunit. The MvhADG-HdrABC hydrogenase, which is so far specific to anaerobes[7], provides additional evidence for Loki's hydrogen dependence.

We detected no genes for membrane complexes involved in chemiosmotic energy coupling, including those typical for anaerobic autotrophs: Rnf/Nqr complexes[7], the Mrp/hydrogenase family of complexes that includes complex I, Ech[7] and Mbh, were missing, as were components of mitochondrial-type respiratory chains as well as the HdrE/NarI/FdnI cytochrome-containing enzymes typical of anaerobic respiratory chains. An incomplete A_0A_1 ATP-synthase (subunits *a*, A(β), B(α) and D(γ)) was present, but a eukaryotic-type vacuole-acidifying (protease-activating) vacuolar ATPase, required in free-living eukaryotes for acidifying food vacuoles to activate digestive enzymes during phagotrophy[10], was not. Neither quinone interacting proteins nor haem-containing enzymes were detected, although sirohaem proteins are present in the current Loki genome, suggesting that it might be capable of sulfur reduction[11].

The enzymes of Loki's H_4MPT-dependent Wood–Ljungdahl pathway were most similar to methanogen homologues (Supplementary Table 2), providing important evidence for the existence of methanogen-like metabolism outside the euryarchaeotes[2]. Metagenomic data have uncovered the existence of further methanogen-like metabolisms outside the euryarchaeotes[9], but, as with Loki, the energy metabolism of the organisms behind these new metagenomic lineages is unknown. Loki's central intermediary metabolism (Supplementary Table 2) resembles that of *Candidatus* Methanoplasma termitum[12].

Our characterization of Loki as an anaerobic, hydrogen-dependent autotroph, as predicted by the hydrogen hypothesis, does not support the view that the host for the origin of mitochondria was a phagotroph[2]. This distinction needs to be seen in light of three decades of dispute about the identity of the host cell that acquired mitochondria[13,14]. The archezoa hypothesis argued that the host cell was a phagotroph that engulfed and digested food particles such as bacteria[13,14]. It predicted that at least some modern archezoa

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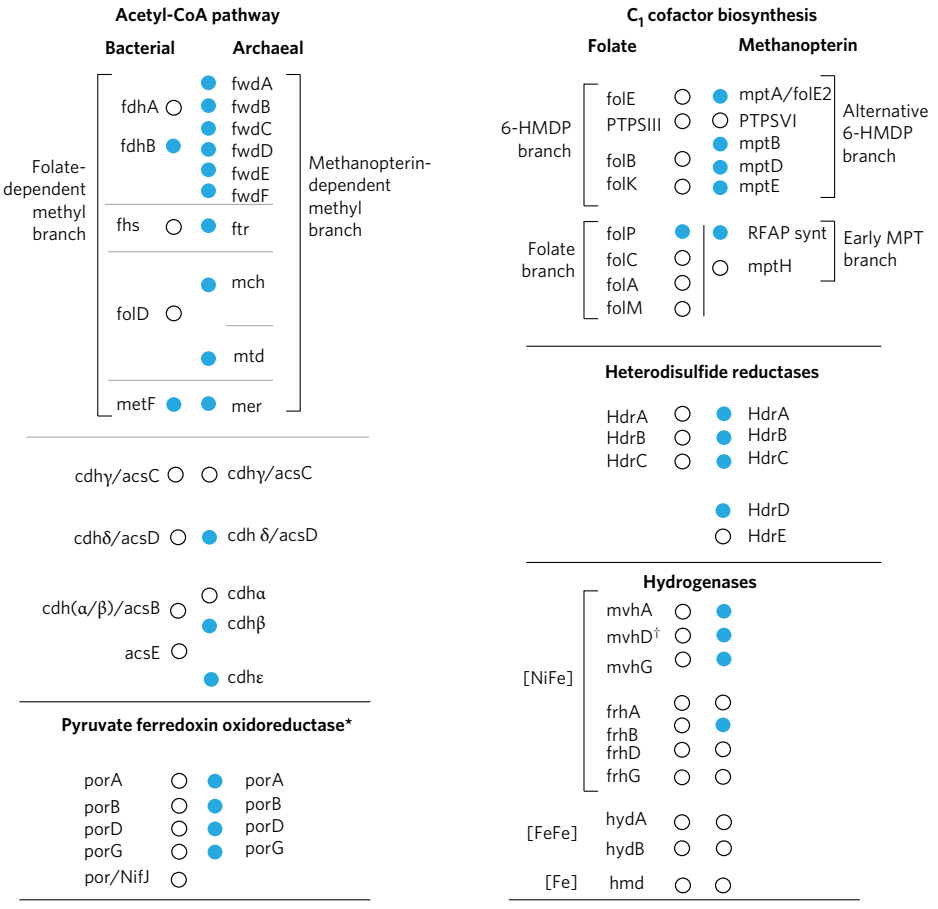

Figure 1 | Identification of typical genes of H_2-dependent, anaerobic autotrophs. Typical genes were identified using query sequences for well-characterized proteins specific to the pathways indicated in the figure in sequence comparisons, then scoring whether the proteins in the Lokiarchaeon genome annotation[2] were more similar to archaeal or bacterial homologues (Supplementary Tables 1 and 2). Filled circles indicate that the gene is present in the Lokiarchaeon genome at an *E*-value threshold of 10^{-10} and at least 25% local amino acid identity. Open circles indicate that the gene was missing at that threshold or that the Lokiarchaeon sequence was more similar to homologues from the domain indicated. fdhAB, formate dehydrogenase; fhs, 10-formyl-H_4F-synthetase; folD, 5,10-methenyl-H_4F cyclohydrolase/dehydrogenase; metF/MTHFR, 5,10-methylene-H_4F reductase; acsE, corrinoid iron–sulfur protein methyltransferase; cdhδ/acsD, corrinoid iron–sulfur protein CoFeS; cdh(α/β)/acsB, cdhγ/acsC carbon monoxide dehydrogenase/acetyl-CoA synthase; fmd/fwdABCDEF, formyl-methanofuran dehydrogenase; ftr, formyl transferase; mch, 5,10-methenyl-H_4-MPT cyclohydrolase; mtd, 5,10-methylene-H_4-MPT dehydrogenase; mer, 5,10-methylene-H_4-MPT reductase; cdhγ/acsC, methyl-H_4-MPT:corrinoid iron–sulfur protein methyltransferase; corrinoid iron–sulfur protein cdhγδ/acsD; CO dehydrogenase/acetyl-CoA synthase cdhα, cdhβ, cdhε; porABCD, nifJ, pyruvate:ferredoxin oxidoreductase; folE, 7,8-dihydroneopterin triphosphate synthase; PTPSIII, pyruvoyltetrahydropterin synthase; folB, 7,8-dihydroneopterin aldolase; folK, diphosphokinase; folE2, GTP cyclohydrolase IB; mptA, Fe(II)-dependent-GTP cyclohydrolase IB; PTPSVI, pyruvoyltetrahydropterin synthase; mptB, Fe(II)-dependent-cyclic phosphodiesterase; mptD, 7,8-dihydroneopterin aldolase; mptE, 7,8-dihydro-6-hydroxymethylpterin diphosphokinase; RFAP synt, β-D-ribofuranosylaminobenzene-5-phosphate synthase; mptH, dihydropteroate synthase; folP, dihydropteroate synthase; folC, dihydrofolate synthase; folA, folM, dihydrofolate reductase; HdrABC and HdrED, heterodisulfide reductase; mvhADG, methyl viologen hydrogenase complex; frhABG, coenzyme F_{420} hydrogenase; hydAB [FeFe] hydrogenase; hmd, [Fe] H_2-dependent methylene-tetrahydromethanopterin dehydrogenase. *Sequences from this family can include ketoisovalerate oxidoreductase (vorABCD) and 2-oxoacid:ferredoxin oxidoreductase (korABCD). †The detected mvhD gene may be fused with one HdrA domain.

were evolutionary intermediates, survivors from early eukaryotic evolution before the acquisition of mitochondria. This hypothesis has been discounted with the demonstration that all the supposed archezoa in fact arose by reductive evolution from more complex eukaryotic ancestors, with their mitochondria specializing to become hydrogenosomes or mitosomes[14]. Phylogenetic studies, including the Loki study[2], have since established that the host cell was an archaeon and therefore a *bona fide* prokaryote[1]. Yet the idea that the host cell somehow had to be a phagocyte to have acquired mitochondria has risen again with the Lokiarchaeon genome report[2].

How an archaeal host cell, presumably with a cell wall, acquired mitochondria is unknown, but examples of walled prokaryotes that have acquired bacterial endosymbionts do exist[15], so it is plainly possible. There is no need to invoke a phagotrophic host cell and no evidence that one ever existed. On the contrary, bioenergetic considerations indicate that the host cell could hardly have been phagotrophic[15]. Phagocytosis requires a fully fledged eukaryotic cytoskeleton, food-vacuole formation and complex intracellular membrane vesicle trafficking[10], none of which is known among prokaryotes[10]. The machinery of phagocytosis has a high energetic cost, not only in terms of cytoskeletal motility, cell movement and dynamic vesicle trafficking, but even more importantly in its requirement for high expression of many hundreds if not thousands of genes[3]. The energetic costs for the *de novo* evolution of complex traits such as phagocytosis in terms of exploration of protein sequence space and gene expression must be substantially greater than the costs of maintaining the system once it has evolved. Yet bacteria and archaea are strictly limited in their size and energy availability per gene by their bioenergetic architecture, in particular their use of the plasma membrane for chemiosmotic coupling[15].

In contrast, mitochondria internalize respiration and always retain tiny specialized bioenergetic genomes and ribosomes[16]. This uniquely eukaryotic bioenergetic architecture enables the local control of chemiosmotic coupling, increasing the energy availability per gene by orders of magnitude relative to prokaryotes[15]. Although a recent theoretical study of adenosine triphosphate (ATP) cost accounting concluded that mitochondria might not have been essential to the eukaryote origin after all[17], the modelled cell had an unlimited (unconstrained) ATP supply, thereby missing the role of mitochondria at the eukaryote origin altogether[18], while the cost accounting furthermore failed to include ribosomes[17], the energetically most expensive component of eukaryotic cells[18]. Only the enormous energy boost provided by mitochondria could power the *de novo* evolution of the machinery of phagocytosis, explaining why such an apparently advantageous mode of living is completely absent in prokaryotes[15].

The data presented (Fig. 1) here indicate that Loki is hydrogen dependent, strictly anaerobic and probably autotrophic. Its hydrogen dependence needs to be included in discussions about the metabolic context of mitochondrial origin[2,3]. For thermodynamic reasons[19], hydrogen dependence would preclude a heterotrophic carbon metabolism for Loki. Because phagocytosis requires heterotrophy it is therefore unlikely that Loki possesses a level of cellular complexity[2] that would include phagocytotic abilities.

By the measure of ribosomal protein phylogeny, Loki is indeed the closest known relative of the host identified so far[2]. The available genomic evidence suggests that Loki is hydrogen dependent, bearing out central predictions of the hydrogen hypothesis[4] with regard to the nature of the host lineage. The further predictions are clear. Under a microscope, Loki will not be a large archaeon with a eukaryote-like feeding habit[2,3]. It will, instead, be a strictly anaerobic, hydrogen-dependent[4] archaeon.

Methods

Sequences for 394 proteins involved in carbon and energy metabolism across diverse prokaryotic groups were used to query Loki's genome using BLAST (ref. 20) using as threshold 25% amino acid identity and an *E*-value of 10^{-10} (query sequences, references as well as their accession numbers are listed in Supplementary Table 1). Lokiarchaeon genes showing matches at the specified threshold were blasted against RefSeq (version 72) and the first ten matches were parsed against NCBI taxonomy. All results are summarized in Supplementary Table 2.

Accession codes. The master record for the Loki composite genome (JYIM00000000), which links to the 504 contigs that represent the Loki genome (GenBank accession codes JYIM01000001–JYIM01000504) was downloaded from GenBank (September 2015). All the accession numbers of the query sequences, Lokiarchaeon sequences and respective ten first hits are given in Suplementary Tables 1 and 2.

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Author contributions

F.L.S. and W.F.M. designed the research. F.L.S. and S.N. performed the analysis. J.F.A. and N.L. assessed and commented on the results and conclusion. All authors discussed the results. F.L.S., J.F.A., N.L. and W.F.M. wrote the paper.

Additional information

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Competing interests

The authors declare no competing financial interests.