Chapter 12
Recent Developments on Bacterial Evolution into Eukaryotic Cells

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Abstract This article stems from the presentation of Mauro Degli Esposti at the 19th Meeting of Evolutionary Biology held in Marseille and summarizes recent developments that have emerged in regard to the evolution of the eukaryotic cell. After updating the application of recently introduced approaches for evaluating the likely bacterial relatives to the symbionts that originated the mitochondrial organelles, the article examines the possibility that such symbionts might have engaged in a syntrophic association with ammonia-oxidizing archaea. The bacterial ancestors of mitochondria could have provided the ammonia required for chemoautotrophic growth of the archaean host, the nitrite by-product of which could have been subsequently recycled back to ammonia by the pathway of nitrate assimilation that is shared by a few proteobacteria and several eukaryotes. The similarity of this syntrophic association with the symbiosis between nitrogen-fixing bacteria and plants is discussed in detail. The article then explores the possible presence of relics of archaean lipids in current eukaryotes, a fundamental problem that weakens current views on the evolution of the eukaryotic cell.

12.1 Introduction

This article discusses recent developments on the origin of mitochondria and related organelles of eukaryotic cells from bacterial ancestors following the presentation by Mauro Degli Esposti at the Marseille conference of September 2015. According to the current consensus, these bacterial ancestors became symbionts of an archaean host that is considered to be related to a group of archaea (Spang et al. 2015; López-García and Moreira 2015; Koonin 2015) including organisms capable of nitrification, i.e. oxidation of ammonia to nitrite (Brochier-Armanet et al. 2012;

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Hatzenpichler 2012). One peculiar metabolic trait that is present in some fungi entails the opposite reaction, namely the reduction of nitrite to ammonia for re-oxidizing NADH under anaerobic conditions (Takasaki et al. 2004). This reaction of the nitrogen cycle is part of the pathway of bacterial and fungal nitrate assimilation (Moreno-Vivián and Ferguson 1998), the crucial enzyme of which is a large Mo-containing enzyme, NADH-dependent nitrate reductase coded by the *NiaD* gene in fungi (Johnstone et al. 1990) and by the *NapA* gene in enterobacteria (Moreno-Vivián and Ferguson 1998). The multidomain enzyme and its operon constitute crucial clues to identify the closest relatives to proto-mitochondria (Degli Esposti et al. 2014), the bacterial ancestors of contemporary organelles.

The article is structured in three parts. Initially, it surveys recent phylogenomics data that expand and refine previously proposed clues and traits that can be used to identify bacterial relatives of proto-mitochondria, including assimilatory nitrate reduction (Degli Esposti et al. 2014). Subsequently, the article elaborates on the recurrent presence of such traits in bacteria that form mutualistic symbiosis with plants, often producing syntrophic associations based upon the exchange of nitrogen compounds (Martinez-Romero 2012). Finally, it presents a brief overview on the membrane lipids that are present in eukaryotes and may derive from either the bacterial guest or the archaean host that contributed to eukaryogenesis (cf. Koonin 2015).

12.1.1 Part 1. Screening Extant Proteobacteria to Identify Relatives to Proto-Mitochondria

The origin of eukaryotic cells constitutes one of the major transitions in evolution and derives from a unique event of symbiosis between different prokaryotes (Martin et al. 2015). Recent developments have solidified the view that the host in this symbiotic event was an archaean organism related to the TACK superphylum (Koonin 2015), while the guest was a proteobacterial organism that then evolved into current mitochondria. The general consensus is that such a guest belonged to the alpha class of proteobacteria (Müller et al. 2012; Martin et al. 2015; Ku et al. 2016; Gray 2015), even if the exact lineage remains unclear and controversial—for a review, see: Müller et al. (2012), Degli Esposti (2014), Gray (2015). The controversy primarily derives from the intrinsic limits of phylogenetic and phylogenomic analyses applied to such an ancient event as eukaryogenesis, which probably occurred between 1.5 and 2 billion years ago (Müller et al. 2012). Recently, three complementary approaches have been introduced to identify current relatives of proto-mitochondria (Degli Esposti et al. 2014). These approaches stem from a detailed analysis of bacterial genomes available at the beginning of 2014, which is updated here in the light of the additional genomic information accumulated in the last two years. Figure 12.1 illustrates the conceptual basis for the approaches: Mitochondria share genomic signatures and metabolic traits not just with alpha proteobacteria, but also with facultatively anaerobic beta and gamma proteobacteria

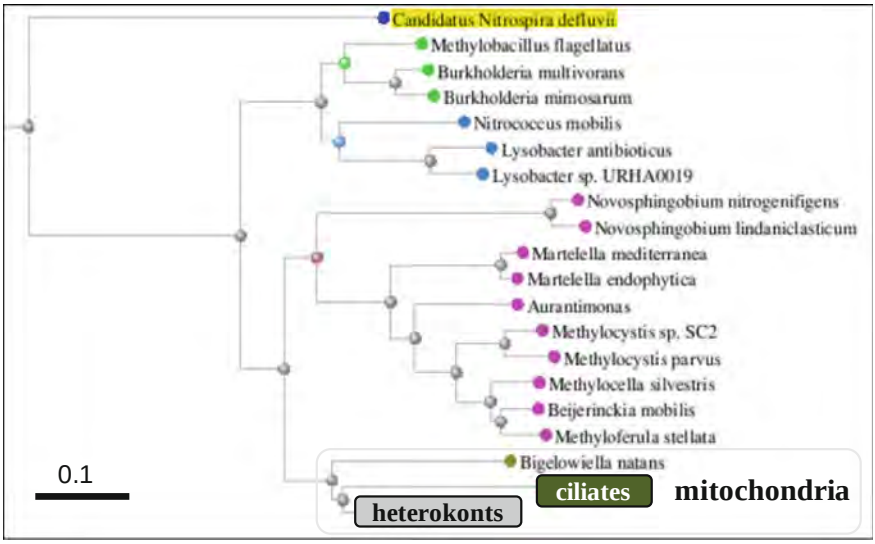

Fig. 12.1 Typical phylogenetic tree of a very conserved subunit of respiratory complex I, NuoD. The neighbor-joining tree of the ubiquinone-binding subunit NuoD (also called Nad7 or 49-kDa subunit in mitochondria) was obtained using the tools of the DELTABLAST facility as described previously (Degli Esposti et al. 2014, 2015). The NuoD protein from the Nitrospirae, *Ca. Nitrospira defluvii* (accession: CBK40015), was used as a query—hence it is highlighted in yellow. This protein appears as the outgroup of the proteobacterial and mitochondrial sequences in the tree view, which was rooted on the homologous large subunit of CO-induced hydrogenase from the rhizobial *Pleomorphomonas* (cut-off from view). Colour codes for the bacterial taxa are as follows: *purple*, alpha proteobacteria; *green*, beta proteobacteria; *pale blue*, gamma proteobacteria. The bottom branch contains the mitochondrial homologues from the following taxa: the Rhizarian *Bigelowiella natans* (Cercozoans), 5 ciliates (*dark green box*) and 4 heterokonts including the brown alga *Ectocarpus silicosus* plus the Ascomycete *Aspergillus flavus* (*grey box*) which have *NiaD* nitrate reductases. Note how the sequences of the mitochondrial and alpha proteobacterial proteins form part of the same clade that is sister to the clade containing the sequences of beta and gamma proteobacteria, as previously reported for COX1 and COX3 (Degli Esposti 2014)

(Degli Esposti 2014), consistent with the mosaic nature of bacterial inheritance in eukaryotes (Gray 2015).

The tree shown in Fig. 12.1 underlines a fundamental aspect of the phylogenesis of mitochondrial proteins, which is often overlooked. Such proteins cluster together in a clade that contains all their close homologues of alpha proteobacteria and is sister to the clade formed by their homologues in beta and gamma proteobacteria. Hence, mitochondrial proteins are effectively related to their homologues from all alpha proteobacterial taxa and not just a specific branch of such bacteria as often claimed, a concept that has been overlooked even in recent studies of phylogenomics (Pittis and Gabaldón 2016). The closeness may derive from the proteins or genes chosen for the phylogenetic analysis and is heavily influenced by phenomena of long-branch attraction for obligate endocellular parasites such as Rickettsiales (Searcy 2003; Brinkmann and Philippe 2007; Rodríguez-Ezpeleta and Embley 2012; Gray 2015).

Table 12.1 Bacteria possessing *NiaD*-like nitrate reductase of fungi and their relatives

alpha with NiaD-like nitrate reductase	COX operons of type a	relatives with COX operon type a-II	nitrogenase	metabolic traits
Methylocystis parvus	a & a-I	Methylocystis sp. SC2	yes	methanotroph, close to *Spagnum* endosymbionts
Beijerinckia mobilis	?	Methylocella silvestris, Methyloferula stellata	yes	methylotroph, sporadic plant endophyte
Martelella endophytica	a-I ?	Aurantimonas coralicida, Aurantimonas manganoxydans	yes	plant endophyte
Martelella mediterranea	a-I ?			
Martelella sp. AD-3	a			
Novosphingonbium nitrogenifigens	COX operon type a-II	Sphingonbium banderi, Sphingomonas	yes	diazotroph
Novosphingobium sp. P6W	COX operon type a-II & a			aromatic degrading, rarely plant endophyte
Novosphingobium lindaniclasticum	COX operon type a-II & a			
beta with NiaD-like nitrate reductase				
Methylobacillus flagellatus	COX operon type a-II			methylotroph, related to plant endophytes
Burkholderia mimosarum	COX operon type a-II		yes	plant N2 fixing symbiont
Burkholderia sp. CCGE1002	COX operon type a-II		yes	plant N2 fixing symbiont
Burkholderia xenovorans	COX operon type a-II		yes	polychlorinatred biphenyl degrading
Burkholderia sprentiae	COX operon type a-II		yes	
Burkholderia sp. JPY251	COX operon type a-II		yes	
Burkholderia sp. WSM4176	COX operon type a-II		yes	
Burkholderia sp. CCGE1003	COX operon type a-II			
Burkholderia sp. 9120	COX operon type a-II			
Burkholderia sp. WSM2230	COX operon type a-II			
Burkholderia multivorans	COX operon type a-II & a			
gamma with NiaD-like nitrate reductase				
Methylomicrobium album BG8	COX operon type a-II	Methylobacter luteus, Methylosarcina fibrata, Methylobacter tundripaludum	in relatives	methanotroph, close relative of endosymbionts of clams in hydrothermal vents
Methylomicrobium agile	COX operon type a-II			

The presence of a precursor form of fungal *NiaD* nitrate reductase, defined as *NiaD*-like, is probably the rarest trait that extant alpha proteobacteria share with eukaryotes (Degli Esposti 2014). Currently, about 30 alpha proteobacterial organisms contain this protein and the associated *NirB* nitrite reductase in their genomes. Besides various members of the Acetobacteraceae family, they include three *Novosphingobium* and *Martelella* organisms, two *Beijerinckia* species and the methanotroph *Methylocystis parvus* (Table 12.1 and data not shown). Conversely, the same proteins are present in many enterobacteria such as *Klebsiella* (Lin et al. 1993), two methanogenic gamma proteobacteria and a variety of beta proteobacteria, predominantly of clade I of the *Burkholderia* group (Sawana et al. 2013) (Table 12.1 and data not shown). Among such organisms, Acetobacteraceae and enterobacteria can be excluded from mitochondrial ancestry because they do not possess active aa3-type cytochrome oxidases (COX). These oxidases are assembled in eight different operon types in proteobacteria: one in some delta, one shared by both beta and gamma, another specific to alpha only (COX operon type b) and the rest present in alpha, beta and gamma proteobacteria (Degli Esposti 2014). Although the mitochondrial subunits of eukaryotic cytochrome oxidase generally resemble those of alpha proteobacterial COX operon type b, a few fungi have mitochondrial COX1 proteins containing an additional transmembrane helix at the C-terminus. The protein sequence around this additional helix shows signatures that are typical of the bacterial COX4 subunit, which is characteristically fused to COX1 in COX operon type a-II (Degli Esposti et al. 2014). Currently, five fungal species belonging to four different orders of Ascomycetes have this usual COX1 protein: the plant pathogen *Zymoseptoria tritici* (Testa et al. 2015), *Sclerotinia borealis* (Mardanov et al. 2014), *Peltigera membranacea* (Xavier et al. 2012), *Paracoccidioides brasiliensis*

(Desjardins et al. 2011) and *Trichoderma reesei* (Martinez et al. 2008), as well as the Heterolobosea *Acrasis kona*.

The presence of mitochondrial COX1 proteins resembling those of COX operon type a-II has previously suggested that the ancestors of mitochondria must have possessed this operon in combination with COX operon type b (Degli Esposti et al. 2014). Therefore, the bacteria that possess in their genome both such COX operons could be considered relatives of proto-mitochondria. If we combine this trait with the presence of *NiaD*-like nitrate reductase, only the organisms listed in Table 12.1 remain, defining a restricted group of taxa that may have the largest probability of being close to proto-mitochondria (Degli Esposti et al. 2014). Intriguingly, many of such organisms can fix N_2, since they possess a complete nitrogenase system (Table 12.1). They often are endophytic or symbionts, or are close relatives of organisms that engage in symbiotic interactions (Table 12.1). Among the taxa exhibiting these traits, the beta and gamma organisms listed in Table 12.1 clearly cannot be close to proto-mitochondria, which are known to be related to alpha proteobacteria (Gray 2015). However, they indicate that proto-mitochondria might have been close to the common ancestors of the alpha, beta and gamma proteobacteria that combined *NiaD*-like-driven assimilatory denitrification with terminal oxidases including COX operons of type a.

The major novelty of the abovementioned analysis regards three strains of *Novosphingobium* (Table 12.1), a metabolically versatile alpha proteobacterium belonging to the order of Sphingomonadales, which has been previously considered among the possible ancestors of mitochondria (Finnegan et al. 2003). However, *Novosphingobium* sequences of the Rieske iron sulphur protein of the cytochrome bc_1 complex, a protein with strong phylogenetic signal hereafter abbreviated as ISP, present a characteristic insert of five amino acids at the C-terminus (Cimit5, Fig. 12.2), which is absent in mitochondrial homologues (Degli Esposti et al. 2014).

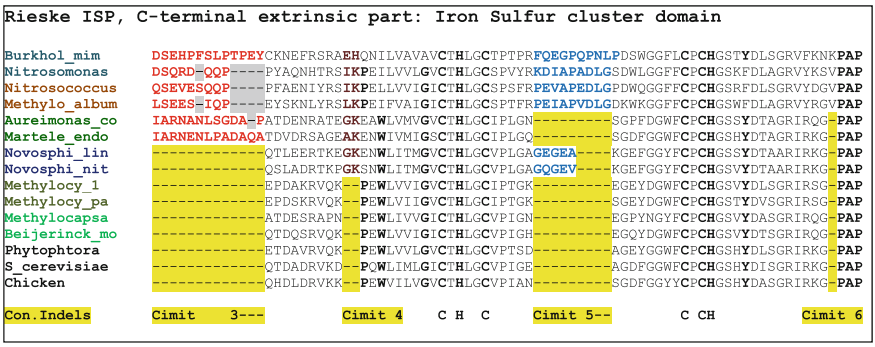

Fig. 12.2 Alignment of the cluster domain of the Rieske ISP of bacteria and mitochondria. The alignment was manually refined using the latest coordinates of the 3D structure of various mitochondrial and bacterial ISP proteins (Berry et al. 2004) as previously reported (Degli Esposti et al. 2014), using the same nomenclature for the conserved insertions and deletions (called Cimit). The residues of the Cimit4 deletion are in *dark purple*, while the residues of the Cimit3 and Cimit5 *insert* are shown in *red* and *blue*, respectively. The ligand residues of the iron sulphur cluster are in bold character and indicated at the bottom of the alignment

Conversely, the ISP sequence of *Martelella* and related organisms presents a larger central insert (Cimit3, Fig. 12.2), which is also absent in mitochondrial homologues as well as in the ISP sequence of *Beijerinckia* and *Methylocystis* (Fig. 12.2 cf. Degli Esposti et al. 2014). Hence, the application of the third approach introduced by Degli Esposti et al. (2014), namely the inspection of conserved INDELs in the ISP sequence (Fig. 12.2), restricts the possible relatives of proto-mitochondria to a few methylotrophs of the genera *Beijerinckia* and *Methylocystis* among all the organisms now found to possess both *NiaD*-like and COX operon type a-II proteins listed in Table 12.1. These findings sustain earlier conclusions that pointed at such organisms as the most likely relatives to the ancestors of mitochondria (Degli Esposti 2014).

It is of interest to consider the potential evolutionary relevance of the preservation of the assimilatory nitrate reductase system in eukaryotes from ancestral proto-mitochondria (Degli Esposti et al. 2014), enabling the pathway of ammonia fermentation present in some fungi (Takasaki et al. 2004). In the framework of syntrophic interactions, alpha proteobacterial symbionts producing ammonia would provide a thriving niche for ammonia-oxidizing archaea, usually abbreviated as AOA and classified among the Thaumarchaeota (Brochier-Armanet et al. 2007, 2012; Hatzenpichler 2012; Raymann et al. 2015). Thaumarchaeota are now included in the TACK superphylum (Guy and Ettema 2011), to which the ancestor of eukaryotic cells belong (Spang et al. 2015; López-García and Moreira 2015; Koonin 2015). Examples of microbial communities of Thaumarchaeota AOA associated with facultatively anaerobic proteobacteria (predominantly gamma, but also alpha) have been documented in diverse environments characterized by gradients of oxic–anoxic conditions (Muller et al. 2012; Hatzenpichler 2012; Tetu et al. 2013; Hawley et al. 2014; Gagliano et al. 2016). In particular, the following genera of alpha proteobacteria have been found in significant abundance within such communities: the methylotrophs *Beijerinckia* and *Methylocystis* in volcanic soils (Gagliano et al. 2016), *Reugeria* and unidentified Rhizobiales in marine low oxygen strata (Hawley et al. 2014). In the latter case, metabolic associations with nitrite-oxidizing organisms of the *Nitrospira* group have been proposed (Hawley et al. 2014). The key role of the abundant *Nitrospira* organisms in the nitrogen cycle has been recently emphasized by the discovery of *Nitrospira* strains capable of carrying out the complete process of nitrification; i.e., they oxidize ammonia to nitrite besides oxidizing nitrite to nitrate (Daims et al. 2015). In theory, the association of AOA with such organisms would be unnecessary if primordial archaean lineages would carry the same capacity of complete nitrification, which might have been subsequently lost along evolution. However, organisms of this kind have yet to be discovered.

Ammonia is produced geochemically in specific hotspots (e.g. see Gagliano et al. 2016); however, in most biological environments, it derives from atmospheric N_2 fixed by bacteria such as rhizobia (Ormeño-Orrillo et al. 2013), while ammonia produced via assimilatory pathways is usually consumed within bacterial cells. Hence, bacterial guests capable of N_2 fixation under low oxygen concentrations, as well as nitrate assimilation under anaerobic conditions, would be ideal syntrophic partners for nitrifying archaean hosts. Ancient organisms close to the root

separating Nitrospirae, which are the dominant nitrite oxidizers in most environments (Hawley et al. 2014; Daims et al. 2015), from (alpha) proteobacteria might have engaged in syntrophic associations with AOA, which can oxidize ammonia only to nitrite. Alternatively, proto-mitochondria might have possessed enzymes for the oxidation of nitrite under aerobic conditions, e.g. the copper-containing *NirK* and nitrite reductase of the *NirB* type as in fungi for the reverse assimilatory reaction under anaerobic conditions. Genomic and bioinformatic analyses indicate that, so far, there is no extant alpha proteobacterium combining nitrogenase with NADH-dependent nitrite reductase for assimilatory production of ammonia, together with nitrite oxidoreductase for the oxidation of nitrite produced by a hypothetical syntrophic archaean. Consequently, it is necessary to consider a syntrophic consortium with *Nitrospira* organisms to support the hypothetical symbiosis between AOA and N_2-fixing, nitrate-assimilating alpha proteobacteria, of which there are several extant examples among currently described organisms (Table 12.1). Moreover, the archaean host might have provided carbon metabolites, e.g. pyruvate, as a fuel for N_2-fixing guests under both high and low concentrations of oxygen, given the versatility of carbon fixation pathways in current AOA (Zhalina et al. 2012). A similar exchange of carbon metabolites exists in the well-studied symbiosis between N_2-fixing bacteria and plants, as discussed below.

12.1.2 Part 2. Syntrophic Associations Between Bacteria and Plants Based on Nitrogen Compounds

Symbiosis of different bacteria and their hosts are widespread in nature and can enhance the carrying capacity of diverse environments (Martinez-Romero 2012). The natural association of N_2-fixing bacteria (diazotrophs) with photosynthetic organisms is fundamentally based on syntrophism, a term that refers to a type of mutualism where the consumption of excreted substances by symbiotic partners can accelerate the metabolism of the donor symbiont (Kouzuma et al. 2015). In common nitrogen-fixing symbiosis, carbon compounds produced by plants are exchanged for fixed nitrogen produced by bacteria, mainly in the form of ammonia (Ormeño-Orrillo et al. 2013). Among diazotrophs, rhizobia are well known for their nitrogen contribution to legumes in root or stem specialized structures called nodules. In such nodules, bacteria differentiate into bacteroides and produce the nitrogenase enzyme for N_2 fixation. In some legumes of temperate climates, there is a terminal differentiation process of bacteroides in response to plant peptides (Kondorosi et al. 2013). A review on the evolution and phylogenies of rhizobia was presented at the 13th Evolutionary Biology Meeting at Marseilles and reported in Martinez-Romero et al. (2010). Since then, a large diversity of new rhizobial lineages has been revealed in various genomic and metagenomic studies (Miranda-Sánchez et al. 2015; reviewed in Ormeño-Orrillo et al. 2015). This is the case, in particular, of *Rhizobium leguminosarum* exhibiting different genomic

lineages co-existing in a single limited habitat, with restricted genetic recombination. These diverse genomic lineages could be considered as different species, though the term 'biovar' was used for their designation instead (Kumar et al. 2015).

Rhizobium legume symbiosis is not an obligate one, and therefore, it has two important evolutionary characteristics: It follows precise recognition and colonization processes at the onset of each nodulation stage and, additionally, the microsymbiont genome does not undergo a reduction process as in the ancestors of mitochondria (Gray 2015). Both legumes and rhizobia can independently live without the corresponding partner. Seemingly, the lack of obligate dependence has hampered the development of N_2-fixing organelles in legumes. In contrast, an equivalent system has evolved in a diatom containing a cyanobacteria-derived organelle (with a reduced genome) that may not be grown in culture media (Prechtl et al. 2004; Kneip et al. 2007). The refined rhizobial colonization process has been extensively studied both in plants and in bacteria (Oldroyd and Downie 2008) and includes an attachment of rhizobia to roots followed by differential gene expression of both the bacterial and plant symbiotic partner in response to each other signals (Oldroyd and Downie 2008; Ramachandran et al. 2011; López- Guerrero et al. 2012).

The most remarkable molecular signal in rhizobial symbiosis is the Nod factor produced by rhizobia, which bear structural similarity to Myc factors produced by arbuscular mycorrhiza (Maillet et al. 2011). Recently, it has been reported that some of the *nod* genes might have emerged in a basal group of actinobacterial N_2-fixing *Frankia* organisms (Persson et al. 2015). *nod* genes resembling those from rhizobia are also found in some beta proteobacteria of the genus *Burkholderia* and *Cupriavidus*, which are capable of nodulating legumes (Andrus et al. 2012; Gyaneshwar et al. 2011). Presumably, *nod* genes, with the exception of *nodIJ* (Aoki et al. 2013), were laterally transferred from alpha to beta rhizobia (Moulin et al. 2011; Martinez-Romero et al. 2010). These genes are considered accessory in rhizobial genomes and consequently are prone to events of lateral gene transfer (López-Guerrero et al. 2012). Indeed, *nod* genes do not have a congruent evolutionary history with respect to housekeeping rhizobial genes (reviewed in Martinez-Romero 2009). Rhizobial *nod* genes are located in symbiosis plasmids or in symbiosis islands as other genes involved in the process of N_2 fixation, such as the *nif* genes responsible for the catalytic process of the nitrogenase reaction.

Rhizobia survive as free-living organisms in soils and associate with different legume and non-legume rhizospheres or may colonize plant internal tissues as endophytes of many diverse plants, including cereals (Chi et al. 2005; Chaintreuil et al. 2000), bananas (Martínez et al. 2003) and other plants (Doty et al. 2005). Consequently, they have a metabolic versatility, backed up by large genomes, which are not found in obligate endosymbionts. Indeed, we have found nitrate reduction genes in rhizobia and related N_2-fixing bacteria (see previous section and Table 12.1). Hence, nitrate assimilation and other pathways that are found in current genomes suggest that rhizobia have retained a reservoir of genes conferring metabolic adaptability (López-Guerrero et al. 2013), as well as syntrophic capacity

that originally emerged during the early evolution of alpha proteobacteria, from the root of which the ancestors of mitochondria diverged (Degli Esposti et al. 2015).

12.1.3 Part 3. How to Bridge the Lipid Divide Between Prokaryotes and Eukaryotes

The initial proposal for the three domains of life, Eukarya, Bacteria and Archaea (Woese and Fox 1977), was soon supported by one of the most remarkable features distinguishing Archaea from Bacteria and Eukarya, which is the lipid composition of the archaeal membrane. In archaea, isoprenoid hydrocarbon side chains are linked via ether bonds to the *sn*-glycerol-1-phosphate backbone. In contrast, in bacteria and eukarya, fatty acyl side chains are linked via ester bonds to the *sn*-glycerol-3-phosphate backbone. Nevertheless, the polar head groups of membrane lipids are globally shared by the three domains of life.

Eukaryotic membranes contain sphingolipids (SL), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cholesterol (CHO) as major (most abundant) lipids. Mitochondrial, lysosomal and smooth ER membranes have phosphatidylglycerol (PG) and cardiolipin (CL) in addition (Nelson and Cox 2013; Ridgway 2016). Among the minor lipids in eukaryotic membranes usually feature phosphatidic acid (PA), diacylglycerol (DAG), phosphatidylserine (PS), phosphatidylinositol (PI), its polyphosphorylated derivatives (PIPs) and numerous isoprenoid derivatives, i.e. ubiquinone. Another general hallmark of eukaryotic membrane lipids is the frequent presence of long (more than 19 carbons) and multiunsaturated fatty acyl residues. Additionally, there is a striking asymmetry in the distribution of phospholipids between the inner and the outer monolayer of the cytoplasmic membrane. Whereas PC and SL are mainly encountered in the outer monolayer, PE, PS, PA, PI and PIPs are predominantly orientated towards the cytoplasmic side of the membrane (Nelson and Cox 2013).

Phospholipids of bacteria share three key features with eukaryote phospholipids: (1) *sn*-glycerol-3-phosphate as the initial building block for lipid formation, (2) fatty acyl hydrocarbon moieties, that are; (3) linked as esters to the glycerol backbone. Although the model bacterium *Escherichia coli* has PG, CL and PE as major phospholipids in its membranes, it is clear by now that there is no such thing as a typical bacterial membrane lipid composition (Sohlenkamp and Geiger 2016). In addition to these three most common phospholipid classes, bacteria contain less frequently PC and PI, as well as a variety of other membrane lipids, such as ornithine lipids, glycolipids, SL, hopanoids or other isoprenoid-derived lipids among others (Sohlenkamp and Geiger 2016). Moreover, DAG, PA and PS are formed in most bacteria, often as intermediates in metabolic pathways (Geiger et al. 2013). Notably, CHO is absent from bacteria, but some of its functions might be taken over by hopanoids or other polyisoprenoids (Sohlenkamp and Geiger 2016; López and Kolter 2010). Fatty acyl chains encountered in bacteria are frequently saturated, monounsaturated or ramified (*iso*- or *anteiso*-) and seldom exceed 19

carbon units. Additionally, one should keep in mind that information on membrane lipid composition is scarce or absent for most of the bacterial phyla. In contrast, membrane phospholipids of archaea differ from the eukaryotic and bacterial versions in the three key features defining the so-called lipid divide (Koga 2011): (1) *sn*-glycerol-1-phosphate as the initial building block for lipid formation, (2) isoprenoid hydrocarbon moieties, that are; (3) linked as ethers to the glycerol backbone.

The opposite stereoconfiguration of the glycerol-phosphate backbone in archaea and bacteria is due to the action of the non-homologous enzymes, glycerol-1-phosphate dehydrogenase (encoded by *egsA* in archaea) (Daiyasu et al. 2002) and glycerol-3-phosphate dehydrogenase (encoded by *glpD* in bacteria), respectively. The C20 unit of geranylgeranyl pyrophosphate is formed from four isoprenoid units and used by geranylgeranyl glycerophosphate synthase to form geranylgeranyl glycerophosphate. In turn, the second alkylation is performed by bis-geranylgeranyl glycerophosphate synthase also using geranylgeranyl pyrophosphate to convert geranylgeranyl glycerophosphate into 2,3-bis-*O*-geranylgeranyl-*sn*-glyceryl-1-glycerophosphate (archaetidic acid). Again, the two enzymes forming the ether bonds are unique to archaea. Steps that follow the formation of archaeal phospholipids are the activation of CDP-archaeol by *CarS* (Jain et al. 2014a) and head group modification by enzymes of the CDP-alcohol phosphatidyltransferases and by enzymes that are homologues of their bacterial counterparts. As in bacteria, the alcohol head groups linked to archaetidic acid can be glycerol, ethanolamine, serine, choline or inositol. Activation of archaetidic acid and modification reactions with CDP-archaeol still occur with the tetra-unsaturated geranylgeranyl residues (Caforio et al. 2015). Some of these pre-existing double bonds might be used to form cyclopentane or cyclohexane ring moieties, which are encountered in some of the archaeal membrane lipids. Saturation of the isoprenoid double bonds probably occurs after the head group activation (Jain et al. 2014b). Tetraether lipid structures with varying numbers of cyclopentane moieties are widespread in archaea, and they are thought to be formed from saturated diethers via head-to-head condensation reactions (Jain et al. 2014b).

Biochemically, Thaumarchaeota are the best characterized organisms of the TACK superphylum from which the ancestral archaean that then formed the eukaryotic nucleus emerged (Sinninghe Damsté et al. 2002a; Pearson and Ingalls 2013; Dang et al. 2013; Zhalnina et al. 2012). Thaumarchaeota have a specific cyclopentane ring-containing dibiphytanyl glycerol tetraether membrane lipid, crenarchaeol (Sinninghe Damsté et al. 2002a). Although such tetraether lipids are unknown outside archaea, ladderane membrane lipids, isolated from the anammoxosome of Planctomycetes, can contain ester or ether bonds and harbour a similar concatenated cyclobutane rings in their hydrocarbon residues (Sinninghe Damsté et al. 2002b). With regard to some modification pathways of the phospholipids' polar head groups, archaea and bacteria are more similar to each other compared to eukaryotes. For example, in eukarya, PI is synthesized from CDP-diacylglycerol and inositol (Carman and Fischl 1992). In contrast, bacteria use inositol 1-phosphate and CDP-DAG to form phosphatidylinositol phosphate (PIP),

whereas in an analogous reaction archaea condense inositol 1-phosphate to CDP-archaeol, forming archaetidylinositol phosphate (AIP) (Michell 2013; Morii et al. 2014). In this context, it is interesting to note that PI-3-phosphate and other phosphorylated forms of PI play crucial roles in the complex membrane traffic machinery of eukaryotes (Lindmo and Stenmark 2006; Michell 2013) and might do so in archaean ancestors too, given the abundance of membrane trafficking proteins in Lokiarchaean genome (Spang et al. 2015). Subsequently, PIP and AIP are dephosphorylated to become PI and archaetidylinositol, respectively (Morii et al. 2014). Notably, eukaryotic PI synthase, bacterial PIP synthase, archael AIP synthase and PG phosphate synthase are homologues to each other (Morii et al. 2014).

Although the three main features mostly hold true to argue for the lipid divide between bacteria and archaea, there are some recent reports that certainly blur the picture. For example, archaea might be able to perform a simplified fatty acid biosynthesis that is independent of acyl carrier protein (Lombard et al. 2012). Another, possibly dogma-breaking example is provided by a novel pathway for inositol phospholipid synthesis in *Rhodothermus marinus* (Jorge et al. 2015). CDP-inositol and dialkylether glycerol are condensed by dialkylether glycerophosphoinositol synthase (BEPIS) to form *sn*-1,2-dialkylether-glycero-3-phosphoinositol (Jorge et al. 2015).

The limited knowledge of the energy metabolism of the proposed Lokiarchaean ancestor of eukaryotic cells (Spang et al. 2015) together with the uncertainty still surrounding the identity of the alpha proteobacterial lineage for the bacterial ancestors of mitochondria (see part 1 above) leaves space to an alternative scenario for the symbiotic event that originated eukaryogenesis (López-García and Moreira 2015). Namely, a small archaean might have become an endosymbiont of a large proteobacterium that subsequently was infected by alpha proteobacterial parasites which evolved into mitochondria (López-García and Moreira 2015 and references therein). Such a scenario would solve a fundamental problem of the popular alternative of an archaean host then ingesting proto-mitochondria: Eukaryotic cells predominantly have proteobacterial types of phospholipids in all their membranes (Nelson and Cox 2013), not just in mitochondria. Where are the relics of the ancestral archaean lipids in current eukaryotic cells? To answer this fundamental question, this article has presented a survey of the membrane lipids that may derive from either the archaean or the proteobacterial partner of the symbiotic event leading to eukaryogenesis. Based on such a survey, we conclude that presently there are no specific relics of ancestral archaean lipids in current eukaryotic cells. Considering that most data about the lipid composition in eukaryotic membranes have been generated several decades ago, it is well possible that minor lipids or difficult-to-detect lipids might have been missed. Therefore, it probably would be worthwhile to reinvestigate lipid composition from the different eukaryotic membranes by modern mass spectrometric technique searching specifically for lipids that are considered as markers of archaea.

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References

Andrus AD, Andam C, Parker MA (2012) American origin of *Cupriavidus* bacteria associated with invasive *Mimosa* legumes in the Philippines. FEMS Microbiol Ecol 80:747–750. doi:10.1111/j.1574-6941.2012.01342.x

Aoki S, Ito M, Iwasaki W (2013) From β- to α-proteobacteria: the origin and evolution of rhizobial nodulation genes nodIJ. Mol Biol Evol 30:2494–2508. doi:10.1093/molbev/mst153

Berry EA, Huang LS, Saechao LK, Pon NG, Valkova-Valchanova M, Daldal F (2004) X-Ray structure of *Rhodobacter Capsulatus* Cytochrome bc (1): comparison with its mitochondrial and chloroplast counterparts. Photosynth Res 81:251–275

Brinkmann H, Philippe H (2007) The diversity of eukaryotes and the root of the eukaryotic tree. Adv Exp Med Biol 607:20–37

Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P (2007) Mesophilic crenarchaeota: proposal for a third archaeal phylum, the Thaumarchaeota. Nat Rev Microbiol 6:245–252. doi:10.1038/nrmicro1852

Brochier-Armanet C, Gribaldo S, Forterre P (2012) Spotlight on the Thaumarchaeota. ISME J 6:227–230. doi:10.1038/ismej.2011.145

Caforio A, Jain S, Fodran P, Siliakus M, Minnaard AJ, van der Oost J, Driessen AJ (2015) Formation of the ether lipids archaetidylglycerol and archaetidylethanolamine in *Escherichia coli*. Biochem J 470:343–355. doi:10.1042/BJ20150626

Carman GM, Fischl AS (1992) Phosphatidylinositol synthase from yeast. Methods Enzymol 209:305–312

Chaintreuil C, Giraud E, Prin Y, Lorquin J, Bâ A, Gillis M, de Lajudie P, Dreyfus B (2000) Photosynthetic bradyrhizobia are natural endophytes of the African wild rice *Oryza breviligulata*. Appl Environ Microbiol 66:5437–5447

Chi Shen SH, Cheng HP, Jing YX, Yanni YG, Dazzo FB (2005) Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl Environ Microbiol 71:7271–7278

Daims H, Lebedeva EV, Pjevac P, Han P, Herbold C, Albertsen M, Jehmlich N, Palatinszky M, Vierheilig J, Bulaev A, Kirkegaard RH, von Bergen M, Rattei T, Bendinger B, Nielsen PH, Wagner M (2015) Complete nitrification by Nitrospira bacteria. Nature 528:504–509. doi:10.1038/nature16461

Daiyasu H, Hiroike T, Koga Y, Toh H (2002) Analysis of membrane stereochemistry with homology modeling of *sn*-glycerol-1-phosphate dehydrogenase. Protein Eng 15:987–995

Dang H, Zhou H, Yang J, Ge H, Jiao N, Luan X, Zhang C, Klotz MG (2013) Thaumarchaeotal signature gene distribution in sediments of the northern South China Sea: an indicator of the metabolic intersection of the marine carbon, nitrogen, and phosphorus cycles? Appl Environ Microbiol 79:2137–2147. doi:10.1128/AEM.03204-12

Degli Esposti M (2014) Bioenergetic evolution in proteobacteria and mitochondria. Genome Biol Evol 6:3238–3251. doi:10.1093/gbe/evu257

Degli Esposti M, Chouaia B, Comandatore F, Crotti E, Sassera D, Lievens PM, Daffonchio D, Bandi C (2014) Evolution of mitochondria reconstructed from the energy metabolism of living bacteria. PLoS ONE 9:e96566. doi:10.1371/journal.pone.0096566

Degli Esposti M, Rosas-Pérez T, Servín-Garcidueñas LE, Bolaños LM, Rosenblueth M, Martínez-Romero E (2015) Molecular evolution of cytochrome bd oxidases across proteobacterial genomes. Genome Biol Evol 7:801–820. doi:10.1093/gbe/evv032

Desjardins CA, Champion MD, Holder JW, Muszewska A, Goldberg J, Bailão AM, Brigido MM, Ferreira ME, Garcia AM, Grynberg M, Gujja S, Heiman DI, Henn MR, Kodira CD, León-Narváez H, Longo LV, Ma LJ, Malavazi I, Matsuo AL, Morais FV, Pereira M, Rodríguez-Brito S, Sakthikumar S, Salem-Izacc SM, Sykes SM, Teixeira MM, Vallejo MC, Walter ME, Yandava C, Young S, Zeng Q, Zucker J, Felipe MS, Goldman GH, Haas BJ, McEwen JG, Nino-Vega G, Puccia R, San-Blas G, Soares CM, Birren BW, Cuomo CA (2011)

Comparative genomic analysis of human fungal pathogens causing paracoccidioidomycosis. PLoS Genet 7:e1002345. doi:10.1371/journal.pgen.1002345
Doty SL, Dosher MR, Singleton GL, Moore AL, Aken B, van Stettler RF, Gordon MP (2005) Identification of an endophytic Rhizobium in stems of Populus. Symbiosis 39:27–35
Finnegan PM, Umbach AL, Wilce JA (2003) Prokaryotic origins for the mitochondrial alternative oxidase and plastid terminal oxidase nuclear genes. FEBS Lett 555:425–430
Gagliano AL, Tagliavia M, D'Alessandro W, Franzetti A, Parello F, Quatrini P (2016) So close, so different: geothermal flux shapes divergent soil microbial communities at neighbouring sites. Geobiology 14:150–62
Geiger O, López-Lara IM, Sohlenkamp C (2013) Phosphatidylcholine biosynthesis and function in bacteria. Biochim Biophys Acta 1831:503–513. doi:10.1016/j.bbalip.2012.08.009
Gray MW (2015) Mosaic nature of the mitochondrial proteome: implications for the origin and evolution of mitochondria. Proc Natl Acad Sci USA 112:10133–10138. doi:10.1073/pnas.1421379112
Guy L, Ettema TJ (2011) The archaeal 'TACK' superphylum and the origin of eukaryotes. Trends Microbiol 19:580–587. doi:10.1016/j.tim.2011.09.002
Gyaneshwar P, Hirsch AM, Moulin L, Chen WM, Elliott GN, Bontemps C, Estrada-de Los Santos P, Gross E, Dos Reis FB, Sprent JI, Young JP, James EK (2011) Legume-nodulating betaproteobacteria: diversity, host range, and future prospects. Mol Plant Microbe Interact 24:1276–1288. doi:10.1094/MPMI-06-11-0172
Hatzenpichler R (2012) Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. Appl Environ Microbiol 78:7501–7510. doi:10.1128/AEM.01960-12
Hawley AK, Brewer HM, Norbeck AD, Paša-Tolić L, Hallam SJ (2014) Metaproteomics reveals differential modes of metabolic coupling among ubiquitous oxygen minimum zone microbes. Proc Natl Acad Sci USA 111:11395–11400. doi:10.1073/pnas.1322132111
Jain S, Caforio A, Fodran P, Lolkema JS, Minnaard AJ, Driessen AJ (2014a) Identification of CDP-archaeol synthase, a missing link of ether lipid biosynthesis in Archaea. Chem Biol 21:1392–1401. doi:10.1016/j.chembiol.2014.07.022
Jain S, Caforio A, Driessen AJ (2014b) Biosynthesis of archaeal membrane ether lipids. Front Microbiol 5:641. doi:10.3389/fmicb.2014.00641
Johnstone IL, McCabe PC, Greaves P, Gurr SJ, Cole GE, Brow MA, Unkles SE, Clutterbuck AJ, Kinghorn JR, Innis MA (1990) Isolation and characterisation of the crnA-niiA-niaD gene cluster for nitrate assimilation in *Aspergillus nidulans*. Gene 90:181–192
Jorge CD, Borges N, Santos H (2015) A novel pathway for the synthesis of inositol phospholipids uses cytidine diphosphate (CDP)-inositol as donor of the polar head group. Environ Microbiol 17:2492–2504. doi:10.1111/1462-2920.12734
Kneip C, Lockhart P, Voss C, Maier UG (2007) Nitrogen fixation in eukaryotes–new models for symbiosis. BMC Evol Biol 7:55
Koga Y (2011) Early evolution of membrane lipids: how did the lipid divide occur? J Mol Evol 72:274–82. doi: 10.1007/s00239-011-9428-5
Kondorosi E, Mergaert P, Kereszt A (2013) A paradigm for endosymbiotic life: cell differentiation of Rhizobium bacteria provoked by host plant factors. Annu Rev Microbiol 67:611–628. doi:10.1146/annurev-micro-092412-155630
Koonin EV (2015) Origin of eukaryotes from within archaea, archaeal eukaryome and bursts of gene gain: eukaryogenesis just made easier? Philos Trans R Soc Lond B Biol Sci 370:20140333. doi:10.1098/rstb.2014.0333
Kouzuma A, Kato S, Watanabe K (2015) Microbial interspecies interactions: recent findings in syntrophic consortia. Front Microbiol 6:477. doi:10.3389/fmicb.2015.00477
Ku C, Nelson-Sathi S, Roettger M, Sousa FL, Lockhart PJ, Bryant D, Hazkani-Covo E, McInerney JO, Landan G, Martin WF (2016) Endosymbiotic origin and differential loss of eukaryotic genes. Nature 524:427–432. doi:10.1038/nature14963
Kumar N, Lad G, Giuntini E, Kaye ME, Udomwong P, Shamsani NJ, Young JP, Bailly X (2015) Bacterial genospecies that are not ecologically coherent: population genomics of Rhizobium leguminosarum. Open Biol 5:140133. doi:10.1098/rsob.140133

Lin JT, Goldman BS, Stewart V (1993) Structures of genes nasA and nasB, encoding assimilatory nitrate and nitrite reductases in Klebsiella pneumoniae M5al. J Bacteriol 175:2370–2378

Lindmo K, Stenmark H (2006) Regulation of membrane traffic by phosphoinositide 3-kinases. J Cell Sci 119:605–614

Lombard J, López-García P, Moreira D (2012) An ACP-independent fatty acid synthesis pathway in archaea: implications for the origin of phospholipids. Mol Biol Evol 29:3261–3265. doi:10.1093/molbev/mss160

López D, Kolter R (2010) Functional microdomains in bacterial membranes. Genes Dev 24:1893–1902. doi:10.1101/gad.1945010

López-García P, Moreira D (2015) Open questions on the origin of eukaryotes. Trends Ecol Evol 30:697–708. doi:10.1016/j.tree.2015

López-Guerrero M, Ormeño-Orrillo E, Acosta JL, Mendoza-Vargas A, Rogel MA, Ramírez MA, Rosenblueth M, Martínez-Romero J, Martínez-Romero E (2012) Rhizobial extrachromosomal replicon variability, stability and expression in natural niches. Plasmid 68:149–158. doi:10.1016/j.plasmid.2012.07.002

López-Guerrero M, Ormeño-Orrillo E, Rosenblueth M, Martínez J, Martínez-Romero E (2013) Buffet hypothesis for microbial nutrition at the rhizosphere. Front Plant Sci 4:1–4

Maillet F, Poinsot V, André O, Puech-Pagès V, Haouy A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A, Martinez EA, Driguez H, Bécard G, Dénarié J (2011) Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 469:58–63. doi:10.1038/nature09622

Mardanov AV, Beletsky AV, Kadnikov VV, Ignatov AN, Ravin NV (2014) Draft genome sequence of *sclerotinia borealis*, a psychrophilic plant pathogenic fungus. Genome Announc 2. pii: e01175-13. doi:10.1128/genomeA.01175-13

Martin WF, Garg S, Zimorski V (2015) Endosymbiotic theories for eukaryote origin. Philos Trans R Soc Lond B Biol Sci 370:20140330. doi:10.1098/rstb.2014.0330

Martinez D, Berka RM, Henrissat B, Saloheimo M, Arvas M, Baker SE, Chapman J, Chertkov O, Coutinho PM, Cullen D, Danchin EG, Grigoriev IV, Harris P, Jackson M, Kubicek CP, Han CS, Ho I, Larrondo LF, de Leon AL, Magnuson JK, Merino S, Misra M, Nelson B, Putnam N, Robbertse B, Salamov AA, Schmoll M, Terry A, Thayer N, Westerholm-Parvinen A, Schoch CL, Yao J, Barabote R, Nelson MA, Detter C, Bruce D, Kuske CR, Xie G, Richardson P, Rokhsar DS, Lucas SM, Rubin EM, Dunn-Coleman N, Ward M, Brettin TS (2008) Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). Nat Biotechnol 26:553–560. doi:10.1038/nbt1403

Martínez L, Caballero-Mellado J, Orozco J, Martínez-Romero E (2003) Diazotrophic bacteria associated with banana (*Musa* spp.). Plant Soil 257:35–47

Martinez-Romero E (2012) How do microbes enhance the carrying capacity of their habitats? Expert Opin Environ Biol 1:1–2

Martínez-Romero E (2009) Coevolution in Rhizobium-legume symbiosis? DNA Cell Biol 28:361–370. doi:10.1089/dna.2009.0863

Martínez-Romero JC, Ormeño-Orrillo E, Rogel-Hernández MA, López-López A, Martínez-Romero E (2010) Trends in rhizobial evolution and some taxonomic remarks. In: Pontarotti PT (ed) Evolutionary biology—concepts, molecular and morphological evolution. Springer, Berlin, pp 301–315

Michell RH (2013) Inositol lipids: from an archaeal origin to phosphatidylinositol 3,5-bisphosphate faults in human disease. FEBS J 280:6281–6294. doi:10.1111/febs.12452

Miranda-Sánchez F, Rivera J, Vinuesa P (2015) Diversity patterns of Rhizobiaceae communities inhabiting soils, root surfaces and nodules reveal a strong selection of rhizobial partners by legumes. Environ Microbiol. doi: 10.1111/1462-2920.13061 (Epub ahead of print)

Moreno-Vivián C, Ferguson SJ (1998) Definition and distinction between assimilatory, dissimilatory and respiratory pathways. Mol Microbiol 29:664–666

Morii H, Ogawa M, Fukuda K, Taniguchi H (2014) Ubiquitous distribution of phosphatidylinositol phosphate synthase and archaetidylinositol phosphate synthase in Bacteria and Archaea,

which contain inositol phospholipid. Biochem Biophys Res Commun 443:86–90. doi:10.1016/j.bbrc.2013.11.054

Moulin L, Munive A, Dreyfus B, Boivin-Masson C (2011) Nodulation of legumes by members of the beta-subclass of Proteobacteria. Nature 411:948–950

Muller F, Brissac T, Le Bris N, Felbeck H, Gros O (2012) First description of giant Archaea (*Thaumarchaeota*) associated with putative bacterial ectosymbionts in a sulfidic marine habitat. Environ Microbiol 12:2371–2383. doi:10.1111/j.1462-2920.2010.02309.x

Müller M, Mentel M, van Hellemond JJ, Henze K, Woehle C, Gould SB, Yu RY, van der Giezen M, Tielens AG, Martin WF (2012) Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. Microbiol Mol Biol Rev 76:444–495. doi:10.1128/MMBR.05024-11

Nelson DL, Cox MM (2013) Lehninger: principles of biochemistry, 6th edn. WH Freeman and Company, New York

Oldroyd GE, Downie JA (2008) Coordinating nodule morphogenesis with rhizobial infection in legumes. Annu Rev Plant Biol 59:519–546. doi:10.1146/annurev.arplant.59.032607.092839

Ormeño-Orrillo E, Hungria M, Martínez-Romero E (2013) Dinitrogen-fixing prokaryotes. In: Rosenberg E et al (eds) The prokaryotes, prokaryotic physiology and biochemistry. Springer, Heidelberg, pp 427–451. ISBN: 978-3-642-30140-7

Ormeño-Orrillo E, Servín-Garcidueñas LE, Rogel MA, González V, Peralta H, Mora J, Martínez-Romero J, Martínez-Romero E (2015) Taxonomy of rhizobia and agrobacteria from the Rhizobiaceae family in light of genomics. Syst Appl Microbiol 38:287–291. doi:10.1016/j.syapm.2014.12.002

Pearson A, Ingalls AE (2013) Assessing the use of archaeal lipids as marine environmental proxies. Annu Rev Earth Planet Sci 41:359–384. doi:10.1146/annurev-earth-050212-123947

Persson T, Battenberg K, Demina IV, Vigil-Stenman T, Vanden Heuvel B, Pujic P, Facciotti MT, Wilbanks EG, O'Brien A, Fournier P, Cruz Hernandez MA, Mendoza Herrera A, Médigue C, Normand P, Pawlowski K, Berry AM (2015) Candidatus Frankia Datiscae Dg1, the Actinobacterial microsymbiont of Datisca glomerata, expresses the canonical nod genes nodABC in symbiosis with its host plant. PLoS ONE 10:e0127630. doi:10.1371/journal.pone.0127630

Pittis AA, Gabaldón T (2016) Late acquisition of mitochondria by a host with chimaeric prokaryotic ancestry. Nature 531:101–114

Prechtl J, Kneip C, Lockhart P, Wenderoth K, Maier UG (2004) Intracellular spheroid bodies of Rhopalodia gibba have nitrogen-fixing apparatus of cyanobacterial origin. Mol Biol Evol 21:1477–1481

Ramachandran VK, East AK, Karunakaran R, Downie JA, Poole PS (2011) Adaptation of Rhizobium leguminosarum to pea, alfalfa and sugar beet rhizospheres investigated by comparative transcriptomics. Genome Biol 12:R106. doi:10.1186/gb-2011-12-10-r106

Raymann K, Brochier-Armanet C, Gribaldo S (2015) The two-domain tree of life is linked to a new root for the Archaea. Proc Natl Acad Sci USA 112:6670–6675. doi:10.1073/pnas.1420858112

Ridgway ND (2016) Phospholipid synthesis in mammalian cells. In: Ridgway N, McLeod R (eds) Biochemistry of lipids, lipoproteins and membranes, 6th edn. Elsevier, Amsterdam, pp 209–236

Rodríguez-Ezpeleta N, Embley TM (2012) The SAR11 group of alpha-proteobacteria is not related to the origin of mitochondria. PLoS ONE 7:e30520. doi:10.1371/journal.pone.0030520

Sawana A, Adeolu M, Gupta RS (2013) Molecular signatures and phylogenomic analysis of the genus Burkholderia: proposal for division of this genus into the emended genus Burkholderia containing pathogenic organisms and a new genus Paraburkholderia gen. nov. harboring environmental species. Front Genet 5:429. doi:10.3389/fgene.2014.00429

Schweizer H, Larson TJ (1987) Cloning and characterization of the aerobic sn-glycerol-3-phosphate dehydrogenase structural gene glpD in Escherichia coli K-12. J Bacteriol 169:507–513

Searcy DG (2003) Metabolic integration during the evolutionary origin of mitochondria. Cell Res 13:229–238

Sinninghe Damsté JS, Schouten S, Hopmans EC, van Duin AC, Geenevasen JA (2002a) Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota. J Lipid Res 43:1641–1651. doi:10.1194/jlr.m200148-jlr200

Sinninghe Damsté JS, Strous M, Rijpstra WI, Hopmans EC, Geenevasen JA, van Duin AC, van Niftrik LA, Jetten MS (2002b) Linearly concatenated cyclobutane lipids form a dense bacterial membrane. Nature 419:708–712

Sohlenkamp C, Geiger O (2016) Bacterial membrane lipids: diversity in structures and pathways. FEMS Microbiol Rev 40:133–159. doi:10.1093/femsre/fuv008

Spang A, Saw JH, Jørgensen SL, Zaremba-Niedzwiedzka K, Martijn J, Lind AE, van Eijk R, Schleper C, Guy L, Ettema TJ (2015) Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature 521:173–179. doi:10.1038/nature14447

Takasaki K, Shoun H, Nakamura A, Hoshino T, Takaya N (2004) Unusual transcription regulation of the niaD gene under anaerobic conditions supporting fungal ammonia fermentation. Biosci Biotechnol Biochem 68:978–980

Testa A, Oliver R, Hane J (2015) Overview of genomic and bioinformatic resources for *Zymoseptoria tritici*. Fungal Genet Biol 79:13–16. doi:10.1016/j.fgb.2015.04.011

Tetu SG, Breakwell K, Elbourne LD, Holmes AJ, Gillings MR, Paulsen IT (2013) Life in the dark: metagenomic evidence that a microbial slime community is driven by inorganic nitrogen metabolism. ISME J 7:1227–1236. doi:10.1038/ismej.2013.14

Woese CR, Fox GE (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc Natl Acad Sci USA 74:5088–5090

Xavier BB, Miao VP, Jónsson ZO, Andrésson ÓS (2012) Mitochondrial genomes from the lichenized fungi Peltigera membranacea and Peltigera malacea: features and phylogeny. Fungal Biol 116:802–814. doi:10.1016/j.funbio.2012.04.013

Zhalnina KV, Dias R, Leonard MT, Dorr de Quadros P, Camargo FA, Drew JC, Farmerie WG, Daroub SH, Triplett EW (2012) Genome sequence of *Candidatus* Nitrososphaera evergladensis from group I.1b enriched from Everglades soil reveals novel genomic features of the ammonia-oxidizing archaea. PLoS One 9:e101648. doi:10.1371/journal.pone.0101648