 DOI 10.1002/bies.200900018

Predation between prokaryotes and the origin of eukaryotes

*Yaacov Davidov and Edouard Jurkevitch**

Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot, Israel

Accumulating data suggest that the eukaryotic cell originated from a merger of two prokaryotes, an archaeal host and a bacterial endosymbiont. However, since prokaryotes are unable to perform phagocytosis, the means by which the endosymbiont entered its host is an enigma. We suggest that a predatory or parasitic interaction between prokaryotes provides a reasonable explanation for this conundrum. According to the model presented here, the host in this interaction was an anaerobic archaeon with a periplasm-like space. The predator was a small (facultative) aerobic α-proteobacterium, which penetrated and replicated within the host periplasm, and later became the mitochondria. Plausible conditions under which this interaction took place and circumstances that may have led to the contemporary complex eukaryotic cell are discussed.

Keywords: eukaryotic origin; mitochondrial origin; predatory bacteria

Introduction

The origin of eukaryotes has remained one of the most enigmatic, controversial and challenging questions in evolution. Eukaryotic cells differ from prokaryotes by a variety of key features: a nucleus, mRNA splicing, endoplasmic reticulum (ER), Golgi complex, dynamic cytoskeleton with internal filaments, peroxisomes, mitochondria, a large cell volume and additional traits. Since the recognition of the prokaryotic–eukaryotic transition as 'the greatest single evolutionary discontinuity' of life about half a century ago,[1] prokaryotes with some typical 'eukaryotic features' have been found, including some possessing membranes defining intracellular compartments and structures resembling a nucleus.[2] Nevertheless, true intermediate forms representing transitional structures between prokaryotes and eukaryotes were never found. Nucleus-like structures, as well as other typical 'eukaryotic features' like giant cell size and internal membranes, which are found in some prokaryotes, have most probably evolved independently, and do not stand for a true continuum in complexity between prokaryotes and eukaryotes.[3–5] In parallel, the absence of mitochondria, peroxisomes, Golgi complex, introns or other eukaryotic features in proposed primitive eukaryotes all appear to be better explained *via* secondary losses.[6–9] As recently summarized by Embley and Martin:[9] 'the evolutionary gap between prokaryotes and eukaryotes is now deeper... than ever before'.

Accumulating data suggest that the most parsimonious scenario explaining this evolutionary gap is a merger of two prokaryotes – an archaeal host and a bacterial endosymbiont – at the origin of the eukaryotic cell. However, since prokaryotes are unable to perform phagocytosis, the means by which the endosymbiont got access into its host poses a mechanistic challenge to this scenario. In this essay, we revive a previously proposed hypothesis, *i.e.* that a predatory interaction between prokaryotes may have stood at the very origin of the eukaryotic cell, providing a starting point from which two prokaryotes became one endosymbiotic entity. Evolutionary circumstances that may have led to the contemporary complex eukaryotic cell are discussed.

> *We used to think that if we knew one, we knew two, because one and one are two. We are finding that we must learn a great deal more about 'and'.*
>
> *Sir Arthur* Eddington (1882–1944).

Models for the origin of eukaryotes: a historical perspective

The acquisition of the mitochondrion was certainly a defining event in the evolution of the eukaryotic cell. The principle of endosymbiosis was suggested more than a century ago (for recent historic review, see Sapp[10]) but was generally considered as 'entertaining fantasy' or 'too fantastic to mention in polite society'[11] until the discovery of mitochondrial and plastid DNA in the 1960s. Phylogenetics based on mitochondrial DNA later demonstrated that mitochondria evolved only once, from an α-proteobacterium.[12,13] The identity of the primordial protomitochondrion host and how the association between the two evolved are still hotly debated.

***Correspondence to:** E. Jurkevitch, Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot, Israel.
E-mail: jurkevi@agri.huji.ac.il

About four decades ago, Margulis[14,15] renewed the endosymbiotic theory and suggested that eukaryotes formed as a result of a gradual multi-endosymbiotic union of prokaryotes. In contrast, others, like de Duve and Stanier,[16,17] proposed that phagotrophy, which requires a dynamic cytoskeleton, an endomembrane system and the loss of the prokaryotic rigid cell wall, evolved prior to endosymbiosis, enabling later the engulfment of prokaryotic endosymbionts. These developments occurred as Carl Woese's trichotomous tree of life was taking roots, leading to a 'phylogenetic revolution'. The basic prokaryote–eukaryote dichotomy of life was challenged by the three domains concept, which includes the Bacteria, Eukarya and Archaea, with the last two domains forming a monophyletic group.[18,19]

Models assuming 'mitochondrion-late origin' (*i.e.* postulating the engulfment of the protomitochondrion by an early eukaryote) were strongly supported by the observations of anaerobic amitochondriate eukaryotes, and by phylogenetic analyses that placed these organisms, collectively named 'Archezoa', at the base of the eukaryotic tree.[20,21] Accordingly, the hypothesis that the protomitochondrion was an aerobic α-proteobacterium that had been engulfed by a strictly anaerobic primitive eukaryote became widely accepted (the 'Archezoa model'). During the 1990s, new data questioned the dogma: in addition to genes phylogenetically similar to archaeal ones, numerous eukaryotic genes, many of which not being relevant to the mitochondrial function, were found to be of bacterial origin. On this basis, a number of models suggesting that early eukaryotes evolved as the result of a fusion between archaeal and bacterial cells were formulated (*e.g.* Refs[22,23]). In parallel, Archezoa were found to be polyphyletic, with many of these putative early eukaryotes forming late branches in the eukaryotic tree. Further, in all the investigated cases, Archezoa were found to descend from mitochondrion-bearing ancestors.[9]

About a decade ago, Martin and Muller[24] published the 'hydrogen hypothesis'. This proposes that the initial symbiosis that led to the eukaryotic cell occurred between an archaeal host and a bacterial endosymbiotic protomitochondrion. The excretion of hydrogen by the protomitochondrion and its consumption by the archaeal host were suggested to be the selective principle driving the evolution of this symbiosis. Many other hypotheses on the origin of eukaryotes have also been put forward (reviewed in Refs[9,25,26]). In the following sections, we will examine, with the help of recently published literature, two of the most fundamental and controversial questions concerning the different eukaryogenesis models: Was the protomitochondrion acquired by a prokaryotic archaeal host or was the host a eukaryote, and did this acquisition occur under anaerobic or under aerobic conditions? But first, we will address the question of the phylogenetic origin of the mitochondria.

The phylogeny and the nature of the mitochondrial common ancestor

It is now established that mitochondria evolved from a single α-proteobacterium.[13] However, the exact position of the mitochondrial branch within the α-proteobacteria is still not settled. The α-proteobacteria include many metabolically and morphologically different bacteria with varied life cycles and habitats. Despite this wide diversity, a feature common to many α-proteobacteria is a close association with eukaryotes, as intra- or extracellular, obligate or facultative, mutualists, commensals or parasites.[27] Among the main subgroups or orders within this class, Rickettsiales, obligate intracellular parasites or symbionts of eukaryotes, form the earliest branch, and are frequently considered as most relevant to the origin of the mitochondrion. The second deepest order among the α-proteobacteria is the Rhodospirillales. Other orders that form later branches seem to be less relevant to the mitochondrial origin.[28–30]

Gene concatenation and supertree approaches with tens of protein-coding genes were recently used to reconstruct the phylogeny of the mitochondria within the α-proteobacteria.[28,30–32] The emerging conclusion from these studies is that among the currently defined α-proteobacterial orders, the Rickettsiales are the closest to the mitochondria, with the Rhodospirillales coming next. This by no means supposes that the mitochondrion evolved from a Rickettsia-like bacterium as both are the result of independent reductive evolutionary paths originating in free-living ancestors. While the estimated upper limit for the origin of known Rickettsiales is about 400 million years ago,[33,34] the origin of the mitochondrion is estimated to be four to six times older.[35–37] It is thus unfounded to assume a close similarity between known Rickettsiales and the protomitochondrion.

Six hundred thirty nuclear eukaryotic genes were identified as derived from the protomitochondrion and used to reconstruct the promitochondrial metabolism.[38,39] The cognate proteome was found to contain a complete set of enzymes for β-oxidation and oxidative phosphorylation, and many metabolite transporters, including transporters for lipids, glycerol and amino acids. In contrast, ATP transporters and numerous pathways for amino acid anabolism were missing. These data collectively suggest that the protomitochondrion was a (facultative) aerobic organism with host dependency.[38,39] A computational inference of the genome of the ancestor of the α-proteobacterial clade further pointed to various functions required for interactions with a host cell.[40] Therefore, if the protomitochondrion was host-dependent, and if eukaryotes were not yet present at the time of its acquisition (next section), one has to conclude that the protomitochondrion's host had to be a prokaryote and that the protomitochondrion could have been a parasite or a predator of this host.

'Mitochondrion-early' *versus* 'mitochondrion-late' models: the nature of the protomitochondrial host

The hypotheses for the origin of eukaryotes can be divided into two main categories: those that assume that the primary, triggering event of subsequent cellular transformations leading to the extant eukaryotes was the endosymbiotic acquisition of the protomitochondrion by a prokaryotic archaeal host ('mitochondrion-early' models), and those that suggest that eukaryotic features such as a complex cytoskeleton, endomembranes, a nucleus and phagotrophy evolved first, enabling later engulfment of the protomitochondrion ('mitochondrion-late' models).[9,37] In this section, we suggest, in the light of the recently published literature, that 'mitochondrion-early' models provide the most plausible and parsimonious explanation for the origin of eukaryotes.

Unicellular anaerobic eukaryotes often lack mitochondria. Instead, these organisms harbour reduced mitochondrion-like organelles: hydrogenosomes or mitosomes. Hydrogenosomes produce ATP by substrate-level phosphorylation and generate H_2. Mitosomes seem not to have any direct role in ATP synthesis, and their function is unclear. Data accumulating during the last few years provide strong evidence for a shared origin between these organelles and mitochondria in a common endosymbiotic event.[9,41–44] The phylogenetic distribution of these reduced organelles strongly suggests that they do not represent an ancestral pre-mitochondrial state, but rather arose independently many times as the result of convergent reductive evolution.[41,43] We can thus summarize that in the broad sense of the term, all currently known eukaryotes contain mitochondria.

In addition to these new exciting findings, several recent genomics and phylogenetic analyses indicate that the eukaryotic cell contains a mix of bacterial and archaeal genes.[31,45–48] The exact origin of the archaeal signal is debated, as some analyses suggest that it forms a sister group to the Crenarchaeota,[45,49] while others propose that it originated from within the Euryarchaeota,[47] or from an ancient, unknown archaeal lineage.[48,50] Most of these studies strongly support, while none of them challenge, the most parsimonious scenario that the protomitochondrial host was a bona fide archaeon.

Other studies addressing the origin of specific subcellular components (*e.g.* the nuclear envelope, nucleolus, ER and peroxisomes) found a mixed contribution from Bacteria (including α-proteobacteria) and Archaea,[3,7,51–53] and suggest their endogenous origin after the protomitochondrial acquisition. Although the exact phylogenetic placement of these components is difficult to trace, their chimeric pattern is most parsimoniously explained as resulting from an endosymbiotic relationship between a protomitochondrial symbiont and an archaeal host.

To conclude, scenarios based on a mysterious amitochondriate eukaryote ('mitochondrion-late' models) appear to be less plausible when compared to the more parsimonious 'mitochondrion-early' models. The main argument of the 'mitochondrial-late' models, *i.e.* phagotrophy must precede endosymbiotic acquisition, is discussed in the next section.

New α-proteobacterial killers revive an old hypothesis

A major issue that 'mitochondrion-early' models generally fail to address is the mechanism by which the protomitochondrion entered its prokaryotic host. Prokaryotes cannot perform phagocytosis, and there is not any reason to believe they ever had such abilities. Phagotrophy requires: a flexible cell wall, a dynamic internal cytoskeleton with motor proteins that interacts with a complex endomembrane system, lysosomes that bud from the Golgi complex and are targeted to food vacuoles, and particles enclosed in a phagocytotic cup that are based on the spatially controlled polymerization of actin. These characters are absent from prokaryotes.

Moreover, recent studies suggest a late origin of phagocytosis in eukaryotes. The phylogeny of small GTPases suggests that the first endomembranes to evolve in eukaryotes had secretory, and not phagocytotic, functions,[54] while the phylogeny of endocytic components showed that certain gene duplications needed for the evolution of the endocytic system, previously considered ancestral for all eukaryotes, actually occurred much later.[55] An analysis of proteins involved in phagocytosis in different eukaryotic groups reveals an extreme diversity and suggests that phagocytosis evolved independently at least three times.[56] A late origin of phagocytosis contradicts scenarios which postulate phagotrophy as a prerequisite for any endosymbiosis.[57,58] Since many data indicate an early origin for the protomitochondrion, there seems to be a conundrum: How could the protomitochondrion enter its host if not through phagocytosis?

The notion that endosymbiosis must occur through a phagocytotic process seems to be widespread, and this may have its deep roots in the 'Archezoa model'. Statements like 'One thing at least is agreed: the mitochondrion, powerhouse of the eukaryote cell evolved from an engulfed bacterium'[58] are common but inaccurate. We suggest that, contrarily to the intuitive, common notion that a large predator engulfed a small prey, the penetration of a small bacterial predator into a larger archaeal cell was the cornerstone event of the evolution of the eukaryotic cell.

Predatory interactions between prokaryotes are widespread and ubiquitous.[59] In the pre-eukaryotic era, when competition from eukaryotic predators did not yet exist, these predators may have been even more common than they are

today. It seems that although these organisms were discovered almost half a century ago, some authors are unaware of the existence of non-phagocytotic predatory interactions between prokaryotes. The best known obligate predatory prokaryotes are the *Bdellovibrio* and like organisms (BALOs), small and highly motile bacteria that feed on Gram-negative cells. Most BALOs are δ-proteobacteria and exhibit a dimorphic life cycle (Fig. 1), during which they invade and replicate within the periplasm of their prey. However, the BALOs *Micavibrio* spp. were recently demonstrated to form a deep branching group within the α-proteobacteria.[60]

The α-proteobacterial *Rickettsia*-like *Midichloria mitochondrii* represents another interesting case in which a presumably obligatory parasite of eukaryotic cells became a *Bdellovibrio*-like predator of mitochondria.[61,62] Since no free-living rickettsiales are known, nothing can be inferred on their ancient trophic behaviour, but one may speculate on possible predatory capacities in these bacteria. These examples are conjectural but bring to light the existence of predatory bacteria in the clade from which the protomitochondrion evolved, which by itself is characterized by the disposition of many of its members to interact with other cells.

Hypotheses that are discarded and years later rediscovered are common in science, and the history of ideas about mitochondrial endosymbiosis provides a particularly good example of such cycles.[10] While at first stating that prokaryotes do not engage in predator-prey relationships,[14] Margulis later proposed predation between a *Bdellovibrio*-like bacterium and an archaeal host as a potential mechanism to explain the penetration of the protomitochondrion into its host.[63,64] This idea was then discarded, for two good reasons: the phylogenetic incompatibility between mitochondria (α-proteobacteria) and *Bdellovibrio* (δ-proteobacteria), and the discovery of 'amitochondriate' eukaryotes that led to the Archezoa (phagocytotic) hypothesis. However, this rationale is not pertinent any longer as all studied 'amitochondriate' eukaryotes were found to bear mitochondrion-related features, and *Bdellovibrio*-like predators belonging to the α-proteobacteria have been discovered.

We therefore propose that predation or a form of microbial parasitism played a major role in the evolution of the eukaryotic cell. Accordingly, the ancestor of the mitochondrion was able to attach to a prey or to a host cell and develop a parasitic or a predatory relationship with it. Such an organism would have been endowed, like today's predators (BALOs)[59] or parasites (*Nanoarchaeum* spp.),[65] with the ability to breach the prey cell barrier (an outer membrane and/or an S layer) while conserving its host's cellular integrity. A number of alternative mechanisms leading to the inclusion of the protomitochondrion within its host can be formulated based on this hypothesis. Here, we propose that at the start of this interaction, the infecting organism was able to gain access to

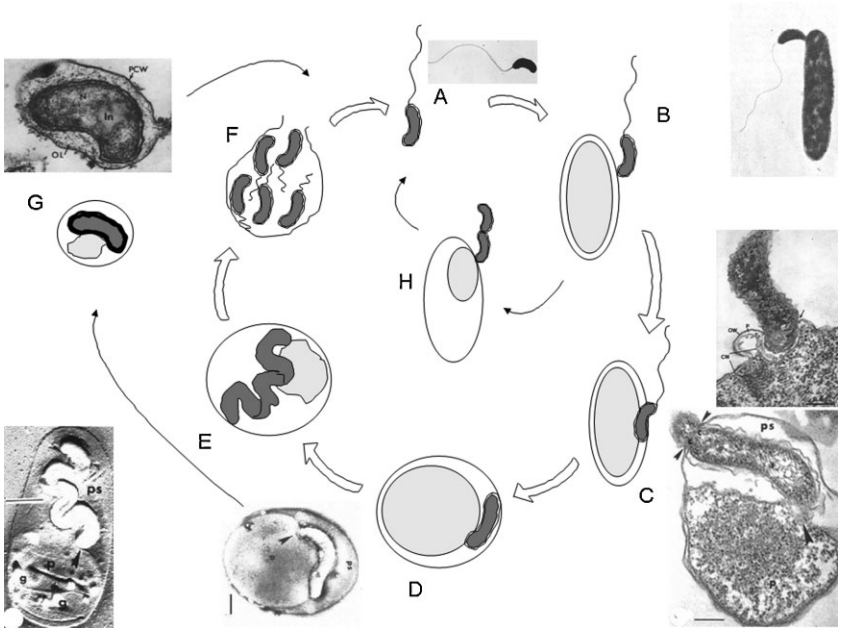

Figure 1. Life cycle of *Bdellovibrio* and like organisms (BALO). In a typical life cycle (**A**–**F**), free attack-phase cells (**A**) swim rapidly until they encounter a potential prey. They attach to the prey (**B**) and penetrate (**C**) its periplasmic space through a temporary cavity in the prey cell wall. The invaded prey typically becomes spherical, and is called a bdelloplast (**D**). The predator grows at the expense of the prey's cytoplasmic content, forming a long filament (**E**). Finally, the filament splits into flagellated progeny cells that burst out of the host's remains (**F**). In some BALO strains, the predator can form a resting stage called a bdellocyst (**G**). In other strains the predators do not penetrate their host but rather remain attached and divide by binary fission (**H**). Pictures were taken with permission from J. Bacteriol.

the periplasmic (or a periplasm-like) space of its host. Recent studies in the growing field of archaeal research have brought forward hitherto unsuspected features in these organisms, such as the existence of parasitism, outer membranes, periplasmic vesicles, protein domains with homology to key components in the eukaryotic vesicle transport system and cell division machinery with homology to eukaryotic sorting proteins.[65,66] The possible nature of the symbiotic association between the two partners, and the conditions that may have favoured such an association, are discussed below.

Was the common ancestor of eukaryotes aerobic or anaerobic?

Most models of the origin of the eukaryotic cell postulate that the common ancestor of eukaryotes was an anaerobic organism.[37] However, our interpretation of present data leads us to favour an aerobic origin of the mitochondrion, and a primordial aerobic or microaerophilic, chimeric common ancestor to eukaryotes.

Life emerged more than 3.5 billion years ago (Ga).[67,68] Geochemical data indicate that until 2.45 Ga, earth's atmosphere was essentially devoid of oxygen, and with the probable exception of small oxygen oases in the shallow oceans, conditions on the planet were anoxic. Between 2.45 and 2.0 Ga, atmospheric O_2 levels raised drastically ('the great oxidation event').[69–72] As a result, continental and ocean surfaces became oxic, while deep oceans probably remained anoxic for more than another billion years.[69,72] Estimates for the timing of the origin of the eukaryotic cell vary from more than 2.5 to 1.5 Ga[35–37] or even less.[57] Since the environments in which these cells evolved could have been almost anywhere from continental surfaces to deep oceans, these data alone are clearly not sufficient to solve the question whether eukaryotes evolved under oxic or anoxic conditions.

Analyses of the distribution of amino acids using an oxyphobic index, and of the abundance of metal-binding structures in archaeal, bacterial and eukaryotic proteins suggest that the ancestor of the Eukarya evolved in an aerobic environment, in contrast to the anaerobic ancestors of Bacteria and Archaea.[73,74] The increase in atmospheric O_2 levels was suggested to approximately coincide with a drastic increase in maximum cell size found in the fossil record[75] and with an increase in the complexity of biochemical networks[76] which are thought to have occurred during the prokaryotic–eukaryotic transition.

As mentioned earlier, hydrogenosomes and mitosomes probably developed numerous times independently from aerobic mitochondria as secondary adaptations to anaerobic environments.[41,43,77] Several observations indicate that anaerobic mitochondria that exhibit oxidative phosphorylation also evolved from aerobic types of mitochondria, and did not directly originate from a facultative anaerobic protomitochondrion.[78] Finally, sterols are ubiquitous features of eukaryotic membranes and it is plausible that the initial steps of their biosynthesis already existed in the common ancestor of eukaryotes. Noteworthy, 11 molecules of oxygen are required by four enzymes to produce one cholesterol molecule.[79] Thermodynamics, functional optimization and parsimony all support aerobic conditions as a prerequisite for the biosynthesis of ancient sterols.[79] According to our judgment, collectively the current data better support the hypothesis that mitochondrial acquisition and the origin of the eukaryotic common ancestor occurred under oxic conditions, than alternative hypotheses postulating that these events occurred under anaerobic conditions. Since life first developed under anaerobic conditions, anaerobic metabolism is certainly a primitive state. However, in the case of the evolution of the eukaryotic cell, it seems to be an adaptive trait derived from aerobic or microaerophilic ancestors.

The driving force for endosymbiosis

In the model we support, profound environmental changes brought about by increasing oxygen levels probably provided the impetus for the evolution of the mitochondrial partnership. Nevertheless, other models put forward a number of alternative possible selective forces (for reviews, see Refs[9,25,26]). The 'hydrogen hypothesis',[24] by far the most popular among the 'mitochondrion-early' models, proposes that similar to current hydrogenosomes, hydrogen produced by a protomitochondrion and consumed by a methanogenic archaeal host provided the energy source and the starting point of the mitochondrial symbiosis. Since methanogenic metabolism is inactivated by oxygen, such a scenario is incompatible with an aerobic context for the origin of eukaryotes. Additional concerns related to the 'hydrogen hypothesis' were voiced elsewhere (*e.g.* Refs[26,37,80]) and are beyond the scope of this paper. In our view, while the 'hydrogen hypothesis' was an important contribution in promoting alternatives to the 'Archezoa model', additional 'mitochondrion-early' models are not less plausible. Here, we will focus on two of these models: the oxygen detoxification model and the sulphide syntrophy model.

'The great oxidation event' led to drastic environmental and ecological changes. Anaerobic organisms, for example, became restricted to decreased anoxic environments, evolved protection mechanisms against the toxicity of oxygen or went extinct. Under such conditions, cooperative relationships between anaerobic and aerobic prokaryotes could have developed, the latter consuming the oxygen found in the vicinity of the former, thus enabling its survival. Anaerobic electron transfer enzymes operate at low potentials that facilitate the electron transfer process. When exposed to

oxygen, they may produce levels of reactive oxygen species higher than those produced by their aerobic counterparts.[81] The enzymatic activities needed to scavenge and protect the cell against the effects of oxidative damages, such as peroxidases, catalases, superoxide dismutases, antioxidants and thiol-rich proteins, may have been lacking or may have been poorly adapted in an archaeal obligate anaerobic host, rendering it dependent upon an oxygen-consuming partner. The endosymbiont in turn could have benefited from nutrients provided by the archaeon.

While this oxygen detoxification scenario fits both 'mitochondrion-early'[82,83] and 'mitochondrial-late' models,[80,84] it does not explain how a small endosymbiontic cell found inside a large host cell could protect its host from high external oxygen levels. We propose the contrasting hypothesis that the endosymbiont was found within a periplasm-like compartment inside its host where it could reproduce and actually surround the host's cytoplasm (Fig. 2), thereby protecting the host cell from toxic, external levels of oxygen.

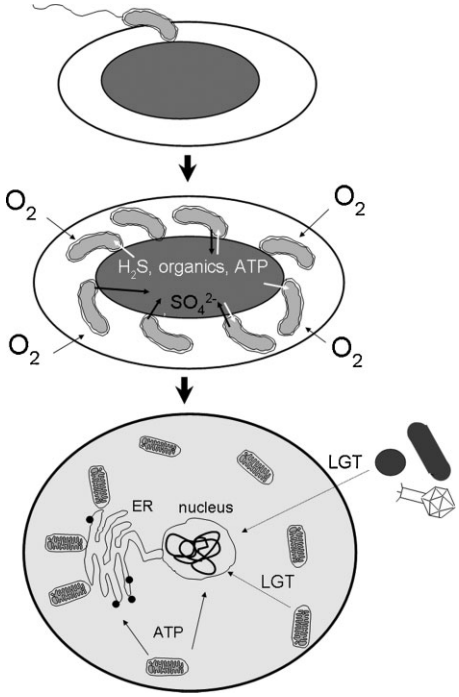

Figure 2. A predation-based scenario for the origin of eukaryotes. **Top**: A small facultatively aerobic predatory bacterium penetrates the periplasm of an anaerobic archaeal host. **Centre**: Under adequate environmental conditions, the predator does not consume it its prey. Rather, it develops a mutualist interaction and multiplies within the living host cell. This mutualism may be based on oxygen detoxification and sulphur syntrophy, thereby enabling the host cell to survive in an increasingly oxic environment. In return, the archaeal host provides the symbiont with shelter, and with an essential resource such as organic compounds and reduced sulphur. **Bottom**: Eventually, the host's cytoplasmic membrane transforms into internal membranes, including the nuclear membrane, while the outer membrane becomes the cell's cytoplasmic membrane. Concomitantly, the host's metabolism (including membrane components) is overtaken by bacterial functions. ATP produced by mitochondria fuelled increased genomes and cell sizes and complexity. Lateral gene transfer (LGT) from the mitochondria as well as from other bacteria and viruses (dashed arrows) contribute to nuclear genetic repertoire.

The sulphide syntrophy model[26,85] proposes that the protomitochondrion performed H_2S oxidation to sulphur while the archaeal host reduced this latter compound back to sulphide. Sulphur was cycled repeatedly, serving as an electron carrier between the two organisms. Such syntrophic interactions have been described.[26] Under low sulphur concentrations, neither species can grow in the absence of a recycling process. In contrast to methanogenesis, H_2S production occurs under aerobic as well as under anoxic conditions, and among phylogenetic basal Archaea, sulphur-dependent H_2S-producing organisms are common. Remarkably, in eukaryotic cells, H_2S produced by the cytoplasm is consumed by mitochondria.[85,86] Periodic cytoplasmic production of H_2S was found to result in an inhibition of respiratory activity and in an increase in O_2 concentration, thereby enhancing H_2S oxidation, leading to a new cycle. It was suggested that oxidative stress acts as a trigger for H_2S production, which itself acts as a redox regulator when the level of oxidative stress exceeds the antioxidant capacity of the cell.[87–89] This tight relationship suggests that the origin of mitochondrial endosymbionts may be based on a syntrophic association between a sulphide-oxidizing α-proteobacterium and an archaeal sulphide-producing host. Temporal separation and compartmentalization of the oxidative and the reductive processes eased the conflicts between the partners, further enhancing cooperation and created a mixed and flexible economy of sulphur cycling and oxygen detoxification well adapted to changing environmental conditions.

From endosymbiont to organelle

The two main partners that led to the formation of the eukaryotic cell were, according to the model we support, an anaerobic archaeal host and a facultatively aerobic protomitochondrion. Since the energetic yield of aerobic respiration is much higher than that of anaerobic metabolism, ATP production by the protomitochondrion is energetically advantageous. Compartmentalization of ATP synthesis requires the existence of an ATP export machinery from the protomitochondrion into the host cytoplasm, which is lacking in bacteria. It was even suggested that proteins for ATP export were lacking in the protomitochondrion.[39,40] In this regard as well, BALOs are exceptional as *Bdellovibrio* import ATP by active, not yet identified permeases.[90] Import systems working in reverse (*e.g.* Ref.[91]) may enable ATP transfer from the

protomitochondrion to its host and the compartmentalization of ATP production. This would be thermodynamically advantageous while concomitantly enabling oxygen detoxification. The evolving chimeric cell would be gradually released from constraints of limited bioenergetic surface area as energy production shifted from the cell wall (typical to prokaryotes) to internal, discrete units, creating ATP pools inside the cell. This 'energetic boost' could sustain the increase in genome size, cell volume and complexity seen in eukaryotes.[82]

The tight contact between the two evolving partners may have facilitated massive lateral gene transfer, as lysis of endosymbionts inside the host can create DNA free to recombine into the host genome, resulting in a continuous gene flow.[92] In contemporary eukaryotes, the large majority of the mitochondrial proteins are transferred from the cytosol to the organelle through transport systems. How these transport systems evolved remains a mystery. Without protein translocation systems, transfer of genes into the host genome seems to be impractical, but on the other hand, without massive gene transfer to the host genome there is no need for such a system to evolve. The unique nature of BALOs suggests a simple starting point to resolve this 'chicken and egg' puzzle: BALOs contain numerous transport systems, and experimental data even suggest that they can transfer outer membrane proteins into their host's membranes.[93,94] This ability may explain how mitochondrial coding genes could have been transferred to the host on the one hand, while nutrients would keep on flowing between the partners on the other hand.

To summarize, we propose that a predatory, facultative aerobic bacterium invaded the periplasm or the periplasm-like space of an archaeal host and that this complex evolved into the ancestor of all eukaryotes. This scheme not only permits tighter contact between the partners, molecular exchanges and gene flow, it also allows the host cytoplasmic membrane to become the progenitor of the ER and of nuclear membranes, cancelling a physical barrier between the endosymbiont and the chimeric cell's cytoplasm (Fig. 2).

Two organisms turn into one: conflicts, cooperation and the Janus paradox

The merger of two very different organisms to form a single complex cell is not a simple process. It is very likely that many conflicts and incompatibilities occurred between the two partners. Functional coordination was essential to form one cooperating, united entity. Solving the numerous problems the partners had to confront required innovations such as clear compartmentalization. Thus, a reason underlying the evolution of the nucleus might be protecting the growing genomic DNA from damages formed by reactive oxygen species created during the mitochondrial respiration.

The merger of bacterial and archaeal partners left its mark in the chimeric phylogeny of the eukaryotic genome. However, the distribution of homologous genes between the domains is strongly biased: eukaryotic informational genes involved in transcription, translation, DNA replication and repair are almost exclusively of archaeal origin, while operational genes coding for metabolic and cell structural functions are almost exclusively of bacterial origin.[31,45,47,50] Understanding why this is so is still challenging. This bizarre correlation was named by James Lake 'the Janus paradox',[95] after the two-faced Roman god.

Informational genes are generally less prone to lateral transfer than operational genes. Lake suggested that because two types of ribosomal genes cannot exist in the same nucleus, the archaeal ribosome may have been the lucky survivor when one of the components in the bacterial ribosome was inactivated.[95] Yet, this may have to do with more than chance. A single, centralized informational centre in the host nucleus could more easily coordinate actions than the multiple genome copies of the many mitochondrial units. Further, the separation of energy production (mitochondrion) and information (host) enabled the development of a balanced and equilibrated partnership that otherwise may degenerate into parasitism. Benefits and trade offs for each of the partners drove them toward cooperation. What were the rules that governed this game is a fascinating question.

The second question is then, why are operational genes of archaeal origin almost completely absent from the eukaryotic genome? David Valentine suggested that adaptation to energy stress dictates the ecology and evolution of archaea.[96] Known archaeal groups can outcompete bacteria in ecological niches in which chronic energy stress is a dominant feature. In contrast, many bacteria became adapted to maximize the availability of energy and other resources.[96] The distinctive archaeal membranes composed of isoprenoid glycerol ether lipids form structures that are less permeable to ions, thereby decreasing the amount of energy lost during maintenance of a chemiosmotic potential.[96] The trade off may be that low-permeability membranes enjoy lower rates of lateral diffusion, a parameter thought to be important for respiration and signal transduction.

As the protomitochondrion became the 'power house' of the eukaryotic cell, adaptations to chronic energy stress became irrelevant and were outcompeted by the 'higher energy'-adapted bacterial metabolism.[97] This provides a reasonable explanation for two unanswered issues: First, the eukaryotic membranes, including the cytoplasmic membrane, are bacterial-like, although the host cell was archaeal or archaeal-like. Second, it provides an explanation for the lack of archaeal operational genes in eukaryotes.

Conclusions

The origin of the eukaryotic cell is one of the most debated and enigmatic issues in evolutionary biology. This debate includes many cycles of discarded and subsequently rediscovered hypotheses. Here, we have addressed a few central questions concerning the different eukaryogenesis models in the light of the recently published literature. Based on the current recognition that all known eukaryotes contain mitochondria (or mitochondrion-like organelles), and on different genomics and phylogenetic analyses of both the whole eukaryotic genome and specific subcellular components, we conclude that 'mitochondrion-early' models that postulate the acquisition of the protomitochondrion by an archaeal host, are more plausible than 'mitochondrion-late' models. However, since prokaryotes are unable to perform phagocytosis, such models failed to suggest a reasonable mechanism by which the endosymbiont got access into its host.

We thus revive a previously proposed hypothesis that a predatory interaction between prokaryotes may have stood at the very origin of the eukaryotic cell, providing a starting point from which two prokaryotes became one endosymbiotic entity. According to the model presented here, the predator was a small (facultative) aerobic α-proteobacterium, which penetrated and replicated within an archaeal host periplasm or a periplasm-like space, and later became the mitochondria. Different lines of evidence suggest that contrary to the common notion, the origin of the eukaryotic cell and its mitochondrion occurred under aerobic conditions. We further suggest that oxygen detoxification combined with sulphur cycling served as the driving force in these endosymbiotic relationships. The merger of two different organisms to form a single cell required many innovations and it left its unique imprints in the developing complex cell.

About three decades ago, Jacob[98] suggested that at each level of a structured system, units associate to form a unit of higher level. Accordingly, Nowak[99] recently suggested adding 'natural cooperation' as a third fundamental principle of evolution in addition to mutation and natural selection. He stated: 'Evolution is constructive because of cooperation. New levels of organization evolve when the competing units on the lower level begin to cooperate'. The origin of the eukaryotic cell might be the most successful example of such a cooperative process. Here, we suggest that an interaction between two prokaryotes which begun as a predatory or a parasitic relation, by overcoming conflicts and incompatibilities, evolved into a symbiotic mutualistic interaction. New features emerged, finally resulting in a higher level of biological complexity. The chimera waned leaving a synergetic new life form that has conquered our planet, and is reaching beyond.

References

1. **Stanier, R. Y., Douderoff, M. and Adelberg, E.,** The microbial world, 2nd ed. Englewood Cliffs, NJ, Prentice-Hall, 1963.
2. **Furest, J. A.,** Intracellular compartmentation in planctomycetes. *Annu Rev Microbiol* 2005. **59**: 299–328.
3. **Mans, B. J., Anantharaman, V., Aravind, L. and Koonin, E. V.,** Comparative genomics, evolution and origins of the nuclear envelope and nuclear pore complex. *Cell Cycle* 2004. **3**: 1612–1637.
4. **Martin, W.,** Archaebacteria (Archaea) and the origin of the eukaryotic nucleus. *Curr Opin Microbiol* 2005. **8**: 630–637.
5. **Lane, N.,** Mitochondria: key to complexity. In: Martin, W. F. and Muller, M. editors. Origin of mitochondria and hydrogenosomes. Heidelberg, Germany, Springer, 2007. pp 13–38.
6. **Vanácová, S., Yan, W., Carlton, J. M. and Johnson, P. J.,** Spliceosomal introns in the deep-branching eukaryote Trichomonas vaginalis. *Proc Natl Acad Sci USA* 2005. **102**: 4430–4435.
7. **Gabaldón, T., Snel, B., van Zimmeren, F., Hemrika, W., Tabak, H. and Huynen, M. A.,** Origin and evolution of the peroxisomal proteome. *Biol Direct* 2006. **1**: 8.
8. **Schlüter, A., Fourcade, S., Ripp, R., Mandel, J. L., Poch, O. and Pujol, A.,** The evolutionary origin of peroxisomes: an ER-peroxisome connection. *Mol Biol Evol* 2006. **23**: 838–845.
9. **Embley, T. M. and Martin, W.,** Eukaryotic evolution, changes and challenges. *Nature* 2006. **440**: 623–630.
10. **Sapp, J.,** Mitochondria and their host: morphology to molecular phylogeny. In: Martin, W. F. and Muller, M. editors. Origin of mitochondria and hydrogenosomes. Heidelberg, Germany, Springer, 2007. pp 57–84.
11. **Wilson, E. B.,** The cell in development and heredity, 3rd ed. Macmillan, New York, 1925.
12. **Gray, M. W. and Doolittle, W. F.,** Has the endosymbiont hypothesis been proven? *Microbiol Rev* 1982. **46**: 1–42.
13. **Gray, M. W., Burger, G. and Lang, B. F.,** Mitochondrial evolution. *Science* 1999. **283**: 1476–1481.
14. **Sagan, L.,** On the origin of mitosing cells. *J Theor Biol* 1967. **14**: 225–274.
15. **Margulis, L.,** Origin of eukaryotic cells. New Haven, Yale University Press, 1970.
16. **de Duve, C.,** Evolution of the peroxisome. *Ann N Y Acad Sci* 1969. **168**: 369–381.
17. **Stanier, R.,** Some aspects of the biology of cells and their possible evolutionary significance. In: Charles, H. P. and Knight, B. C. editors. Organization and control in prokaryotic cells. 20th Symposium of the Society for General Microbiology. Cambridge, Cambridge University Press, 1970. pp 1–38.
18. **Woese, C. R. and Fox, G. E.,** Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc Natl Acad Sci USA* 1977. **74**: 5088–5090.
19. **Woese, C. R., Kandler, O. and Wheelis, M. L.,** Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci USA* 1990. **87**: 4576–4579.
20. **Vossbrinck, C. R., Maddox, J. V., Friedman, S., Debrunner-Vossbrinck, B. A. and Woese, C. R.,** Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. *Nature* 1987. **326**: 411–414.
21. **Cavalier-Smith, T.,** The simultaneous symbiotic origin of mitochondria, chloroplasts and microbodies. *Ann N Y Acad Sci* 1987. **503**: 55–71.
22. **Golding, G. B. and Gupta, R. S.,** Protein-based phylogenies support a chimeric origin for the eukaryotic genome. *Mol Biol Evol* 1995. **12**: 1–6.
23. **Margulis, L.,** Archaeal-eubacterial mergers in the origin of Eukarya: phylogenetic classification of life. *Proc Natl Acad Sci USA* 1996. **93**: 1071–1076.
24. **Martin, W. and Muller, M.,** The hydrogen hypothesis for the first eukaryote. *Nature* 1998. **392**: 37–41.
25. **Martin, W., Hoffmeister, M., Rotte, C. and Henze, K.,** An overview of endosymbiotic models for the origins of eukaryotes, their ATP-producing organelles (mitochondria and hydrogenosomes) and their heterotropic lifestyle. *Biol Chem* 2001. **382**: 1521–1539.
26. **Searcy, D. G.,** Metabolic integration during the evolutionary origin of mitochondria. *Cell Res* 2003. **13**: 229–238.

27. **Batut, J., Andersson, S. G. and O'Callaghan, D.,** The evolution of chronic infection strategies in the alpha-proteobacteria. *Nat Rev Microbiol* 2004. **2**: 933–945.
28. **Fitzpatrick, D. A., Creevey, C. J. and McInerney, J. O.,** Genome phylogenies indicate a meaningful alpha-proteobacterial phylogeny and support a grouping of the mitochondria with the Rickettsiales. *Mol Biol Evol* 2006. **23**: 74–85.
29. **Gupta, R. S. and Mok, A.,** Phylogenomics and signature proteins for the alpha proteobacteria and its main groups. *BMC Microbiol* 2007. **7**: 106.
30. **Williams, K. P., Sobral, B. W. and Dickerman, A. W.,** A robust species tree for the alphaproteobacteria. *J Bacteriol* 2007. **189**: 4578–4586.
31. **Esser, C., Ahmadinejad, N., Wiegand, C., Rotte, C., Sebastiani, F.,** *et al.* A genome phylogeny for mitochondria among alpha-proteobacteria and a predominantly eubacterial ancestry of yeast nuclear genes. *Mol Biol Evol* 2004. **21**: 1643–1660.
32. **Barbera, M. J., Ruiz-Trillo, I., Leigh, J., Hug, L. A. and Roger, A. J.,** The diversity of mitochondrion-related organells amongst eukaryotic microbes. In: Martin, W. F. and Muller, M. editors. Origin of mitochondria and hydrogenosomes. Heidelberg, Germany, Springer, 2007. pp 239–276.
33. **Khachane, A. N., Timmis, K. N. and Martins dos Santos, V. A.,** Dynamics of reductive genome evolution in mitochondria and obligate intracellular microbes. *Mol Biol Evol* 2007. **24**: 449–456.
34. **Darby, A. C., Cho, N. H., Fuxelius, H. H., Westberg, J. and Andersson, S. G.,** Intracellular pathogens go extreme: genome evolution in the Rickettsiales. *Trends Genet* 2007. **23**: 511–520.
35. **Knoll, A. H., Javaux, E. J., Hewitt, D. and Cohen, P.,** Eukaryotic organisms in Proterozoic oceans. *Philos Trans R Soc Lond B Biol Sci* 2006. **361**: 1023–1038.
36. **Roger, A. J. and Hug, L. A.,** The origin and diversification of eukaryotes: problems with molecular phylogenetics and molecular clock estimation. *Philos Trans R Soc Lond B Biol Sci* 2006. **361**: 1039–1054.
37. **de Duve, C.,** The origin of eukaryotes: a reappraisal. *Nat Rev Genet* 2007. **8**: 395–403.
38. **Gabaldón, T. and Huynen, M. A.,** Reconstruction of the proto-mitochondrial metabolism. *Science* 2003. **301**: 609.
39. **Gabaldón, T. and Huynen, M. A.,** From endosymbiont to host-controlled organelle: the hijacking of mitochondrial protein synthesis and metabolism. *PLoS Comput Biol* 2007. **3**: e219.
40. **Boussau, B., Karlberg, E. O., Frank, A. C., Legault, B. A. and Andersson, S. G.,** Computational inference of scenarios for alpha-proteobacterial genome evolution. *Proc Natl Acad Sci USA* 2004. **101**: 9722–9727.
41. **Hackstein, J. H., Tjaden, J. and Huynen, M.,** Mitochondria, hydrogenosomes and mitosomes: products of evolutionary tinkering. *Curr Genet* 2006. **50**: 225–245.
42. Martin, W. F. and Muller, M. editors. Origin of mitochondria and hydrogenosomes. Heidelberg, Germany, Springer, 2007.
43. Tachezy, J., editor. Hydrogenosomes and mitosomes: mitochondria of anaerobic eukaryotes. Heidelberg, Germany, Springer, 2008.
44. **van der Giezen, M.**, Hydrogenosomes and mitosomes: conservation and evolution of functions. *J Euk Microbiol* 2009. **56**: 221–231.
45. **Rivera, M. C. and Lake, J. A.,** The ring of life provides evidence for a genome fusion origin of eukaryotes. *Nature* 2004. **431**: 152–155.
46. **Aravind, L., Iyer, L. M. and Koonin, E. V.,** Comparative genomics and structural biology of the molecular innovations of eukaryotes. *Curr Opin Struct Biol* 2006. **16**: 409–419.
47. **Pisani, D., Cotton, J. A. and McInerney, J. O.,** Supertrees disentangle the chimerical origin of eukaryotic genomes. *Mol Biol Evol* 2007. **24**: 1752–1760.
48. **Saruhashi, S., Hamada, K., Miyata, D., Horiike, T. and Shinozawa, T.,** Comprehensive analysis of the origin of eukaryotic genomes. *Genes Genet Syst* 2008. **83**: 285–291.
49. **Cox, C. J., Foster, P. G., Hirt, R. P., Harris, S. R. and Embley, T. M.,** The archaebacterial origin of eukaryotes. *Proc Natl Acad Sci USA* 2008. **105**: 2035–20361.
50. **Yutin, N., Makarova, K. S., Mekhedov, S. L., Wolf, Y. I. and Koonin, E. V.,** The deep archaeal roots of eukaryotes. *Mol Biol Evol* 2008. **25**: 1619–1630.
51. **Staub, E., Fiziev, P., Rosenthal, A. and Hinzmann, B.,** Insights into the evolution of the nucleolus by an analysis of its protein domain repertoire. *Bioessays* 2004. **26**: 567–581.
52. **Staub, E., Mackowiak, S. and Vingron, M.,** An inventory of yeast proteins associated with nucleolar and ribosomal components. *Genome Biol* 2006. **7**: R98.
53. **Ohyanagi, H., Ikeo, K. and Gojobori, T.,** The origin of nucleus: rebuild from the prokaryotic ancestors of ribosome export factors. *Gene* 2008. **423**: 149–152.
54. **Jekely, G.,** Small GTPases and the evolution of the eukaryotic cell. *Bioessays* 2003. **25**: 1129–1138.
55. **Dacks, J. B., Poon, P. P. and Field, M. C.,** Phylogeny of endocytic components yields insight into the process of nonendosymbiotic organelle evolution. *Proc Natl Acad Sci USA* 2008. **105**: 588–593.
56. **Yutin, N., Wolf, M. Y., Wolf, Y. I. and Koonin, E. V.,** The origins of phagocytosis and eukaryogenesis. *Biol Direct* 2009. **4**: 9.
57. **Cavalier-Smith, T.,** Predation and eukaryote cell origins: a coevolutionary perspective. *Int J Biochem Cell Biol* 2009. **41**: 307–322.
58. **Poole, A. M. and Penny, D.,** Eukaryote evolution: engulfed by speculation. *Nature* 2007. **447**: 913.
59. **Jurkevitch, E. and Davidov, Y.,** Phylogenetic diversity and evolution of predatory prokaryotes. In: Jurkevitch, E. editor. Predatory prokaryotes—biology, ecology, and evolution. Heidelberg, Springer-Verlag, 2007. pp 1–56.
60. **Davidov, Y., Huchon, D., Koval, S. F. and Jurkevitch, E.,** A new α-proteobacterial clade of *Bdellovibrio*-like predators: implications for the mitochondrial endosymbiotic theory. *Environ Microbiol* 2006. **8**: 2079–2088.
61. **Sacchi, L., Bigliardi, E., Corona, S., Beninati, T., Lo, N.,** *et al.* A symbiont of the tick *Inoxes ricinus* invades and consumes mitochondria in a mode similar to that of the parasitic bacterium *Bdellovibrio bacteriovorus*. *Tissue Cell* 2004. **36**: 43–53.
62. **Sassera, D., Beninati, T., Bandi, C., Bouman, E. A., Sacchi, L.,** *et al.* 'Candidatus Midichloria mitochondrii', an endosymbiont of the tick Ixodes ricinus with a unique intramitochondrial lifestyle. *Int J Syst Evol Microbiol* 2006. **56**: 2535–2540.
63. **Guerrero, R., Pedros-Alio, C., Esteve, I., Mas, J., Chase, D. and Margulis, L.,** Predatory prokaryotes: predation and primary consumption evolved in bacteria. *Proc Natl Acad Sci USA* 1986. **83**: 2138–2142.
64. **Margulis, L.,** Symbiosis in cell evolution, 2nd ed. New York, USA, Freeman, 1993.
65. **Podar, M., Anderson, I., Makarova, K. S., Elkins, J. G., Ivanova, N.,** *et al.* A genomic analysis of the archaeal system Ignicoccus hospitalis-*Nanoarchaeum* equitans. *Genome Biol* 2008. **9**: R158.
66. **Samson, R. Y., Obita, T., Freund, S. M., Williams, R. L. and Bell, S. D.,** A role for the ESCRT system in cell division in Archaea. *Science* 2008. **322**: 1710–1713.
67. **Nisbet, E. G. and Sleep, N. H.,** The habitat and nature of early life. *Nature* 2001. **409**: 1083–1091.
68. **Schopf, J. W.,** Fossil evidence of Archaean life. *Philos Trans R Soc Lond B Biol Sci* 2006. **361**: 869–885.
69. **Anbar, A. D. and Knoll, A. H.,** Proterozoic ocean chemistry and evolution: a bioinorganic bridge? *Science* 2002. **297**: 1137–1142.
70. **Canfield, D. E.,** The early history of atmospheric oxygen: homage to Robert A. *Garrels Annu Rev Earth Plan Sci* 2005. **33**: 1–36.
71. **Holland, H. D.,** The oxygenation of the atmosphere and oceans. *Philos Trans R Soc Lond B Biol Sci* 2006. **361**: 903–915.
72. **Scott, C., Lyons, T. W., Bekker, A., Shen, Y., Poulton, S. W.,** *et al.* Tracing the stepwise oxygenation of the Proterozoic ocean. *Nature* 2008. **452**: 456–459.
73. **Di Giulio, M.,** The universal ancestor and the ancestors of Archaea and Bacteria were anaerobes whereas the ancestor of the eukarya domain was an aerobe. *J Evol Biol* 2007. **20**: 543–548.
74. **Dupont, C. L., Yang, S., Palenik, B. and Bourne, P. E.,** Modern proteomes contain putative imprints of ancient shifts in trace metal geochemistry. *Proc Natl Acad Sci USA* 2006. **103**: 17822–17827.
75. **Payne, J. L., Boyer, A. G., Brown, J. H., Finnegan, S., Kowalewski, M.,** *et al.* Two-phase increase in the maximum size of life over 3.5 billion years reflects biological innovation and environmental opportunity. *Proc Natl Acad Sci USA* 2009. **106**: 24–27.
76. **Raymond, J. and Segrè, D.,** The effect of oxygen on biochemical networks and the evolution of complex life. *Science* 2006. **311**: 1764–1767.

77. **Embley, T. M.,** Multiple secondary origins of the anaerobic lifestyle in eukaryotes. *Philos Trans R Soc Lond B Biol Sci* 2006. **361**: 1055–1067.
78. **Tielens, A. G. and van Hellemond, J. J.,** Anaerobic mitchondria: properties and origin. In: Martin, W. F. and Muller, M. editors. Origin of mitochondria and hydrogenosomes. Heidelberg, Germany, Springer, 2007. pp 85–104.
79. **Summons, R. E., Bradley, A. S., Jahnke, L. L. and Waldbauer, J. R.,** Steroids, triterpenoids and molecular oxygen. *Philos Trans R Soc Lond B Biol Sci* 2006. **361**: 951–968.
80. **Andersson, S. G., Karlberg, O., Canbäck, B. and Kurland, C. G.,** On the origin of mitochondria: a genomics perspective. *Philos Trans R Soc Lond B Biol Sci* 2003. **358**: 165–177.
81. **Imlay, J. A.,** Pathways of oxidative damage. *Annu Rev Microbiol* 2003. **57**: 395–418.
82. **Vellai, T., Takács, K. and Vida, G.,** A new aspect to the origin and evolution of eukaryotes. *J Mol Evol* 1998. **46**: 499–507.
83. **Vellai, T. and Vida, G.,** The origin of eukaryotes: the difference between prokaryotic and eukaryotic cells. *Proc Biol Sci* 1999. **266**: 1571–1577.
84. **Andersson, S. G. and Kurland, C. G.,** Origins of mitochondria and hydrogenosomes. *Curr Opin Microbiol* 1999. **2**: 535–541.
85. **Searcy, D. G.** Origins of mitochiondria and chloroplasts from sulphur-based symbioses. In: Hartman, H. and Matsuno, K. editors. The origin and evolution of the cell. Singapore, World Scientific, 1992. pp 47–78.
86. **Searcy, D. G.,** Rapid hydrogen sulfide consumption by Tetrahymena pyriformis and its implications for the origin of mitochondria. *Eur J Protistol* 2006. **42**: 221–231.
87. **Kwak, W. J., Kwon, G. S., Jin, I., Kuriyama, H. and Sohn, H. Y.,** Involvement of oxidative stress in the regulation of H_2S production during ultradian metabolic oscillation of *Saccharomyces cerevisiae*. *FEMS Microbiol Lett* 2003. **219**: 99–104.
88. **Lloyd, D.,** Hydrogen sulfide: clandestine microbial messenger? *Trends Microbiol* 2006. **14**: 456–462.
89. **Lloyd, D. and Murray, D. B.,** Redox rhythmicity: clocks at the core of temporal coherence. *Bioessays* 2007. **29**: 465–473.
90. **Ruby, E. G. and McCabe, J. B.,** An ATP transport system in the intracellular bacterium, *Bdellovibrio bacteriovorus* 109J. *J Bacteriol* 1986. **167**: 1066–1070.
91. **Saier, M. H.,** Protein uptake into *E. coli* during *Bdellovibrio* infection. A process of reverse secretion? *FEBS Lett* 1994. **337**: 14–17.
92. **Martin, W. and Koonin, E. V.,** Introns and the origin of nucleus-cytosol compartmentalization. *Nature* 2006. **440**: 41–45.
93. **Tudor, J. J. and Karp, M. A.,** Translocation of an outer membrane protein into prey cytoplasmic membranes by bdellovibrios. *J Bacteriol* 1994. **176**: 948–952.
94. **Barel, G., Sirota, A., Volpin, H. and Jurkevitch, E.,** Fate of predator and prey proteins during growth of *Bdellovibrio bacteriovorus* on *Escherichia coli* and *Pseudomonas syringae* prey. *J Bacteriol* 2005. **187**: 329–335.
95. **Lake, J. A.,** Disappearing act. *Nature* 2007. **446**: 983.
96. **Valentine, D. L.,** Adaptations to energy stress dictate the ecology and evolution of the Archaea. *Nat Rev Microbiol* 2007. **5**: 316–323.
97. **Davidov, Y. and Jurkevitch, E.,** How incompatibilities may have led to eukaryotic cell. *Nature* 2007. **448**: 130.
98. **Jacob, F.,** Evolution and tinkering. *Science* 1977. **196**: 1161–1166.
99. **Nowak, M. A.,** Five rules for the evolution of cooperation. *Science* 2006. **314**: 1560–1563.