Metabolic symbiosis at the origin of eukaryotes

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Thirty years after Margulis revived the endosymbiosis theory for the origin of mitochondria and chloroplasts, two novel symbiosis hypotheses for the origin of eukaryotes have been put forward. Both propose that eukaryotes arose through metabolic symbiosis (syntrophy) between eubacteria and methanogenic Archaea. They also propose that this was mediated by interspecies hydrogen transfer and that, initially, mitochondria were anaerobic. These hypotheses explain the mosaic character of eukaryotes (i.e., an archaeal-like genetic machinery and a eubacterial-like metabolism), as well as distinct eukaryotic characteristics (which are proposed to be products of symbiosis). Combined data from comparative genomics, microbial ecology and the fossil record should help to test their validity.

Our Origins Have Always Concerned Us – from the speculation of Homo sapiens to the origins of life itself. Between these events, a crucial midpoint is the origin of the eukaryotic cell, the nature of which is still controversial and elusive. Until the mid-1970s, two possibilities were conceivable: either eukaryotes were ancestral, and thus their origin was intimately linked to the origin of life itself, or they derived from prokaryotes (organisms lacking a true nucleus that encloses the genetic material). The evolution of eukaryotes from simple prokaryotes (then called Monera) is implicit in the phylogeny proposed by Haeckel in the 19th century. However, after the demise of the eukaryote/prokaryote dogma (the commonly held belief that all that is not eukaryote is bacterial) – which followed the recognition (based on RNA-sequence comparison) that there are two distinct phylogenetic lineages within the prokaryotes, eubacteria (Bacteria) and archaea (Archaia) – the situation became more complex. These lineages appeared to be as different from each other as they were from eukaryotes; yet, later, the use of several protein gene markers established that archaia are, strikingly, more similar to eukaryotes. Furthermore, studies of paralogous duplications allowed us to place the root of the tree of life tentatively in the eubacterial branch. This explained the archaean-eukaryote similarities but also refined our view of the origin of eukaryotes: these would share a common (prokaryotic) ancestor with archaia. This idea permeated the scientific community rapidly and is now widely accepted.

Many protein trees, however, contain discrepancies that consistently relate either archaia (mostly on the basis of the genetic machinery) or Gram-negative bacteria (mostly on the basis of metabolism) to eukaryotes. Such discrepancies have led several investigators to propose different chimera hypotheses (Fig. 1). Of these, fusion and engulfment models are mechanistically problematic, although they can explain the mosaic distribution of many genes. By contrast, syntrophy models rely on intimate relationships over extended periods of time that allowed symbionts to co-evolve and become dependent on each other. Indeed, the first detailed symbiosis proposal for the origin of eukaryotes, the endosymbiosis hypothesis for the origin of plastids and mitochondria proposed by Margulis, although harshly criticized initially, is supported by extensive evidence and is now accepted as mainstream science. Later, Margulis further proposed that eukaryotes originated through symbiosis between spirochetes and wall-less archaia (Fig. 1), but compelling evidence to support this hypothesis is lacking. Surprisingly, despite the potential of symbiosis to account for mixed characters (which would be a consequence of the contribution of at least two partners to a mutually beneficial association), and the increasingly evident mosaic features of eukaryotic genomes, nobody had proposed other symbiosis hypotheses until recently. In 1998, the 30th anniversary of the endosymbiosis proposal, we, and Martin and Miller, independently, published two novel symbiosis hypotheses: the hydrogen hypothesis and the syntrophy hypothesis. The hypotheses are different but share striking similarities.

Syntrophy and interspecies hydrogen transfer

The hydrogen hypothesis proposed by Martin and Miller states that eukaryotes arose through a symbiotic association (or syntrophy) in anaerobic environments between a fermentative α-proteobacterium that generated hydrogen and carbon dioxide as waste products, and a strict anaerobic autotrophic archaean that depended on hydrogen and might have been a methanogen (Fig. 2). The authors follow a metabolic top-down approach from the observation that amitochondriate eukaryotes possess eubacterial-like metabolic enzymes (in addition to other known eubacterial-like genes) and that hydrogenosomes (hydrogen-producing organelles present in some anaerobic eukaryotes) share a common ancestry with mitochondria. They propose that both symbionts first met in anaerobic environments rich in hydrogen and carbon dioxide, but that soon the host changed its dependence on an exogenous source of these products, becoming dependent on the symbionts supplying them. The archaean increased the cell-contact surface with the symbiont and ended up importing membrane transport systems and carbohydrate metabolism. Finally, to avoid futile cycling of metabolites in its cytoplasm, the host lost its autotrophic pathway. The final organism in this evolutionary process is an irreversible heterotroph that contains ancestral mitochondria and has lost its dependence on hydrogen – hence, the need for anaerobiosis. The more efficient oxygenic respiration was then adopted by many organisms: aerobic mitochondria evolved. Secondary reduction or loss of organelles would explain present-day amitochondriate protists.

The idea that the origin of mitochondria is the key to the origin of the eukaryotic cell is now new in scientific thought. Another hypothesis published in 1998 proposes that an α-proteobacterium was engulfed by an archaean prior to the establishment of
classical endosymbiosis. The brilliant novelty is that Martin and Müller offer a plausible explanation of the process in terms of metabolism, and conclude that the origins of mitochondria and eukaryotes are identical but that anaerobic mitochondria came first.

**The syntrophy hypothesis**

Our syntrophy hypothesis is based on similar metabolic considerations (i.e. we propose that symbiosis was mediated by interspecies hydrogen transfer), but we speculate that the organisms involved were *d*-proteobacteria (ancestral sulphate-reducing myxobacteria) and methanogenic archaea (Fig. 2). The hydrogen and syntrophy hypotheses share several common features (Fig. 3), despite our use of a different approach. From microbial ecology, we know that the most widespread symbiotic association between archaea and eubacteria is syntrophy between sulphate-reducing bacteria and methanogenic archaea. Furthermore, the habitats where these organisms exist are ubiquitous. This gave us a likely initial driving force for symbiosis: syntrophy. The methanogen consumed the hydrogen and carbon dioxide liberated from the sulphate reducer by fermentation. The sulphate reducer also benefited: it could speed up its metabolic rate because it now had a ready hydrogen sink. We based our arguments on a variety of molecular features that, in addition to the classical characteristics that link Gram-negative bacteria or archaea to eukaryotes, connect myxobacteria and certain methanogens with eukaryotes. Myxobacteria display very complex social behaviour and developmental cycles, and many of the genes involved have specific homologues in eukaryotic signalling pathways. Methanogen candidates (Fig. 2) share some homologous lipids or lipid-synthesis pathways with eukaryotes, and their content with respect to many enzymes that interact with DNA (e.g. topoisomerases) is similar to that of eukaryotes. However, their most remarkable feature is the presence of true histones and nucleosomes. Archaeal nucleosomes not only are homologous in sequence and three-dimensional structure to eukaryotic (H3–H4) tetramers, but also experience similar dynamics (D. Musgrave et al., pers. commun.). This strongly suggests that these organisms have eukaryotic-like chromatin (both at structural and regulatory levels), which is found neither in the kingdom Crenarchaeota nor in the halophilic methanogens (Fig. 2).

The latter are endowed instead with small DNA-binding proteins analogous to those of eubacteria. We envisage an evolutionary pathway in which close cell–cell contacts and extensive membrane development in well-established symbiotic consortia led to more highly evolved structures that had primitive eukaryotic features, such as a prokaryotic region (old archaean cytoplasm) defined by membranous structures (Fig. 3). Eubacterial genome extinction could have occurred by progressive transfer to the archaeal genome, where genes adapted to a new genetic environment. Many redundant eubacterial genes (mostly those that encoded the genetic machinery) would have been lost, whereas others would have replaced archaean genes (mainly those that encoded proteins involved in metabolism). Finally, methanogenesis would have been lost in favour of a versatile heterotrophy. Common features Although their starting points differ, the two hypotheses agree in several respects. Their arguments are therefore complementary. Central to both is the metabolic nature of the original symbiotic event; in this sense, both hypotheses involve hydrogen and syntrophy. Martin and Müller have provided a detailed explanation of the metabolic context during the process and give a good reason for the loss of the host autotrophic pathway, whereas we have tried to construct a more global picture that also covers the formation of the eukaryotic genome and membrane systems.

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**Figure 1** (a) A schematic view of the evolution of Eubacteria, eukaryotes and Archaea. (b–d) Previous chimeric models for the origin of eukaryotes (colour coded as in (a)).

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Common ancestor

Eubacteria

Eukaryotes

Archaea

Fusion

Engulfment

Symbiosis

Zillig

Lake and Rivera

Gupta and Golding

Sogin

Margulis

(a)

(b–d)

An archaeon and a bacterium amalgamate to generate the eukaryotic cell

A bacterium engulfs an eocyte (kingdom Crenarchaeota)

A wall-less Gram-negative bacterium engulfs an eocyte

An RNA-based proto-eukaryote phagocytoses an archaeon

Serial endosymbiosis theory
Another common feature is the suggestion that a methanogen was the archaeal partner. Martin and Müller believe that any anaerobic hydrogen-dependent autotrophic archaeon (e.g. a sulphur-dependent archaeon) could have started the process. Methanogens, however, appeal to them because they appear early in the archaeal tree, are autotrophic, anaerobic, and widespread, and can use hydrogen, carbon dioxide and acetate (waste products of the presumptive symbiont)\(^1\). We have several additional reasons for suggesting that the archaeal symbiont must have been a methanogen\(^1\). Finally, both proposals give a plausible explanation for the mosaic nature of eukaryotic genomes without proposing any dramatic event but simply by postulating gene transfer and replacement over a long symbiotic life.

One or two eubacterial symbionts at the origin?

The critical difference between the two hypotheses is the nature of the eubacterial partners (Fig. 2). According to the hydrogen hypothesis, \(\alpha\)-proteobacteria established the symbiosis and, on the way to becoming mitochondria, produced eukaryotes. In our proposal, two eubacterial types were involved. First, sulphate-reducing \(\delta\)-proteobacteria, which also produce hydrogen from fermentation and form syntrophic consortia with methanogens. Second, either at the same time or shortly after, \(\alpha\)-proteobacterial methanotrophs (the progenitors of mitochondria) took part in the symbiotic community. These methanotrophs fed on the methane produced by the methanogen, producing carbon dioxide and thereby permitting an increase in the rate of methanogenesis (Fig. 3). Everybody was happy.

It might be difficult to find out which hypothesis is correct, given that all eubacterial candidates belong to Proteobacteria and that phylogenetic signals that could allow us to differentiate between the two hypotheses might have been erased with time. However, some pieces of evidence, which link \(\delta\)-proteobacteria (especially myxobacteria) to eukaryotes, are worth exploring.

An anaerobic origin for mitochondria

Regardless of whether the \(\alpha\)-proteobacterial bacterium was the primary symbiont (hydrogen hypothesis) or a secondary symbiont (syntrophy hypothesis), we agree that ancestral mitochondria were anaerobic. This contradicts the classical endosymbiosis theory, which assumes that the predecessors of mitochondria were efficient aerobes. As Smith and Szathmary\(^1\) first pointed out, and Martin and Müller\(^1\) emphasize, in the endosymbiosis theory the initial benefit for the host is not clear. No bacterium gives free ATP to the medium. Nevertheless, whereas in the hydrogen hypothesis the presumptive \(\alpha\)-proteobacterial ancestor of mitochondria is a fermentative anaerobe, we suggest that it is an anaerobic methanotroph. Again, choosing between the two possibilities might be difficult, but some indicative evidence should be investigated further. For instance, it is widely assumed that methanotrophs are strict aerobes, because the enzyme that converts methane to methanol, methane monooxygenase, requires oxygen. However, anaerobic methanotrophs that might use sulphate or nitrate instead exist. Interestingly, these are linked to methanogen sulphate-reducer consortia.\(^1\) Also intriguing is the recent discovery of several \(C\_1\)-transfer enzymes and coenzymes (which are required for the interconversion of one-carbon compounds) that link methylo trophic bacteria (which feed on \(C\_1\)
compounds in general) and methanogenic archaea. These enzymes and cofactors previously were thought to be unique to methanogens and the sulfate reducer *Archaeoglobus* (a derived methanogen). Interdomain horizontal transfer of these genes between such intimately associated groups of organisms could be a good explanation.

Mitochondria are believed to be derivatives of *Rickettsia*-like ancestors. The *Rickettsia* are intracellular parasites and seem to be phylogenetically related to mitochondria. However, because of their adaptation to the intracellular environment, endosymbiotic organelles and cytoplasmic parasites have accelerated their evolutionary rates and, consequently, display long branches in phylogenetic trees. The fact that fast-evolving lineages tend to cluster together artefactually because of the long-branch attraction phenomenon is well known. Therefore, the phylogenetic positions of such organisms should be regarded with caution.

Methanotrophs are interesting alternatives to *Rickettsia* as mitochondrial progenitors. Methanotrophy is widely distributed among the *α*-Proteobacteria and probably represents an ancestral phenotype in this group. Hence, even if mitochondria have a rickettsial origin, their ancestor might have been endowed with this metabolic ability. Furthermore, note that methanotrophs are commonly ecto- or endosymbionts. They are found in the cytoplasm of a wide variety of eukaryotes (e.g. they are abundant in the tissues of deep-vent-associated invertebrates) and in bacteria, such as *Beggiatoa* (*a γ*-proteobacterium that usually forms mats around deep hydrothermal vents).

**Insights from comparative genomics**

The impressive developments in genome sequencing over the past few years have already produced enough data to support a mixed heritage for the eukaryotic genome, which contains archaeal-like DNA-processing (informational) genes and Gram-negative bacterial-like metabolic (operational) genes. This can only be explained either by a massive horizontal gene transfer from Gram-negative bacteria to eukaryotic ancestors or by a chimeric origin. The two symbiosis hypotheses marry both possibilities: the chimerism they propose is directional. Either a selective transfer of metabolic genes towards an archaeal host occurred (the hydrogen hypothesis), or a progressive transfer and replacement of non-informational genes occurred (the syntrophy hypothesis).

Of course, the fact that these symbiotic models explain the mosaic character of eukaryotic genomes does not mean that they are correct. Extensive comparison of the increasingly available genome sequences might, however, help to test the hypotheses’ robustness. Two genome types would be particularly interesting: (1) a non-methanogenic archaeal genome, preferably belonging to the Crenarchaeota (*Pyrobaculum aerophilum* should shortly be released, and others will follow); (2) an extreme halophile (the genomes of two *Halobacterium* species are being sequenced). Comparative analysis would show whether methanogens are more closely related to eukaryotes than are other archaea. A myxobacterial genome sequence (the *Myxococcus xanthus* genome project is also under way) could support one symbiosis hypothesis rather than the other, given that the syntrophy hypothesis predicts that eukaryotes contain a mixture of *α*- and *δ*-proteobacterial-like genes.

Increased evolutionary rates and the generation of innovative properties are associated with symbiosis. Indeed,
symbiosis can be regarded as an eco-
nological association that provides most
mechanisms (genomic and spatial com-
partmentation, genomic and physio-
logical redundancy and specialization,
and evolutionary flexibility) for cir-
cumventing or reducing selective con-
straints. It allows an increase in com-
plexity; the hallmark of eukaryotes, that
otherwise would be selected against.22
The detection, through comparative gen-
omics, of eukaryotic molecules that
have adapted to perform functions dif-
ferent from those of their prokaryotic pre-
decessors (and have therefore in-
creased their evolutionary rates) is particu-
larly interesting. One example might be the evolution of cytoskeletal proteins. Tubulin shares some sequence similarity with, and is structurally homologous to, prokaryotic FtsZ, which is involved in cell division. Interestingly, eu- bactera have a single FtsZ copy, but Eurarchaeota at least (and thus meth-
anogens) have two different copies.22
Duplicated genes, which are released
from functional constraints, can evolve
faster and adapt to new needs, and
eukaryotes obviously needed a well-
developed cytoskeleton.

Insights from microbial ecology

New life is unlikely to be originating
nowadays, because proto-organisms
would be outcompeted by efficient life
forms. By analogy, eukaryotic life is
unlikely to be forming anew: proto-
eukaryotes would be outcompeted by
modern well-adapted eukaryotes. None-
theless, the study of present-day anaero-
bic communities might provide interesting
cues to eukaryote evolution.

Molecular ecology might be of further
help. We have identified an enormous
diversity of uncultured microorganisms
by this means and have confirmed the
idea that sulphate reducers and meth-
anogens predominate in many anaero-
bic biotopes in the oceanic crust. Most pres-
ently are found in geothermally heated
thermophilic microbial mats (laminar
stromatolites) or geothermally heated
biotopes in the oceanic crust. These
mats appear first and most strongly in stromato-
lices whereas 27, whereas

communities that have left us fossil
remains (stromatolites and microfossils), whereas

Around 3500 million years ago, the
Earth supported complex prokaryotic
communities that have left us fossil
stomalitites and microfossils, whereas
the first eukaryotic fossils date from
1800–2100 million years ago.23 If the
analysis of microfossils alone does not
reveal decisive information about the origins of eukaryotes, the combination of this approach and physico-chemical
measures of biogenic markers might.
For example, Kral and co-workers have
reported the presence of glycerol tetra-
ethers, which are characteristic of
methanogens, at that time. Ohmoto and Felder report that eu-
bacterial sulphate reduction occurred in Archaean oceans,
which were rich in sulphate, at tempera-
atures up to 50°C. On the basis of the iso-
topic composition of the organic carbon
contained in sediments, Hayes has
pointed out the existence of a historical
peak of methanotrophic activity, which
would be linked to methanogenesis in
global carbon cycling, at the Archaean–
Prototrozoic transition. Interestingly, the
isotopic signal of methanotrophy ap-
ppears first and most strongly in stromato-
lices units.24 By contrast, the extremely
low abundance of steranes (biomarkers
for eubacteria), compared with that of
hopanes (steroid surrogates that are
considered biomarkers for eukaryota),
in mid-Prototrozoic sediments supports
a later rise of eukaryota.25

Finally, because the early Earth was
probably warmer and had much less free
atmospheric oxygen, it is interesting to
study living fossil ecosystems, such as
thermophilic microbial mats (laminar
stromatolites) or geothermally heated
biotopes in the oceanic crust. Most pres-
ent-day thermophilic mats (which are at
temperatures of -50-70°C) are aerobic at
the surface and possess a cyanobacte-
rial layer (the first cyanobacterial mats
would have given eukaryotes the poten-
tial to acquire chloroplasts). However,
the earliest probably were anaerobic and
possessed anaerobic photosynthetic eu-
bacteria instead. In the deeper, anaerobic
layers, methanogens and sulphate reducers dominate.23,24 At 22–55°C,
sulphate-reduction and methanogenesis
also predominate in sea-floor commu-
nities associated with geothermal regions,
whose temperatures would be among the
oldest on the planet.24

Conclusions

The two symbiosis hypotheses for
the origin of eukaryotes try to explain
as much as possible with the minimum
number of assumptions. Thus, although
they differ in the nature and number of
ubacterial original symbionts that are
proposed, both hypotheses convincingly
account for the molecular signature of eu-
kytotic genomes and are based on metabolic interactions that are wide-
spread in nature. Remarkably, both pro-
pose that a methanogen was the archaean
partner and that mitochondria have an
anaerobic origin.

To prove the Martin and Müller hy-
pothesis directly could be difficult. We
already know that the ancestors of mito-
ochondria transferred many genes to their
host, but how can we identify whether
the latter was an archaen or a member
of a third lineage (the classical model)?25
To test the symbiosis hypothesis could be easier, although a recent adaptation
of the classical view would explain the
presence in eukaryotes of eu-
bacterial genes from different taxonomic groups
(the 'you are what you eat' version)26.
However, in our model, we expect traits
common to only a restricted range of organisms (ß-proteobacterial, ß-pro-
tobacterial methanotrophs and some
methanogenic archaenas) to be found to-
gether. We must learn to look at the
molecular level but, at the same time, at
the ecological context and at the fossil
record. Only if data from different ap-
proaches converge, will we be able to
construct a plausible answer to the
question of the origin of eukaryotes.

Acknowledgments

We thank Miklós Müller and William
Martin for critical reading of the
manuscript and helpful comments, and
the European Community and the
Spanish Ministerio de Educación y
Cultura for financial support.
Researchers at the University of Davis in California have used confocal fluorescence and reflection methods simultaneously to produce crisp three-dimensional images of endothelial cells, with no bleed-through problems. This ability to visualize intact vascular architecture and endothelial cell morphology in three-dimensions provides a new way of investigating the response to inflammatory mediators in microvessels.

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