Metabolic symbiosis at the origin of eukaryotes

Purificación López-García and David Moreira

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 speciation 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 similar) – which followed the recognition (based on rRNA-sequence comparison) that there are two distinct phylogenetic lineages within the prokaryotes, eubacteria (Bacteria) and archaea[1,2] – 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 archaea are, strikingly, more similar to eukaryotes[3]. Furthermore, studies of paralogous duplications allowed us to place the root of the tree of life tentatively in the eubacterial branch[4]. This explained the archaea–eukaryote similarities but also refined our view of the origin of eukaryotes: these would share a common (prokaryotic) ancestor with archaea. This idea permeated the scientific community rapidly and is now widely accepted.

Many protein trees, however, contain discrepancies that consistently relate either archaea (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, symbiosis 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[5], although harshly criticized initially, is supported by extensive evidence[6] and is now accepted as mainstream science. Later, Margulis[7] further proposed that eukaryotes originated through symbiosis between spirochetes and wall-less archaea (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[8,9], nobody had proposed other symbiosis hypotheses until recently. In 1998, the 30th anniversary of the endosymbiosis proposal, we, and Martin and Müller, independently, published two novel symbiosis hypotheses: the hydrogen hypothesis[10] and the syntrophy hypothesis[11]. The hypotheses are different but share striking similarities.

Syntrophy and interspecies hydrogen transfer

The hydrogen hypothesis

The hydrogen hypothesis proposed by Martin and Müller[10] states that eukaryotes arose through a symbiotic metabolic 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 archaeon 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 eubacterium supplying them. The archaeon 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 oxigenic 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 not new in scientific thought. Another hypothesis published in 1998, proposes that an aerobic α-proteobacterium was engulfed by an archaeon prior to the establishment of

P. López-García is at the Institut de Génétique et Microbiologie, Université Paris-Sud, bâtiment 409, 91405 Orsay Cedex, France; and **D. Moreira** is at the Institut de Biologie Cellulaire BC4, Université Paris-Sud, bâtiment 444, 91405 Orsay Cedex, France.
Email: puri@igmors.u-psud.fr

0968–0004/99/$ – See front matter PII: S0968-0004(98)01342-5

classical endosymbiosis[12]. 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[11] 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 δ-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 biotopes 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[13]. 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 $(H3–H4)_2$ 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).

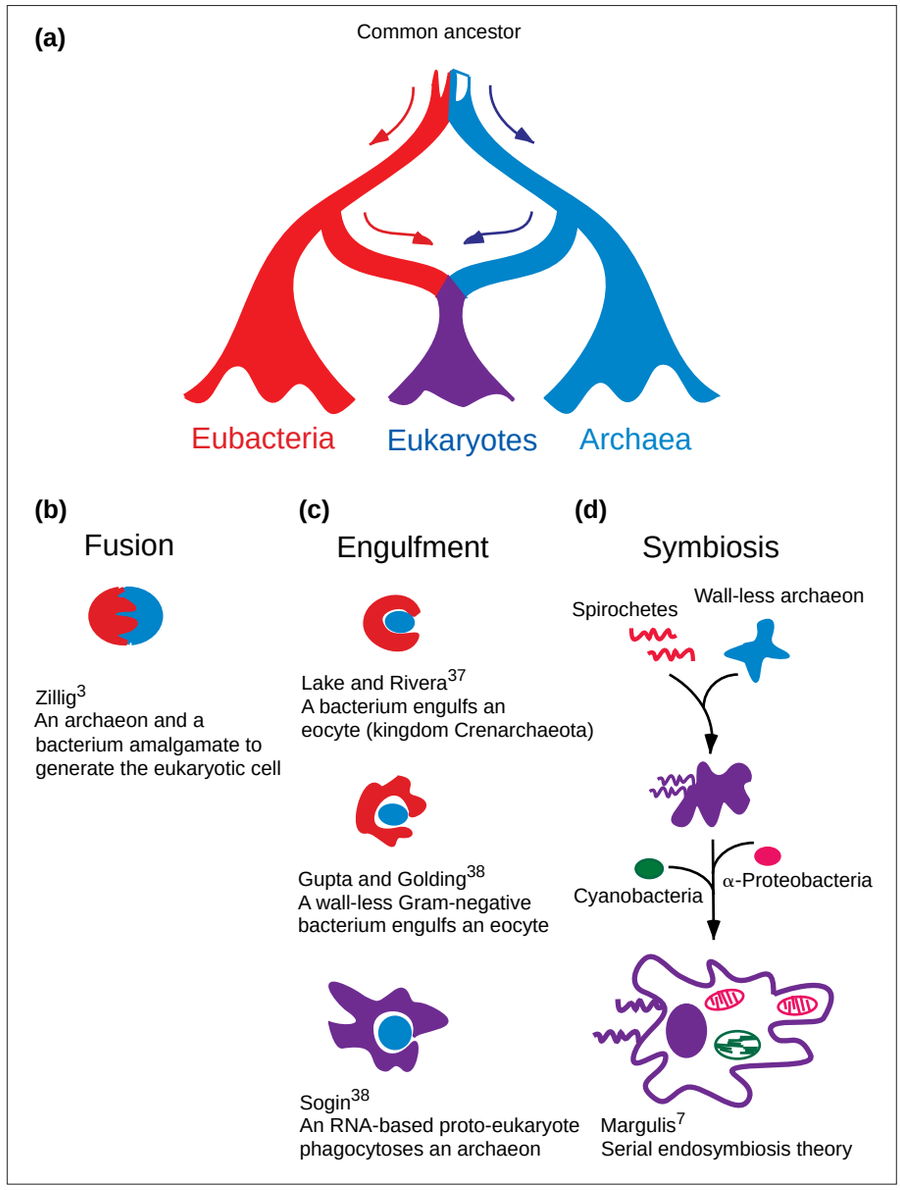

Figure 1

(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)][3,7,37–39].

The latter are endowed instead with small DNA-binding proteins analogous to those of eubacteria[14].

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 protonuclear region (old archaeal 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 archaeal 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.

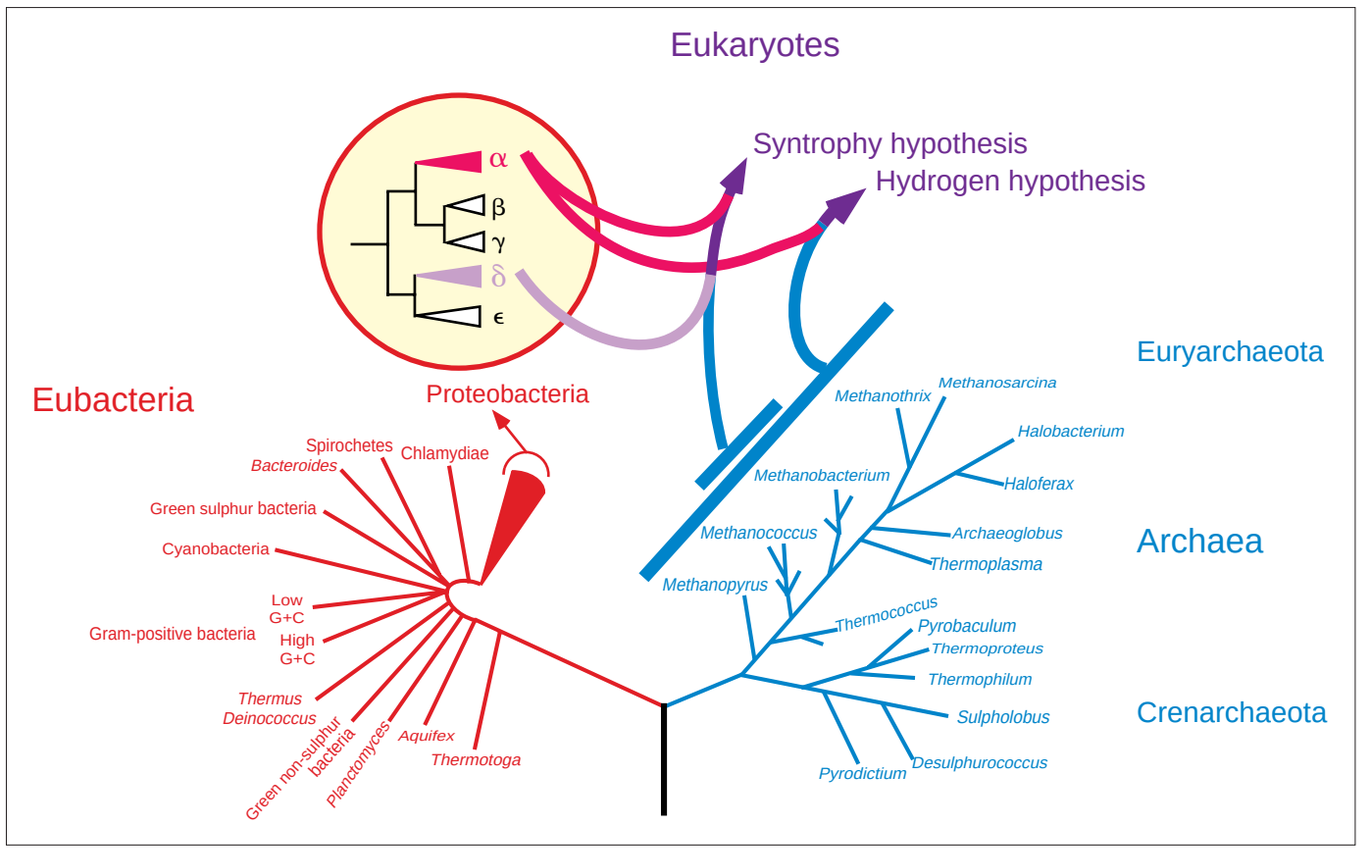

Figure 2

An rRNA-based phylogenetic tree showing the locations of the prokaryotic partners that are at the origin of eukaryotes in the two novel symbiosis hypotheses. Myxobacteria and most sulphate reducers belong to the δ-Proteobacteria.

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)[10]. We have several additional reasons for suggesting that the archaeal symbiont must have been a methanogen[11]. 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, α-Proteobacteria established the symbiosis and, on the way to becoming mitochondria, produced eukaryotes. In our proposal, two eubacterial types were involved. First, sulphate-reducing δ-proteobacteria, which also produce hydrogen from fermentation and form syntrophic consortia with methanogens. Second, either at the same time or shortly after, α-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 effaced with time. However, some pieces of evidence, which link δ-proteobacteria (especially myxobacteria) to eukaryotes, are worth exploring.

An anaerobic origin for mitochondria

Regardless of whether the α-proteobacterium 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[15] first pointed out, and Martin and Müller[10] 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 α-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[16]. 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 methylotrophic bacteria (which feed on C_1

compounds in general) and methanogenic archaea[17]. These enzymes and cofactors previously were thought to be unique to methanogens and the sulphate 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[18]. The *Rickettsia* are intracellular parasites and seem to be phylogenetically related to mitochondria[18]. 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[19]. 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[16] 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 endo-symbionts. 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 eubacteria, such as *Beggiatoa* (a γ-proteobacterium that usually forms mats around deep hydrothermal vents)[16,20].

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[8,9]. This can only be explained either by a massive horizontal gene transfer from Gram-negative bacteria to eukaryotic ancestors[9] or by a chimeric origin[8,9]. 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).

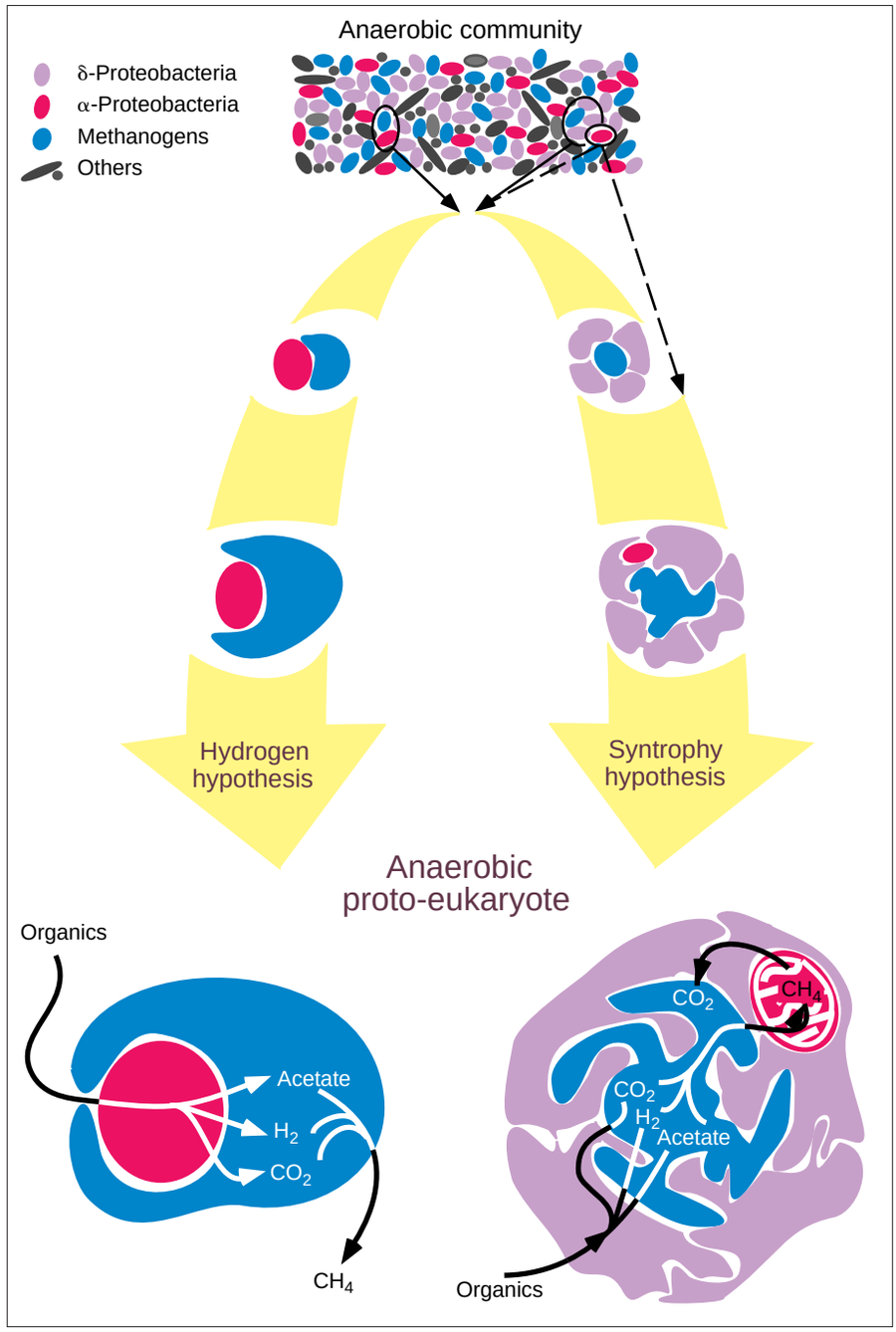

Figure 3

Evolutionary pathways proposed by the hydrogen and syntrophy hypotheses for the origin of eukaryotes. The exchange of products between the different prokaryotic partners is shown at an advanced state of the metabolic symbiosis.

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[7]. Indeed,

symbiosis can be regarded as an ecological association that provides most mechanisms (genomic and spatial compartmentation, genomic and physiological redundancy and specialization, and evolutionary flexibility) for circumventing or reducing selective constraints[21]. It allows an increase in complexity, the hallmark of eukaryotes, that otherwise would be selected against[21]. The detection, through comparative genomics, of eukaryotic molecules that have adapted to perform functions different from those of their prokaryotic predecessors (and have therefore increased their evolutionary rates) is particularly 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, eubacteria have a single *ftsZ* copy, but Euryarchaeota at least (and thus methanogens) 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. Nonetheless, the study of present-day anaerobic communities might provide interesting clues 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 methanogens predominate in many anaerobic meso- and thermophilic environments[23]. In fact, many organisms that are candidates for the eubacteria and archaea in the syntrophy hypothesis had not been identified until recently, because they could not be cultivated (many would be difficult to grow in pure cultures) or simply because they had not been looked for. This was the case for methanotrophic anaerobes, which are now known to form symbiotic consortia with methanogens and sulphate reducers in anoxic environments[16]. Similarly, although myxobacteria generally are thought to be strict aerobes, we have known for a long time that anaerobic myxobacteria exist[24]; an anaerobic sulphate-reducing myxobacterium has even been identified[25].

Communities that might be especially interesting to study are microbial mats, where sulphate reducers and methanogens frequently dominate in the anoxic layers, and strong metabolic interactions exist. Interestingly, myxobacteria are present in microbial mats at active hydrothermal vents[26].

Insights from the fossil record

Not only are the biotopes where methanogens, sulphate reducers and methanotrophs coexist ubiquitous on the planet today, but some might be as ancient as the first living organisms. Around 3500 million years ago, the Earth supported complex prokaryotic communities that have left us fossil stromatolites and microfossils[27], whereas the first eukaryotic fossils date from 1800–2100 million years ago[28]. If the analysis of microfossils alone does not reveal decisive information about the origins of eukaryotes, the combination of this approach and physico-chemical measurements of biogenic markers might. For example, Kral and co-workers[29] have produced evidence for hydrogen consumption by methanogens on the early Earth. Chappe and co-workers[30] have reported the presence of glycerol tetraethers, which are characteristic of methanogens, at that time. Ohmoto and Felder[31] report that eubacterial sulphate reduction occurred in Archaean oceans, which were rich in sulphate, at temperatures up to 50°C. On the basis of the isotopic composition of the organic carbon contained in sediments, Hayes[32] 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–Proterozoic transition. Interestingly, the isotopic signal of methanotrophy appears first and most strongly in stromatolitic units[32]. By contrast, the extremely low abundance of steranes (biomarkers for eukaryotes), compared with that of hopanes (steroid surrogates that are considered biomarkers for eubacteria), in mid-Proterozoic sediments supports a later rise of eukaryotes[33].

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 present-day thermophilic mats (which are at temperatures of ~50–70°C) are aerobic at the surface and possess a cyanobacterial layer (the first cyanobacterial mats would have given eukaryotes the potential to acquire chloroplasts). However, the earliest probably were anaerobic and possessed anaerobic photosynthetic eubacteria instead. In the deeper, anaerobic layers, methanogens and sulphate reducers dominate[23,34]. At 22–55°C, sulphate reduction and methanogenesis also predominate in sea-floor communities associated with geothermal regions, environments thought to be among the oldest on the planet[35].

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 eubacterial original symbionts that are proposed, both hypotheses convincingly account for the mosaic character of eukaryotic genomes and are based on metabolic interactions that are widespread in nature. Remarkably, both propose that a methanogen was the archaeal partner and that mitochondria have an anaerobic origin.

To prove the Martin and Müller hypothesis directly could be difficult. We already know that the ancestors of mitochondria transferred many genes to their host, but how can we identify whether the latter was an archaeon or a member of a third lineage (the classical model[2])? To test the syntrophy hypothesis could be easier, although a recent adaptation of the classical view would explain the presence in eukaryotes of eubacterial genes from different taxonomic groups (the 'you are what you eat' version)[36]. However, in our model, we expect traits common to only a restricted range of organisms (δ-proteobacterial, α-proteobacterial methanotrophs and some methanogenic archaea) to be found together. 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 approaches converge, will we be able to construct a plausible answer to the question of the origin of eukaryotes.

Acknowledgements

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.

References

1 Woese, C. R. and Fox, G. E. (1977) *Proc. Natl. Acad. Sci. U. S. A.* 74, 5088–5090
2 Woese, C. R., Kandler, O. and Wheelis, M. L. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 4576–4579
3 Zillig, W. (1991) *Curr. Opin. Genet. Dev.* 1, 544–551
4 Doolittle, W. F. and Brown, J. R. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 6721–6728
5 Margulis, L. (1970) *Origin of Eukaryotic Cells*, Yale University Press
6 Gray, M. W. (1989) *Trends Genet.* 5, 294–299
7 Margulis, L. (1993) *Symbiosis in Cell Evolution*, W. H. Freeman
8 Rivera, M. C., Jain, R., Moore, J. E. and Lake, J. A. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 6239–6244
9 Ribeiro, S. and Golding, G. B. (1998) *Mol. Biol. Evol.* 15, 779–788
10 Martin, W. and Müller, M. (1998) *Nature* 392, 37–41
11 Moreira, D. and López-García, P. (1998) *J. Mol. Evol.* 47, 517–530
12 Vellai, T., Takacs, K. and Vida, G. (1998) *J. Mol. Evol.* 46, 499–507
13 Dworkin, M. (1996) *Microbiol. Rev.* 60, 70–102
14 Grayling, R. A., Sandman, K. and Reeve, J. N. (1994) *Syst. Appl. Microbiol.* 16, 582–590
15 Smith, J. M. and Szathmary, E. (1995) *The Major Transitions in Evolution*, Freeman, Oxford
16 Hanson, R. S. and Hanson, T. E. (1996) *Microbiol. Rev.* 60, 439–471
17 Chistoserdova, L., Vorholt, J. A., Thauer, R. K. and Lidstrom, M. E. (1998) *Science* 281, 99–102
18 Andersson, S. G. E. *et al.* (1998) *Nature* 396, 133–143
19 Felsenstein, J. (1978) *Syst. Zool.* 27, 401–410
20 Larkin, J. M. and Henk, M. C. (1996) *Microsc. Res. Tech.* 33, 23–31
21 Kirschner, M. and Gerhart, J. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 8420–8427
22 Faguy, D. M. and Doolittle, W. F. (1998) *Curr. Biol.* 8, R338–R341
23 Fenchel, T. and Finlay, B. J. (1995) in *Ecology and Evolution in Anoxic Worlds* (May, R. M. and Harvey, P. H., eds), pp. 85–98, Oxford University Press
24 Sanzhieva, E. U. (1970) *Mikrobiologiia* 39, 817–820
25 Cole, J. R., Cascarelli, A. L., Mohn, W. W. and Tiedje, J. M. (1994) *Appl. Environ. Microbiol.* 60, 3536–3542
26 Moyer, C. L., Dobbs, F. C. and Karl, D. M. (1995) *Appl. Environ. Microbiol.* 61, 1555–1562
27 Knoll, A. H. (1990) in *Paleobiology: A Synthesis* (Briggs, D. E. G. and Crowther, P. R., eds), pp. 9–16, Blackwell Scientific Publications
28 Han, T. M. and Runnegar, B. (1992) *Science* 257, 232–235
29 Kral, T. A., Brink, K. M., Miller, S. L. and McKay, C. P. (1998) *Orig. Life Evol. Biosph.* 28, 311–319
30 Chappe, B., Albrecht, P. and Michaelis, W. (1982) *Science* 217, 65–66
31 Ohmoto, H. and Felder, R. P. (1987) *Nature* 328, 244–246
32 Hayes, J. M. (1994) in *Early Life on Earth* (Bengtson, S., ed.), pp. 220–236, Columbia University Press
33 Jackson, M. J., Powell, T. G., Summons, R. E. and Sweet, I. P. (1986) *Nature* 322, 727–729
34 Ward, D. M., Tayne, T. A., Anderson, K. L. and Bateson, M. M. (1987) in *Ecology of Microbial Communities* (Fletcher, M., Gray, T. R. G. and Jones, J. G., eds), pp. 179–210, Cambridge University Press
35 Delaney, J. R. *et al.* (1998) *Science* 281, 222–230
36 Doolittle, W. F. (1998) *Trends Genet.* 14, 307–311
37 Lake, J. A. and Rivera, M. C. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 2880–2881
38 Gupta, R. and Golding, G. B. (1996) *Trends Biochem. Sci.* 21, 166–171
39 Sogin, M. L. (1991) *Curr. Opin. Genet. Dev.* 1, 457–463