ESSAY

The origin of eukaryotes: a reappraisal

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Abstract | Ever since the elucidation of the main structural and functional features of eukaryotic cells and subsequent discovery of the endosymbiotic origin of mitochondria and plastids, two opposing hypotheses have been proposed to account for the origin of eukaryotic cells. One hypothesis postulates that the main features of these cells, including their ability to capture food by endocytosis and to digest it intracellularly, were developed first, and later had a key role in the adoption of endosymbionts; the other proposes that the transformation was triggered by an interaction between two typical prokaryotic cells, one of which became the host and the other the endosymbiont. Re-examination of this question in the light of cell-biological and phylogenetic data leads to the conclusion that the first model is more likely to be the correct one.

The origin of nucleated cells has long been an object of wonder and speculation. Three events have marked the modern era of research in this field. First, discoveries in the 1950s and 1960s revealed the intricate organization of eukaryotic cells and the functional specializations of each type of structure. Second, the landmark paper by Lynn Margulis (Sagan at the time)[1] revived the endosymbiotic theory of the origin of mitochondria and plastids, and subsequent experiments confirmed her proposal. Third, with the innovative investigations of Woese and Fox[2], molecular phylogenies were extended back to the dawn of life, yielding a wealth of new data that prompted a profusion of new hypothetical models.

Surprisingly, these new models often focus on a single eukaryotic feature, mostly the nuclear genome or the mitochondria, ignoring several other cell parts of comparable importance. My main purpose in this Essay is to restore some balance in the field, reconciling the equally valid demands of cell biology and phylogenies. References to the relevant literature, which has grown to immense proportions, are necessarily selective, but should be sufficient to allow retrieval of further information (see also REF. 3).

The making of a eukaryote

Eukaryotic cells differ from prokaryotic cells by a number of features: a nucleus, fenced off by an envelope and containing elaborately structured chromosomes, along with the main molecular systems responsible for replication and transcription of the chromosomal DNA and for processing of the RNA transcripts; an extensive system of cytomembranes, subdivided into a number of specialized parts; cytoskeletal elements and associated motor systems; peroxisomes and related organelles; mitochondria and the related hydrogenosomes; and, in phototrophic eukaryotes only, plastids (FIG. 1). Another distinguishing feature of eukaryotic cells is that they divide by mitosis. When, how and in what order were these various eukaryotic features acquired, and what evolutionary advantages did they provide?

Time and setting. A crucial date in the history of eukaryotes lies around 2.4 billion years ago, when molecular oxygen started rising in the Earth's atmosphere[4]. Oxygen-related organelles, such as peroxisomes, mitochondria and plastids, must have been acquired after that date. Other eukaryotic features must likewise have developed under aerobic conditions if their acquisition accompanied or followed that of oxygen-related organelles. If acquired earlier, they could have been developed under anaerobic conditions, a point that is relevant to theories that assume eukaryotic transformation was triggered by the adoption of mitochondria. Such theories imply that eukaryotic cells developed within the period between the rise of atmospheric oxygen and the appearance of the first eukaryotic organisms. Unfortunately, estimates of that date vary widely, from as early as 2.7 billion years ago[5] to no more than 0.9–1.3 billion years ago[6,7], or even later[8,9].

The more recent estimates rest almost exclusively on the lack of undisputed fossil evidence of more ancient eukaryotic organisms; which, by itself, is not a strong argument. Earlier organisms might not have left any recognizable fossil remains. Alternatively, they might have occupied a restricted niche that has not yet been searched for microfossils. This could have been the case for the long succession of intermediates in the development of the main eukaryotic features, which most likely preceded the adoption of mitochondria (see below).

Arguments brought forward in favour of a very ancient origin of eukaryotes, long predating the first identified eukaryotic microfossils, perhaps even the appearance of atmospheric oxygen, have been the large number and apparent antiquity of eukaryotic innovations that do not have a prokaryotic counterpart[10,11]. Revealed by molecular studies, these so-called ESPs (eukaryotic signature proteins) number in the hundreds and have led some to contend that the eukaryotic line could date back to as early as 3.5 billion years ago, or perhaps even to the last universal common ancestor (LUCA). Furthermore, what is known of most eukaryotic features allows the assumption that they developed under anaerobic conditions. Remove peroxisomes and mitochondria (and plastids) from a eukaryotic cell, and you are left with what is essentially an anaerobic organism, one in which rare oxygen-utilizing systems, such as cytochrome P_{450} and associated oxygenases, could be late acquisitions. The same could

be the case for the eukaryotic cholesterol, which was most likely preceded by polyisoprenoids that did not require oxygen for their synthesis[12].

Genomic chimerism. Eukaryotic genes seem to be partly of eubacterial and partly of archaebacterial type, implying a mixed ancestry for eukaryotes. The distribution of the two types of genes seems to be non-random. Roughly speaking, genes that have nuclear functions (informational genes) have archaebacterial characteristics; those that have cytoplasmic functions (operational genes) have eubacterial characteristics[13,14]. This genetic mixing has been attributed to the fusion of a eubacterial and an archaebacterial cell[15–18], or to the formation of an endosymbiotic relationship, with either the eubacterial partner acting as host cell for an archaebacterial endosymbiont, destined to become the eukaryotic nucleus[14,19,20], or the archaebacterial partner serving as host cell for a eubacterial endosymbiont, ancestral to the mitochondria[21–24].

Notably, all of these models assume prokaryote–prokaryote interactions that have never been observed. They all also raise a serious problem, related to the chemical composition of the membranes of the two kinds of prokaryotes (see below). Furthermore, it is not clear how the two genomes could have become reorganized into a single genome, with each partner providing a given set of genes and losing the others. This difficulty and other considerations have led some to postulate that eukaryotic cells go back to a very ancient protoeukaryote, or 'urkaryote', that even antedates prokaryotes, which are assumed to have arisen from this ancestral organism by 'reductive' evolution and to have split into two distinct branches only later[25–28]. Others, also invoking very early phenomena, have attributed the genomic chimerism of eukaryotes to lateral gene transfer, a process that is thought to have been much more prevalent among the first primitive cells than it is today[29–32]. Both of these models place the origin of eukaryotic cells long before the acquisition of endosymbionts, pushing the main events of eukaryote genesis even further back than other models.

In sharp contrast, Cavalier-Smith[8,9] has defended the theory that archaebacteria emerged at the same time as eukaryotes, a mere 850 million years ago, from a common ancestor (neomuran) that he believes to have arisen from eubacteria after these had been around for more than 2 billion years. According to this theory, the alleged genetic chimerism of eukaryotes is really a mosaicism, combining genes inherited from the eubacterial ancestor (or derived later from mitochondria) with new genes gained by the common neomuran ancestor of eukaryotes and archaebacteria in the course of its evolution.

A possibility that does not seem to have been considered is that the archaebacterial genes were acquired from an endosymbiont by a eubacterium-related host cell that already possessed some key eukaryotic properties, including a nucleus and an operational phagocytic machinery. Such an endosymbiont could have abandoned a number of genes to the host-cell nucleus, as mitochondria and plastids are known to have done, and could subsequently have disappeared (or been converted into peroxisomes, see below). This possibility has two advantages: it does not require an interaction between prokaryotes of a kind that has never been observed, and it postulates only well-known phenomena that are associated with other instances of endosymbiosis. However, like the other models of genomic chimerism, it fails to explain the mechanism by which the informational genes of the host were selectively replaced by those of the endosymbiont.

There is the even more drastic possibility that the very idea of genomic chimerism might rest on a questionable phylogenetic basis, and that gene transfers from endosymbionts and, perhaps, neighbouring cells might account for the mosaic composition of the eukaryotic genome. This opinion is defended by Kurland *et al.*[11], who have severely criticized all fusion models.

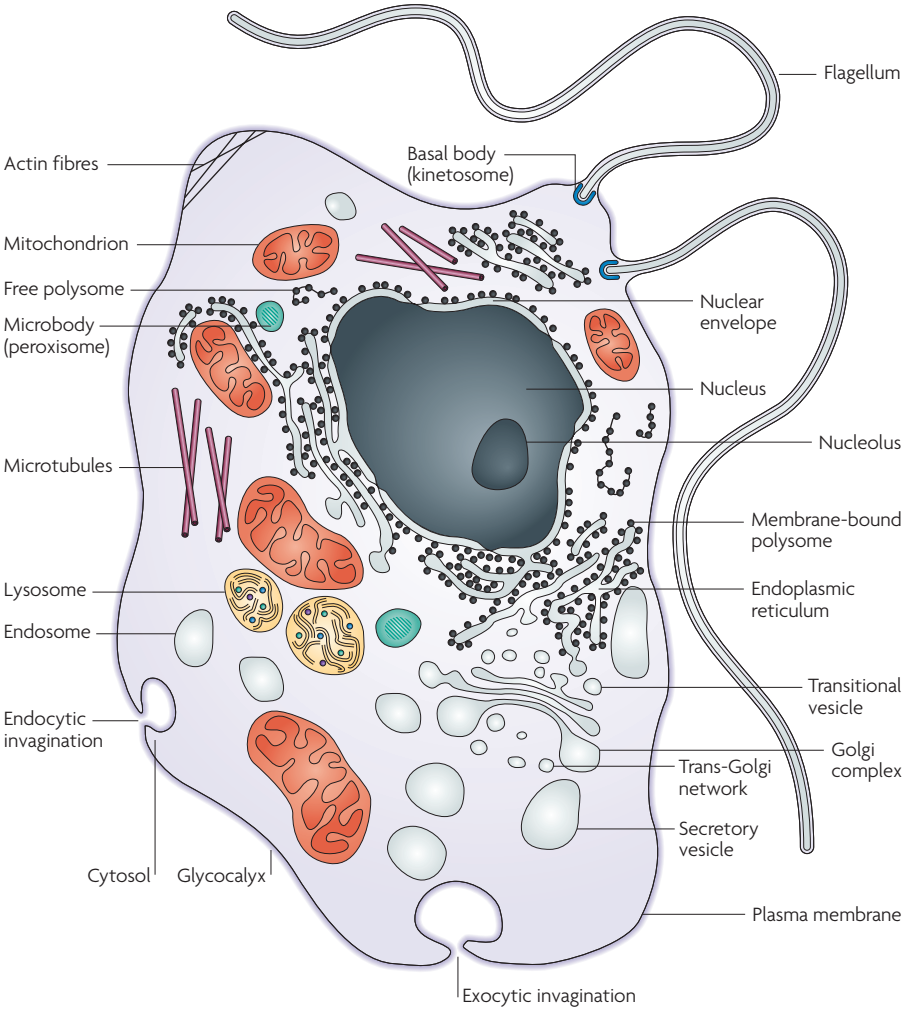

Figure 1 | **The main features of eukaryotic cells.** A hypothetical flagellated protist showing all the components of eukaryotic cells, with the exception of plastids, which are present only in phototrophic eukaryotes. For comparison, the average prokaryote is about the size of a mitochondrion. Modified with permission from REF. 51 © (1991) Neil Patterson Publishers.

Membrane lipids. All biological membranes are built with phospholipid bilayers. In eubacteria and eukaryotes, the phospholipid core consists of long-chain fatty acids linked by ester bonds to L-glycerol-3-phosphate. In archaebacteria, this core is made of long-chain isoprenoid alcohols linked by ether bonds to D-glycerol-3-phosphate. Transient coexistence of the two kinds of phospholipids is implicit in almost any model of genomic chimerism; how was this biochemical difference resolved? Mixed bilayers are unstable and are bound to be eliminated by natural selection in favour of pure types of one kind or the other.

Fusion models are particularly questionable in this context. Even between two kindred prokaryotes, fusion would require special conditions, such as the absence of a cell wall, close proximity, surface-protein compatibility and, perhaps, some joining agent. With the problems of phospholipid chimerism and bilayer instability added to the requirement for these conditions, fusion between a eubacterial and an archaebacterial prokaryote could well become highly improbable, if not impossible.

Another difficulty that is common to fusion and other 'encounter' models concerns the assembly of new membranes. In present-day cells, membranes always arise by the insertion of newly made constituents into pre-existing membranes, eventually followed by fission: "*omnis membrana e membrana*", all membranes arise from membranes[33]. Thus, lipid chimerism and its attendant difficulties seem to pose an intractable problem for all encounter models. The problem is particularly serious in the model that posits the development of a eubacterial endosymbiont within an archaebacterial host cell, implying that the host somehow replaced its own ether lipids with the ester products of enzymes encoded by endosymbiont genes. The proposed assembly of eubacterial-type membranes in the cytoplasm of the host, followed by their substitution for the host's own membranes[34,35], strains credibility. Even an archaebacterial endosymbiont that evolved to become the nucleus of a eubacterial host would probably not be able to readily exchange its lipid bilayers for those of the host.

This issue poses fewer problems for the model of an archaebacterial endosymbiont being adopted by a host cell of eukaryotic character. As pointed out below in connection with the origin of peroxisomes, it is conceivable that such an endosymbiont took residence within the host-cell's cytomembrane system and, thus sheltered, lost its own membrane as well as its genes, leaving as sole vestiges of its erstwhile presence those of its genes that were incorporated into the host's nuclear genome.

The fact remains that the two lipid types must have coexisted at some stage of evolution if the two prokaryotic groups are derived from a common ancestor[36]. A key event in the emergence of archaebacteria might have been the conversion of a eubacterial glycerol dehydrogenase into a D-glycerol-3-phosphate dehydrogenase[37].

The cytomembrane system. The cytomembrane system is a dynamic, elaborate network of differentiated, membranous sacs, intermittently connected with each other and with the plasma membrane by fusion and fission events. Channelled and supported by cytoskeletal and motor elements (see below), this system is involved in the endocytic uptake and digestive breakdown of materials that are imported from the outside, and in the synthesis, processing, transport and exocytic discharge of materials that are destined for export.

The cytomembrane network most likely originated from infoldings of the plasma membrane of some wall-less ancestral cell, probably related to eubacteria, which likewise possess ester phospholipids. As the cell's size increased, the invaginations grew deeper and more convoluted, splitting into vesicles that gradually differentiated into specialized parts, comprising the rough endoplasmic reticulum (ER), the related nuclear envelope, the smooth ER, the Golgi complex, endosomes and lysosomes. Convincing evidence of such an evolutionary process is provided by the close similarities that exist between the co-translational protein translocation systems in bacterial membranes and in the rough ER[38–45].

The above hypothesis was first formulated by Wattiaux and myself[46] at a time when the endosymbiotic origin of mitochondria and plastids was not yet appreciated. Our aim was to account for the origin of lysosomes. We suggested that the highly advantageous conversion from extracellular to intracellular digestion, associated with membrane internalization, acted as the evolutionary driving force of the process (FIG. 2), thereby initiating one of the most fateful events in cellular evolution. This heralded (as was also underlined independently by Stanier[47] and by Cavalier-Smith[48]) "…the beginning of cellular emancipation."[49] Henceforth, heterotrophic cells were no longer obliged to reside within their food supply; they were free to pursue their prey actively, living on endocytized bacteria and other engulfed materials, which they digested intracellularly within their lysosomes. Today, all eukaryotic cells use this process, not only for nutrition, but also for various specialized functions, including the capture and destruction of bacteria. This defence mechanism is occasionally thwarted. In exceptional instances, it is followed by the endosymbiotic adoption of the captured organisms. The many known cases of endosymbiosis are all believed to have occurred in this way.

When the endosymbiotic origin of mitochondria and plastids was recognized, it seemed reasonable to assume that the development of the cytomembrane system preceded the acquisition of these organelles and provided the means for their adoption. The first phylogenetic reconstructions, made on the basis of the comparative sequencing of 16S ribosomal RNA[2,50], provided what appeared to be a clinching confirmation of this hypothesis, showing that the most ancient positions in the eukaryotic tree were occupied by organisms that are devoid of mitochondria, presumably descendants of lineages that had split off from the so-called 'primitive phagocyte' before the acquisition of mitochondria[51,52,53]. Subsequent results have failed to support this idea, instead showing that the organisms in question probably did at some time contain mitochondria or related organelles (see REF. 24 and references within). At present, no known organism qualifies as a direct descendant of the primitive phagocyte.

In itself, this negative finding merely fails to confirm, but does not invalidate, a hypothesis that rests on solid grounds and has considerable explanatory power. Biological evolution is landmarked by 'missing links', of which no living or fossil trace has yet been identified. This fact is rarely used as an argument against an otherwise well-supported evolutionary theory. In this case, however, the missing-link argument has served to bolster the theory that the adoption of mitochondria initiated eukaryote genesis (see below).

The cytoskeleton. Eukaryotic cells contain several specific proteins that are not found in prokaryotes and have the remarkable ability to self-assemble into complex, three-dimensional structures, such as fibres (actin), hollow rods (tubulin) or miniature baskets (clathrin). These cytoskeletal elements serve as props for the massive cell bodies of eukaryotes and, by alternately assembling

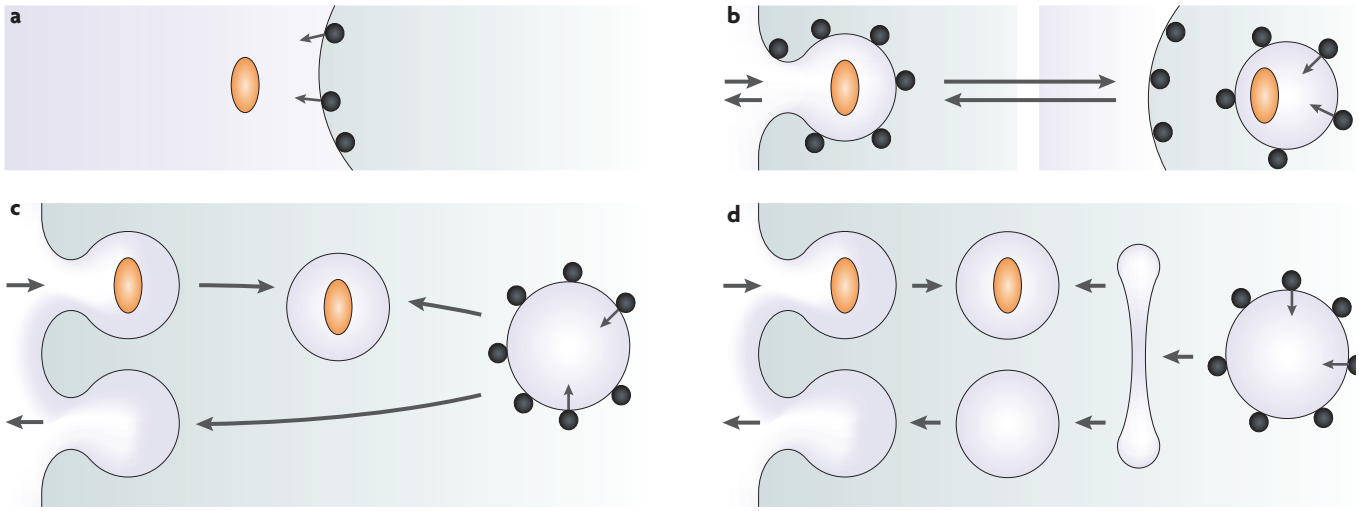

Figure 2 | **Hypothetical steps in the development of the eukaryotic cytomembrane system. a** | A putative heterotrophic prokaryotic ancestor digests its food (represented by an orange oval) extracellularly with the help of exoenzymes, which are discharged by plasma-membrane-bound ribosomes (represented by black circles). **b** | Reversible infolding and vesiculation of the cell's plasma membrane allows intracellular digestion of internalized materials and subsequent excretion of residues; the primitive intracellular vesicle combines the properties of endosomes, rough endoplasmic reticulum (ER) cisternae and lysosomes. **c** | Ribosome-bearing membranes migrate from the surface to the interior of the cell, forming a proto-ER, which secretes its products partly into endocytic vesicles, converting them into lysosomes in which intracellular digestion takes place, and partly outside by exocytosis, allowing extracellular digestion to proceed. **d** | The proto-Golgi, formed by differentiation between the ER and the endocytic system, sorts intracellularly active digestive enzymes, which are delivered into endosomes and lysosomes, from true secretion products, which are discharged outside the cell. Modified with permission from REF. 51 © (1991) Neil Patterson Publishers.

and disassembling or with the aid of motors (myosin, dynein, kinesin), use ATP energy to bring about all kinds of cellular and intracellular movements; they also have an important role in powering and channelling the vesicular traffic that underlies the operations of the cytomembrane system. Some of these structures join with other proteins into edifices of considerable complexity, such as flagella, cilia, the myofibrils of muscle cells and the mitotic spindle.

First believed to be eukaryotic innovations, several key cytoskeletal and motor-protein components have now been traced to prokaryotic precursors[54–56]. These systems must have developed in parallel with the cytomembrane system, under the selective pressure of the growing cell bulk and the spreading membranes, which depended on these cytoskeletal and motor systems for proper functioning[52,56,57].

The nucleus. The eukaryotic nucleus is surrounded by an envelope, typically made of rough ER vesicles and cytoskeletal elements (lamina) that are associated with each other and with pore complexes, which are also related to cytoplasmic components[58]. In the cells of animals, plants and some protists, although not those of most protists or fungi, this complex assemblage dissociates before mitosis and, at the end of the mitotic process, reassembles from pre-existing ER vesicles into distinct envelopes that surround the two sets of daughter chromosomes. The formation of the nuclear envelope must have accompanied or followed the parallel development of the cytomembrane and cytoskeletal systems. Of possible significance is the fact that prokaryotic chromosomes are anchored to the cell membrane. Perhaps a vesicle derived from the piece of membrane that bore the chromosome in the prokaryotic ancestor joined with other vesicles to form a double-membranous envelope around the chromosome[33,48,51,57], thereby initiating the canonical nucleo-cytoplasmic division that characterizes all eukaryotic cells.

The most important consequence of this division was the physical separation of DNA replication and transcription (by nuclear systems) from RNA translation (by cytoplasmic ribosomes). In particular, a much finer and more selective regulation of transcription became possible, and special RNA-processing centres, serving for ribosome assembly (nucleoli) and mRNA splicing (spliceosomes), were created inside the nucleus. As a result, only mature mRNAs that had gone through all transcriptional and spliceosomal regulatory filters were offered to ribosomes for translation. It seems likely that the substantial advantages that were conferred by these innovations provided the evolutionary driving force for their development.

The possibility that kinetic factors might have been involved as well has also been proposed[35].

It is likely that the formation of the nuclear envelope initiated the entire succession of events that culminated into the eukaryotic nucleus[56,57,59]. A primary change that was imposed by the segregation of chromosomes within an envelope was the development of a new mode of cell division, with the help of what might have been the first major tubulin-based structure, the mitotic spindle. Also important was the creation of multiple replicons, which allowed genome enrichment without increasing the duration of replication. Yet another development was the formation of linear chromosomes and their organization with histones into nucleosomes and chromatin fibres, which served as important props for the growing nuclear bulk. Nucleoli, spliceosomes and traffic-regulating pore complexes were other key achievements of this long and complex history, which is only beginning to be unravelled.

Interestingly, the entire process of chromosome construction and subsequent enclosure within an envelope, which takes place at the end of most mitotic divisions, occurs spontaneously by a self-assembly process that can be reproduced *in vitro*, with ATP as source of energy and naked DNA as sole triggering factor[60,61].

Peroxisomes. Peroxisomes are among the most mysterious eukaryotic components. Surrounded by a single membrane, they accomplish many functions that differ from one cell type to another but, collectively, include various reactions involved in the oxidative metabolism of carbohydrates, lipids, amino acids and purines, and the synthesis of certain lipids[62–64]. The oxidations that take place in peroxisomes are not coupled with ATP assembly, and characteristically occur by way of hydrogen peroxide, which is made by type II oxidases and broken down by catalase.

First thought to be offshoots of the ER, peroxisomes were subsequently found to be morphologically[65] and chemically[66] unrelated to this structure, and to take up their proteins from the cytosol by a post-translational mechanism that is different from the co-translational mechanism used by the ER[67–70]. These new findings prompted the suggestion that peroxisomes descend from ancestral endosymbionts, which, unlike mitochondria and chloroplasts, were left with a single peripheral membrane and no residual genome, having lost all their genes to the host-cell's nucleus[51,71–74].

More recently, the ER-offshoot theory has been revived on the strength of new observations that are purported to show that some peroxisomal membrane proteins are assembled in the ER, from which they bud off as vesicles that fuse with each other and with pre-existing peroxisomes[75,76]. Questioned by some investigators on the basis of technical considerations[69,70,77,78], this proposal has received strong support from two recent phylogenetic studies[79,80] that covered more than 25 peroxisomal membrane proteins in a number of organisms, and independently showed that all of these proteins are eukaryotic innovations with no prokaryotic counterpart. Several were found to be related to the ERAD (endoplasmic reticulum associated decay) pathway, which is involved in removing misfolded proteins from the ER and transferring them to cytosolic proteasomes for breakdown.

The possibility that the peroxisomal membrane might be an evolutionary offshoot of the ER raises two questions. First, how did the parts of the ER ancestral to peroxisomal membranes develop their characteristic post-translational protein-translocation system, which is entirely different from the co-translational system used by the ER for the import of its own internal proteins? Admittedly, the peroxisomal mechanism also differs greatly from the post-translational mechanisms of the authentic endosymbiont descendants, mitochondria and plastids; it presumably arose independently in specialized parts of the ER, perhaps from a pre-existing ERAD system. However, the question remains as to what evolutionary advantage could have driven such a transformation if retrieval of essential proteins, as presumably occurred with endosymbionts, had no role.

The second question raised by the theory that peroxisomes arose from the ER concerns the origin and selection of the enzymes that were presumably recruited by the translocation system to form the characteristic H_2O_2-centered, metabolic core of peroxisomes. It is difficult to see how such a coherent collection of enzymes could have been assembled individually, by a mechanism that would have depended on each gene being fitted with a sequence that caused its protein products to be targeted to a newly arising assemblage. Supply *en bloc* by an ancestral organism, followed by piecemeal retrieval of lost enzymes, fits the picture better.

Remarkably, unlike the peroxisomal membrane proteins, all the peroxisomal matrix enzymes that were investigated in the studies described above were found to have prokaryotic homologues[79,80]. Some of them seem to be late acquisitions, mostly from α-proteobacteria (presumably by way of mitochondria), with a small addition from actinomycetes and cyanobacteria[79]. However, the majority of matrix proteins studied form an unidentified group that is merely described as showing "…homology to prokaryotic sequences without a tree that specifically supports a bacterial or archaeal origin."[79]

The proteins of this group might hold the clue to the origin of peroxisomes. They could be simple heirlooms derived from the prokaryotic ancestor of eukaryotes, as postulated by Cavalier-Smith[56], who no longer defends the endosymbiont hypothesis. Alternatively, the peroxisomal proteins of undefined prokaryotic origin could have originated from an endosymbiont that took residence in the host cytomembrane system, either immediately after endocytic uptake, or later, following autophagic segregation[81] (perhaps with participation of the ERAD), eventually losing its own membrane to persist within the membrane provided by the host cell. This loss could have been tolerated if, unlike the membranes of mitochondria and plastids, the endosymbiont membrane did not carry any special selective asset. The loss could even have been favoured if the endosymbiont membrane was incompatible with the membranes of the host, as would have been the case, for example, for an endosymbiont of archaebacterial nature. Such an endosymbiont would have been at constant risk of losing the ability to make the ether phospholipids of its own membrane, making rescue by a host-cell-derived membrane highly beneficial. An intriguing possibility is that peroxisomes might descend from the hypothetical archaebacterial endosymbiont that is suggested above as a possible source of the informational genes of eukaryotes. Of course, the two phenomena are purely conjectural, and furthermore could be entirely unrelated.

When did peroxisomes first appear in the history of eukaryotes? Before the adoption of mitochondria, as I have long advocated[53,62]? After, as claimed by the defenders of the mitochondria-first theory? Or close to concomitantly, as believed by Cavalier-Smith[56]? As I discuss below, I see no reason to change my view. Certainly, the fact that peroxisomes contain a few proteins of possible mitochondrial origin[79] hardly proves that they arose after the mitochondria. The once-held idea[62] of an ancestral organelle that contained all the enzymes found in peroxisomes today and evolved only by attrition is overly simplistic. Peroxisomes have acquired many new components in the course of evolution. Particularly impressive is the cluster of eukaryotic enzymes that the peroxisomes of trypanosomatids have gained, presumably from an endosymbiotic algal cell, on becoming the glycosomes that characterize these organisms[82–84]. Mitochondria have also acquired many new components. Amazingly, no more than about 15% of mitochondrial proteins have been traced to the ancestral α-proteobacteria[11,85]; all others have apparently been recruited later from elsewhere. Without the presence of a residual genetic system of a prokaryotic nature, the endosymbiotic origin of mitochondria might never have been uncovered. Unfortunately, similarly decisive clues are lacking in the case of peroxisomes.

Mitochondria and hydrogenosomes. Mitochondria are the centres of aerobic energy production throughout the eukaryotic world. They are equipped with the most efficient known systems of oxidative phosphorylation, which they have inherited from a possibly photosynthetic[86] endosymbiotic ancestor related to present-day α-proteobacteria.

Margulis first attributed the endosymbiotic uptake of the ancestors of mitochondria to an initial attack on a prokaryote by another prokaryote[1,87–89]. She rejected the possibility of uptake by phagocytosis because "…pinocytosis and phagocytosis have never been seen in prokaryotes."[88] In her view, phagocytosis was developed after the uptake of mitochondria and was responsible for the subsequent adoption of plastids.

As discussed above, consideration of the development of the cytomembrane system has led to the suggestion that the host cell of the mitochondrial endosymbionts, rather than being the victim that was pictured by Margulis, might actually have been a captor that had already acquired a number of eukaryotic properties including phagocytic ability. Phagocytosis could then be responsible for the uptake of the mitochondrial ancestors, as in the case of plastids and of virtually all other known endosymbionts[51,53,56,57].

Originally, Margulis assumed that the prospective host of the mitochondrial endosymbiont was a strict anaerobe, which was actually rescued by its aerobic guest from the widespread extinction of anaerobes believed to have been caused by the rising oxygen content of the atmosphere. However, mitochondria contain the most sophisticated known systems of oxidative phosphorylation, and must be the outcome of a long process of aerobic evolution. Therefore, one would have to assume that the anaerobic host cells survived during all that time in some oxygen-free niche until they met their rescuers and became aerotolerant. It seems more likely, if there was rescue, that it was accomplished by the simpler peroxisomal systems, which could have arisen much earlier than the mitochondrial systems[62]. As to the evolutionary advantage of mitochondria, the enormous gain in energetic efficiency offers the obvious answer.

This theory has been challenged in recent years by two sets of findings, leading to a revival of Margulis's model of an initial encounter between two prokaryotes. First, as mentioned earlier, it seems that the amitochondriate organisms that were thought to be descendants of the proposed primitive phagocyte probably did contain mitochondria at some earlier evolutionary stage, a finding that is put forward as evidence, which it clearly is not, that the primitive phagocyte never existed. Second, hydrogenosomes — membrane-bounded organelles, found in certain protists and fungi, that anaerobically generate molecular hydrogen by a process linked to ATP assembly — are related to mitochondria (for details, see REFS 90,91).

These two findings have inspired the so-called 'hydrogen hypothesis', according to which the development of eukaryotic cells was initiated by an endosymbiotic association between an archaebacterial hydrogen utilizer (for example, a methanogen), which became the host cell, and a hydrogen-producing eubacterium that combined the properties of hydrogenosomes and mitochondria and was ancestral to the two kinds of organelles[21–24]. This association is thought to have been initially favoured by the mutual advantage of a hydrogen-for-food swap and subsequently to have enjoyed an enormous evolutionary success because of its adaptability to both aerobic and anaerobic surroundings. In today's world, oxygen-utilizing mitochondria are clearly dominant, but hydrogenosomes have found a few favourable niches.

This imaginative hypothesis has been well received in the phylogenetic community, despite several serious mechanistic difficulties. The engulfment of one prokaryote by another, as postulated in the model, has never been observed except for a single case[92], which is repeatedly stressed as proof that the phenomenon is plausible[24,35,93], even though the host cell in that case is not a free-living archaebacterium but a eubacterial endosymbiont. Against the many examples of endosymbiont uptake by endocytosis, the proposed mechanism by prokaryote–prokaryote interaction is hardly tenable without strong corroborative evidence, which is missing. In addition, the model implies that the host cell replaced its own membranes with membranes that were constructed under endosymbiont instructions. As discussed above, no credible mechanism has been suggested to account for such an extraordinary event. Finally, the model implies that the primordial function of mitochondria, namely oxidative ATP synthesis, was acquired almost accidentally because it happened to accompany anaerobic hydrogen production, suggested as the true evolutionary motor despite its marginal importance today.

Glossary

Archaebacteria
Archaebacteria are one of the two main groups of prokaryotes (the other being Eubacteria). Thus named because, when discovered, they were believed to be particularly ancient (Greek Archaios), which is no longer unanimously accepted; they share a number of special genetic and metabolic characteristics and have ether lipids in their membranes. They include many extremophiles, microbes adapted to extreme environments.

Endocytosis
The uptake of extracellular materials by cells. The plasma membrane invaginates and vesicles pinch off that contain trapped extracellular materials enclosed within the membrane patch derived from the plasma membrane. Those vesicles, called endosomes, either fuse with lysosomes, within which their contents are digested, or migrate to a distant site, where they fuse with the plasma membrane by exocytosis, discharging their contents outside the cell (vesicular transport).

Endosymbiont
An intracellular organism that contributes to the survival of the host cell and depends on the host for its own persistence. The relationship can be either mutualistic (in which both species benefit) or commensalistic (in which one species benefits, whereas the other is not affected). Some organelles (mitochondria, plastids) are derived from degenerate endosymbionts.

Eubacteria
Eubacteria are one of the two main groups of prokaryotes (the other being Archaebacteria). They share a number of special genetic and metabolic characteristics and have ester lipids in their membranes. They comprise all the commonly known bacteria, including those responsible for diseases.

Exocytosis
A process by which the surrounding membrane of an intracellular vesicle fuses with the plasma membrane, so that the contents of the vesicle (usually secretory products) are discharged into the extracellular membrane.

Heterotrophy
Dependence on organic foodstuffs for survival, as opposed to autotrophy, which describes self-sufficiency, the ability to survive on mineral foodstuffs.

Lateral gene transfer
Horizontal transfer of genes between unrelated species, as opposed to vertical inheritance within a species.

Phagocytosis
A form of endocytosis whereby large particles are taken up.

Pinocytosis
A form of endocytosis whereby droplets of fluid and soluble molecules are taken up.

Polyisoprenoids
A large and diverse class of lipids that are derived from 5-carbon isoprene units and enter into the formation of many natural substances, including cholesterol.

Protists
Unicellular eukaryotes including protozoans, slime molds and certain algae.

Another weakness of the model is that it does not explain the development of the other complex features of eukaryotic cells, or how that development could have been triggered by the assumed interaction between two prokaryotes. In fact, these features are mostly ignored in all relevant discussions. In particular, no mention is made of peroxisomes, even though these organelles are as ubiquitous as mitochondria. Although the possibility that peroxisomes were acquired after mitochondria, which is implicit in the model, is not implausible in itself, it is much less likely than the alternative, considering the simple character of the peroxisomal oxidizing systems. Also unexplained is the manner in which the endosymbiont was sustained during the time it took the host cell to acquire a nucleus to which the endosymbiont genes could be transferred, a major factor in the adoption of the organism. Furthermore, by making eukaryote genesis a consequence of the adoption of mitochondria, the model situates the onset of this whole complex series of events at a time less than 2.4 billion years ago, when the Earth's atmosphere had already gained significant amounts of oxygen. This time frame disagrees with the view proposed above: that eukaryote genesis might have started long before the rise of atmospheric oxygen. Finally, even the common ancestry of mitochondria and hydrogenosomes, the mainspring of the new model, might be questionable[90,94,95]. Hydrogenosomes are almost certainly polyphyletic[96–98], and could have arisen more than once by retargeting to mitochondria of gene products that originated from some other endosymbiont[90].

It is to be hoped that future results will allow a satisfactory solution of the mitochondria and hydrogenosomes conundrum. In the meantime, there seems to be no valid reason to reject the phagotrophic model in favour of the new encounter model solely on the ground of a missing link. The same opinion has been defended by others[11,56,99].

Conclusions

Eukaryotic cells most probably acquired mitochondria after they had developed the cytomembrane and cytoskeletal machineries that are involved in the endocytic uptake of extracellular materials, and not before, as claimed in a number of recent theories. This conclusion would no doubt be stronger if, as was believed at one time, descendants of eukaryotes that had never contained mitochondria had been found in the present-day world. However, the absence of such organisms does not suffice to reject an otherwise eminently plausible theory that is supported by solid circumstantial evidence. Mitochondria confer such tremendous selective advantages on their owners that the extinction of cells that lacked this asset should hardly be surprising. In contrast, the various mitochondria-first theories postulate improbable events that have never been observed, while failing to account for eukaryotic features other than mitochondria.

Given this main conclusion, the manner and order in which the various eukaryotic properties were acquired remain debatable. At the start of the process, there probably existed a wall-less, anaerobic, heterotrophic, eubacterial prokaryote with ester-lipid membranes. According to the proposed view, this organism went through an exceedingly long developmental process that led to the primitive phagocyte, a large cell that was endowed with all the main eukaryotic properties other than oxygen-related organelles, including cytomembranes, cytoskeletal elements, an organized and fenced-off nucleus and the capacity for mitotic division. This process could have been triggered by the loss of the ability to build an outside wall; and, it could have been selectively favoured by the transition from extracellular to intracellular digestion.

This view agrees with the neomuran theory of Cavalier-Smith[8,56], except for the anaerobic character of the ancestral cell and the assumed time frame of the process. Contrary to the opinion, which is vigorously defended by this investigator, that eukaryotes developed no earlier than about 1 billion years ago, the possibility is left open that their development might have started long before the rise of atmospheric oxygen, some 2.4 billion years ago. Such a protracted process would have required an exceptionally stable and sheltered environment, abundantly supplied with food. An attractive possibility is that the whole process took place within giant bacterial colonies of the kind known as stromatolites (REF. 100 and references within). These formations would have provided plenty of food and shelter, and there is evidence that some date back to the dawn of life and might have persisted for hundreds of millions of years, if not more. If the process that my proposed model assumes left any fossil traces, which is far from certain, those traces would be restricted to the inside of some ancient stromatolites, remaining undetected for a very long time. Even the possibility that some 'living fossils' are still going through a similar 'adventure' today, awaiting discovery, cannot be excluded.

The hypothetical primitive phagocyte of my model need not have completed its entire development before adopting mitochondria. But phagotrophic ability and a nucleus that was capable of incorporating endosymbiont genes would have been essential. Another condition would have been an environment that was shared with the α-proteobacterial ancestors of mitochondria, almost certainly implying the presence of oxygen. It is mainly for this reason that I suggest that the acquisition of peroxisomes preceded that of mitochondria, converting the anaerobic phagocyte into an aerotolerant one.

How these organelles appeared remains one of the most mysterious questions raised by eukaryote genesis, involving, as it does, an autogenous envelope with contents of prokaryotic origin. As argued, an endosymbiont origin of peroxisomes can still be contemplated, but is so far unsupported by evidence.

Another unsolved question is the apparent genomic chimerism of eukaryotes. An in-depth discussion of this question, which is related to the origin of archaebacteria and ether lipids, falls outside the limits of both this Essay and the competence of its author.

Perhaps future research will help to answer the questions that remain pending. Much will depend on the reliability of the information that can be extracted from the comparative sequencing of genes. Molecular phylogenetics has revolutionized our ability to probe the history of life on Earth. However, this powerful tool is not without pitfalls, which become increasingly hazardous as the events one tries to reconstruct are more remote[101,102]. When dealing with phenomena that took place more than 1 billion years ago, and that might go back even as far as the earliest manifestations of life on Earth, the possibility that the record has become hopelessly blurred must be seriously contemplated. The enormous degree of gene wandering that is revealed by recent investigations should serve as a warning to this effect, making it imperative that new models be critically confronted with what is known of the properties of extant organisms through other biological disciplines. Many recently proposed models are singularly lacking in this respect. Until this omission is corrected, new models of eukaryote genesis must be viewed with caution.

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1. Sagan, L. On the origin of mitosing cells. *J. Theoret. Biol.* **14**, 225–274 (1967).
2. Woese, C. R. & Fox, G. E. The phylogenetic structure of the procaryotic domain: the primary kingdoms. *Proc. Natl Acad. Sci. USA* **74**, 5088–5090 (1977).
3. de Duve, C. *Singularities* (Cambridge Univ. Press, New York, 2005).
4. Holland, H. D. The oxygenation of the atmosphere and oceans. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* **361**, 903–915 (2006).
5. Brocks, J. J., Logan, G. A., Buick, R. & Summons, R. E. Archaean molecular fossils and the early rise of eukaryotes. *Science* **285**, 1033–1036 (1999).
6. Douzery, E. J. P., Snell, E. A., Bapteste, E., Delsuc, F. & Philippe, H. The timing of eukaryotic evolution: does a relaxed molecular clock reconcile proteins and fossils? *Proc. Natl Acad. Sci. USA* **101**, 15386–15391 (2004).
7. Berney, C. & Pawlowski, J. A molecular time-scale for eukaryote evolution recalibrated with the continuous microfossil record. *Proc. R. Soc. Lond. B Biol. Sci.* **273**, 1867–1872 (2006).
8. Cavalier-Smith, T. The neomuran origin of archaebacteria, the negibacterial root of the universal tree and bacterial megaclassification. *Int. J. Syst. Evol. Microbiol.* **52**, 7–76 (2002).
9. Cavalier-Smith, T. Cell evolution and Earth history: stasis and revolution. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* **361**, 969–1006 (2006).
10. Hartman, H. & Fedorov, A. The origin of the eukaryotic cell: a genomic investigation. *Proc. Natl Acad. Sci. USA* **99**, 1420–1425 (2002).
11. Kurland, C. G., Collins, L. J. & Penny, D. Genomics and the irreducible nature of eukaryote cells. *Science* **312**, 1011–1014 (2006).
12. Ourisson, G. & Nakatani, T. The terpenoid theory of the origin of cellular life: the evolution of terpenoids to cholesterol. *Chem. Biol.* **1**, 11–23 (1994).
13. Lake, J. A., Jain, R. & Rivera, M. C. Mix and match in the tree of life. *Science* **283**, 2027–2028 (1999).
14. Horiike, T., Hamada, K., Kanaya, S. & Shinozawa, T. Origin of eukaryotic cell nuclei by symbiosis of Archaea in Bacteria is revealed by homology-hit analysis. *Nature Cell Biol.* **3**, 210–214 (2001).
15. Zillig, W. Comparative biochemistry of Archaea and Bacteria. *Curr. Opin. Genet. Dev.* **1**, 544–551 (1991).
16. Gupta, R. S., Aitken, K., Falah, M. & Singh, B. Cloning of *Giardia lamblia* heat shock protein HSP70 homologs: Implications regarding origin of eukaryotic cells and endoplasmic reticulum. *Proc. Natl Acad. Sci. USA* **91**, 2895–2899 (1994).
17. Margulis, L. Archaeal–eubacterial mergers in the origin of Eukarya: phylogenetic classification of life. *Proc. Natl Acad. Sci. USA* **93**, 1071–1076 (1996).
18. Rivera, M. C. & Lake, J. A. The ring of life provides evidence for a genome fusion origin of eukaryotes. *Nature* **431**, 152–155 (2004).
19. Lake, A. & Rivera, M. C. Was the nucleus the first endosymbiont? *Proc. Natl Acad. Sci. USA* **91**, 2880–2881 (1994).
20. Moreira, D. & Lopez-Garcia, P. Symbiosis between methanogenic archaea and δ-proteobacteria as the origin of eukaryotes: The syntrophic hypothesis. *J. Mol. Evol.* **47**, 517–530 (1998).
21. Martin, W. & Müller, M. The hydrogen hypothesis for the first eukaryote. *Nature* **392**, 37–41 (1998).
22. Vellai, T., Takacs, K. & Vida, G. A new aspect to the origin and evolution of eukaryotes. *J. Mol. Evol.* **46**, 499–507 (1998).
23. Vellai, T. & Vida, G. The origin of eukaryotes: the difference between prokaryotic and eukaryotic cells. *Proc. R. Soc. Lond. B Biol. Sci.* **266**, 1571–1577 (1999).
24. Embley, T. M. & Martin, W. Eukaryotic evolution, changes and challenges. *Nature* **440**, 623–630 (2006).
25. Forterre, P. Thermoreduction, a hypothesis for the origin of prokaryotes. *C. R. Acad. Sci. III* **318**, 415–422 (1995).
26. Forterre, P. Where is the root of the universal tree of life? *BioEssays* **21**, 871–879 (1999).
27. Poole, A., Jeffares, D. & Penny, D. Early evolution: prokaryotes, the new kids on the block. *BioEssays* **21**, 880–889 (1999).
28. Xu, Y. & Glansdorff, N. Was our ancestor a thermophilic procaryote? *Comp. Biochem. Physiol. A* **133**, 677–688 (2002).
29. Doolittle, W. F. Phylogenetic classification and the universal tree. *Science* **284**, 2124–2128 (1999).
30. Doolittle, W. F. The nature of the universal ancestor and the evolution of the proteome. *Curr. Opin. Struct. Biol.* **10**, 355–358 (2000).
31. Woese, C. R. The universal ancestor. *Proc. Natl Acad. Sci. USA* **95**, 6854–6859 (1998).
32. Woese, C. R. On the evolution of cells. *Proc. Natl Acad. Sci. USA* **99**, 8742–8747 (2002).
33. Blobel, G. Intracellular protein topogenesis. *Proc. Natl Acad. Sci. USA* **77**, 1496–1500 (1980).
34. Martin, W., Hoffmeister, M., Rotte, C. & Henze, K. An overview of endosymbiotic models for the origins of eukaryotes, their ATP-producing organelles (mitochondria and hydrogenosomes), and their heterotrophic lifestyle. *Biol. Chem.* **382**, 1521–1539 (2001).
35. Martin, W. & Koonin, E. V. Introns and the origin of the nucleus–cytosol compartmentalization. *Nature* **440**, 41–45 (2006).
36. Wächtershäuser, G. From pre-cells to Eukarya: a tale of two lipids. *Mol. Microbiol.* **47**, 13–22 (2003).
37. Boucher, Y., Kamekura, M. & Doolittle, W. F. Origins and evolution of isoprenoid lipid biosynthesis in archaea. *Mol. Microbiol.* **52**, 515–527 (2004).
38. Davis, B. D. & Tai, P.-C. The mechanism of protein secretion across membranes. *Nature* **283**, 433–438 (1980).
39. Silhavy, T. J., Benson, S. A. & Emr, S. D. Mechanisms of protein localization. *Microbiol. Rev.* **47**, 313–344 (1983).
40. Lee, C. & Beckwith, J. Cotranslational and posttranslational protein translocation systems in prokaryotic systems. *Annu. Rev. Cell Biol.* **2**, 315–336 (1986).
41. Hortsch, M. & Meyer, D. I. Transfer of secretory proteins through the membrane of the endoplasmic reticulum. *Int. Rev. Cytol.* **102**, 215–242 (1986).
42. Walter, P. & Lingappa, V. R. Mechanism of protein translocation across the endoplasmic reticulum membrane. *Annu. Rev. Cell Biol.* **2**, 499–586 (1986).
43. Randall, L. L., Hardy, S. J. & Thom, J. R. Export of protein: a biochemical view. *Annu. Rev. Microbiol.* **41**, 507–541 (1987).
44. Powers, T. & Walter, P. Co-translational protein targeting catalyzed by the *Escherichia coli* recognition particle and its receptor. *EMBO J.* **16**, 4880–4886 (1997).
45. Halic, M. *et al.* Following the signal sequence from ribosomal tunnel exit to signal recognition particle. *Nature* **444**, 507–511 (2006).
46. de Duve, C. & Wattiaux, R. Functions of lysosomes. *Annu. Rev. Physiol.* **28**, 435–492 (1966).
47. Stanier, R. Y. Some aspects of the biology of cells and their possible evolutionary significance. *Symp. Soc. Gen. Microbiol.* **20**, 1–38 (1970).
48. Cavalier-Smith, T. The origin of nuclei and of eukaryotic cells. *Nature* **256**, 463–468 (1975).
49. de Duve, C. *A Guided Tour of the Living Cell*. (Scientific American, New York, 1984).
50. Woese, C. Bacterial evolution. *Microbiol. Rev.* **51**, 221–271 (1987).
51. de Duve, C. *Blueprint for a Cell* (Neil Patterson, Burlington, 1991).
52. Cavalier-Smith, T. in *Endocytobiology II* (eds Schwemmler, W. & Schenk, H. E. A.) 265–279 (De Gruyter, Berlin, 1983).
53. de Duve, C. The birth of complex cells. *Sci. Am.* **274**, 38–45 (1996).
54. Nogales, E., Downing, K. H., Amos, L. A. & Löwe, J. Tubulin and FtsZ form a distinct family of GTPases. *Nature Struct. Biol.* **5**, 451–458 (1998).
55. van den Ent, F., Amos, L. A. & Löwe, J. Prokaryotic origin of the actin cytoskeleton. *Nature* **413**, 39–44 (2001).
56. Cavalier-Smith, T. The phagotrophic origin of eukaryotes and phylogenetic classification of protozoa. *Int. J. Syst. Evol. Microbiol.* **52**, 297–354 (2002).
57. Cavalier-Smith, T. The origin of eukaryote and archaebacterial cells. *Ann. NY Acad. Sci.* **503**, 17–54 (1987).
58. Devos, D. *et al.* Components of coated vesicles and nuclear pore complexes share a common molecular architecture. *PLoS Biol.* **2**, e380 (2004).
59. Cavalier-Smith, T. Economy, speed and size matter: evolutionary forces driving nuclear genome miniaturization and expansion. *Ann. Bot.* **95**, 147–175 (2005).
60. Newport, J. Nuclear reconstitution *in vitro*: stages of assembly around protein-free DNA. *Cell* **48**, 205–217 (1987).
61. Gerace, L. & Burke, B. Functional organization of the nuclear envelope. *Annu. Rev. Cell Biol.* **4**, 335–374 (1988).
62. de Duve, C. Evolution of the peroxisome. *Ann. NY Acad. Sci.* **168**, 369–381 (1969).
63. Fahimi, H. D. & Sies, H. (eds) *Peroxisomes in Biology and Medicine* (Springer, Berlin, 1987).
64. Masters, C. & Crane, D. *The Peroxisome: A Vital Organelle* (Cambridge Univ. Press, Cambridge, 1995).
65. Shio, H. & Lazarow, P. B. Relationship between peroxisomes and endoplasmic reticulum investigated by combined catalase and glucose-6-phosphatase cytochemistry. *J. Histochem. Cytochem.* **29**, 1263–1272 (1981).
66. Fujiki, Y., Fowler, S., Shio, H., Hubbard, A. L. & Lazarow, P. B. Polypeptide and phospholipid composition of the membrane of rat liver peroxisomes. Comparison with endoplasmic reticulum and mitochondrial membranes. *J. Cell Biol.* **93**, 103–110 (1982).
67. Lazarow, P. B., Robbi, M., Fujiki, Y. & Wong, L. Biogenesis of peroxisomal proteins *in vivo* and *in vitro*. *Ann. NY Acad. Sci.* **386**, 285–297 (1982).
68. Lazarow, P. B. & Fujiki, Y. Biogenesis of peroxisomes. *Ann. Rev. Cell Biol.* **1**, 489–530 (1985).
69. Purdue, P. E. & Lazarow, P. B. Peroxisome biogenesis. *Annu. Rev. Cell Dev. Biol.* **17**, 701–752 (2001).
70. Lazarow, P. B. Peroxisome biogenesis: advances and conundrums. *Curr. Opin. Cell Biol.* **15**, 489–497 (2003).
71. de Duve, C. Peroxisomes and related particles in historical perspective. *Ann. NY Acad. Sci.* **386**, 1–4 (1982).
72. de Duve, C. Microbodies in the living cell. *Sci. Am.* **248**, 74–84 (1983).
73. Borst, P. How proteins get into microbodies (peroxisomes, glyoxysomes, glycosomes). *Biochim. Biophys. Acta* **866**, 179–203 (1986).
74. Cavalier-Smith, T. in *Endocytobiology IV* (eds Nardon, P. *et al.*) 515–521 (INRA, Paris, 1989).
75. Titorenko, V. I. & Rachubinski, R. A. The endoplasmic reticulum plays an essential role in peroxisome biogenesis. *Trends Biochem. Sci.* **23**, 231–233 (1998).
76. Hoepfner, D., Schildknegt, D., Braakman I., Philippsen, P. & Tabak, H. F. Contribution of the endoplasmic reticulum to peroxisome formation. *Cell* **122**, 85–95 (2005).
77. South, S. T. & Gould S. J. Peroxisome synthesis in the absence of preexisting peroxisomes. *J. Cell Biol.* **144**, 255–266 (1999).
78. South, S. T., Sacksteder, K. A., Li, X., Liu, Y. & Gould, S. J. Inhibitors of COPI and COPII do not block *PEX3*-mediated peroxisome synthesis. *J. Cell Biol.* **149**, 1345–1360 (2000).
79. Gabaldon, T. *et al.* Origin and evolution of the peroxisomal proteome. *Biol. Direct* **1**, 8 (2006).
80. Schlüter, A. *et al.* The evolutionary origin of peroxisomes: an ER-peroxisome connection. *Mol. Biol. Evol.* **23**, 838–845 (2006).
81. Checroun, C., Wehrly, T. D., Fischer, E. R., Hayes, S. F. & Celli, J. Autophagy-mediated reentry of *Francisella tularensis* into the endocytic compartment after cytoplasmic replication. *Proc. Natl Acad. Sci. USA* **103**, 14578–14583 (2006).
82. Hannaert, V., Bringaud, F., Opperdoes, F. R. & Michels, P. A. M. Evolution of energy metabolism and its compartmentation in Kinetoplastida. *Kinetoplastid Biol. Dis.* **2**, 11 (2003).
83. Hannaert, V. *et al.* Plant-like traits associated with metabolism of *Trypanosoma* parasites. *Proc. Natl Acad. Sci. USA* **100**, 1067–1071 (2003).
84. Opperdoes, F. R. & Szikora, J.-P. *In silico* prediction of glycosomal enzymes of *Leishmania major* and trypanosomes. *Mol. Biochem. Parasitol.* **147**, 193–206 (2006).
85. Gabaldon, T. & Huynen, M. A. Reconstruction of the proto-mitochondrial metabolism. *Science* **301**, 609 (2003).
86. Cavalier-Smith, T. Origin of mitochondria by intracellular enslavement of a photosynthetic purple bacterium. *Proc. R. Soc. Lond. B Biol. Sci.* **273**, 1943–1952 (2006).
87. Margulis, L. *Origin of Eukaryotic Cells* (Yale Univ. Press, New Haven, 1970).
88. Margulis, L. *Symbiosis in Cell Evolution* (W. H. Freeman & Co., San Francisco, 1981).
89. Margulis, L. & Sagan, D. *Micro-cosmos* (Summit, New York, 1986).
90. Dyall, S. D., Brown, M. T. & Johnson, P. J. Ancient invasions: from endosymbionts to organelles. *Science* **304**, 253–257 (2004).
91. Hackstein, J. H. P. & Yarlett, N. in *Molecular Basis of Symbiosis* (ed. Overmann, J.) 117–142 (Springer, Berlin-Heidelberg, 2005).

92. von Dohlen, C. D., Kohler, S., Alsop, S. T. & McManus, W. R. Mealybug β-proteobacterial endosymbionts contain γ-proteobacterial symbionts. *Nature* **412**, 433–436 (2001).
93. Martin, W. & Russell, M. J. On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. *Phil. Trans. R. Soc. Lond. B* **358**, 59–85 (2003).
94. Akhmanova, A. *et al.* A hydrogenosome with a genome. *Nature* **396**, 527–528 (1998).
95. Dyall, S. D. *et al.* Non-mitochondrial complex I proteins in a *Trichomonas* hydrogenosomal oxidoreductase complex. *Nature* **431**, 1103–1107 (2004).
96. Horner, D. S., Foster, P. G. & Embley, T. M. Iron hydrogenases and the evolution of anaerobic eukaryotes. *Mol. Biol. Evol.* **17**, 1695–1709 (2000).
97. Hackstein, J. H. P. *et al.* Hydrogenosomes: convergent adaptations of mitochondria to anaerobic environments. *Zoology* **104**, 290–302 (2001).
98. Voncken, F. *et al.* Multiple origins of hydrogenosomes: functional and phylogenetic evidence from the ADP/ATP carrier of the anaerobic chytrid *Neocallimastix* sp. *Mol. Microbiol.* **44**, 1441–1454 (2002).
99. Poole, A. M. & Penny, D. Evaluating hypotheses for the origin of eukaryotes. *BioEssays* **29**, 74–84 (2006).
100. Schopf, J. W. *Cradle of Life* (Princeton Univ. Press, Princeton, 1999).
101. Hedges, S. B. The origin and evolution of model organisms. *Nature Rev. Genet.* **3**, 838–849 (2002).
102. Gribaldo, S. & Philippe, H. Ancient phylogenetic relationships. *Theor. Pop. Biol.* **61**, 391–408 (2002).

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