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# A briefly argued case that mitochondria and plastids are descendants of endosymbionts, but that the nuclear compartment is not

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Recent findings are summarized in support of the view that mitochondria (including hydrogenosomes) and plastids (including complex ones) descend from symbiotic associations of once free-living organisms. The reasoning behind endosymbiotic hypotheses stems from a comparison of biochemistry and physiology in organelles with that in free-living cells; their strength is shown to lie in the specific testable predictions they generate about expected similarity patterns among genes. Although disdained for many decades, endosymbiotic hypotheses have gradually become very popular. In the wake of that popularity, endosymbiotic hypotheses have been formulated to explain the origins of eukaryotic cell compartments and structures that have no biochemical similarity to free-living cells. In particular, it has become fashionable in recent years to entertain the century-old notion that the nucleus might also descend from an endosymbiotic bacterium. A critique of that hypothesis is formulated and a simple alternative to it is outlined, which derives the nuclear compartment in a mitochondrion-bearing cell.

**Keywords:** endosymbiosis; mitochondria; chloroplasts; plants; hydrogenosomes; protists

## 1. INTRODUCTION

Biochemical and molecular data attest beyond all reasonable doubt to the view that plastids descend from cyanobacteria and that mitochondria descend from $\alpha$-proteobacteria. However, the evolutionary origin of the rest of a typical eukaryotic cell (the cytosol, cytoskeleton, flagella, endomembrane system and nucleus) is a much more mysterious matter. The fossil record tells us that eukaryotes were in existence by at least around 1.5 billion years ago (Knoll 1992, 1994; Vidal 1994), with molecular data hinting that eukaryotes might have obtained their mitochondria even as early as two billion years ago (Doolittle 1997). Since there are no known fossil or contemporary intermediate forms between prokaryotic and eukaryotic organization (Doolittle 1998*a*; Mayr 1998), explaining the differences between them boils down to a laborious excercise in deduction and inference. However, it is an important exercise, the purpose of which is to understand a critical phase in our own evolutionary history.

Endosymbiotic hypotheses are popular explanatory vehicles for accounting for many of the differences between prokaryotes and eukaryotes. So popular in fact, that almost every internal membrane system eukaryotes possess has been suggested at one time or another to descend from a free-living bacterium. In particular, the notion is often discussed that the nucleus itself arose from an endosymbiotic bacterium, a view that is shown here to have a number of serious flaws. A biochemically simple alternative hypothesis for the origin of the nucleus is outlined which differs substantially from previous models.

## 2. ENDOSYMBIOSIS: A GOOD EXPLANATORY PRINCIPLE IN SOME CASES

In general terms, endosymbiotic hypotheses posit that some membrane-bounded compartments within eukaryotic cells descend from other, once free-living cells that, as endosymbionts, somehow entered into the cytosol of their host and, through reduction, eventually evolved into that cell compartment of their host, the origin of which the specific hypothesis is designed to explain. In addition, in general terms, the alternatives to endosymbiotic hypotheses for the origin of a given cell compartment can be designated collectively as autogenous hypotheses (Doolittle 1980). These posit that the given cell compartment in question arose *de novo* in the cytosol, usually in response to some specifically formulated selective pressure and, thus, entailed no endosymbiotic relationship.

The concept of symbiosis is generally attributed to the 19th-century work of S. Schwendener and A. De Bary, who found that lichens are consortia of a fungus and a photosynthesizer. The concept and the reasoning behind endosymbiotic hypotheses was also developed a century ago (Mereschkowsky 1905): eukaryotic cell compartments that proliferate by division (e.g. plastids) and that share an evident similarity to free-living cells (e.g. cyanobacteria) can be postulated to have been endosymbionts, and evidence can be sought that might be consistent with or might falsify that premise. As elaborated elsewhere (Sapp 1994), endosymbiosis for the origin of chloroplasts and mitochondria, although a popular view 100 years ago, fell into great disfavour in the 1920s and for various reasons remained scorned until popular resurrection of


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the issue in the 1970s. Its resurgence (Sagan 1967; De Duve 1969; Margulis 1970; Raven 1970; Stanier 1970; John & Whatley 1975) was met with stiff resistance and the final defence of autogenous hypotheses for the origins of these compartments (Raff & Mahler 1972; Uzzel & Spolsky 1974; Bogorad 1975; Cavalier-Smith 1975).

The strength of endosymbiotic hypotheses for the origins of chloroplasts and mitochondria lies to a large extent in their specific prediction, based on biochemical observations that the genes in these organelles should be specifically similar to their homologues in cyanobacteria and $\alpha$-proteobacteria, respectively (Doolittle 1980; Weeden 1981; Gray & Doolittle 1982). The study of organelle genomes has solidly borne out that prediction (Lang *et al*. 1997; Unseld *et al*. 1997; Andersson *et al*. 1998; Gray *et al*. 1998; Martin *et al*. 1998), indicating that these compartments do descend from endosymbionts, but, particularly in the case of mitochondria, not necessarily what kind of endosymbiont (Martin & Müller 1998; Gray *et al*. 1999; Müller & Martin 1999). Similar predictions have been borne out for only two other types of cell compartments: complex plastids and hydrogenosomes.

Complex plastids are surrounded by more than two membranes. They occur among a few green protists and among all chlorophyll $a + c$-containing algae. Based to a large extent upon the comparative distribution of pigments among plastids, the additional surrounding membranes were suggested to reflect an origin of such plastids from plastid-bearing eukaryotic endosymbionts (Gibbs 1978) rather than directly from cyanobacteria, and molecular data have strongly substantiated that prediction (McFadden & Gilson 1995; Van de Peer *et al*. 1996; Delwiche & Palmer 1997; Gilson *et al*. 1997; Herrmann 1997; Douglas 1998). Particularly striking evidence for that view has come from the study of nucleomorphs, remnant nuclei of eukaryotic endosymbionts that are found within the complex plastids of cryptomonads (Douglas *et al*. 1991; Maier 1992; Häuber *et al*. 1994) and chlorarachniophytes (Van de Peer *et al*. 1996; McFadden *et al*. 1997). The vast majority of algae with complex plastids do not possess a nucleomorph, but molecular data from their plastid genomes nonetheless suggest that they too descend from eukaryotic endosymbionts, for example the four membrane-bounded plastids of diatoms (Kowallik *et al*. 1995), the three membrane-bounded plastids of euglenophytes (Martin *et al*. 1998) or the curious four membrane-bounded, non-photosynthetic plastids of the malaria-causing parasite and related organisms (McFadden *et al*. 1996; Köhler *et al*. 1997; McFadden & Waller 1997; Waller *et al*. 1998).

Hydrogenosomes occur among a wide spectrum of anaerobic protists and are surrounded by two membranes (Benchimol *et al*. 1997). These organelles, like mitochondria, import pyruvate (and sometimes malate) and generate ATP that is exported to the cytosol (Lindmark & Müller 1973; Müller 1988, 1993, 1998). They differ from mitochondria in that no hydrogenosomes are known that possess pyruvate dehydrogenase, a citric acid cycle or a respiratory chain. A few cases are known where enzymes typical for hydrogenosomes occur in mitochondria, for example acetate–succinate CoA transferase (Van Hellemond *et al*. 1998). Although the biochemistry of hydrogenosomes in various eukaryotic lineages can differ in some aspects, the overall ATP-producing pathway in those few lineages studied to date in detail appears to be relatively uniform (Müller 1998).

Because their ATP-generating biochemistry is very similar to that found in strict anaerobes such as *Clostridia*, it was suspected that hydrogenosomes might descend from anaerboic endosymbiotic bacteria (Whatley *et al*. 1979). Many recent molecular studies have indicated that hydrogenosomes do descend from bacteria, but from the same endosymbiont as mitochondria (reviewed in Embley *et al*. 1997; Embley & Hirt 1998; Müller 1998), suggesting that the common ancestor of mitochondria and hydrogenosomes was a facultatively anaerobic $\alpha$-proteobacterium that possessed the types of ATP-producing pathways that are found in mitochondria and hydrogenosomes (Martin & Müller 1998). Although most hydrogenosomes do not possess a genome, those in the anaerobic ciliate *Nyctotherus ovalis* do contain DNA which encodes genes which identify its hydrogenosome as an anaerobic form of mitochondria (Akhmanova *et al*. 1998; Embley & Martin 1998).

## 3. OVERWORKING A GOOD EXPLANATORY PRINCIPLE

In the wake of success in explaining the origins of plastids and mitochondria, endosymbiosis has been suggested for the origin of almost all of the membrane-bounded compartments that occur in eukaryotes. Endosymbiotic origins have been elaborated for various types of peroxisomes (Cavalier-Smith 1987*b*) including, more recently, glycosomes (Cavalier-Smith 1997). The view that eukaryotic flagella (also called undulipodia) descend from symbiotic bacteria (spirochaetes) is not without vociferous proponents (Sagan 1967; Margulis 1970, 1981, 1996). One can also find arguments about endosymbiosis for the origin of the endoplasmic reticulum (Gupta *et al*. 1994; Gupta & Golding 1996; Gupta 1998) and it has been suggested that the eukaryotic cytoskeleton itself might have been inherited from an endosymbiotic event (Doolittle 1998*b*). Perhaps the only compartment for which an endosymbiotic origin has not been suggested is the vacuole in plants (or lysosomes in animals). However, no convincing molecular or biochemical data have yet been marshalled to substantiate assertions that any eukaryotic compartment other than plastids and mitochondria (including hydrogenosomes) actually descend from endosymbiotic bacteria.

In the case of peroxisomes, it now seems rather clear that these single membrane-bounded organelles are more closely related to the endoplasmic reticulum than they are to prokaryotes. This is because some peroxisomal proteins are now known to pass through the endoplasmic reticulum *en route* to the organelle and because vesicular elements containing peroxisomal proteins bud from the endoplasmic reticulum and can fuse to form peroxisomes (Kunau & Erdmann 1998; Olsen 1998; Titorenko & Rachubinski 1998). However, other considerations figure in this issue as well. In particular, the case argued for an endosymbiotic origin of glycosomes (Cavalier-Smith 1997) deserves inspection. Glycosomes are highly specialized forms of peroxisomes that contain enzymes of glycolysis; they occur in trypanosomes (the agents of

sleeping sickness) and related organisms (Clayton & Michels 1996). Glycosomes import glucose from the cytosol and export phosphoenolpyruvate and, under some conditions, glycerol or 1,3-bisphosphoglycerate as well (Clayton & Michels 1996). The biochemically specialized function of these organelles is twofold. They permit efficient glycolytic flux in the bloodstream forms of these parasites (Clayton & Michels 1996; Blattner *et al*. 1998) but, more importantly, because glycolytic enzymes in trypanosomes are not allosterically regulated, they permit the parasite to adjust its ATP production (and, hence, its growth rate) to the bloodstream glucose concentrations of its host (Bakker *et al*. 1997). No prokaryotes are known that lack allosteric regulation in their glycolytic pathway (although some are known that lack glycolysis altogether; Andersson *et al*. 1998). Thus, the argument for an endosymbiotic origin of glycosomes (Cavalier-Smith 1997) is odd for two reasons: (i) because it adds a glycolytic pathway to a cell that already has one, and (ii) because it adds an unregulated glycolytic pathway from prokaryotic donors that are not known to possess one.

In the case of the endoplasmic reticulum, a reasonable biochemical justification for even entertaining the notion in the first place that this membrane system might descend from once free-living cells is lacking. In the case of flagella, it should be noted that these structures are not really compartments in the sense that they are not completely surrounded by membranes, one of several rather serious theoretical problems for entertaining the notion of endosymbiotic origin of flagella, as Rizzotti (1995) crisply argued. Evidence claimed for DNA in the basal body of flagella (Hall *et al*. 1989) could not be independently substantiated (Kuroiwa *et al*. 1990; Johnson & Dutcher 1991). The suggestion of an endosymbiotic acquisition of the cytoskeleton (Doolittle 1998*b*) neatly solves the problem of where eukaryotes obtained theirs, but neither explains how the cytoskeleton arose, nor why eukaryotes were unable to evolve one by themselves.

From the biochemical standpoint, there is no reason to suspect endosymbiosis for the origin of any compartments in the cytosol other than plastids (including complex ones) and mitochondria (including hydrogenosomes). This is also true for the nucleus.

## 4. AUTOGENOUS MODELS FOR THE ORIGIN OF THE NUCLEAR COMPARTMENT

The nuclear compartment very certainly contains DNA and is, most of the time, surrounded by a membrane system of flattened endoplasmic reticulum vesicles. In eukaryotes that possess closed mitosis, the nuclear envelope remains intact throughout the cell cycle. However, in those eukaryotes that possess open mitosis, the nuclear envelope disintegrates during cell division and arises *de novo* in the daughter cells. Importantly, in contrast to chloroplasts and mitochondria, the nuclear compartment is not surrounded by two membranes. Rather, the nuclear envelope consists of a series of flattened, single membrane-bounded vesicles that are continuous with the endoplasmic reticulum. In essence, there are currently two different types of hypotheses for explaining the origin of the nuclear compartment and its membrane: autogenous and endosymbiotic.

Autogenous hypotheses posit that the nuclear compartment and its membrane results from the reorganization of a pre-existing intracellular membrane system in the common ancestor of eukaryotes. As candidates for such pre-existing intracellular membranes, invaginations of the outer plasma membrane of a primitive phagocytosing cell are by far the most popular (Uzzel & Spolsky 1974; Bogorad 1975; Doolittle 1980; Cavalier-Smith 1987*a*, 1988), but other alternatives involving thylakoids in a cyanobacterium have also been argued (Cavalier-Smith 1975). Autogenous hypotheses generally demand the existence of a cytoskeleton in the cell which evolved the first nucleus as a mechanism for accounting for such membrane restructuring. This is particularly true for hypotheses that envisage the origin of the nuclear compartment in a phagocytosing prokaryotic (but still 'protoeukaryotic') cell, because the cytoskeleton is a prerequisite for phagocytosis as we know it (De Duve 1969; Stanier 1970; Cavalier-Smith 1987*a*; Doolittle 1996). Autogenous hypotheses also tend to forward specific selective advantages for the possession of a nuclear envelope, for example the construable advantages of decoupling translation from transcription or other more complex advantages.

The numerous strengths that autogenous hypotheses possess are adequately discussed in the original literature, but their weaknesses are usually not. One problem is that previous autogenous hypotheses demand the presence of a cytoskeleton in the prokaryotic ancestor of eukaryotes. Prokaryotes are indeed known to possess proteins with structural and functional similarity to important components of the eukaryotic cytoskeleton, for example the prokaryotic cell division proteins FtsZ and FtsA, which are remarkably similar to tubulin and actin, respectively (Vinella & D'Ari 1995; Vicente & Errington 1996; Desai & Mitchison 1998; Lowe & Amos 1998). However, no prokaryotes are known to possess a true cytoskeleton or any process that can be meaningfully homologized to phagocytosis. In addition, if the nuclear compartment differentiated from a pre-existing endomembrane system in ancient prokaryotes, one would expect to observe evidence for the same process (the origin of a nuclear compartment) in contemporary prokaryotes. Since no prokaryotes are known to possess a structure that can be even vaguely homologized to a nuclear envelope, its pore complexes or the mitotic process with which it is associated, autogenous hypotheses have to come up with a satisfactory explanation for why the nucleus arose in a prokaryote, but only in the one that gave rise to eukaryotes.

## 5. ENDOSYMBIOTIC MODELS FOR THE ORIGIN OF THE NUCLEAR COMPARTMENT

Endosymbiotic hypotheses for the origin of the nuclear compartment posit that the nuclear envelope is to be interpreted as a relic of an ancient invasion of a prokaryote into nucleus-lacking cytoplasm. The notion that the nucleus might descend from an endosymbiotic bacterium is usually attributed to Mereschkowsky (1905, 1910) and enjoys resurgent popularity (Schubert 1988; Lake & Rivera 1994; Gupta & Golding 1996; Gupta 1998;

Moreira & Lopez-Garcia 1998; Lopez-Garcia & Moreira 1999), but it is usually overlooked that Mereschkowsky (1905, 1910) argued in favour of an endosymbiotic origin of the nucleus only for most eukaryotes, favouring an autogenous origin of the nucleus in most fungi.

In line drawings, an endosymbiotic origin of the nucleus can look attractive, since a DNA-containing, membrane-bounded compartment results. However, beyond the similarity of being drawable with pencil and paper as processes resulting in membrane-bounded compartments, endosymbiotic hypotheses for the origin of the nuclear compartment have little if anything in common with endosymbiotic hypotheses for the origins of chloroplasts and mitochondria.

One major difference is that endosymbiotic hypotheses for the origins of the latter compartments are founded in their biochemical and physiological similarity to free-living cells, but those for the origin of the nucleus are not. This is because the contemporary nuclear compartment has no role in physiology in the classical sense, rather it is an information-containing and -processing compartment. From the standpoint of function, the nuclear compartment is not homologous to any kind of free-living cell, because it does not possess any trace of an ATP-generating pathway or any other kind of metabolism needed to fuel a free-living cell. In contrast, plastids, mitochondria and hydrogenosomes have abundantly preserved traces of ATP-generating machinery. If anything, the nuclear compartment is functionally homologous not to a prokaryotic cell but rather to a prokaryotic chromosome.

The basis for the hypothesis that the nucleus was once a free-living bacterium lies primarily in its graphically depictable membrane-topological similarity to a free-living cell. However, that topological similarity is altogether superficial. No cell is known that is surrounded by a folded single membrane such as that which surrounds the nucleus. Very importantly, no cell is known that completely disintegrates its surrounding plasma membrane at every cell division, as does the nuclear compartment in eukaryotes with open mitosis. Conversely, no eukaryotic compartments for which strong evidence exists that they indeed descend from endosymbionts (chloroplasts, mitochondria and hydrogenosomes) disintegrate their surrounding membranes during division.

Even in eukaryotes with closed mitosis, the size and type of pore complexes in the nuclear envelope possess nothing even vaguely similar at the level of structure or function to that in prokaryotes. Nuclear pore complexes are typically 100 nm in diameter and allow molecules smaller than 5000 Da to diffuse freely from one compartment to the other. Neither is any eukaryotic organelle that is known to trace to an endosymbiotic event nor any membrane system in a free-living prokaryote similarly permeable.

Proponents of 'nucleosymbiotic origins' need to address the issue of why the nuclear compartment is so fundamentally different from any known free-living cell from the standpoints of (i) ATP-generating physiology (altogether lacking in the nuclear compartment), (ii) membrane topology (no free-living cell is bounded similarly), (iii) permeability (no prokaryotic cytosol is freely contiguous with the environment) and (iv) division (dissolution of a superficial homologue to the plasma membrane once per cell division).

## 6. A PROBLEM COMMON TO ENDOSYMBIOTIC AND PREVIOUS AUTOGENOUS HYPOTHESES

Despite their differences, endosymbiotic and autogenous hypotheses for the origin of the nucleus (except those that also derive plastids and mitochondria autogenously) have one thing very much in common: the eukaryotic cell derived from them does not possess mitochondria. Indeed, many eukaryotes are known to possess a nucleus but to lack mitochondria. Such suspectedly primitive nucleate cells, such as *Giardia lamblia*, were long viewed—and are still viewed by some (Gupta 1998)—as direct descendants of a hypothetical, ancestral phagocytosing, but mitochondrion-lacking group of eukaryotes intermittently known as Archezoa (Cavalier-Smith 1987*a*,*c*; Vossbrinck *et al*. 1987; Sogin *et al*. 1989; Cavalier-Smith & Chao 1996). However, to the great surprise of everyone, all of the mitochondrion-lacking eukaryotes that have been subjected to molecular investigations to date have turned out to contain molecular evidence in their nuclear genes indicating that they possessed a mitochondrion in their evolutionary past, but subsequently lost the organelle (Clark & Roger 1995; Henze *et al*. 1995; Bui *et al*. 1996; Doolittle 1996, 1997; Germot *et al*. 1996, 1997; Horner *et al*. 1996; Müller 1996, 1997, 1998; Roger *et al*. 1996, 1998; Embley *et al*. 1997; Hashimoto *et al*. 1998; reviewed briefly in Embley & Hirt 1998; Keeling 1998; Gray *et al*. 1999).

Such findings are of particular importance for hypotheses concerning the origin of the nuclear compartment. For if a eukaryote that never possessed a mitochondrion cannot be found, then the possession of a mitochondrial endosymbiont at some time in the evolutionary past would, quite curiously, belong to the group of characters that distinguishes contemporary eukaryotes (the ones we have to explain) from prokaryotes. This in turn, would provide an opportunity to readdress the question concerning the order of the origin of unifyingly eukaryotic features: the mitochondria, cytoskeleton, endomembrane system, nuclear compartment and mitosis.

## 7. WHAT CAME FIRST, THE MITOCHONDRION OR THE NUCLEUS?

Traditional hypotheses posit that the host which acquired mitochondria already possessed a nucleus. However, an alternative hypothesis for the origin of the common eubacterial ancestor of hydrogenosomes and mitochondria was recently forwarded which outlines an origin of the organelle in a non-nucleus-bearing archaebacterial host (Martin & Müller 1998). In brief, it was suggested that the symbiont was a facultatively anaerobic $\alpha$-proteobacterium with considerable metabolic flexibility that was able to respire under aerobic conditions, as in classical formulations of the endosymbiont hypothesis (Doolittle 1998*a*), but was also able to synthesize ATP in the absence of oxygen as well, using such anaerobic pathways as are common among free-living proteobacteria. Such pathways include, for example, the $H_2$-producing fermentations found in hydrogenosomes (Müller 1993, 1998; Akhmanova *et al*. 1998) or the ATP-producing pathways found in anaerobic mitochondria (Kobayashi & Shoun 1995; Kobayashi *et al*. 1996; Embley & Martin

1998; Tielens & Van Hellemond 1998; Müller & Martin 1999).

The host of that mitochondrial endosymbiosis was suggested to have been a strictly anaerobic, strictly autotrophic, strictly $H_2$-dependent archaebacterium, possibly similar to contemporary methanogens, that possessed neither nucleus, cytoskeleton or mitosis nor any other typically eukaryotic attribute. The selective pressure posited to have associated the host to the symbiont was suggested to have been the host's strict dependence upon molecular hydrogen produced by the symbiont (Martin & Müller 1998). Due to that dependence and to the posited lifestyle of the host, the origin of the mitochondrial compartment was suggested to have occurred under anaerobic conditions, rather than under aerobic conditions as classical formulations of the endosymbiont hypotheses envisage (Doolittle 1998*a*).

As a possible mechanism for enhancing the physical association between symbiont and host, it was suggested that host cell 'shapes' rather than endocytosis might have played a role, because no prokaryotes that possess a cytoskeleton are known (Martin & Müller 1998). Indeed, some contemporary methanogens with highly irregular shapes, such as *Methanocorpusculum*, are known, which can exist as distinctly 'C-shaped' forms (Zellner *et al*. 1989). It is not known whether cell shape may have (had) a genetic component, but, if so, it should be (have been) selectable. It has been pointed out that methanogens are the only prokaryotes known that possess true histones, lending credence to the view that the host, in principle, could have been a methanogen (Sandman & Reeve 1998; Sandman *et al*. 1998; Vellai *et al*. 1998). In agreement with the view that the ancestor of mitochondria was a facultative anaerobe is, for example, the finding that some eukaryotes possess a nuclear-encoded, mitochondrial-targeted homologue of the TspO protein, which in the $\alpha$-proteobacterium *Rhodobacter* regulates the switch from aerobic to anaerobic metabolism (Yeliseev *et al*. 1997), in addition to data summarized elsewhere (Embley & Martin 1998; Martin & Müller 1998; Müller & Martin 1999). In addition, Chistoserdova *et al*. (1998) reported evidence for an ancient gene transfer between methanogens and $\alpha$-proteobacteria, suggesting that the type of ecological, $H_2$-mediated association posited might have entailed gene transfers in the other direction (from host to symbiont) as well.

The mitochondrion-bearing common ancestor of eukaryotic cells so inferred is suggested to have lacked a nucleus, but was argued to have possessed ample genetic starting material to evolve cytological and genetic traits that are specific to the eukaryotic lineage. Clearly, such traits should also include the nuclear compartment. The premisses outlined elsewhere (Martin & Müller 1998) can be straightforwardly implemented in such a manner as to account for an origin of the nucleus in a mitochondrion-bearing cell, but in a manner that differs substantially from previous views on the topic.

## 8. ACCUMULATION OF EUBACTERIAL LIPIDS AROUND THE SITE OF SYNTHESIS

Gene transfer from the symbiont's genome to the cytosolic chromosomes of the host could have genetically cemented two prokaryotes into a single, biochemically compartmented, but nucleus-lacking common ancestor of eukaryotes (Martin & Müller 1998). Importantly, gene transfer from symbiont to host is not an ad hoc invention of the hypothesis that is designed to explain a particular pattern of genetic compartmentation. Rather, it is a logical consequence that ensues from the selective pressures that arguably would have confronted such an anaerobic, symbiotic pair of cells in the environment (see Blackstone (1995) for a good discussion of selective pressures in endosymbiotic theory). Furthermore, the earliest phases of gene transfer from symbiont to host would not have required the pre-existence of a protein import machinery that directs the products of translocated genes back into the compartment from which the gene was donated. Rather, the transfer of genes from the symbiont's genome to that of the host is posited to have initially resulted in relocalization within the cell of the encoded gene product into the compartment where the gene is expressed. It is not unreasonable to assume that a protein import machinery for mitochondria (Schatz & Dobberstein 1996) arose later, during the process of organelle genome reduction (Martin & Herrmann 1998), possibly from simpler pre-existing components as newer data for plastids would suggest (Bölter *et al*. 1998; Heins & Soll 1998).

Selective pressures that are suggested to have strongly favoured the transfer of genes from symbiont to host specifically for the heterotrophic lifestyle should have carried along many hitchhiking genes as well. Such hitchhikers were suggested to have included the symbiont's genes for the synthesis of eubacterial lipids (Martin & Müller 1998), to account for the finding that eukaryotes possess the eubacterial type and stereochemistry of lipids (glycerol esters of fatty acids), rather than the archaebacterial type (glycerol ethers of isoprenes) (Koga *et al*. 1998).

On the basis of these premises, if copies of the genes for enzymes of eubacterial lipid synthesis were transferred from the symbiont's genome to the cytosolic chromosomes of the host and were expressed there, the immediate result would have been the synthesis of eubacterial lipids in a compartment (the largely archaebacterial cytosol) that was very likely unprepared to accommodate them. In principle, this could have led to the incorporation of eubacterial lipids into the plasma membrane or the accumulation (by simple phase separation) of the eubacterial lipids as droplets, sheaths or vesicles surrounding their site of synthesis in the cytosol (not improbably both). In the event that eubacterial lipid vesicles did accumulate in the cytosol as a fortuitous result of gene transfer from symbiont to host, the further accumulation of such lipids would have led, through vesicle fusions, to the seeds of a primitive (endoplasmic reticulum-like?) endomembrane system that, upon continued accumulation, ultimately would be expected to have surrounded the chromosomes harbouring the genes that encoded the proteins of the pathway.

A vesicular model for a primitive endomembrane system would follow as a consequence of selection for the transfer of eubacterial genes from the symbiont to the archaebacterial chromosomes of the host. This model would not demand (but would not preclude) the

existence of a cytoskeleton prior to the existence of primitive endomembrane vesicles. However, the cell inferred under these premisses would obviously need to evolve some form of primitive cytoskeleton before anything similar to a truly structured endomembrane system and a truly nuclear-like compartment could arise. The backbone of such a cytoskeleton could easily be derived from pre-existing prokaryotic gene products: tubulin-like FtsZ and actin-like FtsA. However, as Blackstone (1995) lucidly argued, limiting factors and immediate selective pressures must be explicitly formulated to account for the fixation of cellular novelties in endosymbiotic theory. Thus, we have to face the difficult question of what sorts of selective pressures would be sufficient to lead to the fixation of a primitive cytoskeleton and an endomembrane system during the phase of evolution before anything similar to what we observe today in eukaryotes (endocytosis, the endoplasmic reticulum and nucleus) arose. This is clearly a difficult issue. However, considering that the cell in which these processes are here assumed to have occurred is a facultatively anaerobic heterotroph (Martin & Müller, 1998), the first factor limiting its survival would therefore have been the ability to obtain sufficient amounts of oxidizable organic compounds to produce ATP for fueling all other cellular processes. This would suggest that primitive endocytosis-like (feeding) processes could have led to the fixation of a cytoskeleton, as De Duve (1969) and Cavalier-Smith (1987*a*) argued, but, under the views stated here, this would have occurred in a mitochondrion-bearing cell. Once the cytoskeleton, endomembrane vesicles and their routing in the cell were in place, then perhaps something similar to a nuclear compartment could have arisen.

This model would have a distinct advantage over endosymbiotic models for nuclear origins in that it would predict that there be no meaningful similarity, either physiologically or topologically, between the nuclear compartment and any known free-living cell. A vesicular model is just as speculative as all other models for nuclear origins, but it differs from them and is not obviously worse. Like all other models, it also ultimately requires the invention of pore-building proteins to permit the expression of genes contained in the nuclear compartment as proteins that are not contained there, otherwise the new compartment would eventually become entirely sealed off from the cytosol through vesicle fusions. In contrast to traditional autogenous (invagination) and symbiotic hypotheses for the origin of the nucleus, it has the curious property that it is distinguished from them in that the mechanism of the inferred origin of the nuclear compartment during evolution and the physical origin of the nuclear envelope during the cell cycle of modern eukaryotes would be very similar—proximal fusion of pre-formed distal vesicles consisting of eubacterial lipids. Under these premises, the eukaryotic endomembrane system could have arisen as a fortuitous result of the strongly selected transfer of genes from the genome of a heterotrophic mitochondrial symbiont to the genome of an chemolithoautotrophic archaebacterial host and, hence, necessarily occurred subsequent and not prior to the origin of mitochondria.

## 9. CONCLUSION

Endosymbiotic hypotheses convincingly explain why mitochondria (including hydrogenosomes) and chloroplasts (including complex ones) are so similar to free-living organisms. Endosymbiotic hypotheses fare best when their justification is founded in biochemical or physiological similarity to a free-living cell. The nuclear compartment bears no such similarity to a free-living cell. The view that it arose from an endosymbiotic bacterium (in whatever kind of host) is fraught with problems that need to be explicitly addressed by its proponents. Many of the attributes that eukaryotes possess are obviously evolutionary inventions, novelties that arose in specific lineages. There is no reason to assume that the common ancestor of contemporary eukaryotes, regardless of how it arose, was unable to bring forth one or the other novelty itself. The nuclear compartment is almost certainly a specifically eukaryotic novelty, but here it is suggested to have arisen in a cell that possessed a facultatively anaerobic, heterotrophic organelle, the common ancestor of mitochondria and hydrogenosomes.

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