Selective forces for the origin of the eukaryotic nucleus

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Summary

The origin of the eukaryotic cell nucleus and the selective forces that drove its evolution remain unknown and are a matter of controversy. Autogenous models state that both the nucleus and endoplasmic reticulum (ER) derived from the invagination of the plasma membrane, but most of them do not advance clear selective forces for this process. Alternative models proposing an endosymbiotic origin of the nucleus fail to provide a pathway fully compatible with our knowledge of cell biology. We propose here an evolutionary scenario that reconciles both an ancestral endosymbiotic origin of the eukaryotic nucleus (endosymbiosis of a methanogenic archaeon within a fermentative myxobacterium) with an autogenous generation of the contemporary nuclear membrane and ER from the bacterial membrane. We specifically state two selective forces that operated sequentially during its evolution: (1) metabolic compartmentation to avoid deleterious co-existence of anabolic (autotrophic synthesis by the methanogen) and catabolic (fermentation by the myxobacterium) pathways in the cell, and (2) avoidance of aberrant protein synthesis due to intron spreading in the ancient archaeal genome following mitochondrial acquisition and loss of methanogenesis.

***BioEssays* 28:525–533, 2006.**

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Introduction

The origin of eukaryotes remains a hotly debated issue. Various models have been suggested, generally explaining only partially how one or more properties of the eukaryotic cell might have arisen from simpler ancestors. However, none of them explains satisfactorily the origin of one of the most crucial eukaryotic features: the nuclear compartment. In particular, no convincing selective forces have been proposed for its formation from nucleus-lacking ancestors, and a definite explanation that satisfies cell biologists is still missing. Knowledge about cell biology and microbial evolution has progressed considerably since the recognition of the nucleus as a key distinctive feature for eukaryotes,[1–3]. In recent years, this progress has been made possible mainly through molecular phylogenetics and comparative genomics, and the resulting information must be taken into account by any hypothesis trying to explain the origin of eukaryotes. From an evolutionary perspective, the most crucial discovery that has to be accommodated in modern models of eukaryotic evolution is the existence of archaea, a group of organisms fundamentally different from bacteria and eukaryotes,[4,5] and the ensuing recognition that eukaryotic genomes possess a mixed heritage. Complete genome sequence analyses, corroborating the first gene comparisons, have indeed uncovered a paradox: eukaryotic genes related to DNA replication, transcription and translation (the basic informational core) resemble archaeal genes, whereas genes involved in energy and carbon metabolism resemble their bacterial counterparts.[6,7]

Current models attempting to address the origin of the nuclear compartment can be divided in two major families (for review, see Refs. 8–10). Autogenous models state that the nucleus originated from invaginations of the plasma membrane of an ancestral prokaryote,[11,12] and are compatible with cell biology knowledge. The nuclear envelope is composed of two parallel lamellar membranes, whose lumen is continuous with that of the endoplasmic reticulum, that are traversed by nuclear pore complexes (NPC) allowing all transport between the cytoplasm and the nucleus. Recent analyses of small GTPases of the Ras superfamily, particularly Ran as key regulator of nuclear function, and NPC components suggest that all of them radiated from a few ancestral genes.[12,13] This and the existence of a possible connection between NPC and coated vesicle formation[14] strengthen the plausibility of models of endomembrane evolution from some kind of simpler ancestor. The nature of this ancestor is seldom discussed in autogenous models, which are often presented in purely mechanistic terms. Nevertheless, recent versions of autogenous models implicitly invoke a member of a third ancestral lineage sister to the archaea.[5,15,16] This would justify the archaeal nature of eukaryotic informational genes, whereas bacterial-like metabolic genes would have been obtained later via the mitochondrial acquisition. However, this view does not explain why there is a whole category of bacterial-like genes involved in eukaryotic cytoplasmic functions related to cellular import and metabolism that appear

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Funding agency: This work was supported by an ATIP grant of the French Centre National de la Recherche Scientifique, section "Dynamique de la biodiversité".

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DOI 10.1002/bies.20413
Published online in Wiley InterScience (www.interscience.wiley.com).

unrelated to the alphaproteobacterial ancestor of mitochondria.[7]

In contrast to autogenous hypotheses, chimeric symbiotic models for the origin of eukaryotes generally reject the existence of an archaeal-like mysterious lineage for which there is no direct evidence. Archaea and bacteria would be the two only survivor lines descending from the last common ancestor and eukaryotes would derive from the merging of the two. The presence of archaeal and bacterial genes in eukaryotic genomes would be a logical outcome of that process. Distinctive eukaryotic traits would be innovations, rather than inherited characters from an unknown hypothetical lineage, as a consequence of gene redundancy and increased evolutionary rate in a symbiosis context.[17,18] However, although these types of models can easily account for the genetic content of eukaryotic genomes, as they are supported to variable extents by comparative phylogenomic data, they have failed so far to provide a plausible mechanistic scenario for the evolution of the nuclear envelope. On the one hand, the most simple and direct chimeric hypotheses, which suggest that the incorporation of the future mitochondrion to an archaeal host triggered eukaryogenesis, do not explicitly propose a pathway for the origin of the nuclear compartment.[19,20] These hypotheses could in principle make use of an autogenous origin of the nucleus by invagination of the archaeal host membrane, but this would not fit with the bacteria-like nature of eukaryotic membranes. Archaeal membrane lipids and topology are indeed drastically different from their bacterial and eukaryotic counterparts, which raises a major puzzle for studies of early evolution.[21] In turn, endosymbiotic models stating that the nucleus derives from the incorporation of an archaeon within a bacterium[22,23] do not make clear why and how bacterial lipids substituted archaeal lipids and the nuclear envelope evolved.

We propose here to reconcile autogenous and endosymbiotic models for the origin of the nucleus by putting forward a two-step hypothesis that goes beyond our previous ideas on the origin of eukaryotes. Based on ecological, genetic and metabolic grounds, our 'syntrophy hypothesis', as originally formulated in 1998, postulated that modern eukaryotes derive from two independent symbiotic events involving three prokaryotic partners: a methanogenic archaeon, a myxobacterium and an alphaproteobacterium—the ancestor of mitochondria.[9,24] A symbiosis based on interspecies hydrogen transfer would have been initially established between a methanogen and myxobacteria (belonging to the deltaproteobacteria) under anaerobic conditions. At an early stage, a metabolically versatile alphaproteobacterium that was able, among others, to oxidise methane would have become a member of the syntrophic consortium and, later, the mitochondrion. After a period of obligatory symbiosis, the evolution of a proto-eukaryotic assemblage took place. In it, the methanogen would have evolved as the future nucleus, providing most of the genetic machinery, while the myxobacteria, characterised by complex life cycles and cell-to-cell communication, would have provided most cytoplasmic features, including cell signalling and import, and part of the central metabolism. We have now considerably extended and refined our ideas to build a model that envisages a concerted metabolic, membrane and genome evolution. In this article, we propose the following key steps for the origin of the nuclear compartment.

1) A true endosymbiotic origin of the eukaryotic nucleus, with a methanogenic archaeon directly incorporated into the cytoplasm of the myxobacterium.
2) Two clear, sequential, selective forces for the origin of the nucleus and the nuclear membrane: (i) metabolic compartmentation to avoid deleterious co-existence of anabolic (autotrophic synthesis by the methanogen) and catabolic (fermentation by the myxobacterium) pathways in the cell and (ii) protection against aberrant protein synthesis due to intron spreading after the loss of methanogenesis by decoupling transcription and translation.
3) The nuclear envelope and the endoplasmic reticulum derived from the myxobacterial plasma membrane in a way that is fully compatible with autogenous models for the origin of the nucleus.

Endosymbiotic origin of the eukaryotic nucleus

The syntrophy hypothesis proposed that the very initial step in eukaryotic evolution was the establishment of an obligatory symbiosis between a methanogenic archaeon and a myxobacterium. Metabolic symbioses (syntrophy) between methanogens and deltaproteobacteria mediated by hydrogen transfer are widespread today in anoxic sediments,[25] and arguably, must have been even more important in the Precambrian prior to the last significant increase of molecular oxygen in the atmosphere. Methanogenic archaea are members of the Euryarchaeota, one of the two major lines within the domain Archaea, and gain energy by the reduction of carbon dioxide (or acetate) with hydrogen under strict anaerobic conditions. The delta subclass of the Proteobacteria contains organisms that can be classified in four major types according to their lifestyle. Some lineages are dissimilatory sulphate- or sulphur-reducers, strict anaerobes widespread in anoxic sediments. Many of these lineages, such as *Geobacter* species, are not only able to reduce sulphur species with hydrogen, but also oxidised metals such as Fe(III) or Mn(IV).[26] Some of these hydrogen-consuming sulphate reducers establish symbioses with methanogen-related archaea probably operating in reverse, i.e. consuming methane, in well-developed consortia. Anaerobic methane oxidation, mediated by these symbiotic consortia, was demonstrated only recently, and, for a long time, gain of energy by this process was thought to be impossible.[27,28] Other

deltaproteobacteria are fermentors that live in syntrophic symbioses with hydrogen-consuming methanogens (e.g. *Syntrophus, Syntrophobacter*). Some others are unusual bacterial predators, such as *Bdellovibrio* spp.[29] Finally, the myxobacteria constitute a unique group of heterotrophs displaying well-developed cell-to-cell communication and complex developmental life cycles, which include cellular differentiation and multicellular stages.[30–32] Although they were thought to be exclusively aerobic for a long time, facultative anaerobic myxobacteria that are able to respire aryl compounds and, similarly to other deltaproteobacteria, Fe(III) have been isolated from various soils, sediments and the subsurface.[33,34]

The type of symbiosis between a methanogen and an ancestral myxobacterium that we propose most clearly resembles that established today by *Syntrophus* spp. The myxobacterium would ferment liberating hydrogen, carbon dioxide and acetate that would be taken up by the methanogen for its metabolism (Fig. 1). Since sulphate reduction was most likely ancestral to the deltaproteobacteria,[35] we cannot exclude that the myxobacterial partner was also a facultative sulphate reducer (although not acting as such in the eukaryogenetic symbiosis). In the original version of the syntrophy hypothesis, the deltaproteobacterium would have developed extensive membrane-to-membrane contact with the methanogen to facilitate metabolite exchange, as happens in nature.[25] The bacterial cell would have ended up encircling the methanogen and fusing its membrane around it, but it was not a true endosymbiosis, since the archaeal cytoplasm would not have been physically incorporated to a cell-like entity until a much later, already proto-eukaryotic, stage.[24] At the time that we first made this proposal, we avoided the formulation of a true endosymbiosis because phagocytosis was unknown in prokaryotes. However, it is now well established that some prokaryotes harbour prokaryotic endosymbionts.[36] Consequently, although the precise mechanism by which they are incorporated to the cell is unknown, some form of "phagocytosis" must exist in prokaryotes. This invalidates the criticisms raised by some authors that prokaryotes could not acquire endosymbionts within their cytoplasm.[37] Taking into account the fact that prokaryotes can harbour endosymbionts, we propose a true endosymbiosis implying the full incorporation of the methanogen within the myxobacterial cytoplasm (Fig. 1).

During evolution, the archaeal endosymbiont became the eukaryotic nucleus, which inherited most informational genes but also some structural features such as nucleosomes based on histones[38] and, possibly, even the occurrence of multiple origins of replication.[39] Furthermore, the eukaryotic nucleolus can be seen as a functional remnant of the ancient archaeal cytoplasm. The nucleolus is the place where rRNAs are synthesised, playing a key role in functions related to the biogenesis of ribonucleosomal particles, and is also important in cell-cycle control.[40] Ribosomal subunits are assembled there, which implies the remarkable import of ribosomal proteins from the cytoplasm into the nucleus to be exported again through the NPC after they have been organised. It is also the place where small nucleolar RNAs and proteins involved in the regulation of ribosomal assembly, such as fibrillarin, Nop56/58, Nop10 and Gar1p, all with archaeal (but not bacterial) homologues, concentrate. In our view, as happened with many other genes and DNA elements, small RNAs present in the archaeal ancestor experienced duplications and proliferated during eukaryotic evolution mainly adapting to regulatory functions.

Selective forces for the origin of the nucleus

The initial symbiosis between the myxobacterium and the methanogen became obligatory when the first essential bacterial genes were transferred to the archaeal endosymbiont and got fixed in its genome, very much like mitochondria and chloroplasts became obligatory endosymbionts later on. At this stage, the incipient nucleus (i.e. the archaeal endosymbiont) had to be surrounded by its archaeal membrane in order to carry out methanogenesis (requiring membrane-bound electron transport systems[41]) and to avoid the occurrence of opposite anabolic and catabolic pathways in the same compartment. Fermentation from organics occurred in the myxobacterial cytoplasm liberating hydrogen, carbon dioxide and acetate. The methanogen gained energy by reducing carbon dioxide with hydrogen to produce methane and also, being a chemoautotroph, it reduced carbon dioxide to synthesise its own organic components (Fig. 1). The presence of a separate compartment was thus selected to avoid the deleterious incessant recycling of molecules by these two opposite pathways. Therefore, metabolic compartmentation was the first selective force in the evolution of the eukaryotic nucleus.

A crucial step early in eukaryotic evolution was the stable acquisition of the alphaproteobacterial ancestor of mitochondria. Neither fermentation nor methanogenesis are very efficient energy-yielding processes compared to oxygen respiration, although both pathways benefit in symbiosis: the fermentor has a sink for hydrogen and the methanogen a ready source for it, both speeding up their metabolic reactions. However, respiration, particularly oxygen respiration, is much more effective, and is a key to the metabolic evolution that followed. In our model, the ancestor of mitochondria incorporated very early to the existing consortium, most likely at the level of the obligatory symbiosis step. It was a highly versatile facultative aerobe, being able to respire a variety of oxidised molecules including fumarate and oxygen, and to oxidise methane and various hydrocarbons, all of them being metabolic capabilities frequent within the alphaproteobacteria. Initially, the alphaproteobacterium incorporated into a symbiotic consortium that was thriving in anoxic or suboxic environments. Methane oxidation was conducted anaerobically, using

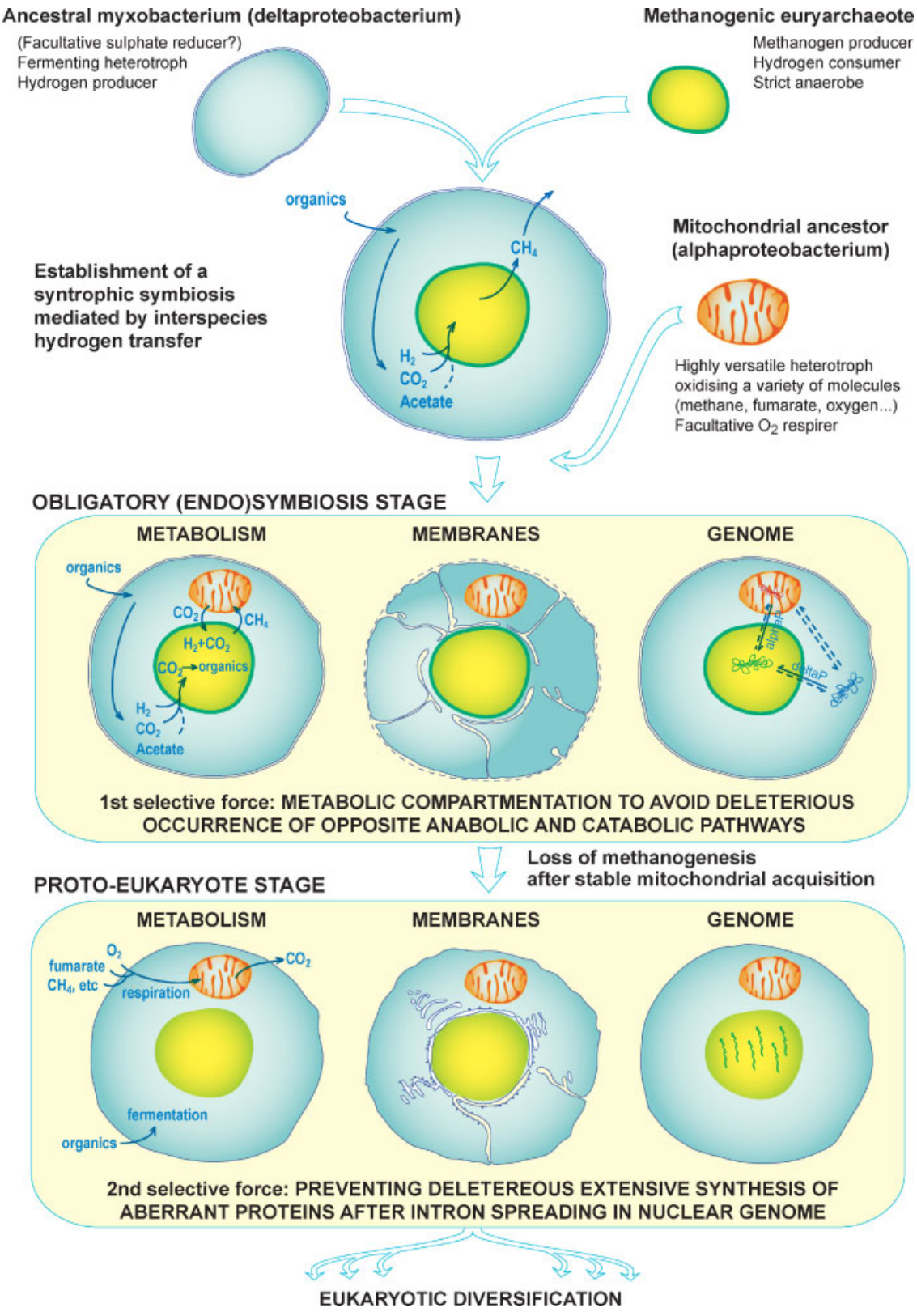
Ancestral myxobacterium (deltaproteobacterium)
(Facultative sulphate reducer?)
Fermenting heterotroph
Hydrogen producer
Methanogenic euryarchaeote
Methanogen producer
Hydrogen consumer
Strict anaerobe
organics
CH4
H2
CO2
Acetate
Establishment of a syntrophic symbiosis mediated by interspecies hydrogen transfer
Mitochondrial ancestor (alphaproteobacterium)
Highly versatile heterotroph oxidising a variety of molecules (methane, fumarate, oxygen...)
Facultative O2 respirer
OBLIGATORY (ENDO)SYMBIOSIS STAGE
METABOLISM
MEMBRANES
GENOME
organics
CO2
CH4
H2+CO2
CO2→organics
H2
CO2
Acetate
alphaP
deltaP
1st selective force: METABOLIC COMPARTMENTATION TO AVOID DELETERIOUS OCCURRENCE OF OPPOSITE ANABOLIC AND CATABOLIC PATHWAYS
Loss of methanogenesis after stable mitochondrial acquisition
PROTO-EUKARYOTE STAGE
METABOLISM
MEMBRANES
GENOME
O2
fumarate
CH4, etc
respiration
CO2
fermentation
organics
2nd selective force: PREVENTING DELETEREOUS EXTENSIVE SYNTHESIS OF ABERRANT PROTEINS AFTER INTRON SPREADING IN NUCLEAR GENOME
EUKARYOTIC DIVERSIFICATION

Figure 1.

nitrate or some other oxidised molecule, or aerobically under microaerophilic conditions if the consortia thrived at the transition between oxic and anoxic zones in sediments. Everybody profited. The alphaproteobacterium had a free source of methane, and the methanogen, in turn, had a sink for methane, an extra source of carbon dioxide, and protection against the possible presence of oxygen. The fermentor benefited from the enhanced methanogenesis rate. Many alphaproteobacterial genes were transferred to the archaeal genome and fixed, which converted mitochondrial ancestors in obligatory symbionts. At this point, the respiration capabilities of the alphaproteobacterium presented extraordinary possibilities to the obligatory myxobacterial–archaeal consortium. Based on the much more energetically efficient oxygen respiration, the tripartite consortium could now colonise oxic environments relying exclusively on aerobic respiration. Thus, once the archaeal genome had taken over the genetic control of the consortium, methanogenesis, which was inefficient and inhibited under oxic conditions, was lost.

This was a decisive step that marked the transition to a true proto-eukaryotic (ancestral eukaryotic) stage. Once methanogenesis was lost, the maintenance of the archaeal membrane and that of the nucleus as an independent metabolic compartment were no longer selectively advantageous. However, during the previous evolutionary period that had accompanied the obligatory symbiosis stage, two important things had happened. First, a system of bacterial endomembranes with extensive growth around the archaeal symbiont developed for secretory functions (see below). Second, the transfer of genes from the bacterial to the archaeal genome was paralleled by the invasion of non-coding DNA elements. These included transposons, repetitive sequences and pseudogenes that accumulated thanks to the gene redundancy between the three symbiotic partners. More importantly, protein-coding genes were invaded by introns, which derived from the self-splicing introns[42] already existing in the tRNA and rRNA genes of the consortium. The archaeal membrane was lost, because it was costly and useless, but the bacterial endomembrane system evolved to form a true nuclear membrane that was selected to prevent massive synthesis of aberrant proteins from intron-containing genes. This was achieved by confining transcription and translation to two different compartments. Hence, the pre-nuclear compartment shifted its function from hosting energy and carbon metabolism to hosting nucleic acid metabolism, a novelty in cellular evolution. The NPC evolved at this point and became involved in the control of the import and export towards this specialised region, which formed the emerging eukaryotic nucleus, and ribosomal subunits began to be exported to the proto-eukaryotic cytoplasm for their final functional assembly. Therefore, prevention of extensive aberrant protein synthesis by decoupling transcription (nuclear) and translation (cytoplasmic) was the second selective force in the evolution of the eukaryotic nucleus.

Once mitochondria incorporated into the consortium and a proto-eukaryotic stage was acquired, the succession of evolutionary events leading to fully developed eukaryotes (loss of methanogenesis, formation of a modern nuclear membrane, emergence of a highly developed cytoskeleton and of protocilia/protoflagella, mitosis and perhaps even meiosis) may have been rapid. This proto-eukaryote readily became the last common eukaryotic ancestor from which contemporary lineages diverged and colonised the wide panoply of novel ecological niches that suddenly opened to its newly acquired adaptive capabilities.

The (simultaneous) origin of the nuclear envelope and the endoplasmic reticulum

As mentioned above, during the co-evolutionary phase that the syntrophic consortium experienced, an extensive system of internal membranes developed by invagination of the myxobacterial membranes. The expansion of these membrane invaginations was selected to secrete hydrolytic compounds from the archaeal endosymbiont towards the surrounding environment. Myxobacteria transferred most of their genes to the archaeal genome to the point of reaching complete genome extinction, and archaeal ribosomes took over most protein-synthesis functions. Hence, many of these hydrolytic enzymes began to be synthesised by the archaeon and required to be exported out of the consortium. The proliferation of small GTPases and, particularly, the evolution of Ran, which later became a key regulator of nuclear membrane transport, made this possible in a way that is fully compatible with the recent proposal by Jékely[12] for the evolution of the modern nuclear envelope. According to him, the original endomembrane system leading to the emergence of the endoplasmic reticulum and the nuclear envelope was primarily secretory, and phagocytosis appeared later, supported by the already existing secretory capacities.

Interestingly, the closest known prokaryotic relatives of small eukaryotic GTPases are myxobacterial MglA-like proteins.[12] Myxobacteria have an extremely well-developed

Figure 1. Schematic pathways of metabolic, membrane and genome evolution leading to the emergence of eukaryotes from symbiotic consortia of myxobacteria (deltaproteobacteria) and methanogenic archaea (syntrophy hypothesis). Myxobacteria fermented liberating hydrogen used by the methanogen for its energetic metabolism. Mitochondria derived from alphaproteobacterial ancestors able to recycle methane within the assemblage. The archaeal membrane is symbolised by a thick green line, the myxobacterial membranes are depicted by blue lines. AlphaP and deltaP indicate transfer of genes from alpha and deltaproteobacteria to the methanogen genome.

secretory system. They not only secrete a large diversity of hydrolytic enzymes for the degradation of macromolecules,[30] but also need it for cell-to-cell communication and gliding motility. Myxobacteria display remarkable communication properties during fruiting body morphogenesis that require, among others, the secretion of soluble signals.[31,43] In addition, myxobacteria glide by displaying two types of motility: social (S) motility, a pulling system based on the protraction of Type IV pili, and adventurous (A) motility, a pushing motor based on the secretion of mucilage.[44] Notably, MglA is an essential regulator in myxobacteria. MglA is a small cytoplasmic GTPase that not only interacts with a membrane-bound tyrosine kinase to regulate the signal transduction pathway controlling both types of motility and development,[45] but also appears to control the polarity of global movement in harmony.[43]

Taking this into account, we propose that an extensive system of myxobacterial endomembranes developed initially and was involved in secretion and connection of the archaeal endosymbiont with the exterior of the symbiotic consortium. Once methanogenesis and the archaeal membrane were lost, the endomembrane system took over the maintenance of a separate compartment while conserving a role in protein transport, thus giving rise to both the endoplasmic reticulum and the nuclear envelope (Fig. 1). During this process, the small GTPase MglA, already involved in secretory and transport regulatory functions, experienced successive duplications and specialised in different cellular functions, including the control of nuclear transport by Ran.[12,13] Thus, contrary to Jékely, who favours the idea that MglA was acquired by myxobacteria by horizontal transfer from eukaryotes,[12] we believe that eukaryotes inherited this and many other eukaryotic-like myxobacterial features from their symbiotic ancestor (see below).

Concerted metabolic, membrane and genome evolution, and some predictions

The emergence of eukaryotes was the result of the symbiosis-promoted co-evolution of metabolic interactions, extensive membrane development and genomic integration, which shaped contemporary eukaryotic genomes, energy and carbon metabolism, and the endoplasmic reticulum and nuclear envelope. Although symbioses, even obligatory, between methanogens and deltaproteobacteria are widespread in nature, the event that led to such level of integration and promoted the passage to a higher level of complexity, that of the eukaryotic cell, was improbable. Therefore, the emergence of eukaryotes was a rare event and, even if identical conditions occurred again and a hypothetical second eukaryotic experiment arose, it would have been certainly outcompeted by the already adapted and successful eukaryotes.

Carbon and energy metabolism in contemporary eukaryotes was inherited mostly from their bacterial ancestors, particularly from the mitochondrial ancestor. The latter could respire not only oxygen but also other oxidised molecules, such as fumarate, to reduce various hydrocarbons, including methane and methanol, activities that appear to be displayed by the mitochondria of different eukaryotes nowadays.[46,47] Nevertheless, some metabolic pathways or enzymes involved in anaerobic and central metabolism from the deltaproteobacterial ancestor may have also been retained in eukaryotes. However, it may be difficult to demonstrate their origin in contemporary eukaryotes because, in general, many genes coding proteins involved in those pathways belong to large multienzymatic families that are difficult to analyse phylogenetically. To date, there are two published examples of eukaryotic enzymes that could have a deltaproteobacterial ancestry. The first corresponds to the pyruvate:ferredoxin oxydoreductase (PFO), which is found in hydrogenosomes of anaerobic protists. Despite the fact that hydrogenosomes and mitochondria share the same bacterial ancestor,[48,49] phylogenetic trees including bacterial and eukaryotic PFOs placed them far from alphaproteobacterial sequences, in disagreement with a mitochondrial ancestry, but sister to deltaproteobacteria, albeit with low statistical support.[50] The second example corresponds to thiolases I and II, which are essential universal enzymes involved in fatty acid oxidation and in various anabolic processes. A recent study showed that cytosolic thiolases from green plants and fungi as well as at least one member of all eukaryotic peroxisomal and mitochondrial thiolases have deltaproteobacteria as their closest relatives. Their phylogenetic distribution in eukaryotes suggests that they were present in the last common eukaryotic ancestor.[51] Regarding the archaeal heritage, although most archaeal metabolic pathways were lost in favour of the more-efficient bacterial ones, some may have been retained, at least partially, and adapted to particular functions. This appears to be the case of the mevalonate pathway leading to the formation of isopentenyl-diphosphate in the biosynthesis of isoprenoids, a route shared by archaea and eukaryotes but not by bacteria, which use mainly the deoxyxyllulose-5-phosphate pathway.[52,53]

In our model, an extensive development of internal membranes leading to the formation of a tubular network, the endoplasmic reticulum, and a flat system bordering the ancient archaeal cytoplasm evolved from the myxobacterial ancestor. The NPC evolved with the nuclear envelope from simpler components to its present-day complexity.[13] Some authors have suggested a planctomycete-like ancestor for eukaryotes[54] based on the occurrence of internal membrane structures that encircle part of the cytoplasm and the genetic material and that, in a few planctomycete species (*Gemmata* spp.), display NPC-like openings.[55,56] However, in addition to the fact that this hypothesis does not explain the archaeal-like content of eukaryotic genomes, the genome of the planctomycete *Pirellula* sp. did not reveal homologues to components

of the nuclear envelope and the NPC.[57] The development of internal membrane systems in bacteria, which always has a metabolic purpose (when known), is rather frequent, for example, the anammoxosome in planctomycetes, and thylakoids in cyanobacteria or multiple membranes in methanotrophic bacteria.[56,58] A compartment similar to that observed in planctomycetes has also been recently detected in the novel candidate bacterial phylum Poribacteria.[59] The existence of NPC-like structures in some planctomycete species illustrates the fact that bacteria can build up large pores in internal membranes. Hence, the development of such pores in internal membranes might have been practised by other bacteria, such as the myxobacterial ancestor of eukaryotes, in their evolution. Myxobacteria are not only endowed with large membrane secretory pores (nozzle-like organelles)[44] but, interestingly, they have been recently found to briefly fuse their outer membranes with other cells.[60] This property might have been crucial for the formation of NPC in internal membranes, by facilitating the fusion of two invaginated parallel membranes at certain points that would have then been stabilised by integral membrane proteins. An important aspect of membrane evolution during eukaryogenesis was the loss of the outer membrane of the myxobacterium. Myxobacteria are Gram-negative bacteria, therefore possessing an internal and an external membrane defining a periplasmic space. Membrane loss has occurred in bacterial evolution (e.g. Gram-positive bacteria). We propose that the loss of the myxobacterial outer membrane improved the exchange of molecules with the external environment and was also linked to the fact that many metabolic reactions that previously took place in the periplasm (a metabolic compartment) were transferred to other membranous compartments. These include the mitochondrion and also peroxisomes, which may have evolved very early,[51] accompanying the development of an endomembrane system and the emergence of phagocytic capacities in the ancestral eukaryote.

The eukaryotic genome would have resulted from the integration of genes from three different origins. Horizontal gene transfer must have happened in all directions, initially bidirectionally between the myxobacterium and the archaeon. However, at some point, the archaeal genome took over the control of the situation. It could have been merely by chance: the first genome that fixed a few essential genes that were lost from the other symbiont became dominant. Thus, the archaeal genome prevailed while the myxobacterial genome became extinct after having transferred many genes to the future nucleus. Once incorporated into the archaeal genome, duplicated genes may have been lost, replaced genes performing equivalent functions or remained as duplicates that could accelerate their evolutionary rate and evolve new functions.[24] When the alphaproteobacterium incorporated into the consortium, it also transferred part of its genes to the nucleus. Some horizontal transfer might have occurred between the two bacterial symbionts if the alphaproteobacterium incorporated prior to the complete genome extinction of the deltaproteobacterium, but this was not relevant for subsequent history.

Finally, during this evolutionary pathway, other typical eukaryotic features evolved, such as a well-developed cytoskeleton, which most likely accompanied the emergence of the endomembrane system, the loss of the outer membrane and the concomitant evolution of phagocytosis. FtsZ and MreB, as bacterial homologues of tubulin and actin,[61,62] did play an important role in this process. Mitosis and, later, meiosis must have evolved tightly linked to the cytoskeletal evolution. Among the key ancestral features that evolved in the ancestral eukaryote are $9+2$ organelles (cilia or flagella). Today, these structures are involved not only in motility but also in various sensory processes. The last common ancestor to eukaryotes most likely possessed one of these organelles as a single posterior flagellum.[37,63] A recent hypothesis postulates that ancestral protocilia determined cell polarity and directed motility, providing the first membrane domain for the localisation of sensorial membrane receptors. According to this hypothesis, the initial motility of protocilia was by gliding rather than undulation.[64] It is interesting to note that both these processes, directionality with cell polarity and gliding, are typical features of myxobacteria.

Conclusions

We have proposed here an evolutionary pathway for the origin of the eukaryotic nuclear compartment (endosymbiotic-first, autogenous-later) from syntrophic bacterial–archaeal consortia, with a clear formulation of the selective forces that led to its emergence. These are indissolubly linked, first, to metabolic compartmentation required for maintaining two opposite metabolic pathways in the same cell-like organism and, second, to gene and genome structural factors (intron appearance). Selective forces for the origin of the nuclear compartment have been rarely articulated. Cavalier-Smith[11,37] suggested that the nuclear envelope appeared to protect DNA from shearing damage caused by the novel molecular motors that allowed cytoskeletal evolution. However, this explanation is unconvincing because eukaryotic chromosomes are able to overcome pulling and other mechanical stresses during mitosis, even in species where they are permanently uncondensed, despite the fact that the nuclear envelope disintegrates at the beginning of mitosis in many contemporary eukaryotes. In contrast, as mentioned above, it is important to note that all membranous compartments described in prokaryotes are involved in energy metabolism. Therefore, conceiving the proto-eukaryotic nucleus as a metabolic compartment seems realistic.

Models for the origin of eukaryotes are now testable, at least partially, by means of comparative genomics and molecular phylogenetics. On the one hand, our model predicts that

eukaryotic informational genes are more similar to their archaeal, and particularly euryarchaeotal, counterparts. A closer relationship of euryarchaeotal, rather than crenarchaeotal, genomes to their eukaryotic counterparts appeared confirmed when the first genome of a crenarchaeote, *Aeropyrum pernix*, was published.[65] Various genes are exclusively shared by eukaryotes and euryarchaeota, but are absent from crenarchaeota, including those encoding FtsZ and some replicative proteins,[66] and some uncharacterised proteins.[67] On the other hand, besides mitochondrial and archaeal genes, the syntrophy hypothesis predicts the presence of deltaproteobacterial, and more precisely myxobacterial, genes. Some genomic comparisons support the involvement of a second bacterium, in addition to the alphaproteobacterial ancestor of mitochondria, whose legacy was mostly related to transport and cytoplasmic functions.[7] A role for the involvement of bacteria other than mitochondrial ancestors in eukaryotic evolution has been suggested in several models: spirochetes, as potential ancestors of eukaryotic flagella and cilia,[68] planctomycetes, because of their nuclear-resembling membrane, and even Verrucomicrobia, possessing true α and β tubulin.[69] However, gene and genome comparisons do not reveal any significant eukaryotic connection with these bacterial groups.[13,57,70,71] In the case of myxobacteria, complete genome sequences have not yet been published, although the sequences of *Myxococcus xanthus* and *Anaeromyxobacter dehalogenans* are awaiting publication. Yet, the number of features known from traditional and molecular biology studies that myxobacteria share with eukaryotes is so remarkable that they must have had some role in the evolution of eukaryotes.[24,30] They include general processes such as the synthesis of steroids, a phosphatidylinositol cycle, the participation of G-proteins in signal transduction, the secretion of many hydrolytic enzymes, the synthesis of antibiotics (many against eukaryotes) and melanin, and also the presence of homologues to eukaryotic proteins. Among the latter, and, in addition to the eukaryotic deltaproteobacterial-like thiolases mentioned above, there are Ser/Thr and Tyr kinases, a calmodulin-type morphogenesis protein, a homologue of the 17 β-hydroxysteroid dehydrogenase, Ras/Rab/Rho-type small GTPases, high mobility group (Y-type) proteins and reverse transcriptase (and retron elements).[30,72] Although the mechanism is not yet well understood, the fact that they are able to briefly fuse their outer membranes with other cells[60] could be seen as an advance of what was later an essential property in eukaryotes, cell–cell recognition and adhesion.[73] Most of these characteristics are related to complex, eukaryotic-like, regulation and signalling[74] that make possible their socially dependent swarming and the transition to higher-level properties such as a cooperative behaviour at a cost to individuals.[75] The analysis of the genome sequence of myxobacteria, in particular the very large genome of *M. xanthus*, may further reveal an interesting eukaryotic connection that helps to shed some light on the origin of eukaryotes.

Acknowledgments

We are grateful to Patrick Forterre and the Fondation des Treilles for their invitation to take part in the workshop on the Origin of the Eukaryotic Nucleus held in Les Treilles (Tourtour, France) in 2004, as well as to the participants in that meeting for stimulating discussions.

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