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Archaebacteria (Archaea) and the origin of the eukaryotic nucleus

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The eukaryotic nucleus is a unique structure. Because it lacks an obvious homologue or precursor among prokaryotes, ideas about its evolutionary origin are diverse. Current attempts to derive the nuclear membrane focus on invaginations of the plasma membrane in a prokaryote, endosymbiosis of an archaebacterium within a eubacterial host, or the origin of a genuinely new membrane system following the origin of mitochondria in an archaebacterial host. Recent reports point to ways in which different ideas regarding the origin of the nucleus might someday be discriminated.

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Current Opinion in Microbiology 2005, **8**:630–637

This review comes from a themed issue on
Growth and development
Edited by John N Reeve and Ruth Schmitz

Available online 20th October 2005

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2005.10.004

Introduction

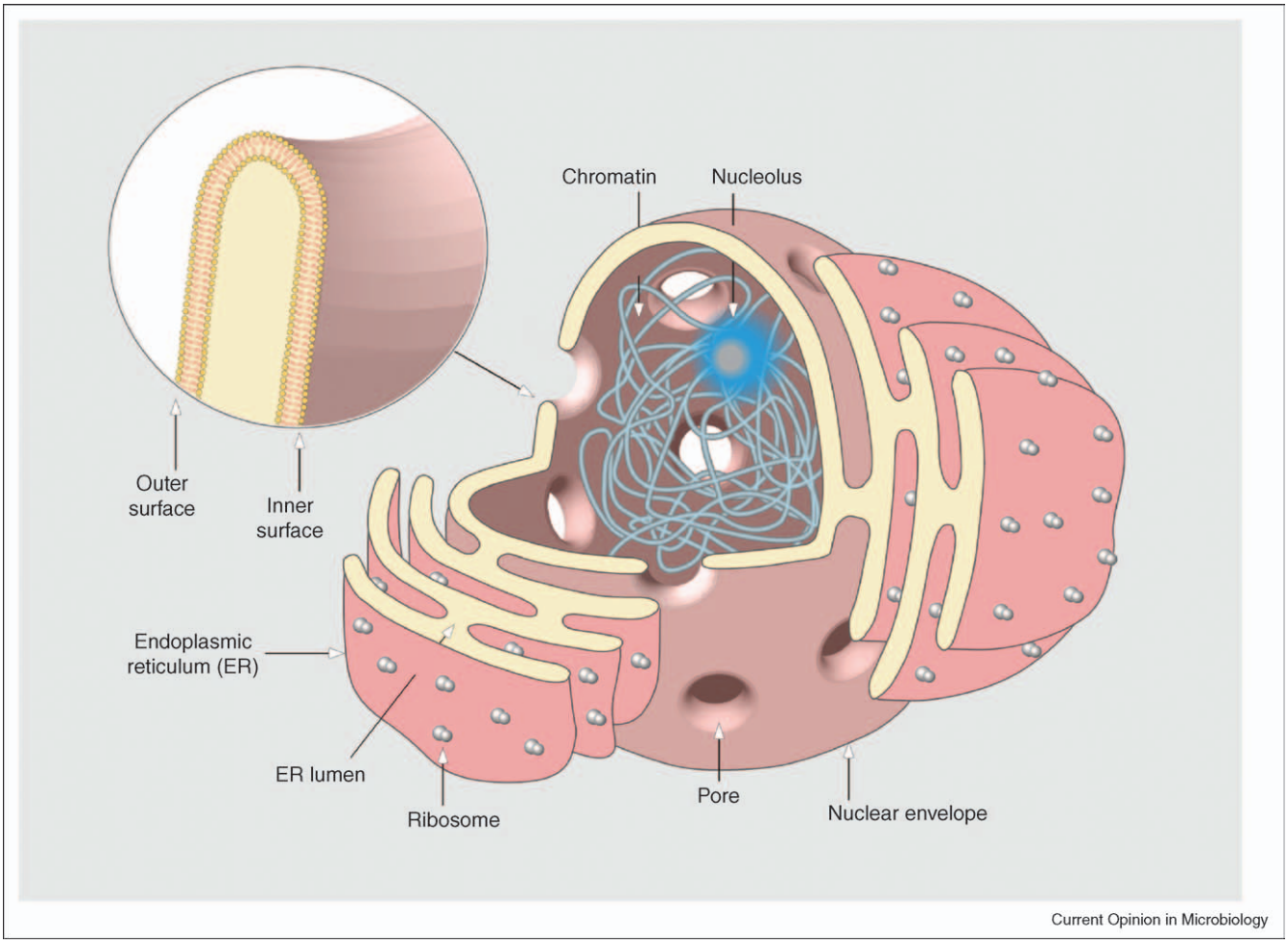
Eukaryotes possess a nucleus; prokaryotes do not. The eukaryotic nucleus contains the chromatin and the nucleolus, the latter of which is also not present in prokaryotes. The membrane topology of the nuclear envelope and its membrane topological relationship to the endoplasmic reticulum (ER) are sketched in Figure 1. The inside of the nuclear compartment, the nucleoplasm, is separated from the cytosol by the nuclear envelope. The nuclear envelope is a single contiguous membrane that has an outer face and an inner face; these meet at the nuclear pore complexes, where the membrane goes around the corner, connecting the inner and outer surfaces [1]. The nuclear membrane is contiguous with the ER [2], rendering the ER lumen contiguous with the space between the inner and outer faces of the nuclear envelope. Although the nuclear envelope is often designated as a double membrane in the literature, according to topological details it is actually a single lipid bilayer. This distinction is important. Chloroplasts and mitochondria are each surrounded by a double membrane (two lipid bilayers); the nucleus is surrounded by one.

There is currently no consensus regarding the evolutionary origin of the nucleus. Although there are rare exceptions (that will not be discussed here), biologists have mostly derived the more complex organizational state of eukaryotic cells from the simpler organizational state of prokaryotic cells [3]. However, there are no cytological structures in prokaryotes that are obvious homologues or precursors to the eukaryotic nucleus. Accordingly, comparative cytology has generated no glaringly obvious route to guide inferences regarding the evolutionary origin of the nucleus. Hence, ideas about the origin of the nucleus — both of the nucleoplasm itself and of the surrounding membrane — go in many different directions.

The archaebacteria figure centrally in the origin of the nucleus because it is the main (and sometimes the sole) information storage and retrieval center of eukaryotic cells. In this review, the term archaebacteria is used instead of the synonymous term Archaea. The reasons for this are that use of the term Archaea implies that an author accepts as correct both the relatedness of prokaryotes to eukaryotes as depicted in the rRNA tree that was used to rename the group [4] and the view that prokaryotes and eukaryotes are of equal rank. The relationship of eukaryotes to archaebacteria and eubacteria is still unresolved [5••,6••,7•,8], as is the issue concerning the origin of the nucleus. Hence, until the general outlines of early cell evolution are resolved to the satisfaction of all, one can write more comfortably about early evolution using the term archaebacteria, which designates exactly the same organisms as the term Archaea does. The term archaebacteria does not imply that a particular view of the relationship between eukaryotes and prokaryotes [4] is demonstrably correct (see also references [5••,6••]). It is now well-known that the molecular machinery involved in information storage and retrieval in eukaryotes shares much more similarity in terms of overall design and sequence conservation to archaebacterial counterparts than to eubacterial counterparts [9–12]. However, whereas the informational genes of eukaryotes (those involved in information processing and expression) reflect an archaebacterial ancestry, the operational genes of eukaryotes (those involved in metabolic and biosynthetic pathways) reflect a eubacterial ancestry [5••,6••,7•,8]. Eukaryotic genomes are thus a chimaera of sorts, and most current ideas on the origin of the nuclear membrane that surrounds them take that into account.

In this review, ideas for the origin of the nucleus are summarized, their strengths and weaknesses are contrasted.

Figure 1



Schematic drawing of the nuclear membrane and its connection to the ER. The proteins that constitute the pore complexes are omitted to stress the membrane configuration at the nuclear pores, the size of which are grossly exaggerated for clarity. The inset at the upper left emphasizes the continuity of the membrane across the inner and outer surfaces of the nuclear envelope. Salient structures are labelled with arrows.

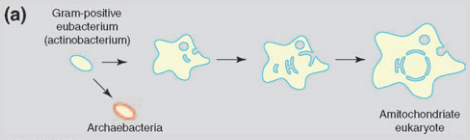
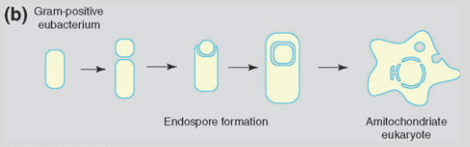
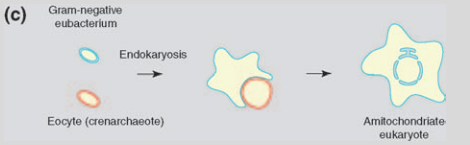
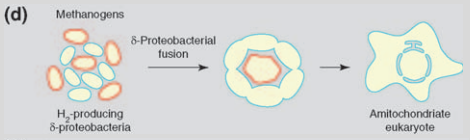
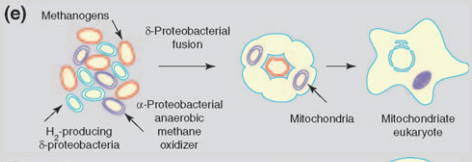
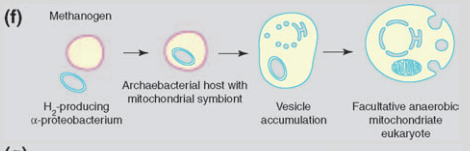
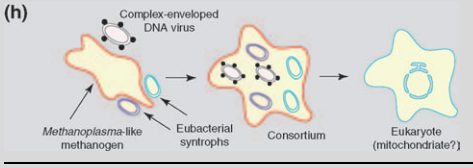
Models for the origin of the nucleus

The most widespread and familiar model for the origin of the nucleus (and the one most often found in textbooks) is the concept that the endomembrane system of eukaryotes, to which the nucleus and ER belong, is derived from invagination of the plasma membrane of a prokaryote (Table 1a). In its most explicit formulations [13–16], the invagination model postulates that a prokaryote lost its cell wall and evolved phagocytosis. Ribosomes that were initially attached to the plasma membrane then became internalized but stayed attached to a membrane, giving rise to a primitive endomembrane system, the rough ER, and finally the nuclear envelope. In phylogenetic terms, this model is more or less congruent with the standard rRNA tree rooted on the eubacterial branch [4], in which eukaryotes appear to be sisters to archaebacteria, and eubacterium is the ancestor of both. However, current formulations of the invagination model differ from the standard rRNA tree [4] as eukaryotes and archaebacteria are both seen as descending specifically from within the actinobacteria, a group of high-GC Gram-positive eubacteria [14,15]. An alternative model that also implicates Gram-positive eubacteria in the origin of eukaryotes suggests that a modification of endospore formation [17] could have given rise to the eukaryote endomembrane system and to the nucleus (Table 1b).

Among the founding fathers of archaebacterial research, Wolfram Zillig interpreted the complicated patterns of characters shared among archaebacteria, eubacteria and eukaryotes as evidence for chimaerism at the root of the eukaryotic lineage. His rationale focussed on gene comparisons rather than on membrane topology. It was envisioned that early on the eubacterial and archaebacterial partners merged, followed by invention of the traits that are specific to the eukaryotic domain, such as the nucleus [18,19]. Although Zillig was not specifically concerned with nuclear origins, the general chimaeric principle that

Table 1

Schematic summary of various models for the origin of the nucleus mentioned in the review.

Schematic model	Membrane that nuclear membrane is derived from and is homologous to	Compartment that the nuclear compartment is derived from and is homologous to	Reference
	Plasma membrane of a eubacterium	Eubacterial cytoplasm	[13–16]
	Plasma membrane of a eubacterium	Eubacterial endospore	[17]
	Plasma membranes of a eubacterium and an archaebacterium	Archaebacterial cytoplasm	[21–23,24•]
	Plasma membranes of several eubacteria	Archaebacterial cytoplasm	[25]
	Plasma membranes of several eubacteria	Archaebacterial cytoplasm	[26]
	Vesicles of eubacterial lipids synthesized in an archaebacterial cytoplasm	Archaebacterial cytoplasm around the chromosome	[29–31]
	Plasma membranes of a eubacterium and an archaebacterium	Spirochaete cytoplasm	[33]
	Viral coat	Viral lumen	[35,36]

he suggested to account for shared characters was well-embraced by others. After Margulis [20] had re-established endosymbiosis as a popular explanatory principle in cell evolution, and at a time at which the unique status of the archaebacteria was beyond doubt, several endokaryotic models for the origin of the nucleus emerged (Table 1c). In all of these models, a eubacterium is seen as the host for an archaebacterial endosymbiont, which becomes transformed into the nucleus [21–23,24•]. Mechanistically, these models lean upon the endosymbiotic origins of chloroplasts and mitochondria, but differ in the details, especially with regard to the resulting membrane topology. Endokaryotic models, similar to all models that involve an archaebacterial and eubacterial partner at eukaryote origins, have little difficulty accounting for the genes that eukaryotes specifically share with both eubacteria and archaebacteria, because eukaryotes are seen as the product of dual inheritance from two prokaryotic lineages [18] rather than being the product of direct filiation from only one prokaryote lineage.

In a variant of endokaryotic models, the principle of hydrogen transfer (anaerobic syntrophy) was suggested to be a selective force that might have given rise to the

nucleus [25]. In this model, the fusion of plasma membranes is invoked among a consortium of eubacteria (δ-proteobacteria), entrapping a methanogenic archaebacterium in the process (Table 1d). A modification thereof suggested that the ancestor of mitochondria might have been an α-proteobacterial anaerobic methane oxidizer; this accounts for the presence of mitochondria early in eukaryotic evolution [26] (Table 1e). A problem with this suggestion is that anaerobic methane oxidizers now appear to be methanogens rather than α-proteobacteria in the phylogenetic sense [27,28].

The vesicle model for the origin of the nucleus begins with a syntrophic symbiosis of prokaryotes. This gives rise to the common α-proteobacterial ancestor of mitochondria and hydrogenosomes (H_2-producing anaerobic forms of mitochondria) in an archaebacterial host that lack a nucleus [29]. This is followed by the transfer (and expression) of genes for eubacterial lipid synthesis to the archaebacterial chromosomes in the cytosol to produce an initially simple system of cytosolic vesicles consisting of eubacterial lipids, which later becomes more complex. From this, a primitive endomembrane system, the ER and then the nucleus arose [30,31] (Table 1f). A problem with the vesicle model is that no archaebacterially related cells (apart from eukaryotes) are known to harbour eubacterial endosymbionts. However, eubacterial endosymbionts that live within other eubacteria have been reported [32], which indicates that phagocytosis is not an absolute prerequisite for the establishment of intracellular endosymbionts, disarming staunch arguments to the contrary [15].

A different variant of symbiosis is shown in Table 1g, which accounts for the origin of the nucleus. In this model, symbiosis between a spirochate and a wall-less *Thermoplasma*-like archaebacterium is suggested to have brought forth the nucleus [33]. A problem with this model is that there is no evidence in the *Thermoplasma* genome that this archaebacterial lineage is specifically related to eukaryotes [6••,7•,24•], and the lineages that Margulis [33] thought were primitively amitochondriate have mitochondria after all [34]. A viral origin of the nucleus has been suggested that involves poxviruses [35], and a variant of that suggestion involves the context of syntrophic consortia involving methanogens [36] (Table 1h). However, it is not obvious why the main gene expression machinery (the chromosomes) would become concentrated within the viral compartment rather than remaining in the prokaryotic cytosol.

More recently, the complex endomembrane systems of planctomycetes [37] have come into play as a possible role model for the origin of the nucleus. The intracellular membrane topology found in some planctomycetes is striking indeed. The most curious membrane structure, which Lindsay *et al.* [37] call the 'nuclear body envelope' in the case of *Gemmata*, is interpreted as consisting of two complete and independent membranes (rather than one as in the eukaryotic nucleus) and containing the DNA of this prokaryote [37]. The outer membrane of the nuclear body envelope of *Gemmata* is contiguous with the intracytoplasmatic membrane (Figure 7b of [37]). However, electron micrographs (Figure 7a and 7c of [37]) leave some doubt as to whether the inner and outer membranes of the nuclear body envelope are indeed independent membranes, or whether they constitute a single invagination that is contiguous with the intracytoplasmatic membrane as in other planctomyctetes such as *Isosphaera* [37]. The planctomycete intracytoplasmatic membrane lies within the plasma membrane and surrounds the cytoplasm.

Lindsay *et al.* [37] are extremely cautious in their evolutionary interpretation of the planctomycete membranes, and do not explicitly suggest a possible homology between the planctomycete endomembranes and the eukaryotic nuclear–ER system. They do, however, note that the example of the planctomycetes shows that the evolution of an endomembrane system superficially resembling that of the eukaryotic nucleus in some respects need not require the workings of an endosymbiotic partner.

In more speculative interpretations [38], the planctomycete structures were designated as 'nuclei' and their membrane as a 'proper nuclear envelope', but this is extremely problematic for four reasons. First, Figure 1 depicts a topological condition that is not fulfilled in planctomycetes. Second, if the term nucleus were applied to some planctomycetes, the presence of a nucleus would no longer be a defining characteristic of eukaryotes. Curiously, however, the presence of mitochondria, once thought to be universal among eukaryotes [39] and later thought to be lacking in some [13], is now thought to be universal among eukaryotes once more [34,40] and is considered to be a defining character of the eukaryotic lineage. Third, many of the planctomycetes that have such unusual membrane configurations perform anaerobic ammonium oxidation (anammox) [37]; their extra membranes might be a specialized physiological adaptation related to anammox physiology (like the thylakoids are specialized for photosynthesis). In line with that view, Damste *et al.* [41] found that anammox planctomycetes contain novel polycyclobutane fatty acid derivatives known as ladderanes, which make these extra membranes particularly dense barriers to diffusion. This property allows them to retain the bioenergetically crucial (and extraordinarily toxic) intermediates hydoxylamine and hydrazine, which are formed during the anammox reaction [42]. Fourth, as pointed out above, although *Gemmata* is sometimes represented as possessing four independent and concentric membranes ([38] and Figure 10 of [37]), electron micrographs of the same organism reveal only two membranes surrounding the cytoplasm, the inner of

which can be invaginated, as in other planctomycetes [37].

The rationale behind having a nucleus

The possible physiological significance of planctomycete membrane configurations raises the question of what evolutionary pressures or selective advantages might have been involved in the origin of the eukaryotic nucleus in the first place. Why would any prokaryote relinquish the obvious regulatory and rapid response advantages associated with coupled transcription and translation? Furthermore, the planctomycetes suggest that prokaryotes have the evolutionary wherewithal to surround their DNA by membranes. Hence, were there a simple evolutionary rationale behind the origin of the nucleus that could be explained by one or a few obvious selective pressures, numerous prokaryotic lineages should have independently evolved nuclei for exactly the same reason(s). The nucleus is complicated and arose only once in evolution, so the underlying reasons cannot be simple and most models take this into account.

Endokaryotic models, which have been criticized [15,30] and defended [43] elsewhere, offer no real solution to the problem of nuclear rationale. They view the origin of the nucleus in the wake of a rare symbiotic event, whereby the nucleus is seen as a frozen accident of sorts, maintained by a rarely specified selective advantage [21–26,44]. By contrast, Cavalier-Smith [15] suggests that the prime selective advantage associated with nuclear origins — also in the wake of a rare event: the origin of phagotrophy — involved physical protection against shearing of chromosomes, the length of which was suggested to have increased [15] during the prokaryote-to-eukaryote organisational transition. This is a reasonable idea and is consistent with the degree of mitotic chromatin packaging that is generally higher in eukaryotes than in prokaryotes, and is required for proper cytosolic sorting of chromosomes during the complex division of their substantially larger cells [15]. The vesicle model [30,31] does not specify any selective advantages for the persistence of either ER or subsequent nucleus and thus falls into the frozen accident category. But as Cavalier-Smith [15] has pointed out, the vesicle model and the invagination model both derive the nucleus from the ER (as it occurs in the cell cycle), such that both would accommodate similar sorts of selective pressures. However, the organizational states of cells in which the nucleus is derived differ fundamentally in the vesicle and invagination models (Table 1). Further differences are that in the vesicle model the ER membrane system is derived *de novo*, and that it strictly requires a mitochondrial symbiont to operate [30,31] (Table 1e). In the invagination model, the ER is derived from the plasma membrane and has been recently adapted to accommodate the presence of a mitochondrial symbiont very early in eukaryote evolution (but after the origin of the nucleus) [15]; however, it does not strictly require one to operate, as older [13,14] and later [16] formulations attest, therefore its depiction in Table 1a is accurate with regard to the amitochondriate state.

What do genome data say about these ideas?

Data that directly address nuclear origins are scarce to date. Staub *et al.* [45••] investigated the evolutionary affinities of the nucleolar proteome. They found that the nucleolus contains some proteins that have archaebacterial affinities and other proteins that have eubacterial affinities, from which they concluded that the nucleolus (a nucleus-specific feature) arose at a time after the eukaryotic lineage had already come to possess eubacterial genes, the donor of which they suggest to have been the mitochondrial symbiont that entered into an archaebacterial host [45••]. Mans *et al.* [46••] investigated the evolutionary affinities of proteins from the nuclear envelope and from the nuclear pore complex. They also found components with both eubacterial and archaebacterial affinities. They interpreted this as evidence that the nucleus arose subsequent to the symbiosis that gave rise to the mitochondrion, involving what they also suggest to have been an archaebacterial (rather than a eukaryotic) host. Both reports concur with the vesicle model (Table 1f) in terms of the kinds of cells involved and the basic order of events at nuclear origins (mitochondria first).

The series of gene duplications documented by Mans *et al.* [46••] that involves proteins common to the nuclear envelope and the ER suggests that the ER arose before the nuclear membrane. This would support both the vesicle model (Table 1f) and the invagination model (Table 1a). The phylogenetic distribution among prokaryotes of the genes that encode evolutionary precursors to the nuclear envelope and the nuclear pore complex proteins — considered as the genetic starting material to evolve the eukaryotic-specific structures — showed no dramatic prevalence among any particular prokaryotic group, barring their notable paucity among the planctomycetes, which are poorly sampled to date [46••].

Some genome-wide studies tend to implicate members of the euryarchaeotes in eukaryote (hence nuclear) origins [7•,24•], whereas other studies tend to implicate members of the crenarchaeotes or eocytes in eukaryote origins [6••]. Histones are manifest in their more or less fully-fledged eukaryotic forms among the methanogens [12,25,26], but have also been found recently among the crenarchaeotes [47••]. The selenocysteine-codon decoding mechanism and the elongation-factor modifying protein deoxyhypusine synthase link eukaryotes to methanogens [48], but individual genes retrace history in a piecemeal manner [7•] and whole-genome phylogenies that take lateral gene transfer into account mathematically are just beginning to be developed [6••].

Additional problems

The origin of nuclear pore complexes is required before the origin of a bona fide nuclear envelope; if this did not occur, the main chromosomes would be physically isolated from the cytosol and therefore useless to the cell [15,46••]. This poses a general problem for the endokaryotic models as they all start off with a fully fledged archaebacterial endosymbiont as the nuclear progenitor, and a functional contribution to the eukaryotic cytosol would depend upon the evolution of pore complexes as the first step for intracellular protein communication. In models in which the nucleus is derived from the ER [13–16,30,31], the evolution of the pore complexes could come late in nuclear origins; this is consistent with the patterns of gene duplications reported by Mans *et al.* [45••].

The vesicle model [30] has been severely criticized as it involves the *de novo* origin of a new membrane system, which was argued to be fundamentally impossible [15]. However, the example of the planctomycetes as well as that of *Ignicoccus* — an archaebacterium in which the cytosol is surrounded by two complete and distinct membranes [49] — indicate that new membrane systems apparently can arise *de novo* in evolution, at least in prokaryotes.

A problem that figured prominently in Zillig's consideration [18,19] was the lipid discrepancy that separates archaebacteria from eubacteria and eukaryotes (isoprene ethers versus fatty acid esters and their glycerol configuration). In the invagination model [15], and implicitly in all models that accept the basic topology of the bifurcating eubacterially rooted rRNA tree [4], this is dealt with by assuming that the common ancestor of eukaryotes and archaebacteria was a eubacterium (e.g. an actinomycete) that lost its cell wall. One descendant lineage thereof evolved phagotrophy to become the first eukaryote; another descendant lineage evolved an isoprene ether plasma membrane and the distinctive murein-lacking archaebacterial cell wall to become the first archaebacterium. The problem here, in this author's view, is that no prokaryotic lineages that represent intermediate stages of either assumed transition (eubacterium-to-eukaryote or eubacterium-to-archaebacterium) have been preserved in modern biota, even though these transitions involve hundreds of genes in total (not just lipids), requiring evolutionary time, prokaryotic progeny, and descendant intermediate lineages. As an illustrative counterexample, intermediate stages in the evolution of oxygenic photosynthesis that only involve either reaction center I or reaction center II are common and can be observed among many contemporary prokaryotes [50••]. Even if two major eubacterial transitions (to archaebacteria or to eukaryotes) went quickly in terms of evolutionary time [15,16], there should be some descendant intermediate lineages around today, however, there aren't. Therefore, a severe corollary assumption of mass lineage extinctions among prokaryotes is required to account for their absence.

Symbiotic models are saltatory in that respect; they do not entail a gradual transition from prokaryotes to eukaryotes, they start with two prokaryotes at eukaryote origins rather than one. Either there is an endosymbiont or there isn't, and only those descendants that solved the cell division problem in this radically chimaeric state (eukaryotes) survived. The lipid issue is dealt with by assuming that the eubacterial membrane synthesis pathway replaced the membrane synthesis pathway of the archaebacterial partner. The archaebacterial isoprene synthesis pathway itself (the pathway of archaebacterial lipid synthesis) was not replaced in eukaryotes — it was retained, but for synthesis of other compounds, such as sterols, quinone tails and dolichol phosphate. This type of combinatorial evolution — mediated by symbiosis and the differential loss of functional redundancy — seemed plausible to Zillig [18] and is integral to many views of cell evolution today [6••,7•,23,24•,45••,46••]. The lipid replacement aspect of models that involve an archaebacterial host [29–31] has been harshly criticized on the grounds that lipid replacement in eukaryotes is fundamentally implausible [15]. However, regardless of whether lipid replacement occurred in ancestral eukaryotes [30,31] or in ancestral archaebacteria [15], lipid replacement has occurred in evolution; the question is therefore not 'if' it occurred, but 'where'.

Conclusions

A fundamental problem that is common to all ideas regarding the origin of the nucleus is that the underlying mechanism has to be plausible enough to have actually occurred, but at the same time so unlikely that it has only occurred once in four billion years, given the adamantine monophyly of eukaryotes. This problem is severe and it applies to all models, hence does not discriminate between them. It is the main reason that they are all coupled to a rare event in evolution, for example: the origin of phagotrophy [13–16], a karyogenic symbiosis that occurred only in the eukaryotic lineage [21–26], or the origin of mitochondria [29–31]. Future study of the evolutionary histories of proteins that are specific to the nucleus [45••,46••] should lead to progress on this issue, which though tough is not, in principle, intractable.

Acknowledgements

This paper is dedicated to the memory of Wolfram Zillig. Work in the author's laboratory is supported by the Deutsche Forschungsgemeinschaft.

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