Think again

Planctomycetes and eukaryotes: A case of analogy not homology

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Planctomycetes, Verrucomicrobia and Chlamydia are prokaryotic phyla, sometimes grouped together as the PVC superphylum of eubacteria. Some PVC species possess interesting attributes, in particular, internal membranes that superficially resemble eukaryotic endomembranes. Some biologists now claim that PVC bacteria are nucleus-bearing prokaryotes and are considered evolutionary intermediates in the transition from prokaryote to eukaryote. PVC prokaryotes do not possess a nucleus and are not intermediates in the prokaryote-to-eukaryote transition. Here we summarise the evidence that shows why all of the PVC traits that are currently cited as evidence for aspiring eukaryoticity are either analogous (the result of convergent evolution), not homologous, to eukaryotic traits; or else they are the result of horizontal gene transfers.

Keywords:
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Abbreviations:
BLAST, basic local alignment search tool; **ER,** endoplasmic reticulum; **HGT,** horizontal gene transfer; **ICM,** intracytoplasmatic membrane; **MC,** membrane coat; **NE,** nuclear envelope; **PVC,** Planctomycetes, Verrucomicrobia and Chlamydia.

Supporting information online

Introduction

In the days before sequenced genomes, speculation on the origin of eukaryotes was seen as '*a relatively harmless habit, like eating peanuts, unless it assumes the form of an obsession; then it becomes a vice*' [1]. Today there is an abundance of genomic and cell biological data that speak to the origin of eukaryotes. If there is any vice left in the topic, it is speculation that does not take the available genomic data into account. Seen from the standpoint of genomes, eukaryotes are chimaeras with genetic attributes inherited both from archaebacteria and from eubacteria [2–6]. A number of studies have explored this issue from a variety of different perspectives and the massive amount of accumulated data consistently points in this direction. This chimaerism is overtly manifest at the level of protein synthesis, as eukaryotes have archaebacterial ribosomes operating in their cytosol and eubacterial ribosomes operating in their mitochondria — and in their plastids, if present — [7, 8] and those eukaryotes that lack mitochondrial ribosomes are secondarily reduced [9–13].

Comparative genomics and phylogenetic tree construction have uncovered large eubacterial and archaebacterial components of eukaryotic chromosomes [2–6, 14–18]. In modern endosymbiotic theory, these genetic components correspond, in phylogenetic terms, to the mitochondrion (proteobacterium) and the host (archaebacterium) of the mitochondrial symbiosis in nonphotosynthetic eukaryotes. In plants they correspond to the chloroplast endosymbiosis (cyano-

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bacterium) [14, 19, 20]. This chimaeric ancestry of eukaryotic cells involving archaebacterial and eubacterial partners is manifest not only in protein sequence conservation, but also in functional categories corresponding to informational and operational genes [2] and in gene expression patterns, protein interactions, and gene essentiality [4]. The intrinsic chimaerism of eukaryotic cells underscores the pivotal role of mitochondria in eukaryote evolution, and is readily explained through bioenergetics: the five orders of magnitude increased power per gene that mitochondria afforded their host was required for the evolution of bona fide cell complexity of the kind that eukaryotes display [21, 22].

Eukaryotes did not, however, inherit all of their attributes directly from their prokaryotic ancestors in ready-made form, because eukaryotes boast many lineage-specific modifications that have no fully fledged homologues in prokaryotes [23], such as the endoplasmic reticulum (ER) and its contiguous nuclear membrane, the Golgi complex, incoming and outgoing vesicles, and digestive vacuoles. Other eukaryotic 'novel entities' include the fully developed eukaryotic cytoskeleton, mitosis, eukaryotic flagella, basal bodies, the cell cycle, and meiosis. Various prokaryotes have made small steps towards complexity [21] and the genetic starting material for some eukaryotic traits such as cytoskeletal components [24], the cell division machinery [25, 26] or the ubiquitin signalling system [27, 28] can be identified in prokaryote genomes. Nevertheless, it remains true that no prokaryote offers anything vaguely similar to the burgeoning complexity of a eukaryotic cell.

Or does it? This brings us to the issue. Several recent high-profile papers, one in *Science* [29], two in *PNAS* [30, 31] and one in *PloS Biology* [32], have brushed aside all genome-based data (and much tradition in evolutionary reasoning) to argue that the Planctomycetes, Verrucomicrobia and Chlamydia (PVC), conscripted together on the basis of gene sequence analysis into the PVC 'superphylum' [33–36], are genuine intermediates in the prokaryote-to-eukaryote transition, that they possess a nucleus and endocytosis, and that many of their unusual traits are homologous to their apparent eukaryotic counterparts [29–32]. On the bottom line, those four papers are saying that the long sought missing links in the prokaryote to eukaryote transition [37] have finally been discovered. Those are exceptional claims by any measure. Exceptional claims demand exceptional evidence, as the recent report of bacteria with arsenate-based nucleic acids attests [38–47]. For those who have been comparing genome sequence data for years in order to illuminate the prokaryotic roots of eukaryotes, the claims that PVC members are the evolutionary forerunners of eukaryotes comes as quite a surprise. Here we inspect the evidence underlying such claims.

Homology and analogy

Proponents of the view that the PVC clade is the missing link in the evolutionary sequence linking prokaryotes and eukaryotes argue for homology of PVC characters with eukaryotic characters. However, the phylogenetic perspectives of those that propose a special role for PVC members in discussion of eukaryotic origins could not be more different. In one view [29], the claim is that 'complex' planctomycetes evolved from simpler prokaryote progenitors, and represent a preserved intermediate stage in the prokaryote-to-eukaryote transition. The other view [30] is that the eukaryotic type of complex cell organisation is ancestral to all life forms and that eubacteria and archaebacteria have undergone simplification. This scenario was originally proposed in the context of the introns early hypothesis 30 years ago [48], long abandoned by its proponents on the strength of multiple lines of evidence against introns early, and later rekindled as thermoreduction [49], as discussed in ref. [18]. Under this scenario, the PVC bacteria are considered to be 'less streamlined' than other eubacteria and archaeabacteria. Thus, despite the polarity of these views, they both maintain that some traits of the PVC bacteria specifically link them to eukaryotes in an evolutionary chain, hence the characters are interpreted as *homologous*.

In the context of genes and proteins, the term homologous means similar in sequence or structure by virtue of common ancestry. In contrast to homology, analogy, sometimes termed superficial, or misleading similarity, is 'the resemblance of structures which depends upon similarity of function' (glossary, page 464, Origin of Species, Penguin Classics 1985. Darwin). Homology in molecular sequences can be examined using a database similarity measure such as that implemented in the basic local alignment search tool (BLAST) suite of programs [50]. Our default position is that, if the level of similarity between two sequences is significantly higher than the chance expectation, then this is most likely to be a consequence of shared ancestry. It is often argued that significant sequence similarity can be caused by functional convergence. However, the actual evidence for convergence in molecular sequences is scarce, and documented cases involve only a very small number of amino acid residues [51]. Moreover, the fact that biochemical functions are performed in different organisms by proteins with dissimilar sequences and even structures [52] is a strong argument against convergence and buttresses significant sequence similarity as evidence of common ancestry. Structural similarity between proteins presents more complicated issues.

Nevertheless, the same argument applies to cases where compared domains are substantially similar, with preserved connectivity of secondary structure elements. Conversely, it is essential to rule out confounding influences such as similar amino acid or nucleotide composition or the high levels of sequence similarity that are often observed between repetitive sequences [50].

Likewise, limited structural similarity should not be over-interpreted. Simplifying, one cannot assume that all alpha-helices or all beta-sheets share common ancestry because the number of fundamental folding patterns of polypeptides is very limited, so at this level, convergence and hence analogy, may be widespread.

In genomic analyses, careful assessment of homology is critical but equally, the route of inheritance is also important. Homologs are often used in botany and zoology as evidence of common ancestry of species. Indeed, in plants and animals, genes/traits are (almost

always) passed from parent to offspring in a vertical fashion, so the presence of vertebrae, for instance, is used as a synapomorphy, or shared-derived character for all vertebrates. In prokaryotes the story is not so simple. Horizontal gene transfer (HGT) is frequent [53] and the presence of a particular trait in two organisms does not guarantee that these organisms are each other's closest relatives. A variety of mechanisms are known to facilitate the horizontal acquisition of genes [54] and assessment of homology for the purposes of inferring organismal relationships is useful only in the context of knowing how the homologs have been inherited. HGT is usually identified either by examination of the distribution of homologs among a large collection of organisms where a sparse, patchy distribution might indicate HGT, or by identification of unusual placement of certain homologs in phylogenetic trees, or most convincingly, by a combination of both approaches. One consequence of HGT is that, depending on the characters that are being analysed, different relationships might be inferred, a reality that has confounded microbiologists in search of a natural systematics for prokaryotes for more than a century [55].

An alternative explanation for a sparse, patchy distribution of characters might be that there is differential loss of genes in different lineages. There is no doubt that such events are common but their inference requires careful phylogenetic analysis. If, for instance, two very different kinds of organisms have a single similar trait, one probably has to invoke multiple independent trait losses or homoplastic convergent evolution or HGT as the explanation. Which of these three is the best explanation for the observed pattern must be decided on a case-by-case basis.

In any analysis of the relationship between eukaryotes and the PVC group of eubacteria one must examine whether the traits that appear similar are in fact real homologs and if they are homologs, whether they have been inherited vertically or horizontally. In general, if we wish to make statements concerning the origin of eukaryotes, then the study of characters or genes is likely to be useful only if they are indeed homologous – similar by virtue of common descent.

A list of issues with the purported PVC-eukaryote connection

Devos and Reynaud [29] made a list of the traits which, in their opinion, link PVC members to eukaryotes. These traits are recapitulated in Table 1. The first of these is called a 'compartmentalised cell plan,' which they suggest to be homologous to that in eukaryotes. The term 'compartmentalised cell plan' would appear to designate configurations of the innermost PVC membrane, called the intracytoplasmatic membrane (or ICM) [32, 34–36], as seen in the electron micrographs of the planctomycete *Gemmata obscuriglobus* shown in Figure 7 of ref. [56] or in Figure 3 of ref. [32]. In a few images this might look, to some, like a nucleus [30, 57]; but it can hardly be a *nucleus* because that would make *Gemmata* a eukaryote, which nobody is claiming. Or are they? For example, in Figure 2C of Lonhienne et al. [31], a membrane is indicated that they label and define as the 'nuclear envelope' (NE) while Forterre and Gribaldo [30] stress the evolutionary significance of the 'double membrane of the *G. obscuriglobus* nucleus'. For most biologists, the terms (i) 'nuclear envelope' and (ii) 'nucleus' have very specific meanings: they designate i) the folded single membrane, contiguous with the ER and bearing nuclear pore complexes that surrounds the active chromatin of eukaryotic cells and thereby separates their ii) true nucleus (eukaryon) from the cytosol. By contrast, the superficially similar planctomycete structures are invaginations of the innermost of the two membranes surrounding the cytoplasm. The use of the terms 'nuclear envelope' or 'nucleus' to describe the planctomycetes 'compartmentalised cell plan' is therefore specious.

Claims that some of the PVC bacteria possess a nucleus raise the question of where their ER resides, the membrane from which the true nucleus is formed.

Neither PVC members nor any other bacteria are (currently) claimed to possess an ER. Also, detectable counterparts to nuclear pore complexes, the elaborate structures that permeate the NE in eukaryotes and mediate nucleocytoplasmic trafficking, are lacking in PVC bacteria. Most importantly, comparative genomic analysis has shown that PVC bacteria encode no homologs of the numerous nuclear pore complex proteins that are conserved among all eukaryotes [58–61]. Thus, all available evidence indicates that despite a superficial cytological similarity between the membrane configurations in the PVC bacteria and eukaryotes, the structures are not homologous.

A similar situation exists with respect to the mechanism of protein uptake recently described in PVC and declared to be homologous to eukaryotic endocytosis [31, 62]. The claim of homology was based solely on misinterpreted results of structural analysis of the eukaryotic clathrin-like membrane coat (MC) proteins [32]. Devos and colleagues were unable to find bacterial homologs of MC proteins using any of the traditional methods of sequence analysis. However, instead of concluding that MC proteins are specific for eukaryotes, they performed a sensitive search for any bacterial proteins that would show even a borderline structural similarity to MC proteins. This search identified a set of multidomain proteins that are found primarily in PVC members but also in several representatives of Bacteroidetes, often in multiple copies per genome, and annotated either as 'membrane-bound dehydrogenase' (after their N-terminal domains) or as 'heme-binding protein' (after their C-terminal domains). Both the N-terminal and C-terminal domains of these bacterial proteins are specific for members of the PVC superphylum and Bacteroidetes. In contrast, the middle domains of these bacterial proteins contain several HEAT repeats, short (~40 aa) α-helical repeats, first identified in 1995 by Andrade and Bork and named after four proteins where these repeats were first detected: huntingtin, elongation factor 3, regulatory A subunit of protein phosphatase 2A, and target of rapamycin protein TOR1 [63].

Although an α-helical superstructure made of multiple HEAT repeats appears to be superficially similar to an α-helical structure made of multiple

Table 1. The status of purportedly eukaryotic features of the PVC bacteria

Features	Found in PVC members	Found in other prokaryotes	Comments
Intracellular compartmentalization	Planctomycetes, Verrucomicrobia	Cyanobacteria, photosynthetic Proteobacteria, spore-forming Gram-positive eubacteria	
Membrane-bounded compartment surrounding genome	Planctomycetes		No homologs of nuclear pore proteins and no endoplasmic reticulum in Planctomycetes
Condensed DNA	Planctomycetes, Chlamydia		
Histone H1	Chlamydia	Histone homologs (not H1) in Euryarchaeota	Purported H1 in Chlamydia represented in many eubacteria but unrelated to H1 (database search artifact)
Division by budding	Planctomycetes		Uncommon in both eukaryotes and prokaryotes
Membrane coats (vesicles)	Planctomycetes	Numerous eubacteria and some archaebacteria [62, 64]	Homologs of eukaryotic vesicle proteins in many eubacteria and archaebacteria (the latter closest to eukaryotes)[17]
Sterols	Planctomycetes, Chlamydia	Alpha-Proteobacteria	Probable LGT among bacteria and from alpha-Proteobacteria to eukaryotes
Absence (loss) of peptidoglycan	Planctomycetes, Chlamydia	Archaebacteria	
Proteinaceous (proteic) cell walls	Planctomycetes	Archaebacteria	
Ether lipids[a)] in biomembranes	Planctomycetes	Archaebacteria	Eukaryotes
Absence (loss of FtsZ)	Planctomycetes, Chlamydia	Many archaebacteria (Crenarchaeota, some Euryarchaeota)[17]	Probably no loss in eukaryotes, rather evolution into tubulin; typical eubacterial FtsZ in organelles
Tubulin	Verrucomirobia		Eukaryotic LGT in Verrucomicrobia
C1 transfer	Planctomycetes	Archaebacteria	Archaeo-bacterial LGT
Endocytosis	Planctomycetes		No homologs of eukaryotic endocytosis proteins in Planctomycetes

The 'eukaryotic' features of the PVC bacteria are from the table published by Devos and Reynaud [29].
[a)] However, the relative abundance, structures, and stereochemistry of ether lipids differ between PVC, archaebacteria and eukaryotes [97, 102].

CLH (Clathrin heavy chain repeat homology) repeats (as shown in Figure 2 of ref. [32]), there are substantial structural differences between them [PMID: 17897938, 18974315], and accordingly, there is no reason to assume common origin of these domains, to the exclusion of other α-helical repeats. This difference in the structure of the repeat regions combined with the differences in the domain architecture rule out common ancestry of the planctomycete membrane-bound dehydrogenase and eukaryotic clathrin-like MC proteins. Thus, the inference of a common origin of protein uptake systems of PVC and eukaryotic endocytosis stemmed from a common error of sequence analysis when distant similarity between repetitive structures was taken as evidence of homology. Unfortunately, in subsequent publications [31, 62], presence of MC homologs in the members of the PVC phylum was assumed to be proven and served as the basis for some far-reaching speculations.

Even less relevant to eukaryote origins are examples of cell division by budding [29], which are as rare in eukaryotes as they are in prokaryotes. Similarly irrelevant are proteinaceous (proteic) cell walls [29], which are present in some derived eukaryotes such as the protist *Euglena* and in virtually all archaebacteria, which possess proteinaceous S-layers [64]. By contrast, eukaryotic cell walls, when present, consist of cellulose or chitin [65].

Another important point in regard to the 'compartmentalised cell plan' of Planctomycetes, Verrucomicrobia, and eukaryotes, which Devos and Reynaud present as a shared derived character [29], is that intracellular compartmentalisation in prokaryotes is by no means unique to the PVC bacteria. Regardless of exactly how many membranes and how many compartments different PVC bacteria actually have — either two or three membranes as in Figure 1 of [66] or two membranes as in Figure 3 of [32] — it should be stressed that

internal membrane-bound compartments are also found in various proteobacteria, for example magnetosomes in *Magnetospirillum magnetotacticum* [67], acidocalcisomes in *Rhodospirillum rubrum* [68], chromatophores in *Rhodobacter sphaeroides* [69], in cyanobacteria, in the form of thylakoids [70], and in Gram positive bacteria (*Bacillus*) in the form of endospores [71]. Even within the PVC group, another membrane bound compartment exists that has been omitted from evolutionary discussion. This is the anammoxosome of anaerobic ammonium-oxidising (anammox) bacteria of the phylum *Planctomycetes* [66]. The anammoxosome is an organelle with membrane-embedded ATPases that appears to have evolved for one specific cellular function – energy metabolism [66]. Just as the mitochondrion provides energy in a eukaryote, this compartment provides energy in anammox bacteria. However, this is where the similarity ends.

Anammoxosomes are not mitochondria or an intermediate step to mitochondria, and lack an associated genome equivalent to mitochondrial DNA. Thus, the 'compartmentalised cell plan' [29, 31] hardly links eukaryotes to any particular group of bacteria; instead, one has to conclude that compartmentalisation evolved independently, and in substantially different ways, in several bacterial lineages and in eukaryotes.

Similarly, Lonhienne et al. [31] write about 'endocytosis-like' processes in Gemmata, then later about 'Endocytosis as found in planctomycetes'; is it endocytosis or is it endocytosis-like?

We would say that what Lonhienne et al. (2010) showed was not endocytosis (a vesicle-generating process of membrane traffic), but rather the uptake of protein from the medium, which is interesting, but not evidence for endocytosis. As the simplest interpretation, that uptake could involve specific or non-specific importers, functionally analogous to the ones that naturally competent prokaryotes use to uptake DNA harbouring uptake sequences [72], for example.

Other cases of apparent support for a PVC-eukaryote relationship listed by Devos and Reynaud, namely sterol biosynthesis which is also present in alphaproteobacteria [73], C1 transfer enzymes that link the PVC bacteria to methanogenic archaebacteria [74], and the bacterial tubulin gene appear to reflect horizontal gene transfers (HGT). In particular, the tubulin present in *Prosthecobacteria* (but not in other PVC bacteria) is a horizontal acquisition of a eukaryotic gene, not a missing link in the prokaryote-eukaryote transition [75, 76].

Regarding arguments for shared losses of FtsZ and peptidoglycan [29], convergent loss of genes and traits is common in evolution. Specifically, FtsZ seems to have been lost in parallel in some of the PVC bacteria and archaebacteria but not in the ancestral eukaryotic lineage in which tubulin in all likelihood evolved from FtsZ, with a dramatic acceleration of evolution caused by major functional changes [26]. In addition, and contrary to the statement of Devos and Reynaud [29], numerous eukaryotes possess FtsZ genes via acquisition from endosymbionts [77]. The case of peptidoglycan is similar: it has been lost in several lineages of bacteria, e.g. Mollicutes, in addition to some of the PVC members, but peptidoglycan is synthesised in certain eukaryotes, for example in the glaucophyte alga *Cyanophora*, also as a result of endosymbiosis [20].

The claim of histone H1 homologs in *Chlamydia* seems to stem from another common error of sequence database searches, namely, inference of homology from spurious similarities between unrelated proteins caused by similar compositional biases. Chlamydia have been claimed to encode two 'histone-like' proteins, HctA and HctB [78, 79]. An iterative database search using the PSI-BLAST program with the composition-based statistical correction [80] reveals no similarity between *Chlamydia* HctA sequences and histone H1. Instead it is possible to detect HctA homologs in the bacteria of the phylum Bacteroidetes but not in members of the PVC superphylum other than Chlamydia or any other bacteria or eukaryotes (Supplementary Material).

The N-terminal region of HctA is predicted to adopt an unknown globular fold whereas the C-terminal region consists of lysine-rich repeats (Supplementary Material) which produce spurious hits to a variety of proteins in database searches (although in our current analysis, histone H1 was not among them, probably due to the huge increase in the database size and diversity since the time the histone annotation was published). The HctB protein consists mostly of lysine-rich repeats and database searches with composition-based corrections show no homologs outside Chlamydia (Supplementary Material). An HHPred search for possible structural similarity [81] failed to show any similarity to the histone H1 fold for either HctA or HctB. Thus, as in the case of clathrin-like MC protein, the persistent mis-annotation of Chlamydial 'histone-like' proteins is the result of a sequence analysis error. It has been shown that the HctA protein of *Chlamydia trachomatis* binds DNA and apparently contributes to nucleoid condensation [82, 83], so it seems to show general functional analogy with histones. However, this is yet another case of loose functional analogy that is not based on homology.

Mitochondria, sine qua non of eukaryogenesis

In terms of complexity, some of the PVC members, as well as other bacteria that possess various intracellular compartments, have made many small steps for a bacterium, in particular with respect to intracellular membranes, cytoskeleton, and cell division, but none of these bacteria is poised for a giant leap to the eukaryotic state. The prime reason is their lack of mitochondria, which provided the cellular power underpinning the origin and expansion of eukaryote-specific gene families that shouldered the prokaryote-to-eukaryote transition [21, 23, 84]. From this perspective, prokaryotic genome sizes are limited for bioenergetic reasons [21], and PVC members are no exception, with even the largest genomes (e.g. *Gemmata obscuriglobus* at 9 Mb) falling well within the bacterial range, four orders of magnitude less than larger protists [21]. The same applies to metabolic rates per cell, which again fall comfortably within the prokaryotic range [85, 86]. Thus, the energy availability per gene in the PVC is archetypically prokaryotic, 3–5 orders

of magnitude less than that for an 'average' protist [21], and is in no sense intermediate between prokaryotes and eukaryotes. The critical point about mitochondria is that they have retained a tiny genome of their own, along with the ribosomal machinery required to express mitochondrial genes on site [7]. These genes enable rapid responses to changes in redox state, and are necessary for oxidative phosphorylation across a wide area of internal membranes: eukaryotes have 3 to 5 orders of magnitude more internal bioenergetic membrane than *Planctomycetes*, corresponding to their 3 to 5 orders of magnitude more power per gene. The mitochondrial genomes, of course, are not derived autogenously, but from the critical endosymbiotic event that gave rise to eukaryotes in the first place. Lacking these dedicated bioenergetic genomes, *Planctomycetes* cannot expand in either cell volume or genome size to remotely eukaryotic proportions [21, 22]. The origin of mitochondria was thus a crucial step in the prokaryote-to-eukaryote transition, and the symbiosis it involved increasingly appears to be the preeminent process behind eukaryogenesis, seen from comparative [10–13, 23] and bioenergetic [21] standpoints.

The PVC bacteria also lack spliceosomes, a possible key to the origin of the eukaryotic nuclear membrane that could have evolved primarily as a means of separating the nuclear compartment in which the slow process of splicing takes place from the cytosol, which is the site of the fast process of translation. By disrupting the transcription-translation coupling, which is a hallmark of gene expression in prokaryotes [87, 88], the nuclear membrane prevents translation of intron-containing transcripts that would have been fatal for eukaryotic cells; the introns most likely originated from the protomitochondrial genome [87, 89].

Following others [90, 91], Devos and Reynaud suggest a scenario for eukaryote origins that does not involve the participation of archaebacteria: the host that they propose for the origin of mitochondria is a PVC bacterium [29]. Hence they, like others propounding similar scenarios, offer no account of the obvious and extensive sequence similarity that many eukaryotic genes share with archaebacterial homologues nor of numerous, essential genes that are shared by archaebacteria and eukaryotes to the exclusion of eubacteria [2–4, 92]. This component of homology between archaebacteria and eukaryotes is naturally explained by the hypothesis that the host for the origin of mitochondria was an archaebacterium, a view that readily accounts for the most pressing observations [3–5, 10, 21, 93].

Devos and Reynaud argue for the monophyly of the PVC group based on 'various phylogenetic trees derived from molecular sequence comparison' [29], indicating that they accept molecular sequence similarity as evidence of evolutionary relationships. There are also numerous published phylogenetic and comparative genomic analyses that address the relationships of prokaryotes to eukaryotes [3–6, 15], and of the PVC bacteria to eukaryotes [58, 60, 61] in particular. None of these studies has ever uncovered a specific PVC-eukaryote link. By contrast, evolutionary links of eukaryotes to archaebacteria and proteobacteria (and algae and plants to cyanobacteria [77]) are readily and reproducibly found in genome data, with a variety of methods [3–6, 15, 93, 94].

Noting the lack of an archaebacterial connection in Devos and Reynaud's model, it has recently been suggested in a new model [95] that a PVC eubacterium was the host for the origin of the nucleus via endosymbiotic acquisition of a mesophilic crenarchaeote (crenarchaeotes are one of the two main divisions within the archaebacteria). The sequence similarities between eukaryotes and archaebacteria are thus accounted for. Like other endosymbiotic models that assume that the nucleus is a modified prokaryote intruder into a eubacterium [59, 96, 97], the new model predicts the existence of primitively amitochondriate eukaryotes (never yet found despite a prolonged search), thus requiring a second endosymbiosis with an alphaproteobacterium. This scenario is more complicated than an existing scheme that adequately accounts for the acquisition of mitochondria, namely via an archaebacterial host [3–6, 15, 59]; in terms of parsimony – according to Occam's razor – the archaebacterial host theory should, therefore, be favoured. Like all other models that derive the nucleus from an endosymbiont, the new model suffers from the absence of any topological equivalence between the endosymbiont plasma membrane and the eukaryotic nuclear membrane [96, 98]. Finally, as in the original suggestion of Devos and Reynaud, a specific evolutionary relationship is predicted between eukaryotes and PVC bacteria [95], one that is not supported by comparative genomic data.

We cannot sufficiently stress: genome sequence data analyses uncover readily evident sequence similarity between thousands of homologous genes that clearly and reproducibly link eukaryotes to protobacteria and archeabacteria (and plants to cyanobacteria [19, 20]) as modern endosymbiotic theory predicts. Those results have been obtained independently in many separate studies by many independent laboratories [3–6, 10, 14, 15, 17, 18, 20, 37, 59, 93, 99–102]. However, comparative gene and genome sequence analyses have never, so far, linked eukaryotes to planctomycetes. Planctomycetes remain interesting prokaryotes, but are irrelevant to eukaryote origins from the standpoint of homologous sequences.

Conclusion

Members of the PVC bacterial assemblage possess several interesting and distinctive traits, but any evolutionary links with similar eukaryotic traits are misconstrued. These characters are analogous not homologous. Comparative study of the convergent processes that have led to the emergence of cell compartmentalisation in PVC, other eubacteria, archaebacteria, such as *Ignicoccus hospitalis*, and eukaryotes have the potential to reveal important general aspects of cell evolution. However, by phylogenomic standards, PVC are no more intermediates in the prokaryote-to-eukaryote transition than dragonflies are intermediates in the evolutionary sequence linking bony fish and birds.

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