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The first eukaryote cell: an unfinished history of contestation

Maureen A. O'Malley

ESRC Research Centre for Genomics in Society (Egenis), University of Exeter, Byrne House, St. Germans Road, Exeter EX4 4PJ, UK

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ABSTRACT

The eukaryote cell is one of the most radical innovations in the history of life, and the circumstances of its emergence are still deeply contested. This paper will outline the recent history of attempts to reveal these origins, with special attention to the argumentative strategies used to support claims about the first eukaryote cell. I will focus on two general models of eukaryogenesis: the phagotrophy model and the syntrophy model. As their labels indicate, they are based on claims about metabolic relationships. The first foregrounds the ability to consume other organisms; the second the ability to enter into symbiotic metabolic arrangements. More importantly, however, the first model argues for the autogenous or self-generated origins of the eukaryote cell, and the second for its exogenous or externally generated origins. Framing cell evolution this way leads each model to assert different priorities in regard to cell-biological versus molecular evidence, cellular versus environmental influences, plausibility versus evolutionary probability, and irreducibility versus the continuity of cell types. My examination of these issues will conclude with broader reflections on the implications of eukaryogenesis studies for a philosophical understanding of scientific contestation.

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1. Introduction

> It might have happened thus; but we shall surely never know with certainty. Evolutionary speculation constitutes a kind of metascience, which has the same intellectual fascination for some biologists that metaphysical speculation possessed for some mediaeval scholastics . . . The most appropriate response to such speculations (if they are plausible and logically consistent) is an Italian rejoinder . . . Se non è vero, è ben trovato [Even if it's not true, it's nicely conjectured]. (Stanier, 1970, p. 31)

We humans are eukaryotes. At the heart of any evolutionary story we tell about ourselves lies the origin of the eukaryote cell. This remarkable evolutionary event consisted of a revolution in cell type. It is matched only in momentousness by the arrival on the biological scene of the eukaryote's 'other', commonly called the prokaryote. Nobody can say exactly how the eukaryote cell came into being, and adherence to different models of its origin is strong and uncompromising. And yet, despite the ontological wonder and epistemological mystery of the eukaryote's origins, its scientific investigation has received limited historical and even less philosophical treatment. The aim of this paper is to outline what is known about the evolution of eukaryotic cells by looking briefly at the field's history and then comparing two recent major models of the genesis of the first eukaryote.

These two models represent different approaches to eukaryogenesis. They not only propose different organisms as starting points and different events as catalysts and contributors, but also emphasize different bodies of data and different epistemological strategies to make their cases. The first group I will call the 'phagotrophy' modellers (phagotrophy being the ingestion of cells); the second, the 'syntrophy' modellers (syntrophy referring to metabolic cooperation between different cells). The two accounts foreground different strategies of making a metabolic living: by wholesale consumption of other organisms, or by shared exploitation of chemical resources and metabolic pathways. But the dispute between the two models is more fundamental than this: it is about whether major transitions in evolution occur from within or without. Phagotrophy modellers argue for the autogenous or self-generated origin of the eukaryote, whereas syntrophy

E-mail address: M.A.O'Malley@ex.ac.uk

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modellers argue for its exogenous ('xenogenous') formation (Taylor, 1974).[1] The first group is concerned to show that cellular constraints and potentials lie behind this revolution in cell type. The second group makes a case for radical innovation that is driven by environmental forces and symbiotic opportunism located outside any single cell's potential. It takes extraordinary events to make a eukaryote from the syntrophy perspective, whereas from the phagotrophy perspective, the transition to a radically new cell type can be represented as more of a cumulative process.

This distinction between autogenous and exogenous evolution guides each model in diverse and usually oppositional ways. Both narratives make their evolutionary case for particular candidate organisms by marshalling different combinations of genetic, biochemical, cell-biological, phylogenetic and geochemical data. Each group has to put into explanatory perspective the origin of the mitochondrion, the nucleus and other organelles, plus cellular capacities of various kinds and structural changes involving the cytoskeleton, cell walls and internal membranes. But also, a variety of strategies of argumentation are at play in this debate, and many of the claims of each model appeal to explanatory abstractions in the contest for model dominance. The virtues of cell-biological evidence are pitted against those of molecular evidence, and the explanatory role of cellular constraints is evaluated against that of environmental factors. The plausibility of evolutionary scenarios is weighed up in relation to their probability, and the irreducible nature of eukaryote cells is contested in regard to their continuity. By working our way through these evaluative strategies we may understand more about the astonishing confluence of events that resulted in the creation of eukaryote cells, and more about how scientific disagreements persist despite the conviction that increasing bodies of evidence will bring about reconciliation.

In what follows, I will introduce the debate with a brief outline of how eukaryote cells are different from prokaryote cells (Sect. 2). Section 3 addresses early accounts of eukaryote evolution, and these lead into Section 4, which outlines the rise of endosymbiont explanations of the mitochondria and other organelles. However, the understanding that some organelles have endosymbiont origins is not a complete account of the first eukaryote, and Sections 5 and 6 provide overviews of the two main competing models of eukaryogenesis: phagotrophy models and syntrophy models. As well as showing how these models deploy claims about autogeny and exogeny, I discuss the multi-faceted nature of the disagreements between the models as I examine their grounds of contestation (Sect. 7). Finally, I conclude with reflections on further issues for philosophy and history of biology to examine in light of the still unfinished debate about the origins of the first eukaryote cell.

2. The eukaryote cell

It is a well known fact that philosophers, if they care about biology at all, care mostly about animals. Plants make occasional appearances in philosophy of biology, but fungi and unicellular organisms do so rarely. This inattention does not mean that philosophers think these sorts of organisms are uninteresting; they simply have not been trained to think about such lifeforms and are unaware of their diversity (O'Malley & Dupré, 2007). One reason to broaden this outlook is that the biological nature of animals is eukaryotic, and eukaryotes began and persist in unicellular forms (many of which are called protists). To understand how multicellular eukaryotes function, proliferate and evolve requires a background understanding of eukaryote cell biology. One of the best ways in which to gain this knowledge is to reflect on the origins of eukaryotic life, which in turn, rests on background knowledge of predecessor cells. Cell biology today is predominantly the biology of eukaryotes because their cells are highly compartmentalized and—in the era of light microscopy in which cell biology emerged—eukaryotes, not prokaryotes, had more to offer.[2] The science of cell biology might, in fact, just as well be known as organelle and cytoskeleton biology (see Bechtel, 2010, this issue). Amongst eukaryotes, the unicellular forms, such as baker's yeast, *Saccharomyces cerevisiae*, are model organisms for cell biology because they share the same or very similar structures to those in the cells of multicellular eukaryotes (Figs. 1 and 2).

Eukaryote cells are distinguished from prokaryote cells by a number of features. One is size, with eukaryote cells most often being tens to hundreds of times greater in diameter and at least 1000 times greater in volume (de Duve, 1996).[3] More important than size is what is packed into that bigger space. A key feature is the nucleus. Eukaryote chromosomes are linear (most prokaryote genomes are circular) and wound up in proteinaceous nucleosomes in separate chromosomes inside the nucleus, the porous membrane of which separates DNA from the rest of the cell. This separation requires translation to occur outside the nucleus. Inside the nuclear compartment, transcription is controlled by a myriad of transcription factors. Further regulation of transcript processing is carried out by spliceosomes and the polyadenylation machinery before the mature mRNAs pass through the nuclear pore complex into the cytoplasm. Thousands of other membrane-bound structures such as lysosomes, peroxisomes and vacuoles perform specialized functions in the eukaryote cell to do with digestion and waste degradation. The endoplasmic reticulum (rough and smooth) and the Golgi body produce, package, store, and post-translationally modify and transport proteins. Mitochondria in their textbook form metabolize carbohydrates to fuel a proton-pumping process that ultimately generates ATP as energy for the cell (see, however, Sect. 7.3). Exclusively eukaryotic protein complexes (e.g., tubulin, kinesin, dynein, actin, myosin) structure processes that depend on the cytoskeleton, such as movement, vesicle transport and cell division. Eukaryote cells replicate through mitosis and cytokinesis, not prokaryotic binary fission, and can often engage in fully sexual reproduction via meiosis and syngamy. Prokaryotes have no equivalent to meiosis, although they do have mechanisms for exchanging genes. No intermediate forms between prokaryotes and eukaryotes have yet been detected or accepted as such, although increasingly complex structures (including potential 'proto-nuclei') and processes are being found in prokaryote cells as molecular cell biology gains ground (for example, Fuerst, 2005; Errington, 2003; Thanbichler et al., 2005).

However, in terms of gene content and genome dynamics, the discontinuity between prokaryotes and eukaryotes is less well defined.[4] The eukaryote genome, according to most analyses, is a chimera of genes that are most closely related to either bacterial or to archaeal homologues (i.e., genes from the two kinds of prokaryotes). For some analysts, this apparent chimerism indicates that the

[1] Syntrophy modellers do not explicitly foreground concepts of autogeny and exogeny, but the framework still fits the emphases of their models.

[2] Prokaryote cell biology now exists, due to technological advances such as fluorescence microscopy (for example, Gitai, 2005; Thanbichler et al., 2005), as does prokaryote developmental biology (for example, Dworkin, 1985; Brun & Shimkets, 2000), but these are quite separate fields of inquiry from mainstream eukaryote cell and developmental biology. There is considerable expectation they will become more integrated as complex structures are increasingly detected in prokaryotes (Gitai, 2005; Errington, 2003).

[3] Exceptions are the picoeukaryotes, which are less than two micrometres in size. They are dwarfed by a few bacteria that are larger than 100 micrometres, including *Beggiatoa*, *Thiomargarita* and *Epulopiscium*.

[4] Some aspects of eukaryotic genomes are very clearly different from prokaryotes. Amongst them are the presence of multiple linear chromosomes capped by eukaryotic-type telomeres and telomerases, spliceosomal (nuclear) introns, and many other tracts of non-coding DNA.

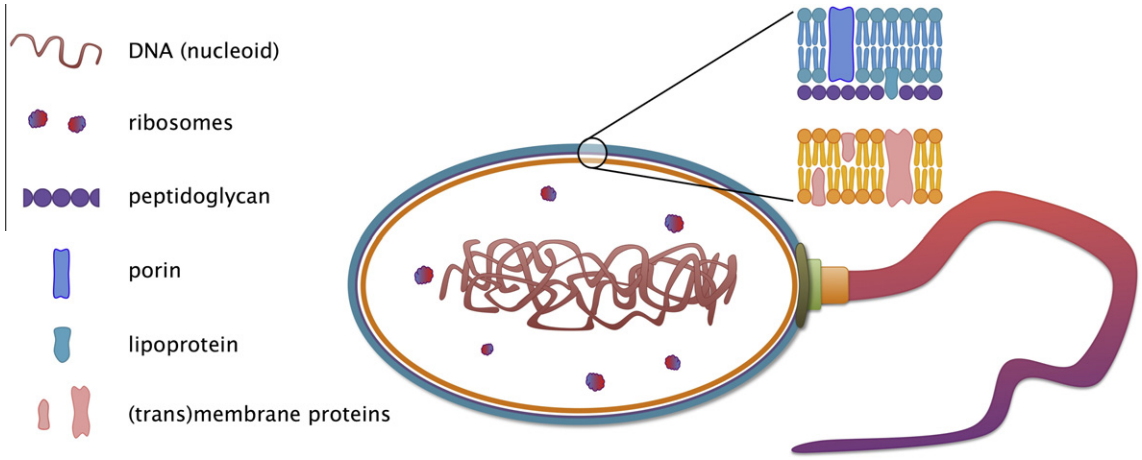

Fig. 1. Prokaryote cell (illustration courtesy of Michel Durinx). This figure depicts schematically the internal structures of a prokaryote cell, in this case (because of cell wall differences) a gram-negative bacterium such as *Escherichia coli*.

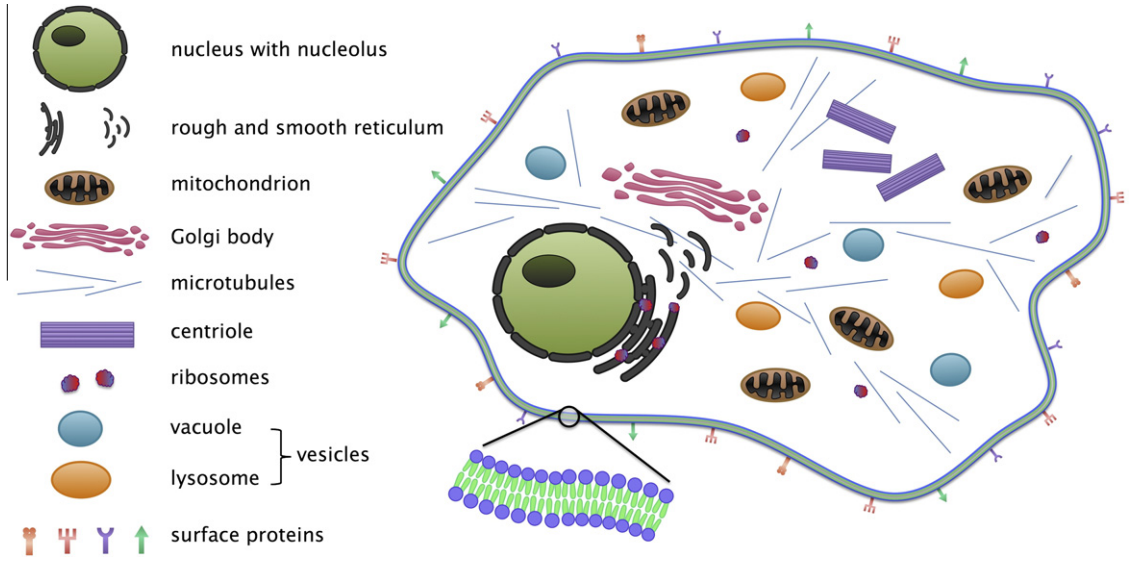

Fig. 2. Eukaryote cell (illustration courtesy of Michel Durinx). A schematic diagram of a generalized eukaryote cell (without a cell wall), depicting some specialized compartments and outer membrane structures. Structural features such as flagella, tubulin and actin are not shown (see Sect. 2). This eukaryote cell's size is not proportionate to the prokaryote in Figure 1.

eukaryotic nuclear genome was formed by a merger between bacterial and archaeal genomes, whereas for others, it indicates the ancient existence of a protoeukaryote (emerging within or beside the archaea) that engaged freely in lateral gene transfer with bacteria and thereby gained a mosaic genome (Lester et al., 2005). But despite this common genetic ancestry, and despite the gene elimination processes that attend prokaryote genome evolution (Kuo & Ochman, 2008), eukaryote genomes are almost always far larger than prokaryote genomes. Scattered throughout eukaryotic genomes are gene duplications, transposable elements and introns of an exclusively eukaryotic sort (i.e., spliceosomal introns). Spliceosomes and the introns they splice out of pre-messenger RNAs are a eukaryote invention.

Any viable account of the origin of eukaryote cells has to explain eukaryote cell biology and genomes in relation to the putative environments in which they evolved (oxic or anoxic, for example), the physiological and metabolic capabilities of early eukaryotes (e.g., aerobic or anaerobic; autotrophic or heterotrophic), and the gene content of the putative ancestral eukaryote genome. The diversity of explanatory factors required to construct the origin story has resulted in multiple competing accounts. It is this contestation, past and present, that provides a rich entry point for philosophical analysis. Some of this philosophical richness arises in relation to how explanations rise to dominance, and which aspect of explanation is the most important for attracting supporters. Other angles of inquiry involve how a field of inquiry copes with decades of epistemological tension between contested and unresolved explanations, and whether, in fact, such tension is a problem or an asset for explanatory puzzle-solving.

3. Early accounts of eukaryote origins

Historically, limited attention was paid to the origin of eukaryote cells until the 1970s. This was in part because the similarities between eukaryote and prokaryote cells were more overwhelming than their differences until the 1940s. The nucleus, often considered to be the defining feature of the eukaryote cell, was still being sought or inferred in bacterial cells right up until the middle of the 1960s (Brock, 1988; Robinow & Kellenberger, 1994). It took the combination of ultrastructural and molecular studies to put an

end finally to the discussion of 'two kinds of nuclei' (for example, Ris, 1966, p. 246). Nor were mitochondria and other organelles seen as exclusive features of eukaryotic cells (Mudd et al., 1951; Bisset, 1953). Although major differences in size and organization between cell types were considered important (for example, Copeland, 1938; Stanier & van Niel, 1941), these distinctions were not formulated in evolutionary terms—as a question of where and how basic cell types had originated—until the 1960s (Stanier & van Niel, 1962; Taylor, 1976). In part, this limited attention to eukaryogenesis was due to disciplinary boundaries. Cell biology was not (and is perhaps still not fully) evolutionary (Nick, 2009), probably because evolutionary studies have traditionally focused on organisms and molecules. And due to the limitations of microscopy, the more visible eukaryote cell was established early on as 'the' cell of scrutiny (Bechtel, 2010, this issue). Molecular cell biology is a fairly recent phenomenon, and because molecular studies tend to enable evolutionary analysis, cell biology is becoming ever more broadly comparative (Mowbrey & Dacks, 2009; Dacks & Field, 2007; Wickstead & Gull, 2006, 2007).

It took questions about the origins of organelles to stimulate more extensive evolutionary inquiries into eukaryote origins, and these were carried out on the basis of increasingly sophisticated biochemical and ultrastructural studies of mitochondria, lysosomes and peroxisomes (for example, de Duve & Wattiaux, 1966; de Duve, 1996). Detailed studies of the origin of the nucleus were and still are scarce. In part this is because evidence is very limited, and analogies to other structures cannot be made with great conviction because of the unusual membrane, for example, that defines the nucleus, as well as its unique contributions to the biology of cells (Martin, 2005a). As the mitochondrion was accepted in the 1960s as an endosymbiont with its own DNA, it became common to hypothesize not only the function but the evolutionary origins and relationships of such organelles, including the identification of their precursors in bacteria (de Duve, 1969). Molecular studies greatly advanced such evolutionary explorations because they allowed more precise calculations of relationships between cells and their organelles.

The advent of the molecular sequencing of genes and proteins brought about a classification revolution in microbiology. Molecular phylogeny, the discipline that uses molecular data to infer evolutionary histories of organisms, was a godsend for microbial systematics, which had struggled for decades to find appropriate tools and a justification for a 'natural' classification (Stanier & van Niel, 1962; Woese, 1987). Whether any such classification has since been achieved for prokaryotes is questionable (for example, Doolittle, 2009; Dagan & Martin, 2006), but there is no doubt at all that molecular phylogeny has opened up a vast realm of evolutionary understanding in microbiology that would not have been dreamed of otherwise. Because protists are richer in morphological and other characters, protistology's reliance on molecular data has been tempered by its trust in microscopy, especially the electron microscope (Patterson, 1999; Cavalier-Smith, 2004). Nevertheless, major evolutionary and classificatory claims to do with protists have risen and sometimes fallen in the short period of constructing evolutionary trees through analysing molecules. These overthrows include the complete revision of hypotheses about whether all eukaryotes have mitochondria, major relocations of the root of the eukaryote tree, massive rethinking of the evolutionary impact of endosymbiosis, and the total overhaul of the classification of major eukaryote groups (Patterson, 1999; Adl et al., 2005; Roger & Hug, 2006). The quest to understand the early evolution of eukaryotes has formed the concerted research focus of many microbiologists and molecular evolutionists for decades now, and enormous amounts of molecular, phylogenetic, biochemical and cell-biological knowledge have been generated while frameworks of interpretation have been constructed, interrogated, rebuilt and sometimes abandoned.

4. Endosymbiont theory

The most well known of the older speculations about the origin of organelles is Constantin Mereschkowsky's endosymbiont hypothesis (1905, in Martin & Kowallik, 1999). He suggested that chloroplasts, the organelles allowing plants to photosynthesize, were obtained initially from unicellular organisms that had been captured and 'enslaved' as endosymbionts (Sapp, 1994). This proposal languished for lack of evidence and from the excoriation it received at the hands of cell biologist Edmund Beecher Wilson in the 1920s. He declared Mereschkowsky's theory 'fantastic' in the sense of being a product of fantasy (Wilson in 1925, in Martin & Kowallik, 1999). Although this general idea of the endosymbiotic origins of organelles was picked up in the mid-twentieth century by Ivan Wallin, who argued for the endosymbiotic origins of mitochondria, it only became more disreputable as Wallin's claim to have grown mitochondria in culture was found to be unreplicable (Wallin, 1924; Hackstein et al., 2006). It took until the late 1960s for another unconventional microbiologist, Lynn Margulis, to provide some correlative cell-biological data to support such a theory, and to argue for it on plausibility grounds (Sagan, 1967;[5] Margulis, 1970; see also Goksøyr, 1967). Unfortunately for the theory's ongoing respectability, she couched it in a much bigger account of serial endosymbiosis, in which the genesis of the cilium and other important cellular machinery were directly and indirectly attributed to the extraordinary evolutionary importance of endosymbiosis (Margulis, 1970). These extended claims have not been widely accepted, even by those willing to accept the importance of endosymbiosis in relation to mitochondrion and plastid origins (for example, Cavalier-Smith, 1987, 1992; Martin et al., 2001; Roger, 1999).

Although Margulis is given full credit for the revival of the endosymbiont theory in regard to mitochondrion and chloroplast origins, what cemented its acceptance for these two organelles was strictly molecular data that filled in all the evidential and inferential gaps to the satisfaction—eventually—of most of the relevant communities of scientists (Gray & Doolittle, 1982). Notable dissenters, such as developmental biologist Rudolf Raff, biochemist Henry Mahler (Raff & Mahler, 1972), and botanist Allan Allsopp (1969), argued for wholly autogenous non-endosymbiotic accounts of the origins of mitochondria and chloroplasts, but their evidence was as circumstantial as Margulis's and could not hold out against the rising tide of molecular data. Although Margulis herself later spoke out against the 'tyranny' of molecular data, as she continued to seek ever more elusive evidence for the endosymbiotic origins of other eukaryotic features (Margulis et al., 2000), molecular support for the endosymbiont theory achieved a victory as far as the mitochondrion and chloroplast (and later, other plastids) were concerned (Sapp, 1994). Nobody now doubts that they were acquired through endosymbiosis, even if the evolutionary importance of such events might be downplayed. The major question that revolves around the acquisition of the mitochondrion in particular is *when* it occurred and *what sort of host* it involved. There are two main contenders for answers to these questions (Figs. 3 and 4). The first, the phagotrophy model (Sect. 5), involves the existence of a recognizably eukaryotic cell prior to the acquisition of the mitochondrion; the second, the syntrophy model (Sect. 6), involves the integration of two types of prokaryote cells, with one incorporating the other.

[5] Sagan was Margulis's married name until around the end of the 1960s.

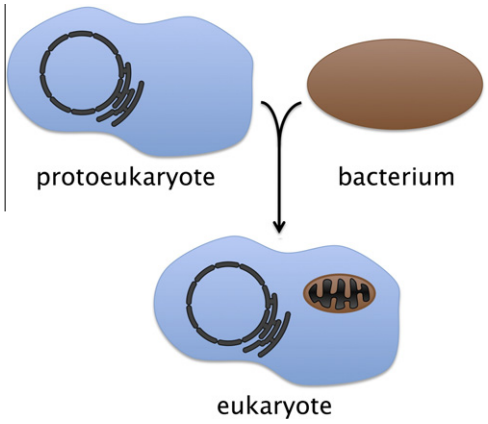

Fig. 3. The phagotrophy model (illustration courtesy of Michel Durinx). This image depicts schematically the main elements of the phagotrophy model of eukaryogenesis. It shows a protoeukaryote, already in possession of a nucleus (plus other non-illustrated structures), in the vicinity of an organism that is then engulfed. It becomes the mitochondrion, as the protoeukaryote becomes a fully fledged eukaryote. Time flows from top to bottom.

5. Phagotrophy models

Early in the acceptance of the endosymbiotic origins of the mitochondrion, the assumption grew that the acquiring organism was already well along the road to becoming a eukaryote. Early versions of such accounts focused on the autogenous differentiation of a cyanobacterium into a eukaryote with a chloroplast (Klein & Cronquist, 1967; Allsopp, 1969; Cavalier-Smith, 1975; Taylor, 1974). Some of these eukaryotes supposedly lost the chloroplast later as they gained the ability to consume other organisms. Other formulations of autogenous eukaryogenesis, willing to accept that endosymbiosis contributed to the evolution of the already evolved protoeukaryote, presumed that amitochondriate eukaryotes still existed (for example, Whatley, 1976; Stanier, 1970). One candidate for the primordial eukaryote was the huge amoeba, *Pelomyxa palustris*. It hosts numerous bacterial endosymbionts (*Paracoccus denitificans*, considered in this hypothesis to be ancestral to aerobic mitochondria), and appeared to undergo unusual nuclear division processes without mitosis (Whatley et al., 1979). Ultrastructural analyses allowed inferences of structural homologies to be drawn between this 'primitive', still transitional eukaryote, and evolutionarily advanced eukaryotes (Whatley, 1976). Other prime candidates, proposed by Tom Cavalier Smith in particular, were anaerobic organisms that seemed to be basal in the eukaryote tree (although not monophyletically), and when analysed further, exhibited no trace of mitochondrial structures or DNA (Cavalier-Smith, 1987). Organisms in this category included *Giardia lamblia* (also known as *G. intestinalis* and *G. duodenalis*), *Entamoeba histolytica*, *Trichomonas vaginalis* (all animal parasites) and Microsporidia. Collectively, they were called the Archezoa (ancient animals), and were considered to be living fossils of the earliest eukaryotes (ibid.).

The Archezoa scenario enjoyed considerable popularity in the 1980s and early 1990s and became part of the standard textbook account of eukaryote origins. But quite rapidly, the category of organisms and associated evolutionary hypothesis of Archezoa were demolished by a slew of papers that found traces of mitochondrial proteins and genes in almost all amitochondriate organisms, thereby indicating that they had once possessed such organelles (Clark & Roger, 1995; Henze et al., 1995; Bui et al., 1996; Germot et al., 1996; Horner et al., 1996). Molecular studies collected and analysed a body of genetic data that indicated the cryptic or vestigial existence of mitochondria in all the purportedly basal 'amitochondriate' eukaryotes, such as *Entamoeba* and *Trichomonas* (Roger et al., 1996; Roger, 1999; Embley & Hirt, 1998; Tovar et al., 1999). The earliest evidence was molecular; subsequent supporting evidence was morphological (Keeling, 1998; van der Giezen, 2009; Martin & Müller, 2007). In addition, the deep divergence in the eukaryote tree of these putatively amitochondriate lineages was increasingly interpreted as an artifact of phlyogenetic methods (Roger, 1999; Embley & Hirt, 1998).

The explanation for the apparently amitochondriate state of the putative Archezoa (a label now used only historically, despite occasional efforts to revive the term for different uses) was that these organisms had in fact lost fully functioning mitochondria and mitochondrial DNA, but retained remnant organelles called mitosomes or hydrogenosomes (Martin & Müller, 1998; Tovar et al., 1999; Embley & Martin, 2006). Even Cavalier-Smith, the architect of the research programme searching for ancient amitochondriate organisms, accepted the evidence against the existence of the Archezoa (for example, Cavalier-Smith, 2002). This revision, however, has by no means upset his and other evolutionary biologists' adherence to the basic phagotrophic protoeukaryote hypothesis of eukaryogenesis. And some protoeukaryote proponents also raise the possibility that the 'missing link' of amitochondriate protists may be out there waiting to be found, either having gone extinct or simply lacking the sorts of features that would make it visible to today's science (for example, de Duve, 2007). Occasionally, new lineages are detected and tentatively proposed as the missing Archezoa (for example, López-Garcia et al., 2001), but multiple errors are usually found to underlie such interpretations (Berney et al., 2004; Cavalier-Smith, 2004).

Despite the demolition of the Archezoa hypothesis, the protoeukaryote component of this model—which is argued to be logically independent of it (for example, Poole & Penny, 2006)—survived and persisted. Cavalier-Smith crystallized his own and others' intuitions that an ancestral phagocyte was the starting place for the first eukaryote, and that it arose between 800 and 850 million years ago (Cavalier-Smith, 2002, 2009).[6] The core claim is that the already existing protoeukaryote (now conceived as an aerobic heterotroph) in possession of a highly developed cytoskeleton, acquired, through engulfment, an endosymbiont that became the mitochondrion. The failure to find an amitochondriate eukaryote is irrelevant in this formulation. Such a finding would have been convenient support, but the protoeukaryote account did not require it. Archaea and eukaryotes are sister taxa in this model, with the key difference being that the archaea retained cell walls and therefore could not evolve phagotrophy (Cavalier-Smith, 2002).

The central biological support for this model revolves around phagocytosis and phagotrophy, which are the processes of enclosing an organism within a cell and consuming it. For many proponents of this account of eukaryogenesis, the ability of eukaryotes to internalize cell-sized objects by endocytosis is *the* fundamental biological difference between eukaryotes and prokaryotes (for example, de Duve, 1969; Stanier 1970, p. 15; Cavalier-Smith, 2009). This activity requires that the consuming cell has a non-rigid cell wall, cytoskeletal and endomembrane interactions, transfer mechanisms from food vacuoles to energy-producing parts of the cell, and other classically eukaryotic capabilities (Richards & Cavalier-Smith,

[6] Some phagotrophy supporters posit a far earlier evolution of eukaryotes (for example, Kurland et al., 2006; de Duve, 2007), but there is no fossil or strong molecular evidence to support this claim yet, says Cavalier-Smith (2009). However, most palaeontologists are willing to accept an earlier origin for eukaryotes than Cavalier-Smith, on the basis of what is interpreted as a Bangiophyte red algal fossil more than 1.2 billion years old (Butterfield, 2000). Cavalier-Smith believes this fossil must be a cyanobacterium, not a eukaryotic alga. See Sect. 7.1 for discussion.

2005). The protoeukaryote hypothesis claims that once phagocytosis had evolved, it provided a mechanism for the endosymbiosis of the mitochondrial ancestor (Cavalier-Smith, 2002; Kurland et al., 2006). Obtaining the endosymbiont was just an accidental by-product of much more fundamental eukaryotic activities (Cavalier-Smith, 1975).

Not all versions of this model involve the same entities as Cavalier-Smith's scenario, itself undergoing considerable conceptual evolution (towards greater autogeny), but they all posit a protoeukaryote with many standard eukaryotic features and suggest it subsequently acquired prokaryote endosymbioses for various organelles, including the mitochondrion and sometimes the nucleus (for example, Hartman & Fedorov, 2002). According to the Cavalier-Smith model, the nucleus was derived autogenously, from an invagination of the plasma membrane, and was selected for its capacity to protect the chromosome from shearing damage caused by the emerging cytoskeleton and molecular motor movements of subcellular components (Cavalier-Smith, 1975, 2002). Elaborations of this model also address the evolution of nuclear pores and the traffic through them (Cavalier-Smith, 1988). Other organelles, such as the peroxisome and endoplasmic reticulum, have an oscillating history of being treated autogenously or exogenously (as endosymbionts), with autogenous-origin scenarios now dominating accounts of all organelles except for the mitochondrion and chloroplast (for example, de Duve, 2007; Cavalier-Smith, 2002). But overall, the many complexities of organelle evolution mean that 'decisive clues are lacking' (de Duve, 2007, p. 399) and exogenous origins cannot be wholly ruled out, even for phagotrophy supporters.

One problem that is often attributed to the phagocytic protoeukaryote model is that phagotrophic behaviour is exceedingly common in many protists, and yet it has only once produced a mitochondrion, and only once a primary symbiosis of a plastid.[7] The frequency of the underlying behaviour and the uniqueness of its evolutionary consequences need reconciliation. It also takes numerous additional evolutionary steps to explain why eukaryotes have membranes similar to bacteria, and why their genomes reflect a dual ancestry of bacteria and eukaryotes (Cavalier-Smith, 2002). A great deal of the Cavalier-Smith model hangs on preceding explanatory steps, and some of them go back so far in the evolutionary record that any chance of finding definitive evidence is accepted as unlikely by proponents and opponents alike. Moreover, large-scale analyses of multiple trees of genomes have been interpreted as supporting not a protoeukaryotic ancestral lineage, but instead, the emergence of eukaryotes from an exogenous symbiosis of archaea and bacteria (Pisani et al., 2007). But lateral gene transfer from non-endosymbiont bacteria to the protoeukaryote is still a contender to explain the bacterial genes in eukaryote genomes, and novel eukaryote genes (e.g. tubulin, actin, and myosin) cannot be simply explained as archaeal or bacterial (Richards & Cavalier-Smith, 2005).

Phagotrophy opponents think that knowledge of the diversity of organelles derived from the ancestral mitochondrion is crucial for understanding eukaryogenesis. These critics argue that phagotrophy models neglect this aspect of how eukaryotes evolved, even though phagotrophy modellers claim to be taking a cell-biological (i.e., organelle-oriented) perspective (Mentel & Martin, 2008; Martin et al., 2001). And from a geochemical point of view, the same opponents think that to suggest the protoeukaryote was an aerobically respiring organism is too oxygen-centric (Mentel & Martin, 2008). Assuming oxygenated oceans, as was common prior to the late 1990s, is the 'mistaken' underpinning of the idea of an aerobic mitochondrion and amitochondriate anaerobic eukaryotes. Now the latter hypothesis has been abandoned, say these critics, the same should happen to the associated metabolic bases of phagotrophy. If the host (the protoeukaryote) was already making ATP, it would have gained nothing from an ATP-generating endosymbiosis (Martin et al., 2001). In return, phagotrophy supporters argue that ATP generation came later, and that the original endosymbiont's consumption of oxygen conferred a protective advantage (Kurland & Andersson, 2000). Eventually, according to this reasoning, the endosymbiont converted to an organelle and acquired new features from the protoeukaryote nucleus, such as ATP-production capabilities. Cavalier-Smith also argues for an increase in metabolic efficiency with a persistent symbiont (2006). The host would develop an ability to 'farm' the endosymbiont, resulting in an increasingly interdependent relationship.

Phagotrophy, a relatively recent innovation in the Cavalier-Smith model, is a very costly one, say critics (Vellai & Vida, 1999). As well as abandoning the cell wall and periplasm (a process that has occurred to some extant bacteria, but which has simultaneously resulted in the reduction of cell and genome size to a level well below that of most prokaryotes, let alone eukaryotes), the protoeukaryote would have already had to evolve an endomembrane system, including a digestive apparatus, and a cytoskeleton. The complex cascade of associated innovations all this requires would have needed a major catalytic event of some sort, argue syntrophy proponents (for example, ibid., p. 1576). They believe it would be considerably less question-begging to think this cascade was in fact initiated by the revolutionary acquisition of an endosymbiont, and the intracellular organization this then necessitated. This criticism lies at the heart of the autogenous versus exogenous debate, and its claims underpin syntrophy models.

6. Syntrophy models

The main competitors to phagotrophy models come from syntrophy hypotheses, in which metabolic interdependence between different prokaryote cells eventually results in an even tighter liaison: the 'hybrid' eukaryote cell. The most well known syntrophy model is the 'hydrogen hypothesis', which was proposed by biochemists Miklós Müller and William Martin in the late 1990s (Martin & Müller, 1998).[8] It is based on the discovery of hydrogenosomes, which are organelles of bacterial origin that produce hydrogen within their anaerobic hosts (Lindmark & Müller, 1973). Their ancestral free-living form entered into a permanent relationship with anaerobic methanogens, which dwelt in the murky sulphidic depths of the anoxic Canfield ocean more than 1.5 billion years ago (Poulton et al., 2004; Martin et al., 2003). The oxidation of the atmosphere and ocean surface was increasing, but oxygen levels were still low elsewhere. Anoxia is important to this model because methanogenesis cannot occur in the presence of oxygen. It also requires hydrogen as a donor molecule, and hydrogen is what the endosymbiont has to offer (along with carbon dioxide), first independently and then dependently, as the ancestral mitochondrion. As Martin and Müller argue, 'Hydrogen is the key. It is the bond that forges eukaryotes out of prokaryotes' (Martin & Müller, 1998, p. 40). This capability depends on biochemical and membrane-bound interactions between host and endosymbiont, and presupposes no pre-existing intracellular structures in the host. The host's dependence

[7] Secondary endosymbioses of plastids have occurred several times (Archibald, 2009). Because the primary endosymbiosis occurred after the acquisition of the mitochondrion, I have not paid much attention to it, but this history is also worth more historical and philosophical attention (for example, Godfrey-Smith, 2009). There are also claims of a least one more primary endosymbiosis of a cyanobacterium in an amoeba (Bodyt et al., 2007).

[8] Alternative syntropy models are based on different combinations of ancestral organisms, different metabolic pathways, different adaptive benefits and additional scenarios of organelle genesis (López-Garcia & Moreira, 1999, 2006; Vellai & Vida, 1999; Searcy, 2003).

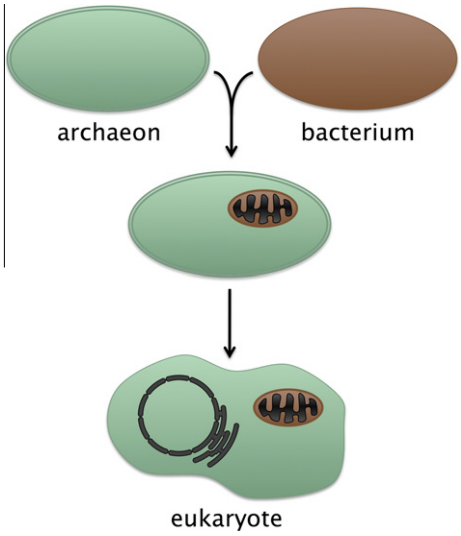

Fig. 4. The syntrophy model (illustration courtesy of Michel Durinx). Figure 4 represents schematically the syntrophy model, in which an archaeon and bacterium exchange metabolites in syntrophy. The archaeon then incorporates the bacterium, syntrophy continues, endosymbiotic gene transfer ensues, and the bacterium becomes the mitochondrion. Once the endosymbiont has been acquired, the rest of the eukaryotic structures evolve (e.g., nucleus, endoplasmic reticulum—see Fig. 2 and Sect. 2 for other intracellular structures). Time flows from top to bottom.

on the hydrogen that its new endosymbiont produces allows major metabolic reorganization. The host can eventually give up on methanogenesis and autotrophy to become an aerobic heterotroph, with the endosymbiont by-product of hydrogen no longer needed (Martin & Müller, 1998). The proto-mitochondrion that was facultatively anaerobic can become an aerobic metabolizer.

Not all mitochondria today are aerobic, however: many unicellular and multicellular organisms possess anaerobic versions of these organelles and there is considerable variation in the morphological, physiological and genetic characteristics amongst these different mitochondria (Tielens et al., 2002; van der Giezen & Tovar, 2005). Molecular studies of mitochondria have shown that as endosymbioses persist over evolutionary time, much of the endosymbiont DNA moves to the host genome. The endosymbiont genome is further modified by acquisition of foreign DNA and mutation, and protein transport mechanisms evolve in the cell to supply the diminished endosymbiont, now an organelle (Timmis et al., 2004; Embley et al., 2003a; Gray et al., 1999).

In addition to mitochondria, the syntrophy model seeks to explain similar organelles. Hydrogenosomes are anaerobic hydrogen and ATP-producing organelles that possess vestiges of the electron transport chain found in mitochondria. They do not usually possess a remnant genome. They are found in anaerobic ciliates, trichomonads and a few fungi (Müller, 1993; Embley et al., 2003a; Martin, 2005b). Mitosomes, on the other hand, do not produce hydrogen or ATP, have no electron transport chain, and never possess a genome. They do have morphological characteristics, such as double membranes, that are similar to mitochondria and hydrogenosomes (Embley & Martin, 2006; van der Giezen & Tovar, 2005). They are found in parasitic protists, such as *Entamoeba* and *Giardia* (the 'amitochondriate' eukaryotes). Despite many biochemical and functional differences between mitosomes, hydrogenosomes and the many varieties of mitochondria, they are all believed to have a common endosymbiotic origin (Embley et al., 2003b; Martin, 2005b; van der Giezen, 2009) and this has been a central tenet of the hydrogen hypothesis. However, recent analyses are suggesting that hydrogenosomes may have had a different origin from mitochondria (for example, Hug et al., 2010; Dyall et al., 2004), thus complicating this aspect of the hydrogen hypothesis. There is consensus about the original endosymbiont, the ancestral mitochondrion. It is believed to have been an alpha-proteobacterium, although its precise capabilities are disputed.

The hydrogen syntrophy model has also addressed the origin of the nucleus, which is envisaged as a solution to the slow splicing-out of introns (Martin & Koonin, 2006; Martin, 2005a). The nucleus effectively decouples transcription and translation, thus allowing introns to be spliced out in the nucleus to protect protein production. Introns came into existence before the fully formed eukaryote, according to this scenario, but only became a problem when they proliferated in the transfer of genes from the new mitochondrion organelle to the host nucleus (Martin & Koonin, 2006; Dagan & Martin, 2007; Koonin, 2006). Consequently, spliceosomic functions are adduced as the evolutionary reason for the nucleus to evolve. Some phagotrophy supporters see archaeal–bacterial eukaryotes as unlikely candidates for the origins of the nucleus and spliceosome, because they think that the proliferation of introns and the selective pressure this produced could only occur to an already sexually reproducing protoeukaryote (for example, Poole, 2006). Asexual organisms cannot tolerate the proliferation of selfish elements and introns will be largely if not entirely selected against. But in most syntrophy and phagotrophy models, the nucleus is endogenously derived.[9] The crucial question becomes one of whether it evolved before or after the acquisition of the mitochondrial symbiont (Martin, 1999), and the exact nature of the host.

Numerous complications arise for the postulated host in the hydrogen version of the syntrophy model because of the difficulties in finding traces of the appropriate pathways in candidate organisms. These absences have to be addressed by additional explanations of why such capabilities are missing in those organisms. While extensive analyses and re-analyses of genomic data are often argued to support syntrophy hypotheses rather than phagotrophy ones (for example, Cox et al., 2008; Pisani et al., 2007), they do not locate the host in or near existing clades of methanogenic archaea, and find little relationship between bacterially derived genes in eukaryote genomes and the alpha-proteobacterial genes that are supposed to have been gained from the endosymbiont. In addition, the internal transformations that occur in the host cell seem to require considerable additional explanation in order to account for their structural and functional complexities (Dacks & Field, 2007; Wickstead et al., 2010). A further problem for any syntrophy hypothesis with an archaeon as the host arises in regard to membrane chemistry and structure. Eukaryote membranes are similar to bacterial membranes, both in regard to lipids and membrane structure. If the putative host cell is archaeal then a complex change must have occurred. The difficulty of exchanging one sort of lipid for another leads phagotrophy critics of syntrophy models to claim such scenarios are mechanistically improbable and 'evolutionarily onerous' (Cavalier-Smith, 2009, p. 312; Poole & Penny, 2007). Syntrophy supporters are unperturbed by such criticisms. They see the replacement of lipid biosynthesis pathways as perfectly feasible, and as a highly plausible instance of 'combinatorial evolution', in which endosymbiotic imports create a context for the functional redundancy of the original host cell machinery (Martin, 2005b).

For phagotrophy proponents, the major failing of syntrophy models is the inability of prokaryotes to ingest other prokaryotes. Prokaryote cell walls simply do not enable the sorts of activities

[9] Only in some peripheral scenarios is the nucleus modelled as exogenously derived, in some cases from the inclusion of a virus in an ancient cell (for example, Bell, 2009; Takemura, 2001), where it may even have become the nucleus in the progenitor of all cells (bacteria and archaea subsequently losing their nuclei); in other cases, the nucleus was generated from a prokaryotic endosymbiont (for example, López-Garcia & Moreira, 2006; Lake & Rivera, 1994; Horiike et al., 2001).

that allow the engulfment of other cells, even though there are prokaryote predators that invade larger cells or hunt them down in packs (Berleman & Kirby, 2009). There are two main examples of prokaryote–prokaryote endosymbioses: a cyanobacterium with a bacterial endosymbiont (Wujek, 1979); and mealybug endo-endosymbionts, in which gamma-proteobacterial endosymbionts live within a beta-proteobacterial endosymbiont of the mealybug (van Dohlen et al., 2001).[10] But for syntrophy modellers, the non-phagocytic behaviour of prokaryotes can be turned into an epistemological *asset* for their account. They reason that if phagotrophy were as common as it is argued to be for the protoeukaryote, then events such as the emergence of the ancestral mitochondrion should have happened multiple times in evolutionary history (Dagan & Martin, 2007). They have not, although ordinary endosymbioses are fairly common. Another line of potential support comes from new findings of a now extinct (or perhaps undiscovered) branch of archaea with capacities for particle engulfment (Yutin et al., 2009). And while prokaryote cells might not have phagotrophic capacities, say other syntrophy supporters, eukaryotes could not have had them at the relevant evolutionary moment either. They might have evolved them later, after the incorporation of the proto-mitochondrial cell. If neither eukaryotes nor prokaryotes had phagocytic advantages, predatory (i.e., invading) prokaryotes could just as well have become endosymbionts, and contemporary capacities for phagocytosis have no role in the explanation (Davidov & Jurkevitch, 2009).

A final issue is that although syntrophy models potentially account for the archaeal–bacterial chimerism of eukaryote genomes (Pisani et al., 2007), they do not explain clearly why this chimerism is so split. Eukaryotic informational genes (those involved in processes such as transcription, translation, DNA replication and repair) tend to be archaeal in origin, whereas operational genes (metabolic and structural) tend to be bacterial in origin (Ribeiro & Golding, 1998; Lester et al., 2005). A proposed explanation for the bacterial genes in eukaryotes is ongoing phagocytosis, in which ingested DNA replaces bit by bit the genes in the nucleus (Doolittle, 1998). Because prokaryote prey are not necessarily related to mitochondria, this 'gene transfer ratchet' might also explain why bacterial-like genes are not very similar to alpha-proteobacterial genes, which might be expected because of the postulated symbiont (Timmis et al., 2004; Lester et al., 2005). But exactly as for phagotrophy models, syntrophy models are missing crucial bodies of evidence, unassailable inferences, comprehensive explanations and knock-down counter-criticisms. As these models contest each other in the explanatory domain of eukaryogenesis, they also advance epistemological claims and argumentative strategies to enhance their chances of dominance.

7. Contested grounds

On one level, the contestation between these two models of eukaryogenesis revolves around the mitochondrion and its centrality to the evolution of the eukaryote cell. Phagotrophy supporters see the mitochondrion as important but just one step in the lengthy and complex emergence of eukaryote cells (Cavalier-Smith, 2002; de Duve, 1996). Syntrophy supporters see the mitochondrion as utterly crucial to the evolution of eukaryotes, and cast it as both the instigator and defining feature of this radical evolutionary transition. These perspectives fit the autogenous versus exogenous framing of the two main models, in which eukaryote cells are either an innovation in their own right, or a totally novel rearrangement of prokaryote cells (Taylor, 1974). This complicates any sense of discontinuity between prokaryotes and eukaryotes. On the one hand, phagotrophy supporters see a major evolutionary divide between the two cell types but posit a more gradual process of evolution. On the other hand, syntrophy supporters posit a dramatic transition—'unspeakably rare' (Martin et al., 2003, p. 197)—that occurred through the continuous evolution of the same cell types after they adapted to their dramatically new relationship.

But at another level, this debate is not simply about fundamental biological processes and entities. It is about justifying certain models, methodological choices, disciplinary commitments, epistemic strategies and ontological assumptions. Autogeny and exogeny may guide these choices, but as is clear from the outlines in the preceding sections, autogenists can tolerate some exogeny (e.g., mitochondria and plastids) and exogenists some autogeny (e.g., the nucleus, peroxisomes). Moreover, it would seem that any complete scenario of eukaryogenesis requires major aspects of each model, and that the two could not be mutually exclusive. Nevertheless, each side in the eukaryogenesis arena continues to argue its own contested position despite limited evidence and frequent challenges regarding data and interpretation. These gaps and challenges, however, are what keep the debate alive. Protecting and advancing a favoured model requires constant inventive gap-filling and rebuttal of criticisms. The argumentative and epistemological strategies deployed in the eukaryogenesis contest are a general characteristic of scientific debate, but they take a particular form here because they are ultimately framed by the autogeny–exogeny distinction.

7.1. Cell-biological versus molecular and biochemical evidence

Each model evaluates its own and the other's strengths on the basis of a hierarchy of evidence that can be understood in the form of a disciplinary divide. Syntrophy scenarios tend to be more biochemical and genetic, whereas phagotrophy models rely more heavily on cell-biological data and interpretations. Christian de Duve, the Nobel Prize-winning biochemist and cell biologist who discovered lysosomes and peroxisomes, suggests that too much of the discussion of the origin of the first eukaryote has been focused on the nuclear or mitochondrial genome (de Duve, 2007). He argues that a broader synthesis of cell biology and physiology is essential for a better understanding of the early evolution of eukaryotes (ibid.). Cavalier-Smith is the epitome of this cell-biological approach. He explicitly claims that his eukaryogenesis account will be seen as obviously correct if the right hierarchies of evidence are imposed (for example, Cavalier-Smith, 2010). From his perspective, molecular and biochemical evidence must be thought of as supplements to cell-biological data.

A key example of a cell-biological approach to eukaryote evolution can be found in Cavalier-Smith's suggestion that cell walls are far more crucial to evolutionary modelling than transcription and translation machinery (Cavalier-Smith, 2002, 2006). The nature of the cell wall is such that retaining it, as did archaea and bacteria, but not eukaryotes, closes off major avenues of morphological and physiological innovation (Cavalier-Smith, 2002). Prokaryotes that had earlier lost a second outer membrane, according to this model, were 'in preparation' for eukaryogenesis (Cavalier-Smith, 2009, p. 310). Although prokaryotes may be biochemically diverse, the very fact they held on to their exoskeletons meant they bound themselves to a certain range of evolutionary possibilities. Taking epistemological counsel from this evolutionary interpretation, Cavalier-Smith proposes that cell biological evidence necessarily outranks genetic and other molecular evidence (Cavalier-Smith,

[10] A recent proposal of a very early endosymbiosis between two bacteria to form another bacterium with a double membrane (Lake, 2009) is a hypothetical (rather than accepted) instance of prokaryote–prokaryote 'engulfment'.

2002, p. 300). Eukaryogenesis is about cellular change, he argues, and only secondarily genomic change (ibid., p. 301). Genome evolution can only occur in relation to cellular properties, and biologists who study genome evolution would be well advised to consider genomes in relation to organismic evolution (Cavalier-Smith, 1997). In this hierarchy of evidence, symbiogenesis is ranked as an important but far less radical event. Autogenous contributions are more significant than exogenous or extracellular ones, and of these autogenously generated properties, phagotrophy is the most remarkable for eukaryogenesis (Cavalier-Smith, 2002, 2009, 2010). Unlike prokaryotes, stuck in a trophic niche that leads 'merely' to exogenously driven biochemical flexibility, eukaryotes begin endogenously to revolutionize the very structure of nutrition by ingesting and consuming food sources internally.

Syntrophy modellers draw heavily on molecular biology, especially for phylogenetic reconstruction, and biochemistry. However, to say syntrophy modellers are not cell-biological would be a mistake. Clearly, they are concerned with the function and integration of the mitochondrion as an organelle, not just as a site of biochemistry. But these modellers are more likely to focus on lipid biosynthesis and mitochondrial biochemistry in order to understand the functionality of such cellular entities (see Bechtel, 2010, this issue). Genomic analyses are also more of a focus amongst syntrophy advocates, on the grounds that molecules are the only way to track cell biology in a quantifiable and computationally analysable way (Embley & Martin, 2006). However, Cavalier-Smith counters such claims with the argument that cell properties can be quantified, and that gene-based analyses will be incomplete and misleading without them (Cavalier-Smith, 1997). Molecular phylogeny can order the history of evolutionary innovation, he says, but only fossils can provide the anchoring dates (Cavalier-Smith, 2006). But in a further twist of the argument, Cavalier-Smith's own dates for calibrating the origin of eukaryotes (800–850 million years ago) have been disputed by palaeontologists who think there are much earlier eukaryote fossils (for example, Butterfield, 2000; see n. 6). There is also considerable room for error in fossil dating. This occurs for different reasons than errors in molecular dating, but is of no less a misleading nature (Roger & Hug, 2006). Contestation in the eukaryogenesis debate is not, obviously, restricted to claims made on the basis of data, but also in regard to the most appropriate disciplinary source of evidence for revealing the deep evolutionary past of cells.

7.2. Cellular constraints versus environmental context

Degrees of support for scenarios of different metabolic capabilities are frequently linked to geochemical scenarios preceding and subsequent to eukaryogenesis. Syntrophy advocates are usually very concerned to locate their putative ancestral organisms in a geochemical context, and to support this contextualization with molecular phylogenies of pathways that 'fit' those ancient environments. 'Nothing in the evolution of eukaryotic anaerobes makes sense except in light of Proterozoic ocean chemistry', quip Mentel and Martin (2008, p. 2718). The hydrogen-driven version of the syntrophy hypothesis emphasizes that although eukaryotes probably evolved 1.5 billion years ago, and an oxygen-rich atmosphere was generated about 2.4 billion years ago, eukaryote evolution occurred not in response to an increasingly oxic atmosphere but to anoxic sulphidic marine environments, such as the persisting Canfield Ocean. Traces of pathways to deal with the sulphidic content of the ocean are still visible in many eukaryotic lineages (Martin et al., 2003). But alternative environments existed at the same period, including much more oxidated zones, or oxic-anoxic interfaces (Hug et al., 2010), and such niches cannot be ruled out as the initial site of eukaryogenesis. Consequently, geochemical reasoning often works to provide additional support to a favoured host, which is usually chosen on the basis of molecular phylogenetic analyses (i.e., for specific inferred ancestral pathways).

Geochemical context is reasoned about somewhat differently in relation to the phagotrophic protoeukaryote. One line of argument, as already noted, was that the primordial adaptive role of the mitochondrion was to protect against the 'oxygen holocaust', rather than to confer metabolic benefits per se (de Duve, 1996, p. 56; Kurland & Andersson, 2000). But for Cavalier-Smith's model, in accordance with its focus on autogenous evolution, environmental geochemistry is conceived merely as a background and not as a driver of evolutionary processes. 'No specific environmental stimulus or external intervention is needed to explain that uniquely revolutionary internal cellular upheaval', he claims (Cavalier-Smith, 2009, p. 320). In this model, novel body plans create adaptive zones and then shape the environment, rather than the other way around (Cavalier-Smith, 2006, p. 999). Cavalier-Smith believes that biologists think too much about external environments because of a focus on selective pressures, and that this leads them to neglect internal organization (Cavalier-Smith, 1997). These so-called 'environmental determinists' should instead focus on endogenous limitations, which—according to Cavalier-Smith's causal hierarchy—are the main factors governing evolutionary timing (Cavalier-Smith, 2006, p. 997). The fossil record is much more important for supporting evolutionary events than geochemistry, because it—unlike planetary geology and chemistry—reveals internal organization (Cavalier-Smith, 2002, p. 319). This epistemological tension, between external and internal explanatory factors, thus plays a major role in both justifying certain evidence hierarchies and supporting autogenous or endogenous inclinations.

7.3. Plausibility versus probability of evolutionary events

Phagotrophy advocates often resist syntrophy hypotheses on the grounds that such events would require highly improbable events, such as lipid replacement (from the host to the endosymbiont) and endomembrane re-assembly within the new host cell (de Duve, 2007; Cavalier-Smith, 2002; Poole & Penny, 2006). Although syntrophic metabolic dependencies between archaea and bacteria are common occurrences between archaeal methanogens and bacterial hydrogen generators, phagotrophy supporters do not think metabolic arguments can overcome cell-biological absences (Poole & Penny, 2006). To imagine that one prokaryote could engulf another prior to the innovation of phagotrophy, says Cavalier-Smith, is so 'mechanistically implausible' that it invokes 'magic, not science' to comprehend it (Cavalier-Smith, 2002, p. 313).

The tangled nature of events such as the evolution of phagotrophy means that a total reconstruction of its pathways will be impossible, says Cavalier-Smith (2009, p. 313). So many things happened, many of them simultaneously, and evolutionary processes produce kludges or makeshift innovations, rather than elegant designs (ibid., p. 319). All that can be done is to focus on 'the central logic' of such evolutionary transitions and 'suggest plausible key intermediates' (ibid., p. 313). But even while accepting the need for 'messier' explanation, Cavalier-Smith can reject syntrophy models on the grounds of being *too* complex, because they require major, multiple metabolic transfers and transformations. Phagotrophy models, however, can account more straightforwardly and 'simply' for the main features of eukaryogenesis (Cavalier-Smith, 2002, p. 318). According to this reasoning, the more factors involved, the more improbable such a scenario becomes.

But demands for elegant and tidy explanations may not match complicated biological reality, says Müller, the discoverer of the hydrogenosome and a major proponent of the hydrogen hypothesis (Lindmark & Müller, 1973). He once cautioned against seeking a common origin for all hydrogenosomes because of their presence

in such diverse and unrelated eukaryote groups (Müller, 1993, p. 2885). While common origins are now generally accepted (but see Hug et al., 2010; Dyall et al., 2004), it took the accumulation of enormous amounts of comparative data to allow the biological communities involved to reach this unstable agreement. It may be the case that messier explanations are most acceptable in the early stages of a research programme, when diverse data are yet to be fully categorized. With the ability to systematically analyse large amounts of information, the quest for a complete and straightforward explanation may arise again, when it is less risky to rule out more ornate accounts of diverse phenomena. Take, for example, the multiple but not obligatory functions of the mitochondrion, which include oxidative phosphorylation, hydrogen generation, haem biosynthesis and the production of iron sulphur clusters.[11] The only all-encompassing statement that can be made about mitochondrial function is that 'the chief function of [such] organelles is to be a membrane-bound compartment' for diverse biochemistry (Douglas, in Embley et al., 2003b, p. 203). While this satisfies what is known about mitochondria, broad but safe claims are not ranked very highly on the explanatory score-chart. A delicate balance of risk is often struck between plausibility and probability in relation to existing data in order to produce a more compelling evolutionary model.

This balance of reasoning can also work in other ways, *against* the most plausible chain of evolutionary events. One such scenario involves the suggestion that eukaryote cells evolved first and that some of them then lost their specialized cellular compartments (and acquired other things, such as cell walls and diverse biochemical pathways), thus making eukaryotes *ancestral* to all prokaryotes (for example, Reanney, 1974; Darnell, 1978; Forterre, 1995; Glansdorff et al., 2008). These biologists say there are good theoretical reasons behind such a counter-intuitive hypothesis. First, it counters 'biased' and 'prejudicial' views of prokaryotes as primitive and their evolution as a matter of simple to complex (for example, Forterre & Prangishvili, 2009), and, second, the evolutionary record is too sketchy to rule out life having been more complex in the deep past (Doolittle, 1978). But genetic and fossil data give few indications of the origins of life being eukaryotic; geochemistry and the fossil record are also negative or silent about such possibilities. Conversely, fossil evidence and geochemical inferences strongly suggest prokaryote-first scenarios. Even though the payoff for radically counter-intuitive hypotheses is very high, the cost is also extreme even if the hypothesis is partly vindicated (e.g., the Margulis case).

In less risky prokaryote-first strategies, phylogenetic plausibility is an important ground of evaluation for both models. The early basal position of supposedly amitochondriate eukaryotes lost much of its remaining appeal when revealed as a potential phylogenetic artefact (Embley & Martin, 2006). One group of the deposed Archezoa, Microsporidia, is now not even near the base of eukaryotes but has been placed phylogenetically in a slot in the fungal tree of life (Keeling, 1998). The very plausibility of placing any anaerobic organisms deep in the eukaryote tree is therefore questionable (Embley & Martin, 2006), meaning that any inferences drawn about eukaryote origins on the basis of these supposedly early eukaryotes is highly suspect too. A major problem arises every time plausibility is invoked, however, no matter which model of eukaryogenesis is involved. Not only do evidence and reasons have to be put forward for the possibility of particular events occurring, often on the basis of similar events elsewhere or at other times, but then a contradictory set of reasons has to be proposed for why key events in eukaryogenesis occurred so very rarely (Martin, 2005b). Plausibility arguments are made on the basis of such features being common now and thus being retrodictively possible in the past. But the nature of eukaryogenesis, accepted on both sides, is that it occurred once and never happened again. These particular epistemological tensions, between plausibility and improbability, are always at work in the background of eukaryogenesis models and other evolutionary hypotheses.

7.4. Irreducibility versus continuity of cell types

As Sections 5 and 6 make clear, neither side is able to claim that autogenous or exogenous factors exclusively construct the eukaryotic cell. However, each model deploys rather different claims about the nature of the evolving eukaryote cell. A few phagotrophy supporters (for example, Kurland et al., 2006) have argued vigorously that eukaryote cells cannot be reduced explanatorily to archaeal cells because eukaryotes share a unique, primordial and essentially eukaryotic nature. This claim by no means denies evolutionary transformations, but it does refuse the possibility that organisms with a prokaryote 'nature' and features could give rise to those with eukaryotic capabilities, such as phagotrophy. Autogeny and irreducibility work together in this line of reasoning. The eukaryote is irreducible in nature; its innovations come from the inside, not the outside. Often (sometimes implicitly), this argument is also making an epistemological case that biochemistry is reductionist, and that a model prepared to reduce organisms to metabolism and biochemistry is the type of model more likely to produce exogenous explanations of eukaryogenesis.

Irreducibility is a risky argument, not only because it is the main basis of the arguments made by 'Intelligent Designers' against evolution, but because it is always a suspect metaphysical claim and a refutable epistemological one. Complex cellular machinery, such as the mitochondrion and flagellum, can indeed be shown to evolve from very simple and limited components (Clements et al., 2009). There need not be any gaps or special pleading in the chain of connected components and events. But the main proponents of the irreducibility argument think that this risky strategy is worth it because what they are countering is 'a rigidly monotonic progression from simple to more complex states ... tacitly favoured by molecular biologists ... who view evolution as an irreversible march from simple prokaryotes to complex eukaryotes, from unicellularity to multicellularity' (Kurland et al., 2006, p. 1013). In other words, these phagotrophy supporters are suggesting that the very idea of prokaryotes giving rise to eukaryotes is an old-fashioned and discredited *scala naturae* kind of account.

Pitched against autogenist arguments is the syntrophy modellers' attribution of crucial importance to the initial process of endosymbiosis. The acquisition of the mitochondrion, and the subsequent evolution of endosymbiotic gene transfer and host protein transport apparatus to the proto-organelle, can be interpreted as 'a powerful and chimera-generating mechanism of natural variation that is truly unique to the eukaryote lineage' (Timmis et al., 2004, p. 133). This is the fundamental 'nature' of the eukaryote for the syntrophy modellers—an evolutionary process to be sure, but of similarly irreducible and unrepeatable importance to eukaryogenesis. Each group thus relies in complex ways on both the continuity and discontinuity of prokaryotes and eukaryotes as they make their arguments. For the phagotrophy supporters, the divide is between basic cell types, whereas for syntrophy supporters, the divide occurs on the basis of a continuity of integrated cell types giving rise to a new and fundamentally different mode of organismal organization. Overall, however, these are loose and sometimes post-hoc reflections, rather than rigid model-building rules. The debate is set up so that each side can be flexible in its justifications

[11] Production of FeS clusters appears to be the only activity carried out by all mitochondria, hydrogenosomes and mitosomes (van der Giezen, 2009).

and interpretations, while at the same time, efforts are continuously made to distinguish each model from the other.

8. Concluding reflections

The evolutionary gulf between prokaryote and eukaryote cells is a truly remarkable phenomenon, whether it is examined scientifically or philosophically. As new cell biology questions the fundamental nature of the differences between prokaryote and eukaryote cells (for example, Fuerst, 2005), and as other microbiologists issue increasingly strident calls for the rejection of the term prokaryote (for example, Pace, 2006; Glandsdorff et al., 2008), the very distinction between the two cell types becomes itself an object of contestation. Rather than pursuing that debate, my discussion has accepted an organizationally based classification of prokaryote and eukaryote, and focused on the two modes of eukaryogenesis. Each model makes appeals to autogeny and exogeny that not only hark back to older debates about preformationism and epigenesis (see Müller-Wille, 2010, this issue), but also to larger philosophical debates such as reductionism and anti-reductionism. It is important to note, however, the strategic uses of such epistemological claims, and how they help define competing interpretations of evidence in pragmatic and self-advancing ways.

A central question on which to reflect further is whether the epistemological tensions between the two models are harmful to competitive research programmes, or whether these disputes in fact enhance research efforts. It is certainly possible to think that the tensions in eukaryogenesis research are at least a little artificially constructed in order to make particular models seem to offer more, and for any finding to be seen as a solution to a major problem that is not only evolutionary but epistemological—about the best ways of doing evolutionary biology, and the best ways of justifying particular proposals. Although these different models are passionately advocated, keeping them separate and maintaining a debate may be highly constructive, because those activities keep open a diversity of channels of research and prevent a premature settling of consensus.

Due to the deeply historical and inferential nature of each eukaryogenesis model, and the admission on both sides that data will never be sufficient, epistemological claims are central to the debate and any evaluation of models. Such claims are always double-edged swords, however, working in one context for a model and in another against it. Scientists clearly recognize this potential, and so it is in their interests not only to keep their side of the sword sharp but to use the other side on their own work. Consequently, this particular history of contestation reveals many reversals of opinion and softening of central claims, as the influx of new data, new interpretations and new disciplinary formations shift the balance of acceptance and rejection. And for all those involved, premature resolution is avoided, because there is more to be gained from trying to fill evidential and inferential gaps than there is by setting them aside in gap-ridden agreement. The ongoing nature of contestation in eukaryogenesis research means there continue to be multiple avenues of study for philosophers and historians of biology, as they expand their horizons and develop methods for approaching recent and incomplete scientific histories.

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