A fungal root for the eukaryote tree

David Moore

Abstract

I offer a new interpretation of the early radiation of eukaryotes based on the emergence of major innovations in cell biology that apply uniquely to present day fungi. These emphasised increasingly detailed management of the positioning and distribution of membrane-bound compartments (vacuoles, vesicles and microvesicles) by the filamentous components of the cytoskeleton (microfilaments, intermediate filaments and microtubules); culminating, as far as filamentous fungi are concerned, with emergence of the Spitzenkörper and apical hyphal extension. I interpret *Tappania* fossils to be fully differentiated sclerotia of filamentous fungi, and so believe that the earlier, most ancient, stem eukaryotes exhibited characteristics of primitive (chytrid) fungi, emerging between 2000 and 1500 million years ago. The primitive eukaryotic stem featured primitive nuclear structures (including the nuclear membrane remaining intact during progress of the division; a characteristic of present day fungi), added the mitochondrion by enslavement of a bacterium, and evolved those aspects of the endomembrane system and cytoskeletal architecture that are also unique characteristics of present day fungi, in the following probable temporal sequence.

(**a**) **Free cell formation**, by managing positioning of wall- and membrane-forming vesicles to enclose volumes of cytoplasm to subdivide sporangia into spores, with adoption of a chitinous cell wall, possibly as an adaptation of muramopeptide oligosaccharide synthesis from the wall of an actinobacterial ancestor. This is a possible branch point to plants if the phragmoplast is assumed to be a vestige of free cell formation and the cell wall was adapted to be a polymer of glucose rather than *N*-acetylglucosamine, possibly for economy in usage of reduced nitrogen in organisms abandoning heterotrophy. Plants also evolved a means to disassemble the nuclear envelope to form the division spindle. (**b**) **Filamentous growth**, first to make rhizoids then apically-extending with the Spitzenkörper as the organising centre for hyphal extension and morphogenesis to make nucleated hyphae to explore and exploit the then extant biofilm and terrestrial debris of 2 billion years of prokaryote growth. (**c**) **Hyphal/cell fusion**, with associated cytoplasmic (vegetative) and nuclear (sexual) compatibility/incompatibility systems, hypha to hypha communication/recognition systems, autotropism, gravitropism, and intrahyphal communication using secondary metabolites, including the evolution of gametes. (**d**) **Hyphal septum formation**, initially dependent on a contractile ring of actin as a way to seal the membrane of damaged filaments rapidly, later developing ingressive wall synthesis to strengthen the seal, and ultimately cross-wall formation at regular intervals to initiate multicellular development. Possibly combined with the (accidental?) fixation on ergosterol as the quantitatively predominant sterol involved with controlling membrane fluidity in fungi. This is a possible branch point from chytrid level fungi to animals (choanozoa), with the animal stem gradually losing wall and adapting cytoskeletal organisation/vesicle trafficking originally used in wall synthesis to the new function of phagocytosis, and developing disassembly of the nuclear envelope to form the division spindle, cholesterol as the predominant sterol for membrane fluidity, and equatorially contractile cell division. Through this sequence of events filamentous fungi emerged 1.5 billion years ago as the first crown group of eukaryotes. They emerged to exploit the debris left by 2 billion years of prokaryote growth and they've been cleaning up the planet ever since.

1. Introduction

The rhythm of life on Earth includes several strong themes contributed by Kingdom Fungi. So why are fungi ignored when theorists ponder the origin and early emergence of life on this planet? From this review of the wide range of new material dealing with new experiments and concepts about the emergence of life on Earth that has become available in the last ten years or so I conclude that a coherent case can be made for an evolutionary process in which the fungal lifestyle or body plan features strongly.

I suggest that the last universal common ancestor (LUCA) was a heterotrophic, mesophilic prokaryote, essentially a bacterial cell with the cell enveloped by two distinct lipid bilayer membranes. Early prokaryotes used prebiotically synthesised organic carbon compounds as

nutrients but, as these supplies diminished, were outstripped by the anoxygenically photosynthetic Chlorobacteria as the most primitive surviving prokaryotic phylum. This interpretation follows the most recently-published deep phylogeny of the tree of life (Cavalier-Smith, 2006, 2010a) which considers thermophiles to have evolved late, making Archaebacteria the youngest bacterial phylum and the sisters (rather than ancestors) of eukaryotes, which diverged from actinobacterial ancestors.

Prokaryotes have dominated the Earth for the bulk of its history; LUCA must have emerged close to the start of the Archaean Eon, about 3.8 billion years ago, because some of the oldest microbial fossils are fully differentiated, photosynthetic bacteria (cyanobacteria) found in Western Australian sediments that are 3.5×10^9 years old (Schopf, 1993; Derenne *et al*., 2008; Boal and Ng, 2010). On the other hand, eukaryotes are generally thought to have appeared no earlier than about 1.5 billion years ago (and some people put their emergence somewhat later than that). So, for at least 2 billion years the only living organisms on the planet were prokaryotes together, presumably, with their associated viruses.

The abundant biological activity in the deep ocean volcanic hydrothermal systems of the present day, most of it being dependent on chemosynthesis rather than photosynthesis, has stimulated the widespread appeal of theories of a 'deep-hot' origin of life (Wächtershäuser, 2006; Alpermann *et al*., 2010). This implies that the pioneer organisms were hyperthermophiles (Stetter, 2006), a notion which builds upon Carl Woese's conclusion that the three domains, now called Eubacteria, Archaea and Eukaryota diverged from the universal ancestor of all organisms alive today (Woese, 1987; Woese *et al*., 1990). Emerging from these arguments we have what might be called a conventional, or 'textbook' phylogenetic tree of life (for example see Moore *et al*., 2011; p. 24). Unfortunately, gene trees are ambiguous and the root of the universal tree of life remains controversial (Penny and Poole, 1999). A significant aspect of the controversy is the origin of the defining characteristic of the eukaryotic cell, its nucleus; eukaryotes have one, prokaryotes, don't. Pennisi (2004) outlines the major theories that have been proposed to explain the origin of the nucleus. Some of these ideas strongly imply that the nucleus could date back to the LUCA, from which eukaryotes, bacteria, and archaea eventually diverged. If this is the case, some features of LUCA, such as the nucleus, were retained in eukaryotes but lost to various degrees in most archaea and bacteria. For my current argument I find it interesting that Penny and Poole (1999) dismiss fusion of a bacterium and an archaean (the archaean then evolving into the nucleus) on the grounds that it does not explain the origin of the nuclear membrane "…which is assembled and disassembled during cell division, quite unlike organellar membranes …". Of course, this criticism cannot apply to Kingdom Fungi. ***Characteristically***, nuclear divisions in fungi take place within the parental nuclear membrane. Consequently, by whatever route the eukaryotic nucleus arose, its most primitive expression survives in present day fungi. Perhaps, then, this is the first hint that present day fungi are the survivors of the most primitive eukaryotes.

The most complete reworking of the tree of life is that recently published by Tom Cavalier-Smith (Cavalier-Smith, 2006, 2010a & b). Cavalier-Smith's approach is to integrate palaeontology with comparative study of present day organisms, emphasising key steps in molecular and cellular evolution. Cavalier-Smith (2010a) identifies five successive kinds of cell: (i) The first cells were negibacteria, with cells bounded by two acyl ester phospholipid membranes, divided into the primitive anaerobic Eobacteria without lipopolysaccharide in the outer membrane and more advanced Glycobacteria with lipolysaccharide (e.g. oxygenic Cyanobacteria and Proteobacteria); (ii) unibacteria, with one bounding and no internal membranes, divided into desiccation-resistant posibacteria, ancestors of eukaryotes, and archaebacteria as the youngest bacterial phylum and a sister group (not an ancestor) of eukaryotes; (iii) eukaryotes with endomembranes and mitochondria, (eukaryotes plus archaebacteria make up the neomura); (iv) plants with chloroplasts; (v) chromists with plastids inside the rough endoplasmic reticulum.

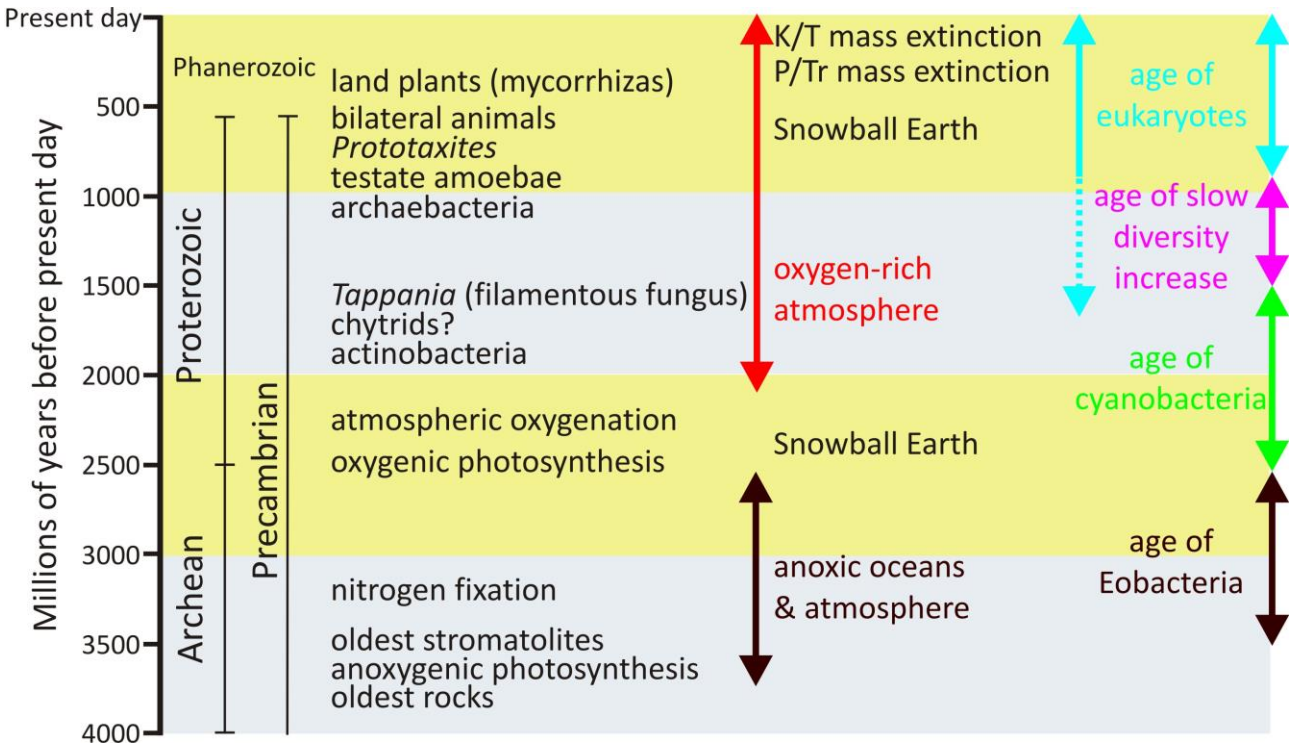

Fig. 1. A geological timescale covering from the time of the oldest rocks (3.8 billion years ago) to the present highlighting major geological and evolutionary events and features mentioned in the text, including Cavalier-Smith's four ages of life at extreme right. Note that Cavalier-Smith's age of eukaryotes starts 850-800 million years ago, but as I interpret *Tappania* fossils to be fully differentiated sclerotia of filamentous fungi I place the origin of stem (chytrid) eukaryotes between 2000 and 1500 million years ago. Modified and redrawn from Cavalier-Smith, 2010a.

These types of cell are placed into four ages of life as follows (Cavalier-Smith, 2010a; see Figs 1 and 2): (i) the **age of Eobacteria**, an anaerobic phase in which photosynthetic non-sulphur bacteria (and before them extinct stem negibacteria) were the major primary producers. Exclusively anaerobic life probably persisted from about 3.5 billion years ago to just under 2.5 billion years ago (the best date for the origin of photosystem II and start of oxygenic photosynthesis). (ii) The **age of cyanobacteria** (about 2.5-1.5 billion years ago) during which cyanobacteria were the major primary producers (and are now the dominant morphological fossils). Convincing fossils of various cyanobacteria have been dated to the later part of this period, including complex filamentous forms, some with heterocysts (= nitrogen fixation?). Extensive anaerobic habitats probably remained, especially in the deep ocean. The origin of eubacterial flagella was a major innovation during this age (enabling planktonic existence), and substantial metabolic diversification of chemotrophic and heterotrophic negibacteria. (iii) The **age of slow diversity increase** (1.5-0.85 billion years ago) features increasing morphological complexity and colonisation of continental surfaces by both Cyanobacteria and, following loss of the outer membrane, Posibacteria and the actinomycete Actinobacteria; the latter displaying the greatest morphological complexity. Some of the largest microfossils from this part of the middle Proterozoic have been attributed to eukaryotic algae, filamentous fungi or stem eukaryotes of undefined affinity, but Cavalier-Smith is sceptical of all such fossil identifications in this period. (iv) The **age of eukaryotes** and obvious macroorganisms (850-800 million years ago to the present). Cavalier-Smith (2006) argues that eukaryotes derived from an actinobacterial ancestor on the grounds (among others) that current Actinobacteria are the only eubacteria having phosphatidylinositol, which is one of the most important eukaryote phospholipids, required for eukaryote specific cell signalling. "...Thus, eukaryote membrane lipids probably came vertically from an actinobacterial ancestor, archaebacterial lipids originating in their [last common ancestor] after it diverged from eukaryotes." A further aspect of this argument is that

shortly after they diverged from eukaryotes, archaebacteria colonised hot, acid environments by evolving the ancestrally hyperthermophilic archaebacteria and later, one archaebacterial lineage evolved biological methanogenesis (Cavalier-Smith, 2006; pp. 977-978).

2. Towards eukaryotes

This last item (iv) encapsulates the revolutionary differences between the Cavalier-Smith model and the 'standard' three domain model based on Woese *et al*. (1990).

- The standard model perceives the archaebacteria as an ancient (over 3.5 billion years old) group of prokaryotes which was the ancestor of eukaryotes.
- The Cavalier-Smith model sees the Archaebacteria as sisters to eukaryotes, rather than their ancestors.

This difference also has major implications for the last universal common ancestor (LUCA). The standard three domain model gives credence to the belief that LUCA emerged from the iron-sulfur world of deep hot hydrothermal vents, which specifically means that LUCA was a hyperthermophile. But in the Cavalier-Smith model this cannot be true because hyperthermophiles are assumed to have appeared for the first time less than 800 million years ago; so this leaves open the possibility (which I believe to be true) that LUCA was a mesophile that arose in a temperate environment (Fig. 2).

Generally speaking I find the Cavalier-Smith model much more convincing because it is based on integration of such a broad range of data. So I accept Cavalier-Smith's narrative from the first appearance of living cells about 3.5 billion years ago (though I believe LUCA was a heterotroph) to the emergence of eukaryotes from an actinobacterial ancestor about 1 billion years ago (both dates give-or-take a few 100 million years). I part company with his version of the origin of eukaryotes which I think is wrong because it is: totally dismissive of fungi, and so animal centric that it equates the origin of phagocytosis with the origin of eukaryotes (e.g. "...the origin of phagocytosis by prey engulfment (which indirectly made the eukaryote cell...)..." Cavalier-Smith, 2010a, p. 123). This extreme position is taken without suggesting what selective advantage there might be in the essential intermediate steps towards phagocytosis.

Phagocytosis requires water management, precise membrane management of endocytosis and exocytosis, and full cytoskeletal management of enzyme, vesicle and vacuole movement and distribution. Although the selective advantage of such a process is self-evident now; I can't see how any advantage can be realised by some distant animal-ancestor that is just embarking on acquiring these many characters. But I think I can see how a fungus might do it, and Martin *et al*. (2003) saw at least part of the way:

> "…The view that osmotrophy had to precede phagotrophy in eukaryotic evolution is compelling because without importers, food vacuoles are useless… all fungi are osmotrophs…" (Martin *et al*., 2003; p. 199).

3. Rise of the fungi

Although fungal hyphae have few unique morphological features and most fungal structures are poor candidates for preservation over long periods of time as fossils, a respectable fossil record for fungi has been assembled in recent years. The most impressive of these are the nematophytes (particularly the fossil genus *Prototaxites*) which were terrestrial fungi found from the mid-Ordovician (460 million years ago) to the early Devonian, suggesting that they lasted a period of at least 40 million years (Hueber, 2001; Boyce *et al*., 2007). These fossils are among the 'nematophyte phytodebris' that constitutes the earliest evidence for terrestrial organisms. They were extremely large: "…specimens of *Prototaxites* over a metre wide have been reported...' (Wellman and Gray, 2000), and Francis Hueber has been photographed alongside specimens that are 2 to 3 m tall (illustrated in Moore *et al*., 2011; see pp. 33 & 34); but *Prototaxites* was also so common that it was a major component of these early terrestrial ecosystems, both in terms of abundance and diversity.

Prototaxites was by far the largest organism present in these ancient habitats; environments that did not include vascular plants, but were still dependent on the more ancient primary producers, cyanobacteria (blue-green algae), eukaryotic algae, lichens and mosses, liverworts, and their relatives (bryophytes). Carbon isotope ratios of individual *Prototaxites* fossils varied too much for them to be photosynthetic primary producers (Boyce *et al*., 2007). Instead, *Prototaxites* was a consumer, and taken together with direct microscopic observation of their anatomy (Hueber, 2001) it is concluded that these enormous fossils, the largest land organisms to have lived up to their point in time, were actually giant fungi. So the current understanding is that the first large terrestrial organisms were multicellular fungi that presumably developed to take advantage of 2 billion years' worth of accumulated bacterial, and eventually eukaryote, protist and bryophyte debris.

Other ancient fungal fossils are found in the exquisitely-preserved Devonian Rhynie Chert of Aberdeenshire in the north of Scotland (400 million years old); easily recognisable mycorrhizal fungi from the Glomeromycota and several other fungi have been found associated with the preserved tissues of early vascular plants (Taylor *et al*., 1997, 2004, 2006). Glomeromycotan fossils have also been found in mid-Ordovician rocks of Wisconsin (460 million years old). The fossilised material consisted of entangled, occasionally branching, nonseptate hyphae together with globose spores. The age of these fossil Glomeromycotan fungi indicates that such fungi were present before the first vascular plants arose, when the land flora consisted of bryophytes, lichens and cyanobacteria. Today, the Glomeromycota form the arbuscular mycorrhizal symbiosis, which is ubiquitous in modern vascular plants and has also been reported in modern hepatics and hornworts. It is reasonable to suppose that arbuscular mycorrhizas played an important role in the success of early terrestrial plants (Blackwell, 2000; Redecker *et al*., 2000).

So, convincing fossil evidence shows that fungi were important, even dominant, members of terrestrial ecosystems at least 500 million years ago. Well-developed filamentous fungi must have first appeared a long time before that, however. How long would it take the ancestors of *Prototaxites* to evolve the capability to produce organised mycelia structures several metres high; or the ancestors of the Rhynie Chert Glomeromycota to evolve the capability to form arbuscular mycorrhizas microscopically indistinguishable from those of the present day? Guessing at maybe 100 to 200 million years pushes 'well-developed filamentous fungi' back in time to about 700 million years ago. But there are much older (though disputed) fossils than that.

Butterfield (2005) assigned fossils extracted from formations in northwestern Canada, the deposition of which has been dated to between 800 and 900 million years ago, to the form-genus *Tappania*; describing the organism as:

> "…an actively growing, benthic, multicellular organism capable of substantial differentiation. Most notably, its septate, branching, filamentous processes were capable of secondary fusion, a synapomorphy of [trait shared by] the 'higher fungi' [of today]. Combined with phylogenetic, taphonomic and functional morphologic evidence, such 'hyphal fusion' identifies *Tappania* reliably, if not conclusively, as a fungus, probably a sister group to the 'higher fungi', but more derived than the zygomycetes." (Butterfield, 2005; abstract).

The form genus fossil *Tappania* is widespread, having been found in ancient shoreline carbonaceous shale deposits in Australia, Canada, and China. Specimens fossilised nearly 1.5 billion years ago in shales in northern Australia have been described as:

> "… *Tappania* populations consist of irregularly spheroidal organic vesicles up to 160 μm in diameter … distinguished by bulbous protrusions and from zero to twenty hollow, cylindrical processes … The processes have closed, slightly expanded terminations and may branch dichotomously … processes are distributed irregularly and asymmetrically on the vesicle surface … the irregular number and length, asymmetric distribution, and branching of processes in *Tappania* suggest an actively growing cell or germinating cyst. The bulbous protrusions in some specimens further suggest vegetative reproduction through budding…" (Javaux *et al*., 2001).

The asymmetric branching of processes and bulbous protrusions are interpreted as representing dynamic cell remodelling of a sort which is only made possible by the cytoskeleton and signalling pathways of eukaryotes. Javaux *et al*. (2001) go no further than to state that the systematic relationships of *Tappania* are uncertain, but its distinctive morphology indicates that "…the cytoskeletal architecture and regulatory networks that characterize living [eukaryote] protists…" were in place in organisms fossilised 1.5 billion years ago. However, Butterfield (2005) discusses these and other putative pre-Devonian fungi and concludes that "…there is a case to be made for an extended and relatively diverse record of Proterozoic fungi." Cavalier-Smith (2006; pp. 983-984) agrees with Butterfield's (2005) identification of *Tappania* as sporangial entities broken from a branching trophic hyphal network, but does not agree that these fossils are probably fungi. He suggests they could instead be actinobacterial pseudosporangia; I do not find this very convincing.

The large spheroidal microfossils shown in these *Tappania* papers are usually described as 'vesicles'. Butterfield's (2005) specimens, after being dissolved into slurry with 30% HF and filtered through a 62 μm mesh sieve, are described as follows:

> "…The fossils described here constitute a highly variable, bimodal continuum of forms. Those of the principal mode are based on a central vesicle bearing a variable number of irregularly distributed processes and occasional larger-scale outgrowths. The central vesicle ranges from spheroidal to elongate, and from 30 μm … to over 400 μm … in transverse dimension … Processes are typically heteromorphic and range from 0.3 μm … to >4 μm … in diameter. In some instances, simple cylindrical processes may be distributed relatively uniformly over the vesicle surface …; in others, they occur as isolated knoblike buds … or elongate filamentous extensions …. In most cases, however, the processes are further distinguished by distal branching … and a capacity to form closed loops through secondary fusion. This fusion appears to be relatively indiscriminate and gives rise to a wide range of expression: occasionally the processes return directly to the vesicle to form simple loops …; in other cases they have fused either with themselves … or, more commonly, with other processes …, resulting in a distally interconnected network …. Multiple layers of process networks are also developed, sometimes to the extent of obscuring the central vesicle … Such variability, combined with a recurrence of unfused buds-on both the vesicle … and processes … attests to the actively growing habit of these structures." (Butterfield, 2005; p. 167).

This is quoted in detail because I have spent most of my research life cultivating a basidiomycete fungus (*Coprinopsis cinerea*) which, in common with many other present day ascomycete and basidiomycete soil fungi produces abundant sclerotia in and on mycelial cultures:

> "…Mature aerial sclerotia were dark brown to black, more or less spherical and variable in size although most were in the range 100-250 μm in diameter. … three tissue layers were apparent - the outer diffuse layer, the rind and the medulla. The outermost diffuse layer … was composed of apparently dead hyphal cells whose cytoplasm was reduced to membrane fragments and vesiculate structures. Many had crenulate cell walls which may indicate they were damaged during preparation for sectioning. This outer layer, though only loosely attached and often sloughed off during fixation, was always present in mature aerial sclerotia and is therefore regarded as an integral part of their structure." (Waters *et al*., 1975a; p. 201; see also Waters *et al*., 1975b).

I have seen and handled a great many '*Coprinus*' sclerotia; fresh, in actively growing cultures including microcosms, desiccated in old stored cultures with collapsed and twisted outer-layer hyphae, fixed for LM and TEM, critical-point dried for SEM and, though I've never seen them after a billion years of preservation followed by dissolution into hydrofluoric acid, I would be willing to hazard the opinion that the *Tappania* 'vesicles' illustrated by Javaux *et al*. (2001) and Butterfield (2005) are all at least the sclerotia of filamentous saprotrophic moulds and soil fungi. I say 'at least' because in *C. cinerea* the same genetic pathway produces sclerotia (as vegetative survival structures) and/or the initials/primordia of the (mushroom) fruit body depending on temperature and illumination during cultivation (Moore, 1981). So the *Tappania* 'vesicles' may also be sclerotia or the initials of ascomata or basidiomata fruit bodies. Potentially, this interpretation means that

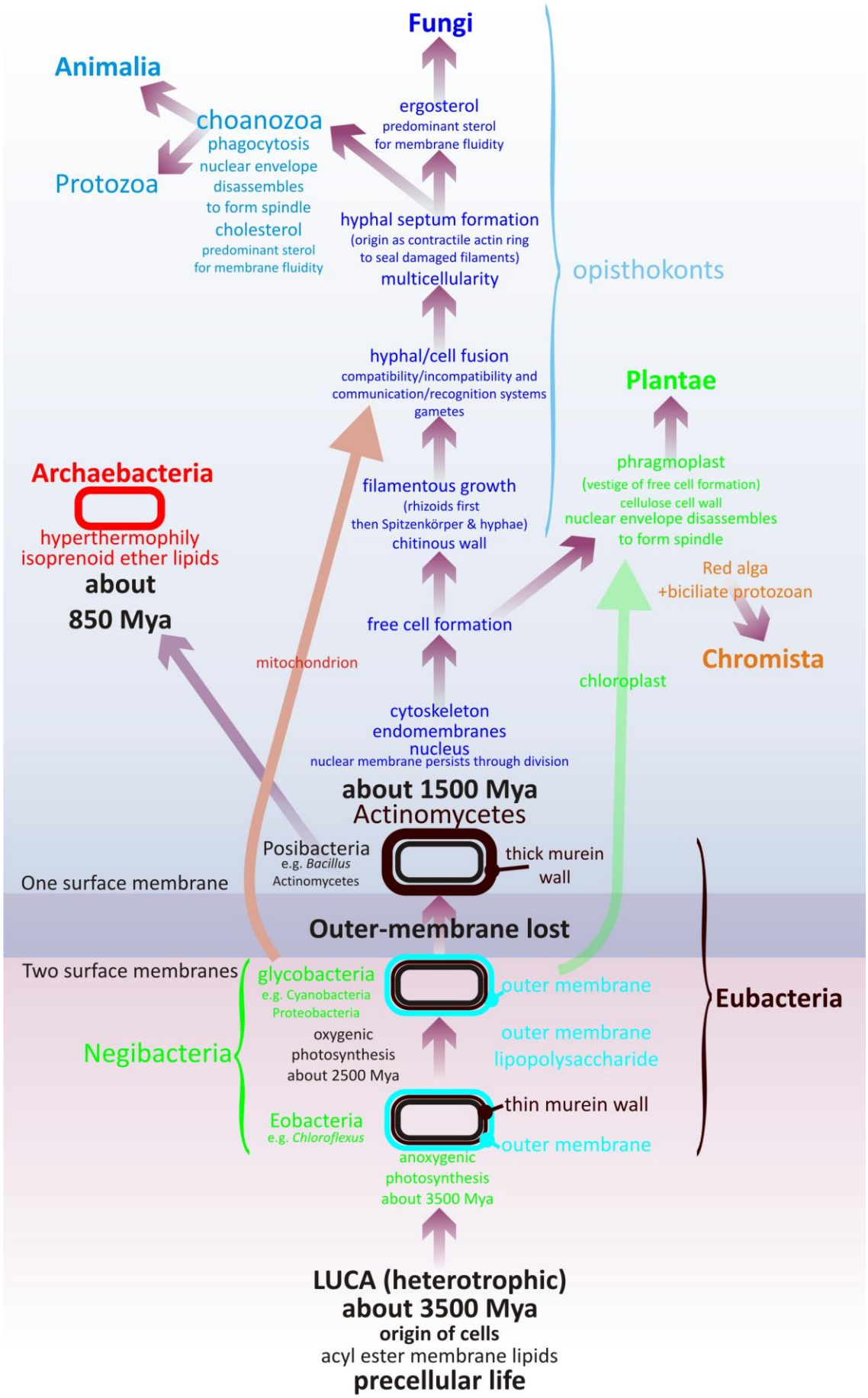

Fig. 2. The tree of life. The lower part of this diagram is based on Cavalier-Smith's tree of life (Cavalier-Smith, 2010a; his Fig. 6), which emphasises major evolutionary changes in membrane

topology and chemistry, except that the most ancient bacteria are shown here to be heterotrophic descendants of LUCA (the last universal common ancestor). Eukaryotes diverge from actinobacterial ancestors about 1500 Mya (million years ago) and the bulk of this illustration deals with eukaryote evolution. The most ancient stem eukaryotes are considered to exhibit characteristics of primitive fungi. Their evolution emphasises increasingly detailed management of the positioning and distribution of membrane-bound compartments (vacuoles, vesicles and microvesicles) by the filamentous components of the cytoskeleton (microfilaments, intermediate filaments and microtubules); culminating, as far as filamentous fungi are concerned, with emergence of the Spitzenkörper and apical hyphal extension.

filamentous moulds able to regulate hyphal branching and hyphal interactions with sufficient finesse to assemble multicellular survival and, perhaps, reproductive structures, were common and widespread 1.5 billion years ago.

One way to achieve this is to suggest, as did Martin *et al*. (2003), that a eukaryotic phylogenetic tree with fungi first would make sense (Martin *et al*., 2003, p. 197). These authors based their overall tree of life on the standard three-domain model and showed the stem eukaryotes as emerging from within the archaebacteria. I would adhere, as above, to the four ages of life as set out by Cavalier-Smith (2010a) but would start the age of eukaryotes about 1.5 billion years ago and amend the origin of eukaryotes as follows (Fig. 2).

The eukaryotic stem added the mitochondrion by enslavement of a bacterium (and perhaps added the nucleus by enslavement of an archaean, depending on the timing of divergences of prokaryote groups), and later evolved the endomembrane system and cytoskeletal architecture. The following features, which in the present day are characteristics of fungi, emerged in this temporal sequence:

1. **Free cell formation**, the cytoskeletal organisation to manage vesicle and organelle trafficking and particularly the positioning of wall- and membrane-forming vesicles to enclose volumes of cytoplasm to subdivide sporangia into spores (see discussion in (Moore *et al*., 2011, pp. 48-50), with adoption of a chitinous cell wall, possibly as an adaptation of the ancestral actinobacterial mechanism for addition of oligosaccharides containing N-acetylglucosamine to surface proteins (muramopeptide wall precursors).
    - After this process is established, this is a potential branch point for divergence to plants with phragmoplast formation left as a vestige of free cell formation specifically localised at the division spindle equator, and the early cell wall adapted to be a polymer of glucose rather than *N*-acetylglucosamine, possibly to economise on the demand for reduced nitrogen in an organism that is abandoning heterotrophy.
2. **Filamentous growth**, first to make rhizoids in chytrids then apically-extending with the Spitzenkörper as the organising centre for hyphal extension and morphogenesis to make nucleated hyphae to explore and exploit the then extant biofilm and accumulated terrestrial debris of 2 billion years of prokaryote growth. Limiting extension growth to the hyphal apex involves creation of a coordinated production and distribution system for wall and membrane precursors and enzymes; together with a cytoskeletal delivery system *and* a cytoskeletal tethering system to stabilise the wall, weakened by insertion of new precursors, against osmotic stress (see discussion of the consensus model of tip extension in Moore *et al*., 2011, pp 137-144; Steinberg, 2007; Read *et al*., 2009, 2010; Riquelme *et al*., 2007; Riquelme and Bartnicki-García, 2008).
3. **Hyphal/cell fusion**, evolved to convert the otherwise radially-arranged hyphae in the central regions of a maturing colony into a fully interconnected network through which materials and signals can be communicated efficiently. The selective advantage here is that the physical integration allows the vegetative mycelium to make best use of the resources its exploration has discovered. Fusion primarily involves joint adaptation of Spitzenkörper function to enable organised disassembly of two hyphal walls in contact (without risking osmotic stress to either

hypha) and their two cell membranes to make the two cytoplasms coextensive (Glass *et al*., 2004). Once the process of hyphal fusion has been established as a means of enhancing the efficiency of the mycelium it could be adapted to other functions within and between mycelia. This would include the creation, for the first time, of multicellular structures and provision of a route for intrahyphal communication for their regulation using secondary metabolites; the emergence of cytoplasmic (vegetative) and nuclear (sexual) compatibility/incompatibility systems (self/non-self recognition) which on the one hand would allow cytoplasmically compatible mycelia to exchange of nuclei and form heterokaryons and on the other hand select exchange of dissimilar nuclei as a prelude to sexual reproduction and all that that means for evolutionary progress. Evolution of autotropism, gravitropism, and other tropisms can be seen as part of this evolutionary thread, although the fundamental basis of a fungal tropism is the directional steering of the Spitzenkörper.

4. **Hyphal-septum (cross wall) formation**, is primarily a way of protecting the exploratory extending hyphal filaments from the hazard of loss of cytoplasmic contents following puncture of the osmotically pressurised hydrostatic system. There is, consequently, selective advantage in developing a contractile ring of actin as a way to seal damaged filaments rapidly; and then to elaborate this with ingressive wall formation, first to make complete (imperforate) septa to isolate particular parts of the hyphal network (spore-forming branches, for example) and then to refine this to regularly deployed perforate septa that allow longitudinal communication along the hypha to be maintained but, combined with a rapidly-deployed septal pore plug also save punctured hyphae from leaking to death (Moore *et al*., 2011, pp. 144-150; and see Steinberg and Schuster, 2011, for illustration of the dynamic behaviour of major cytoskeletal elements and organelles in fungal cells).
    - After these processes are established this becomes a potential branch point for divergence to animals (choanozoa), gradually losing the rigid wall and adapting the cytoskeletal organisation/vesicle trafficking originally used in wall synthesis and stabilisation to new functions of phagocytosis, locomotion and contractile cell division.
5. This branch event could also have been the point in time when fungi became (possibly accidentally) fixed on ergosterol as the quantitatively predominant sterol involved with controlling membrane fluidity in contrast to the cholesterol used in animals.

This sequence of events (Fig. 2) allowed filamentous fungi to emerge about 1.5 billion years ago as the first crown group of eukaryotes. They developed to exploit a particular environment: the debris left by 2 billion years of prokaryote growth. Above the strand lines of oceans, lakes and rivers dead and dying prokaryote microbial mats had been tossed by storm and tempest, dried in the unfiltered rays of a brightening sun, and cracked and broken by wind and rain until covered by the detritus thrown up by the next storm. For two thousand million years. This is what awaited the first filamentous fungi; probably the first instance of an oft-repeated feature of fungal evolution, namely that fungi benefit from wide-scale extinction events. The period 800 to 600 million years ago featured three successive virtually global glaciations (snowball Earth episodes). Cavalier-Smith (2010a, p. 127) suggests these "…surely would have retarded early protist diversification…" but I can see these episodes prompting and benefitting diversification of fungi in general and filamentous fungi in particular to exploit the death and destruction of other organisms in the same way that fungi benefitted at later extinction events.

Analysis of the Permian-Triassic (P-Tr) extinction event that occurred approximately 251 million years ago (known as the Great Dying and the Earth's most severe extinction event so far) includes the quotation:

> "…sedimentary organic matter preserved in latest Permian deposits is characterised by unparalleled abundances of fungal remains, irrespective of depositional environment (marine, lacustrine [= lake sediments], fluviatile [=river/stream deposits]), floral provinciality, and climatic zonation." Visscher *et al*. (1996, quotation comes from the abstract).

Much the same is true for the Cretaceous-Tertiary (K-T) extinction of 65 million years ago, the result of a meteor collision that caused the Chicxulub crater in Mexico, which is blamed for the extinction of the dinosaurs. There was also widespread deforestation right at the end of the Cretaceous, which is assumed to be due to post-impact conditions. However, coincident with all this death and destruction of animal and plant life at the K-T boundary there is a massive proliferation of fungal fossils:

> "…This fungi-rich interval implies wholesale dieback of photosynthetic vegetation at the K-T boundary in this region. The fungal peak is interpreted to represent a dramatic increase in the available substrates for [saprotrophic] organisms (which are not dependent on photosynthesis) provided by global forest dieback after the Chicxulub impact." (Vajda and McLoughlin, 2004).

So it is the same story as at the other extinction boundaries: while the rest of the world was dying, the fungi were having a party!

But that Chicxulub meteor might not have had the last word on dinosaur extinction, because the massive increase in the number of fungal spores in the atmosphere of the time may have caused fungal diseases that "…could have contributed to the demise of dinosaurs and the flourishing of mammalian species…" Casadevall (2005). A reminder, perhaps, that the fungi started the eukaryote journey by spring-cleaning the early Earth, and they've been cleaning up and modifying the planet and its biosphere ever since.

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