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Abstract

Eukaryotic organisms radiated in Proterozoic oceans with oxygenated surface waters, but, commonly, anoxia at depth. Exceptionally preserved fossils of red algae favor crown group emergence more than 1200 million years ago, but older (up to 1600-1800 million years) microfossils could record stem group eukaryotes. Major eukaryotic diversification ca. 800 million years ago is documented by increase in the taxonomic richness of complex organic-walled microfossils, including simple coenocytic and multicellular forms, as well as widespread tests comparable to those of extant testate amoebae and simple foraminiferans and diverse scales comparable to organic and siliceous scales formed today by protists in a number of clades. Mid-Neoproterozoic establishment or expansion of eukaryophagy provides a possible mechanism for accelerating eukaryotic diversification long after the origin of the domain. Protists continued to diversify along with animals in the more pervasively oxygenated oceans of the Phanerozoic Eon.

Introduction

Eukaryotic organisms have a long evolutionary history, recorded, in part, by conventional and molecular fossils. For the Phanerozoic Eon (the past 542 million years), eukaryotic evolution is richly documented by the skeletons (and, occasionally, non-skeletal remains) of animals, as well as the leaves, stems, roots and reproductive organs of land plants. Phylogenetic logic, however, tells us that eukaryotes must have a deeper history, one that began long before the first plant and animal fossils formed. To what extent does the geological record preserve aspects of deep eukaryotic history, and can the chemistry of ancient sedimentary rocks elucidate the environmental conditions under which the eukaryotic cell took shape?

EXPECTATIONS FROM COMPARATIVE BIOLOGY

The diversity of eukaryotic organisms observable today makes two sets of predictions for the fossil record, one phylogenetic and the other preservational. Phylogenies suggest the relative timing of diversification events through Earth history, and when incorporated into molecular clocks, provide quantitative estimates of divergence times. In turn, experimental and observational studies of post-mortem decay indicate that only a subset of eukaryotic clades are likely to be represented in the geologic record and these only under selected environmental circumstances. Together, insights into phylogeny and preservation provide an empirical guide to paleobiological exploration.

Molecular sequence comparisons have revolutionized our understanding of evolutionary relationships among eukaryotes, but consensus on eukaryotic phylogeny remains elusive. Most workers recognize a limited number of major clades, including the opisthonkonts, amoebozoans, excavates, plants (*sensu lato*), and a SAR clade containing the stramenopiles, alveolates, and

rhizarians (e.g., Katz 2012), and many recognize the potential for as yet poorly characterized taxa to expand that roster (e.g., Patterson 1999, Adl et al. 2012). Persistent uncertainties include the position of the root; placement of groups such as centrohelid heliozoans, haptophytes and cryptomonads; and both the monophyly and relationships of photosynthetic lineages commonly grouped as Plantae.

Molecular clocks calibrated by phylogenetically well-constrained fossils have been used to estimate the timing of early eukaryotic diversification. Choice of algorithm can strongly influence these estimates (Roger and Hug 2006), but sensitivity tests suggest that for a given set of sampled taxa at least some estimates are broadly robust to tree topology and calibration choices (e.g., Parfrey et al. 2011). Molecular clock estimates generally agree that much protistan diversification has taken place during the Phanerozoic Eon, paralleling the diversification of animals, plants and fungi. They also agree on an earlier, Neoproterozoic radiation within the major eukaryotic clades, beginning perhaps 800 million years ago (Ma). Where clocks disagree is on the date for the last common ancestor of extant eukaryotes, with some positing a long interval of eukaryotic evolution before Neoproterozoic radiation (e.g., Yoon et al. 2004; Hedges et al. 2004; Parfrey et al 2011), while others suggest a shorter fuse (e.g., Douzery et al. 2004, Berney and Pawlowski 2006; Chernikova et al. 2011; Shih and Matzke 2013). The differing predictions of these clock estimates can be tested against the Proterozoic fossil record.

How do protists impart a paleobiological signature to sedimentary rocks? Mineralized protistan skeletons can form significant sedimentary accumulations – the White Cliffs of Dover, for example, consist mostly of small calcitic scales made by coccolithophorid algae. Tests and scales of calcite and silica document Phanerozoic evolutionary histories for diatoms, chrysophytes, coccolithophoids, foraminiferans and radiolarians, but do not extend into

Proterozoic rocks (Knoll and Kotrc, in press). Organic cell walls, tests, and scales can also survive bacterial decay, depending on molecular composition, and in Phanerozoic rocks these remains record clades that include dinoflagellates and prasinophyte green algae, among other groups. As we shall see, decay-resistant organic walls, tests and scales also document aspects of Proterozoic protistan evolution, although it can be challenging to relate preserved fossils to extant clades.

Preservation, of course, is not the only hurdle in paleobiological investigations of Proterozoic rocks. There is also the challenge of recognition. In the first instance, how do we identify a fossil as eukaryotic rather than bacterial? Given that the record is one of morphology and not DNA or cytology, diagnostic characters must be sought in the size, shape, ultrastructure and preservational circumstances of microfossil populations. Eukaryotic cells are commonly larger than bacteria and archaeons, but are not invariably so. Conversely, cyanobacteria commonly form extracellular sheaths and envelopes that may encompass many cells; this being the case, burial can preserve a hundred micron cyanobacterial envelope that, in life, surrounded numerous micron-scale cells. By itself, then, size is commonly an insufficient criterion for eukaryotic attribution. Cyst walls associated with resting stages in eukaryotic life cycles commonly have spines or other ornamentation, and they commonly have complex ultrastructure as observed via TEM (e.g., Javaux et al. 2004). Bacteria can have large envelopes (and more rarely cell size; Schultz and Jørgensen 2001), but they rarely if ever combine large size, ornamented walls, complex ultrastructure and a preservable composition; thus, fossils that display all of these characters are widely regarded as eukaryotic.

Molecular fossils provide another means by which eukaryotic organisms can impart a signature to the geologic record. Proteins and nucleic acids have a low probability of

preservation, but lipids can preserve well, and sterols in particular have been used to investigate the deep history of eukaryotes. Abundant steranes (the geologically stable derivatives of sterols) extracted from petroleum document a Phanerozoic history of primary producers in the oceans that, to a first approximation, parallels the histories inferred from microfossils and molecular clocks (Knoll et al. 2007). Molecular fossils extend that record into Proterozoic and, more controversially, late Archean rocks.

Together then, phylogenies and preservation potential furnish guides to the paleobiology of early eukaryotic evolution, providing hypotheses of evolutionary history. How well do fossil fit these predictions?

ARCHEAN EUKARYOTES?

The three domain view of life, predicated on comparisons of SSU rRNA gene sequences, posited that eukaryotes are sister to the Archaea (Woese et al. 1990). Given this relationship and isotopic evidence for methanogenesis and methanotrophy in the late Archean carbon cycle (Hayes 1994), logic would dictate that the Eukarya existed no later than about 2700 Ma. This logic, however, is challenged by alternative phylogenies that nest eukaryotes within the Archaea (Williams et al. 2012) and by models for eukaryogenesis that rely on archaeal-bacterial symbiosis (Moreira and Lopez-Garcia 1998, Martin and Müller 1998). Even the most generous molecular clock estimates place the last common ancestor of extant eukaryotes within the Proterozoic Eon (Hedges et al. 2004), requiring that any Archean eukaryotes be stem groups.

Unfortunately, the record of life in Archean rocks is sparse and subject to conflicting interpretations. Early diagenetic cherts, a rich source of microfossils in Proterozoic strata, are largely barren, perhaps reflecting the strong influence of hydrothermal fluid flow and iron

deposition on the silica cycle of Archean oceans (Fischer and Knoll 2009; Chakrabarti et al. 2012). Shales, in turn, contain abundant organic carbon, but few structurally preserved or morphologically distinctive microfossils. One of the few well documented and widely accepted fossil occurrences in earlier Archean rocks, and perhaps the only one that potentially bears on stem group eukaryotes, comes from 3200 Ma shales that contain large (30-300 μm) spheroidal vesicles (Javaux et al. 2010). These appear to be genuine fossils, and they could, in principle, be eukaryotic; however, their simple ultrastructure and ready comparison to the extacellular envelopes of some bacteria saps confidence from such an interpretation.

In the absence of a convincing microfossil record, geobiologists have turned to molecular fossils. Steranes of hypothesized eukaryotic origin have been reported from late Archean sedimentary rocks (Brocks et al. 1999, Waldbauer et al. 2009), potentially documenting early eukaryotes, but raising incompletely resolved environmental, phylogenetic, and geological issues. The environmental concern is that sterol biosynthesis requires molecular oxygen, yet geochemical data consistently indicate that the Archean atmosphere and oceans were anoxic (Holland 2006). Sterol synthesis is possible at nanomolar oxygen tensions (Waldbauer et al. 2011), and so a plausible but unproven solution holds that early cyanobacteria could have generated local oxygen oases within mats or sediments long before O_2 began to accumulate in the atmosphere (e.g., Anbar et al. 2007).

The second issue is phylogenetic. A limited number of bacteria synthesize sterols (e.g., Pearson et al. 2003), raising the concern that preserved biomarkers could be of prokaryotic origin. In general, however, bacterial sterol synthesis is limited to simple products such as lanosterol, and so it is fair to consider more complex steranes of the type found in late Archean rocks as eukaryotic until and unless complex sterol synthesis is demonstrated in free-living

bacteria. The third concern, however, is not so easily dismissed. Steranes in late Archean rocks occur in part per billion concentrations, so geological or modern contamination must be considered. Fluids flow through sedimentary rocks throughout their history, and biomarkers can also be emplaced during the processes of drilling and sample processing. Arguments in favor of an indigenous origin for Archean steranes stress the care taken in sample preparation and the varying biomarker composition of different beds, consistent with the expectation of ecological heterogeneity at time of deposition (Brocks et al. 2003; Waldbauer et al. 2009). Further support comes from steranes and other biomarkers in fluid inclusions from quartz particles in 2400 Ma sedimentary rocks (Dutkiewicz et al. 2006). Critics counter that biomarker heterogeneity could reflect bed by bed variations in porosity and permeability, channeling later flow fluid, and note that steranes occur mostly near the surface of drill samples (Brocks 2011) and have a carbon isotopic composition distinct from the bulk of the organic matter in the samples (Rasmussen et al 2008). The debate continues, commendably fueled by new sampling programs marked by stringent protocols for drilling and sample preparation.

PROTEROZOIC ESTABLISHMENT

As noted above, molecular clocks suggest that regardless of any deeper stem group history, crown group eukaryotes emerged during the Proterozoic Eon. (In this discussion, the term “crown group” is used in its broadly accepted phylogenetic sense to indicate the last common ancestor of all extant members of a clade and its descendants. Earlier, informal usage to denote a diverse subset of eukaryotes has been abandoned.) The last common ancestor of extant eukaryotes possessed a mitochondrion capable of aerobic respiration, consistent with geochemical evidence for the permanent oxygenation of Earth’s atmosphere and surface ocean

about 2400 Ma (Holland 2006). Quantification of Proterozoic oxygen levels is difficult, but the persistence of anoxic water masses beneath the surface mixed layer of the oceans suggests that pO_2 remained low, perhaps no more than a few percent of present day atmospheric levels (e.g., Brocks et al. 2005, Canfield 2005, Scott et al.2008, Johnston et al. 2010, Frei et al. 2013). The chronic challenge of anoxic waters mixed upward from the oxygen minimum zone is consistent with the widespread occurrence in mitochondria of genes for anaerobic metabolism (Müller et al. 2012).

Bangiomorpha pubsecens (Fig. 2E, Butterfield 2000) plays a key role in evaluating crown group-early and –late hypotheses based on molecular clocks. An exceptionally well preserved population of filamentous microfossils found in silicified peritidal carbonates from Arctic Canada, *Bangiomorpha* displays a number of morphological features that collectively place it within the red algae. These include overall morphology, details of thallus development and reproductive biology, cellularly differentiated holdfasts, life cycle characteristics, and details of preservation that differ markedly from those characteristic of silicified cyanobacteria. Many specimens are preserved in life position, rising vertically from attachment sites on the ancient seafloor (Butterfield 2000). Thus, *Bangiomorpha* is reasonably interpreted as a rhodophyte, although it may branch earlier within the clade than extant Bangiales. Multiple geochronological and stratigraphic constraints indicate that *Bangiomorpha* lived ca. 1100-1200 Ma (summarized in Knoll et al. 2013). Neither its age nor its phylogenetic attribution are likely to change markedly with continued study, and so *Bangiomorpha* favors molecular clocks that place the both the last common ancestor of extant eukaryotes and the acquisition of plastids before about 1200 Ma.

Some paleontologists propose that crown group eukaryotes can be traced further back in time. For example, Moczydłowska et al. (2011) have argued that microfossils of green algae

occur in rocks as old as 1800 Ma. Accepting that green algae (and land plans) are sister to the red algae, greens must have lived contemporaneously with 1100-1200 Ma *Bangiomorpha*, and molecular clock estimates not precluded by *Bangiomorpha*'s age suggest that the green-red split occurred up to several hundred million years before this (Wang et al. 1999, Yoon et al. 2004, Parfrey et al. 2011). (If, as sometimes proposed, red algae are sister to greens plus glaucocystophytes, the green clade could have radiated later.) The older fossils in question, however, are simple spheroids whose affinities are not easily ascertained (Fig. 1A and B). Moczydłowska et al. (2011) maintain that only algae make resistant cysts with ornamentation and well-defined excystment structures, but resting cysts have been well described in various heterotrophic protists, including, for example, ciliates that fashion large, spheroidal, and sometimes ornamented cysts with pylome-like excystment structures (e.g., Beers 1948, 1966, Foissner et al. 2007, Verni and Rosati 2011). Wall ultrastructure might provide more definitive evidence of green algae, especially should TEM reveal the distinctive trilaminar sheath structure characteristic of cell walls in some chlorophytes (Allard and Templier 2000). TLS has been demonstrated in Cambrian green algae (Talyzina and Moczydłowska 2000), but not in the older microfossils under consideration here (Javaux et al. 2004). Biomarker molecules might also provide insight, but steranes are rare in mid-Proterozoic rocks (Brocks et al. 2005; Pawlowska et al. 2013; see below), and growing evidence suggests that algaenan, an aliphatic polymer known to be synthesized by a limited diversity of green algae (Kodner et al. 2009), can also form during diagenesis (Gupta et al. 2009) – molecular clocks suggest that TLS and algaenan-synthesizing green algae doubtfully extend much below the Proterozoic-Cambrian boundary. Thus, it remains uncertain whether earlier Proterozoic microfossils record crown group green algae, stem group greens (or Plantae), another crown group clade, or stem group eukaryotes (Knoll et al. 2006).

In general mid-Proterozoic sedimentary rocks contain abundant, but only modestly diverse fossils of probable eukaryotic origin (Javaux 2011). Shales up to about 1600 Ma contain microfossils that combine large size (> 100 μm) with complex ultrastructure, structurally complex ornamented or tessellated cell walls, and surface processes of varying form (Fig. 1C and D, Xiao et al., 1997; Javaux et al. 2001, 2003, 2004; Yin and Yuan 2007, Nagovitsin et al. 2010). Equally large vesicles with less distinctive surface morphology or ultrastructure occur in rocks as old as 1800 Ma (Fig. 1A, Yan 1995, Lamb et al. 2009). These may well be eukaryotic, especially those with corduroy-like, raised parallel ridges on wall surfaces (Fig. 1B, Yan 1995). For most, however, a lack of diagnostic features underscores residual uncertainty at the domain level. Macroscopic impressions and compressions whose regular morphology suggests a eukaryotic origin also occur in rocks of mid-Proterozoic age (Fig. 1E, Grey et al. 1990, Walter et al. 1990, Retallack et al. 2013), with the oldest overlapping in age with possible eukaryotic microfossils (Hofmann and Chen 1981; Han and Runnegar 1992). Most of this record comes from marine rocks, but a rare glimpse of life in Proterozoic lakes has been reported from 1200-1000 Ma beds in Scotland that preserve a moderate diversity of microfossils likely sourced by protists but otherwise of problematic origin (Strother et al. 2010).

From the preceding paragraphs we can draw two conclusions. First, while one might hope for a clean paleobiological boundary between worlds with and without eukaryotic cells, the geologic record actually presents a sliding scale of certainty, from confidently interpreted protists in 1400-1600 Ma rocks to more ambiguously interpreted remains at 1800 Ma and even more debated morphological and molecular signatures in older successions: paleobiological evidence of eukaryotic cells does not so much bottom out as fade away. The second conclusion is that the early eukaryotic record could be dominated by stem group taxa. Stem groups are a logical

necessity in biology, but an empirical challenge for paleontologists. The fossil records of plants and animals contain diverse stem group taxa at varying hierarchical levels, but their characters cannot always be inferred from comparative biology alone – what biologist would have predicted that stem group birds include quadrupeds up to 30 meters long? At present, the inference that early protistan fossils might record stem group eukaryotes (or unrecognized stem groups of major eukaryotic clades) owes more to the absence of diagnostic characters than it does to readily interpreted character combinations.

NEOPROTEROZOIC RADIATION

Fossil diversity increased only moderately over the first half of recorded eukaryotic history. Then, about 800 Ma, things changed in the oceans: both molecular clocks and fossils indicate pronounced diversification within major eukaryotic clades at this time. Organic-walled fossils preserved as compressions in shallow marine mudstones show unprecedented taxonomic richness, including both resting cysts and vegetative cells with complex morphologies, as well as an increased diversity of coenocytic and simple multicellular populations (Fig. 2A-C, Butterfield et al. 1994; Butterfield 2004, 2005a, 2005b). In early interpretations many of these fossils were assigned to specific eukaryotic clades, including xanthophytes, green algae, and fungi. Molecular clocks suggest that some of these attributions reflect convergence, but morphology, molecular clock estimates, and preservational potential all support the interpretation of distinctive coenocytic fossils in 750-800 Ma rocks as Cladophoralean green algae (Fig. 2C, Butterfield et al. 1994; Graham et al., 2013). The number of well preserved fossil assemblages in rocks of this age remains small, and it is possible that continuing exploration will pull the record of accelerating diversification deeper into the past. At present, however, exceptionally preserved

microfossil assemblages in older rocks do not record the diversity documented in their 750-800 Ma counterparts (Knoll et al 2006). Nor is the increase in eukaryotic diversity matched by a jump in observed cyanobacterial diversity, again suggesting that the observed record is not simply an artifact of sampling.

Two classes of eukaryotic fossils are completely unrecorded before ca. 800 Ma. Vase-shaped microfossils comparable to tests made by testate amoebans and some simple foraminiferans occur abundantly in mid-Neoproterozoic rocks around the world (Fig 2D, Porter and Knoll 2000). Their distinctive mode of preservation, most commonly as casts and molds conspicuous in petrographic thin sections of shale, carbonate and chert, lowers the probability that these fossils have a deeper history yet to be discovered. More than a dozen taxa have been distinguished, and at least some bear close comparison to the tests made today by arcellid amoebozoans (Porter et al. 2003). Others have been compared to euglyphid rhizarians, but young molecular clock estimates for euglyphid diversification suggest, once again, that observed similarities may reflect convergence (Berney and Pawlowski 2006).

The other novel class of microfossils is 10-30 μm scales preserved in ca. 800 Ma rocks from northwestern Canada (Fig. 2F-H). Originally reported by Allison and Hilgert (1986), the fossils were observed in thin sections of chert nodules and interpreted as siliceous scales broadly comparable to those of extant chrysophytes. The discovery, however, that the scales are preserved by mineral phosphate (Cohen et al. 2011) prompted a restudy in which thousands of specimens were recovered by the dissolution of encompassing limestones in weak acid. Some 38 distinctive scale types have been documented in exceptional morphological detail (Cohen and Knoll 2012), making these the most diverse eukaryotic fossils known before the Ediacaran diversification of animals. The fossils are assuredly eukaryotic and bear functional comparison

to organic or siliceous scales synthesized by diverse protists today. Phylogenetically, however, it is challenging to place any of these taxa within specific eukaryotic clades. Uncertainty remains, as well, as to the original composition of the scales: does the observed phosphate record *bio*mineralization (Cohen et al. 2011; interesting if correct, as phosphatic biomineralization is extremely rare among living protists) or early diagenetic phosphate replication within sediments (Cohen and Knoll 2012)? In either event, the scale assemblage from northwestern Canada is, for now, unique.

Fossils, then, record an apparent burst of Neoproterozoic diversification. This paleontological expansion is mirrored by molecular clock estimates, but not, intriguingly, by molecular biomarkers in Neoproterozoic rocks (Pawlowska et al. 2013). Should we interpret the dearth of steranes in pre-Ediacaran sedimentary rocks as evidence of absence or an absence of evidence? Given the close stratigraphic correspondence between the microfossil and biomarker records of Phanerozoic primary producers (Schwark and Empt 2006; Knoll et al. 2007), the lack of eukaryotic biomarkers in older strata has commonly been taken to indicate bacterial (especially cyanobacterial) dominance of primary production. Pawlowska et al. (2013), however, argue that this pattern owes more to preservation than production.

In Pawlowska et al.'s (2013) view microbial mats that covered Proterozoic seafloors were the primary sources of sedimentary organic matter preserved in Proterozoic shales. Moreover, they note that the aggressively oxidizing environments generated diurnally by cyanobacterial oxygen production within mats (e.g., Gingras et al., 2011) would have destroyed lipids sourced from the overlying water column. In consequence, the lack of steranes in most Proterozoic shales may simply reflect preservational circumstances common before the Ediacaran Period, when evolving animals dramatically restricted the distribution of benthic mat communities.

Without question, mats were major contributors of organic matter to Proterozoic sediments, and regardless of other influences, abundant cyanobacteria and other bacteria would have diluted molecular signatures of eukaryotes living in mats or the water column. That said, many of the organic-rich shales sampled for biomarker analysis come from relatively deep basins in which bottom waters were anoxic, mooting the destructive impact of oxygen-rich mat interiors. Petrological examination of Proterozoic shales also suggests the need for a more nuanced view of Proterozoic sediment accumulation. In many Proterozoic shales, mat horizons are separated by variably thick event beds that record pulses of mud deposition. These mud layers are rich in organic matter, providing an avenue for phytoplankton to evade the mat-seal effect of Pawlowska et al. (2013). Sedimentological observations also suggest that the aerial extent of benthic mats began to decline well before the Ediacaran Period; the case is best made for carbonates, where microbial lamination is much less common in later Neoproterozoic beds than it is in older successions (Knoll and Swett 1990).

Phanerozoic examples show that bacterial primary production was transiently high during episodes of widespread subsurface anoxia in the world's oceans -- for example, at the beginning of the Triassic Period (Grice et al., 2005). Thus, we might expect that in Proterozoic oceans with persistent subsurface anoxia, cyanobacteria and other photosynthetic bacteria would dominate primary production (Johnston et al. 2009), in part because of low fixed nitrogen abundances in surface waters (Fennel et al. 2005). Indeed, Boyle et al. (2013) have argued that in Proterozoic oceans sulfidic subsurface water masses could only develop beneath surface waters dominated by nitrogen-fixing primary producers. Conversely, biogeochemical study of Silurian microbial mats shows a strong presence of eukaryotic biomarkers (Bauersachs et al. 2009), suggesting that mat-seals provide imperfect barriers to the burial of eukaryotic lipids.

Thus, while Pawlowska et al.'s (2013) hypothesis usefully urges caution in the interpretation of Proterozoic steranes (or their absence), its applicability to the broad Proterozoic record remains uncertain. To date, few analyses have targeted 850-650 Ma black shales in which molecular biomarkers might be expected to mirror evident microfossil diversification. By the Ediacaran Period, however, steranes are relatively abundant in carbonaceous shales (Knoll et al., 2007). In fact, microfossils suggest a diversification of green algal phytoplankton at about the time when C29 sterols (principally sourced by green algae; Kodner et al. 2008) became abundant constituents of sedimentary organic matter; some 20% of the microfossil taxa in Early Cambrian rocks are confidently interpreted as the phycomata of prasinophyte greens, and additional chlorophyte diversity may be recorded by other preserved cysts (e.g., Moczydłowska 2010).

What might have driven the observed Neoproterozoic diversification of marine eukaryotes? Perhaps we can take a lesson from Cambrian animal radiation. Molecular clocks suggest that animals began to diverge about 800 Ma; fossils in turn indicate the presence of metazoans 40-100 million years before the Cambrian explosion (Erwin et al. 2011). While both genetics and environmental change played a role in animal diversification, the evolution of carnivory is thought to have set off an ecological arms race between predators and prey that fueled the observed Cambrian diversification of animals (Stanley 1973; Bengtson and Conway Morris 1992; Sperling et al., 2013) and algae (Knoll 1994; Vidal and Moczydłowska-Vidal 1997). Might the evolution of eukaryophagy have had a broadly comparable effect on Neoproterozoic ecosystems?

The ability to phagocytose bacteria and other small particles appears to be plesiomorphic among the Eukarya. Predation on large cells however, is commonly derived and focused within a relatively small number of clades. Preliminary analysis of molecular clocks suggests that

eukaryophagy evolved in several clades (including ciliates, dinoflagellates, amoebozoans and rhizarians) during the Neoproterozoic Era, and the same logic that underpins ecological amplification of Cambrian animal diversification applies to this event. Indeed, Stanley's (1973) early formulation of the predation hypothesis can be applied at least as well to eukaryophagy in protists as it has been to carnivory in animals. Experiments indicate that protistan and micrometazoan grazers both result in increased growth rates and biomass for eukaryotic phytoplankton, but not cyanobacteria (Trommer et al. 2012; Ratti et al. submitted); thus, eukaryophagy could, in principle, have facilitated the rise of eukaryotic phytoplankton to ecological prominence.

Porter (2011) was the first to propose that protistan predation might have driven the observed Neoproterozoic expansion of eukaryotic fossils. Does this hypothesis make specific predictions that might be tested against the record? The vase-shaped tests introduced earlier provide three lines of evidence consistent with the eukaryophagy hypothesis. The first is phylogenetic: some of the testate microfossils in 750-800 Ma rocks can be allied a eukaryophagic amoebozoan clade (Porter et al. 2003). Then there is functional evidence: by their nature, these tests would have provided protection against eukaryophagic predators, so the mid-Neoproterozoic radiation of such structures is, again, consistent with the eukaryophagy hypothesis. Moreover, some preserved tests have regular half-moon perforations, thought to reflect attack by vampyrellid or other protistan predators (Porter et al. 2003).

Other evidence for protective armor comes from the ca. 800 Ma scale microfossils introduced earlier (Cohen and Knoll 2012). And then, there is the expansion of multicellular and coenocytic fossils. Both theory and experiment suggest that multicellularity provides protection against protistan predators (e.g., Boraas et al. 1998). Intriguingly, as noted above, molecular

clocks suggest that animals date from the mid-Neoproterozoic Era, as well (Erwin et al. 2012), perhaps implicating eukaryophagy in the origin of animal multicellularity. At present, the idea that the establishment or expansion of eukaryophagy drove mid-Neoproterozoic eukaryotic diversification in the oceans remains a hypothesis to be tested by the careful integration of function and phylogeny, as well as continuing paleontological research. It does, however, have the merit of accounting for a broad spectrum of paleontological observations.

Lastly, we can ask about the environmental context of Neoproterozoic eukaryotic diversification. Increased microfossil diversity immediately preceded an interval of global glaciations, popularly known as the Snowball Earth (Hoffman et al. 1996). Changes in both export fluxes and mean Redfield ratios of an increasingly eukaryotic phytoplankton have been implicated in the CO_2 drawdown that initiated glaciation (Tziperman et al. 2012), and decreasing pCO_2 has, in turn, been postulated to drive adaptive evolution in Rubsico, the key enzyme in CO_2 fixation by algae and cyanobacteria (Young et al. 2012). Tectonic changes also characterized the later Neoproterozoic Earth, and these also influenced atmospheric chemistry and climate. The key point for ongoing research is that an expanding ecological presence of eukaryotes in marine ecosystems may have provided important new feedbacks in the integrated Earth system, both facilitating and reflecting changes in the physical environment.

CONCLUDING REMRKS

Of course, eukaryotic diversification did not end with the close of the Proterozoic Eon. Indeed, most eukaryotic diversity is a product of Phanerozoic evolution. Fossils conspicuously record the radiations of complex multicellular clades, first animals in the oceans and later

embryophytic land plants, land animals, and morphologically complex fungi (e.g., Knoll 2011). The chemistry of sedimentary rocks indicates that the transition from Proterozoic to Phanerozoic ecosystems also involved environmental change: animals radiated in Cambrian oceans richer in oxygen than their Proterozoic counterparts, with pO_2 increasing to levels that matched or exceeded those of the present during the later Paleozoic Era (Berner 2009, Dahl et al. 2010). Protists diversified as well: mineralized skeletons document the Paleozoic diversification of radiolarians and benthic foraminiferans followed by Mesozoic radiations of coccolithophorid algae, dinoflagellates and diatoms, not to mention the expansion of foraminiferans into the planktonic realm (Lipps 1993).

Those radiations, however, are only the latest chapter in a much longer history of eukaryotic evolution. Careful field and laboratory investigations of Proterozoic sedimentary rocks are yielding increasing evidence of earlier eukaryotic diversification and its environmental context (Fig. 3). Uncertainties abound, but present evidence suggests that crown group eukaryotes radiated into a world quite distinct from today's, with moderately oxic surface oceans and, commonly, anoxia in subsurface water masses. As phylogenies, molecular clocks, paleoenvironmental reconstructions, and geochronological calibration all continue to improve, our interpretations of the early fossil record will become richer and better integrated with inferences from comparative biology.

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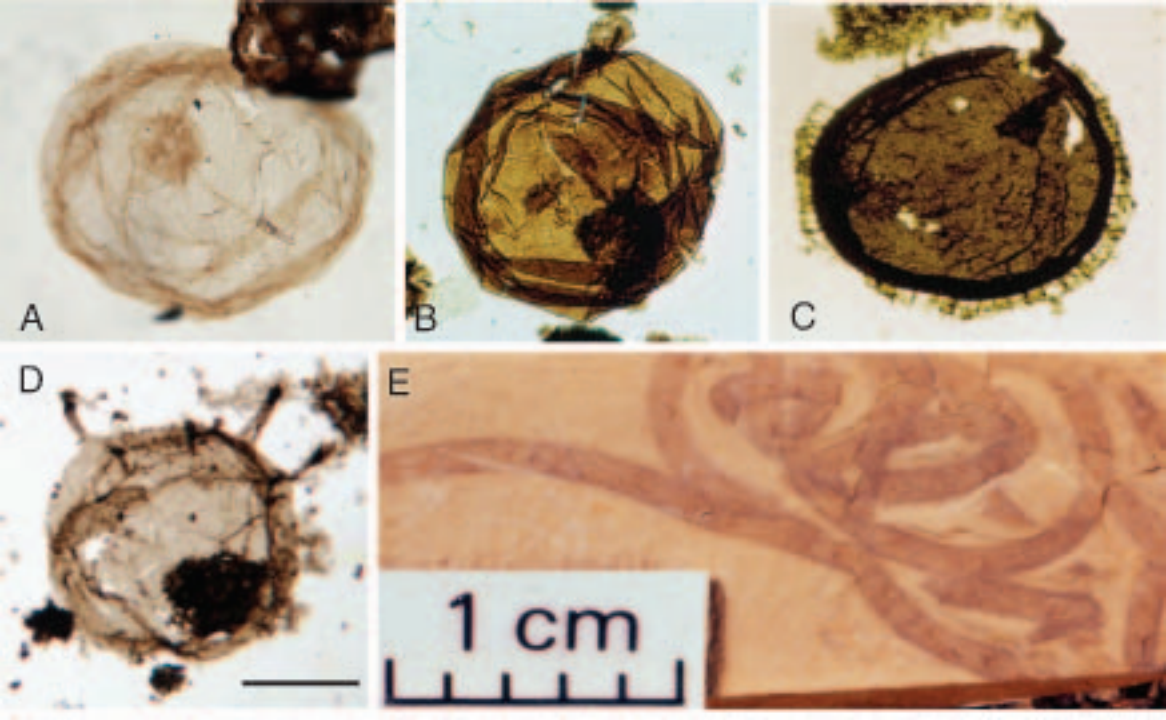
Figure Legends

Figure 1. Mesoproterozoic fossils interpreted as eukaryotic. (A-D) Preserved spheroidal microfossils interpreted as the vegetative or resting walls of unicellular protists, arranged from lowest confidence (A) to highest (C and D). (A). Unornamented spheroidal vesicle, 1400-1500

Ma Roper Group, Australia. (B) Spheroidal vesicle with corduroy-like ornamentation of vesicle wall, Roper Group. (C) Spheroidal microfossil with surface divided into small fields and ornamented with cylindrical processes that expand distally; TEM of walls shows complex multi-layered wall ultrastructure, >1250 Ma Ruyang Group, China. (D) Spheroidal vesicle with asymmetrically placed cylindrical processes; TEM shows complex wall ultrastructure, Roper Group (courtesy of E Javaux). (E) Macroscopic compressions assigned to the form taxon *Grypania*, ca. 1400 Ma Jixian Group, China (courtesy of M Walter). Bar in D = 20 μm for A, = 75 μm for B and D, and = 120 μm for C. Note 1 cm bar in E.

Figure 2. Late Mesoproterozoic and Neoproterozoic fossils interpreted as eukaryotic. (A) Irregularly spheroidal microfossil with long cylindrical processes, 750-800 Ma Svanbergfjellet Formation, Spitsbergen. (B) Large microfossil with opaque inner wall bearing small spines and longer cylindrical processes, within encompassing smoothly spheroidal vesicle, Svanbergfjellet Formation. C. *Cladophora*-like branching filamentous microfossil with apparently coenocytic subunits, Svanbergfjellet Formation. (D) Three-dimensionally preserved mineral replicate of testate eukaryote, Chuar Group, Grand Canyon (courtesy of S Porter). (E) *Bangiomorpha*, interpreted as an early-branching red alga, 1100-1200 Ma Hunting Formation, Arctic Canada (courtesy of N Butterfield). (F-H). Scale microfossils preserved three-dimensionally in ca. 800 Ma carbonate rocks of the Fifteenmile Group, Yukon Territory, Canada (courtesy of P Cohen). Bar in H = 60 μm for A and C, = 120 μm for B, = 10 μm for F, and = 14 μm for G and H. Note scale bars for D and E.

Figure 3. A summary of early eukaryotic evolution. Solid bars denote confident interpretation of geologic record; dashed bars indicate uncertain or controversial extensions of the record. Phan = Phanerozoic Eon (literally, the age of visible animal life). See text for references.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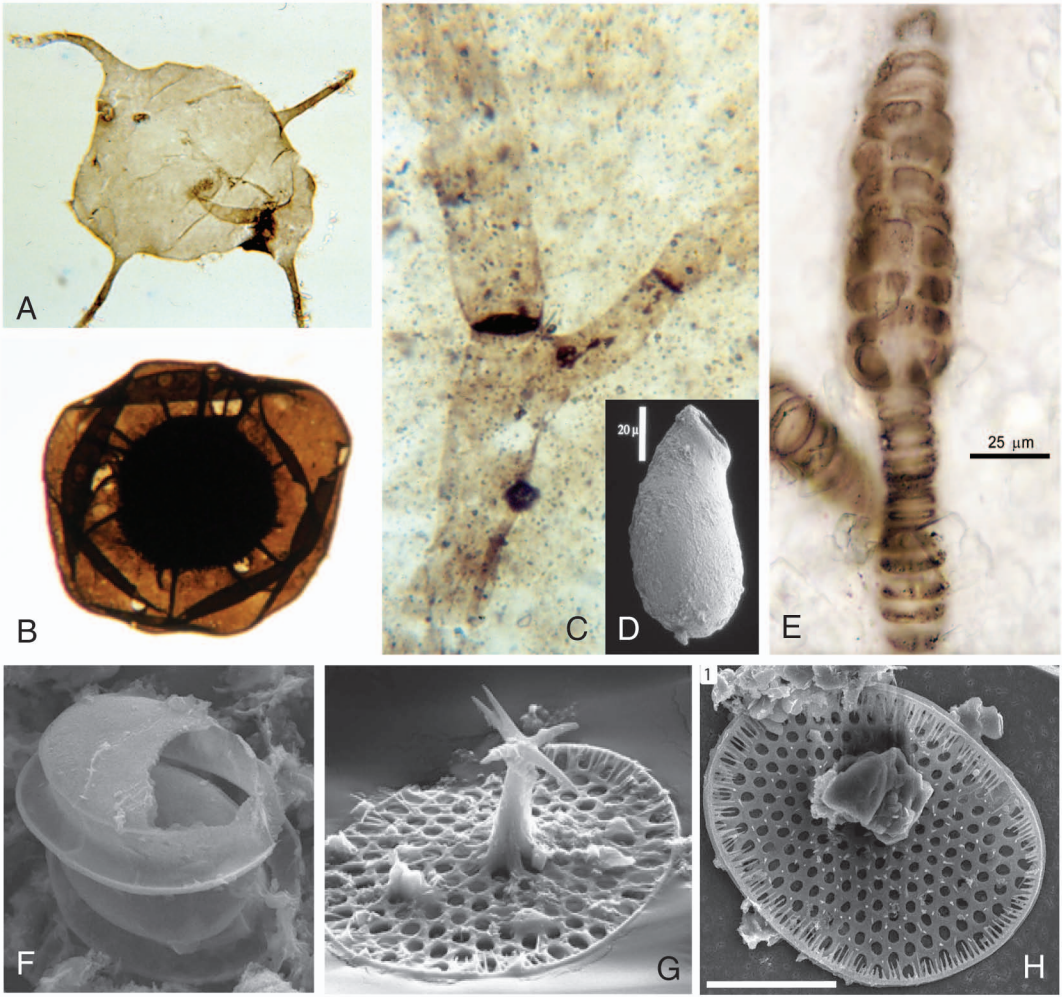
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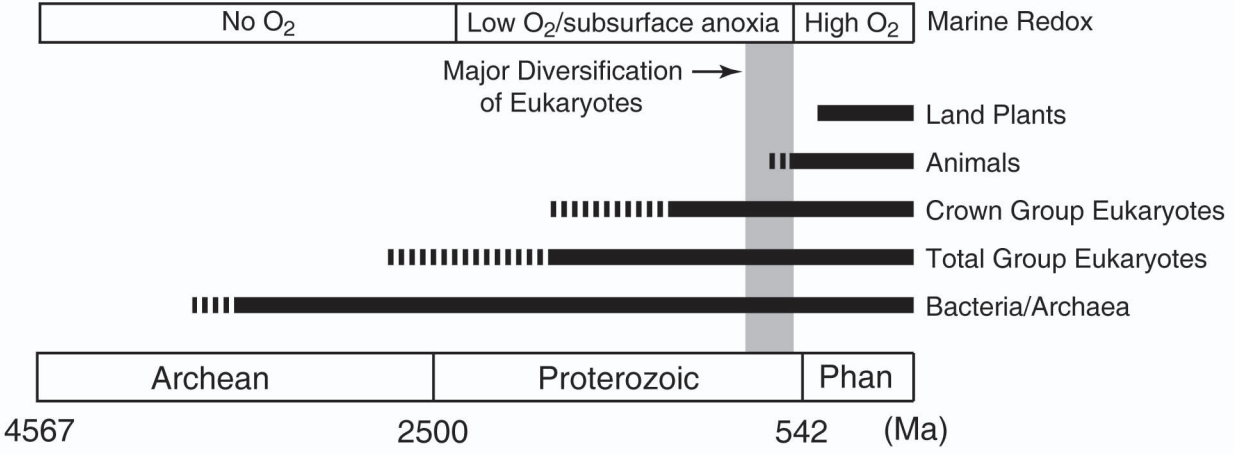
No O_2
Low O_2/subsurface anoxia
High O_2
Marine Redox
Major Diversification of Eukaryotes
Land Plants
Animals
Crown Group Eukaryotes
Total Group Eukaryotes
Bacteria/Archaea
Archean
Proterozoic
Phan
4567
2500
542
(Ma)