ANNUAL REVIEWS

Annual Review of Microbiology

Frameworks for Interpreting the Early Fossil Record of Eukaryotes

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Annu. Rev. Microbiol. 2023. 77:173–91

The *Annual Review of Microbiology* is online at micro.annualreviews.org

https://doi.org/10.1146/annurev-micro-032421-113254

Keywords

Precambrian, evolution, Proterozoic, eukaryote, eukaryogenesis, Great Oxidation Event

Abstract

The origin of modern eukaryotes is one of the key transitions in life's history, and also one of the least understood. Although the fossil record provides the most direct view of this process, interpreting the fossils of early eukaryotes and eukaryote-grade organisms is not straightforward. We present two end-member models for the evolution of modern (i.e., crown) eukaryotes—one in which modern eukaryotes evolved early, and another in which they evolved late—and interpret key fossils within these frameworks, including where they might fit in eukaryote phylogeny and what they may tell us about the evolution of eukaryotic cell biology and ecology. Each model has different implications for understanding the rise of complex life on Earth, including different roles of Earth surface oxygenation, and makes different predictions that future paleontological studies can test.

Contents

1. INTRODUCTION

Extinction is pervasive in the history of life. Over time, it culls large parts of life's tree, resulting in gaps in modern diversity (87)—some so large it can be difficult to make sense of how the modern groups evolved. This is the case for eukaryotes, whose cells are so different from those of the prokaryotes that it is tempting to invoke some abrupt, transformative event during which the eukaryotic cell was formed. There is no need to do so, however: The chasm between modern eukaryotic and prokaryotic cells can be explained simply by evolutionary changes that accrued over a billion years in innumerable intervening taxa that have since gone extinct. The gift of the fossil record is the ability to glimpse these intervening lineages and decipher, if only barely, the steps by which the modern eukaryotic cell evolved, and more broadly, the processes by which complex life can emerge on a planet. This is not a straightforward task, however, because many of these lineages would not have appeared particularly eukaryote-like, some may have convergently evolved traits that today we consider the singular hallmarks of modern eukaryotes, others may have evolved traits that are not known among the modern biota, and none will fit easily into our concept of what a eukaryote is, as defined by the modern taxa.

Here we provide a brief overview of these early fossil eukaryotes (and eukaryote-like organisms) that arose before and just after the modern clade evolved. We focus on key features of early fossils and what they tell us about the stages in the evolution of the modern eukaryotic cell, and, more generally, the emergence of complex life—not just eukaryotic life—on this planet. We frame our discussion within the context of total-, stem-, and crown-group concepts, which are crucial for interpreting the early fossil record of any taxon (**Figure 1**; see the sidebar titled Crown, Stem, and Total Eukaryotes). Note there are several excellent reviews of the early eukaryote fossil record that go into much greater detail than we have space for here. The reader is directed in particular to several recent reviews (2, 3, 28) and several older but influential reviews (20, 61, 62, 67, 73, 75).

2. PREDICTIONS FOR THE FOSSIL RECORD OF EARLY EUKARYOTES

Before we discuss the fossil record of eukaryotes, it is instructive to consider what we might expect that record to comprise based on first principles. The crown, stem, and total group framework (see the sidebar titled Crown, Stem, and Total Eukaryotes) allows us to make several predictions (**Figure 2*a***):

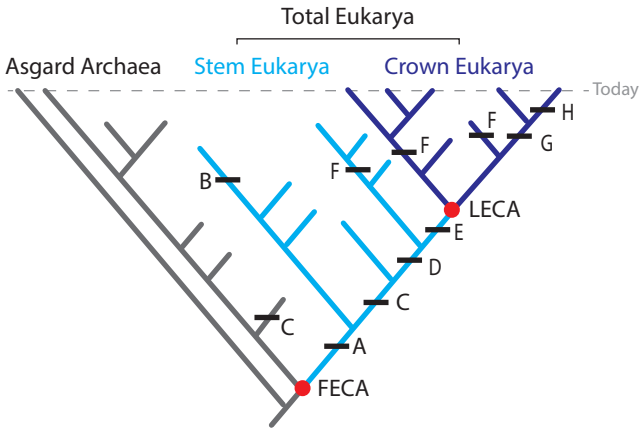

Figure 1

Cladogram illustrating the concepts of total, stem and crown Eukarya. Letters A–H are character traits. Crown Eukarya includes the last common ancestor of all living eukaryotes [the last eukaryotic common ancestor (LECA)] plus all of its descendants. Total Eukarya includes the first eukaryotic common ancestor (FECA) and all of its descendants; it comprises crown and stem Eukarya.

- Stage 1: Just after total eukaryotes emerge, they are unlikely to possess a sufficient number of characters that would allow them to be identified as eukaryotes. Together with the fact that they emerged so long ago, during an interval for which we have very little sedimentary rock record, it seems almost certain that we will not be able to identify fossil evidence for these basal eukaryotes. However, it also seems possible that we encounter fossils exhibiting characters similar to, but convergent with, those we find in eukaryotes, particularly in now-extinct lineages that were much more closely related to total group eukaryotes than anything alive today. Thus, we predict that during this time, we might find fossil organisms that exhibit eukaryote-grade characteristics, but that are not obviously eukaryotic themselves, and that have since gone extinct. There will be a tendency to want to shoehorn these fossils into the eukaryotic clade, because we know of nothing else alive today that is similar.
- Stage 2: After total eukaryotes emerge but before crown eukaryotes appear, only stem eukaryotes will be present. Although they will have characters shared with living eukaryotes, none will have characters that obviously ally them with specific clades within the crown group. They may, however, show evidence of convergent evolution with some crown-group members (e.g., multicellularity, photosynthetic metabolism). As we approach the origin of crown eukaryotes, there should be an increase in the number of lineages that are recognizably eukaryotic. Those stem taxa that branch very late will have nearly all of the characters of the crown group, and there will be a tendency to assume they are in fact crown eukaryotes.
- Stage 3: At the time crown eukaryotes appear, and for some time thereafter, stem eukaryotes will make up the bulk of eukaryotic lineages. As time goes by, however, an increasing proportion of eukaryotic fossils will be members of the crown group. Some models suggest that this increase could happen rapidly and within a relatively short time after the crown group emerges [e.g., crown lineages accounting for 90% of the total group's diversity after the first ~16% of its existence (17)]. If these models are correct, we might expect to see a variety of crown-group forms appearing in the fossil record around the same time (i.e., within ~200–300 million years).

CROWN, STEM, AND TOTAL EUKARYOTES

Critical to the interpretation of the early fossil record of eukaryotes are the concepts of crown, stem, and total groups (16, 36, 69). Crown eukaryotes encompass all living members of Eukarya, their last common ancestor [often referred to as the last eukaryotic common ancestor (LECA)], and all of LECA's descendants, including those that have gone extinct. Stem eukaryotes are, by definition, extinct and encompass all of those lineages that are more closely related to crown eukaryotes than to any other living group; together they form a paraphyletic group. Total Eukarya refers to all eukaryotes: both stem- and crown-group forms. That first lineage from which total Eukarya is descended is often referred to as the first eukaryotic common ancestor (FECA).

These definitions provide a way to make sense of fossil taxa and their characters, and where they fit in the tree. Crown eukaryotes, for example, possess all of the characters that define the living clade (e.g., **Figure 1**, characters A, C–E), unless they were lost later in evolutionary history. [In the case of crown eukaryotes, these characters include the nucleus, mitochondria, a complex cytoskeleton, an endomembrane system, the Golgi apparatus, and eukaryotic sterols (34, 45, 77).] Members of the crown also, of course, possess whatever characters that define the particular clades within the crown to which they belong (e.g., **Figure 1**, F or G and/or H), and identification of these characters in a fossil is critical for making a convincing assignment to the crown group. [In contrast, the presence of only the characters found in LECA (e.g., **Figure 1**, A, C–E) is not enough to identify a crown member: The logic of the tree is such that it is possible a lineage with any or all of those characters could have diverged prior to LECA. Thus fossils that exhibit evidence for the presence of a complex cytoskeleton, intracellular vesicles, and any other number of characters diagnostic of eukaryotes could nonetheless belong to stem Eukarya.]

Stem eukaryotes possess some, but not all, of the characters that define crown eukaryotes. The number of characters shared will depend on how closely related a particular stem taxon is to the crown clade: Very early diverging stem taxa will have very few characters in common and therefore may not look at all like what we imagine eukaryotes to be. Rather, they will likely appear indistinguishable from prokaryotes—just as very early stem metazoans will appear indistinguishable from protists because they have not yet evolved multicellularity, a character that evolved in the metazoan stem (130). Because stem lineages continue to evolve after their divergence from the crown lineage, they will also possess characters of their own—characters not shared with the crown group (e.g., **Figure 1**, B). These characters may be difficult to understand because they have no modern homologs [e.g., fractal architecture in Ediacaran rangeomorphs, thought to be stem eumetazoans (21, 33, 38)].

In addition, stem eukaryotes may possess characters that also evolved (independently) within the crown group, perhaps multiple times (e.g., **Figure 1**, F), including complex multicellularity [which evolved at least six times among living eukaryotes (72)], biomineralization [which evolved independently dozens of times among living eukaryotes (71)], and endosymbiont-derived organelles that impart secondary metabolisms such as photoautotrophy [which also evolved several times (70)]. These characters may mislead us into designating these stem taxa, incorrectly, as crown eukaryotes. Similarly, characters that evolved in the stem lineage of eukaryotes may have analogs in other clades (e.g., **Figure 1**, C), potentially misleading us into assigning noneukaryotic taxa as eukaryotes.

The membership of crown, stem, and total eukaryotes is, in a sense, arbitrary, in that it is tied to the current time slice in which we live and our knowledge of modern diversity. If, one million years from now, the basal-most branching eukaryotic clade alive today goes extinct, that clade will become a stem eukaryote, and the definition of crown eukaryotes, as well as the identity of LECA, will shift. Similarly, if—and probably when—we discover living taxa more closely related to eukaryotes than the Asgard Archaea known today, then the membership of total eukaryotes will shift, as will the set of characters that define FECA. A recent example of such a shift in our understanding of FECA is the discovery of actin-encoding cytoskeletal genes, including those that, in eukaryotes, code for a dynamic actin skeleton that allows growth and retreat of the cytoskeletal framework, and thus, in principle, active changes in cell shape (123)—a finding bolstered by the report of branching filamentous processes in cultured Asgard Archaea (59). Thus FECA probably possessed at least a primitive cytoskeleton, and it may have appeared more similar to very ancient process-bearing microfossils than we had previously imagined (e.g., *Tappania plana*; see Section 5).

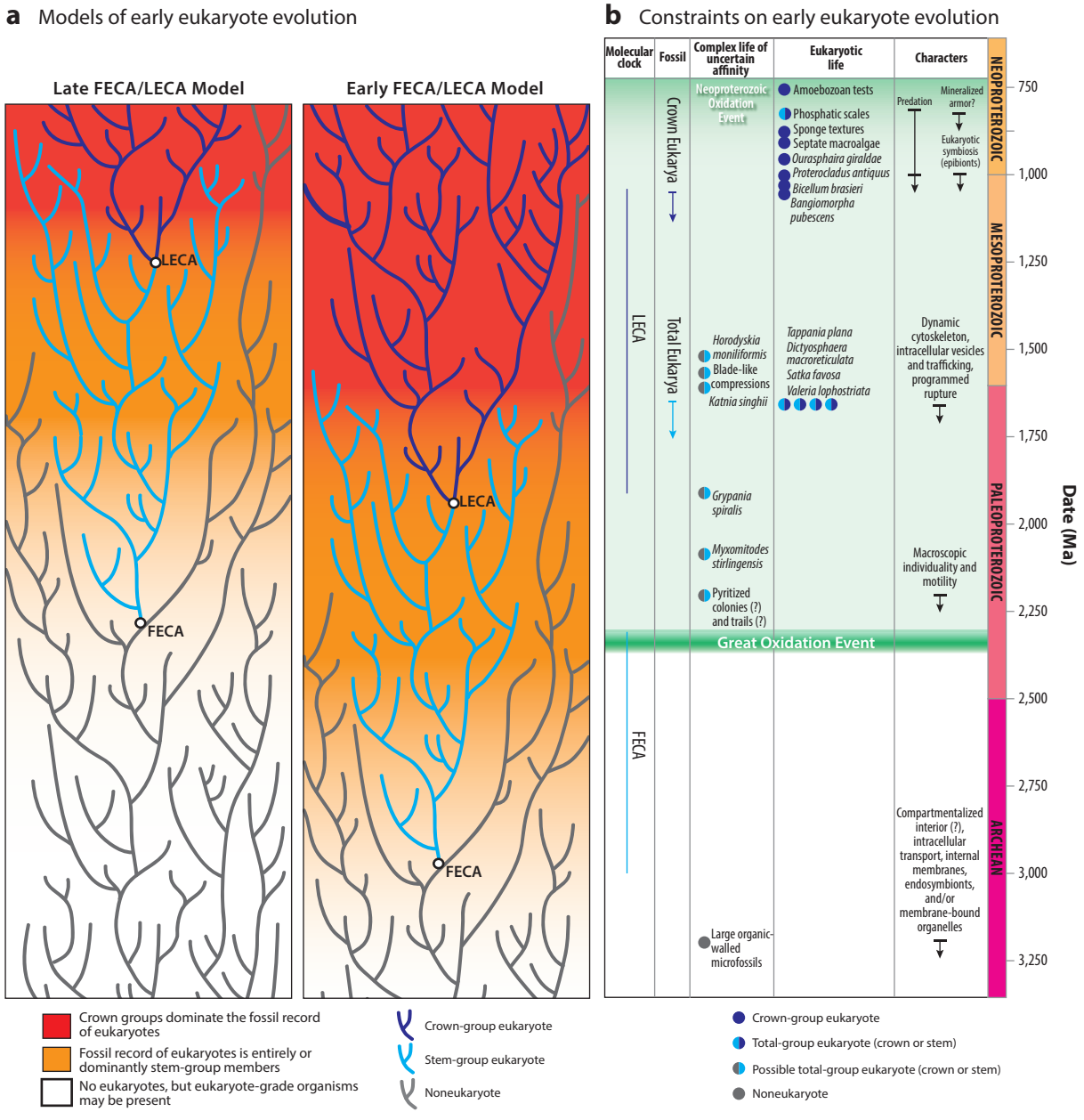

Figure 2

(*a*) End-member models of eukaryote evolution. In the late FECA/LECA model, total eukaryotes do not appear until after the Great Oxidation Event, and the Mesoproterozoic record is dominated by stem eukaryotes. In the early FECA/LECA model, total eukaryotes appear well before the Great Oxidation Event, and the Mesoproterozoic record is dominated by crown eukaryotes. (*b*) Constraints on the evolution of early eukaryotes from molecular clocks and the fossil record. Dates for the FECA, from Reference 12, and for the LECA, from References 25, 44, and 97. Fossil constraints on divergences based on *Dictyosphaera macroreticulata*, *Satka favosa*, *Tappania plana*, and *Valeria lophostriata* (total Eukarya) and *Bangiomorpha pubescens* (crown Eukarya). Dates from the Great Oxidation Event and the Neoproterozoic Oxidation Event based on Reference 84. Abbreviations: FECA, first eukaryotic common ancestor; LECA, last eukaryotic common ancestor.

3. PUTTING A DATE ON THESE STAGES: WHEN DID TOTAL AND CROWN EUKARYA APPEAR?

Assuming they are correctly interpreted, fossils provide hard minimum age constraints on divergences in the eukaryotic tree and suggest that total eukaryotes had evolved by the late Paleoproterozoic time (>1,650 Ma) and crown eukaryotes by late Mesoproterozoic time (>1,050 Ma) (**Figure 2*b***). However, these constraints may underestimate these branching events by hundreds of millions of years. Molecular clock analyses target the age of the divergences themselves [i.e., first eukaryotic common ancestor (FECA) and last eukaryotic common ancestor (LECA)] and thus provide a better way to estimate the origin of total and crown Eukarya. To our knowledge, Betts et al. (12) have provided the only molecular clock estimate of the age of total group eukaryotes that incorporates the Asgard Archaea, the closest known living relatives of eukaryotes (122, 134). Using the planet-sterilizing, moon-forming impact (4,510 ± 10 Ma) and the appearance of eukaryotic organic-walled microfossils in the Changcheng Group, China [1,619 Ma (78, 90)], as the upper and lower bounds on this event, they estimate an origin sometime in the late Archean or early Paleoproterozoic (~3.0–2.3 Ga; estimated from Reference 12, figure 3). Interestingly, nearly all of their models indicate a long stem lineage—500 to 1,800 Myr—between the origin of total and crown Eukarya (i.e., between FECA and LECA), a result that is robust to taxon sampling (12). If correct, it suggests that the features that distinguish modern eukaryotes from their living relatives were acquired over an enormous amount of time, an interval equivalent to, and perhaps as much as three times longer than, the entire history of crown Metazoa.

Estimates for the origin of crown eukaryotes are numerous and wide-ranging, spanning the Paleoproterozoic through Mesoproterozoic (~1,900–1,000 Ma) (25, 44, 97, 104). This is partly due to different model assumptions, but it also reflects different choices of fossils used to calibrate the clock, including some with now-outdated age constraints (see 44, 104 for more details). Recently an even older estimate (2,386–1,858 Ma) was reported for LECA (124), presumably driven in large part by the inclusion of *Rafatazmia* and *Ramathallus*, interpreted as red algal fossils from rocks reportedly 1,600 Ma (11). However, both their age and taxonomic affinities are in question (e.g., 23, 48).

In summary, these molecular clock constraints allow for two end-member models of early eukaryote evolution (**Figure 2*a***). At one extreme, FECA and LECA evolved late, appearing ~2,300 Ma and ~1,100 Ma, respectively. At the other, FECA and LECA evolved early, appearing ~3,000 Ma and 1,900 Ma, respectively. Given these different models, we can roughly match the stages identified above with intervals in the fossil record. In the late FECA/LECA model, stage 1, when total eukaryotes emerge, corresponds to the early Paleoproterozoic; stage 2, when stem groups dominate the fossil record, begins in the late Paleoproterozoic and continues nearly to the end of the Mesoproterozoic; and stage 3, when crown eukaryotes emerge and not long after dominate the fossil record, starts in the latest Mesoproterozoic. In the early FECA/LECA model, stage 1 corresponds to the mid- to late Archean, stage 2 corresponds to the late Archean through mid-Paleoproterozoic, and stage 3 corresponds to the mid- to late Paleoproterozoic and thereafter. Either of these models—or some other model in between these extremes—could be correct, and the model chosen has a strong influence on how the fossil record of early eukaryotes is interpreted. Our goal is not to argue for one versus another, but to provide alternative frameworks within which the fossil record can be interpreted. In the sections below, we discuss key fossils from the late Archean through the early Neoproterozoic, how they might be interpreted, and what they tell us about steps in the evolution of complex life.

4. THE LATE ARCHEAN THROUGH MID-PALEOPROTEROZOIC RECORD: A SPOTTY RECORD OF EUKARYOTE-GRADE ORGANISMS

There are no convincing Archean or early Paleoproterozoic eukaryote fossils, though they may be among the variety of organic microfossils known from rocks of this age (67), hidden by their lack of diagnostic features. There are, however, a few notable reports of fossils or fossil-like structures that exhibit characters consistent with a eukaryote-grade level of complexity, as we might expect for this interval (see Section 2).

Among the oldest of these are hollow organic vesicles from ~3,200 Ma fine-grained shallow water deposits in the Moodies Group of South Africa (68). These are remarkable both for their size (~30 to 300 μm in diameter) and for the fact that they are preserved at all: Their walls, 120 nm thick and homogeneous in transmission electron microscopy (TEM) images, must have been at least somewhat resistant to degradation. These could represent either colonial envelopes or the remains of a single cell. If the latter, they would indicate that by 3,200 Ma at least one lineage of cells had found a solution to the challenges of large size faced by typical modern prokaryotic organisms, including the limitations of diffusion in transporting nutrients in and waste out (119), and a decrease in energy efficiency associated with a relatively limited membrane area for respiration (80, 117). Both eukaryotes and several different bacteria (119, 129) have solved these challenges in a variety of ways, for example, through cell compartmentalization via the presence of a large, inert interior space that reduces cell volume to a layer a few micrometers thick; a system of intracellular transport; an interior membrane system where respiration can occur; the presence of multiple ATP-producing endosymbionts; and/or compartmentalization of DNA and ribosomes into membrane-bound organelles (117, 119, 129). It is reasonable to assume that these early organisms also possessed one or more of these features.

Much larger fossil-like pyritized structures occur in relatively high abundance on the bedding planes of organic-rich shales deposited in a quiet, offshore oxygenated marine environment 2,100 Ma in Gabon (41–43). Of particular note are lobate forms, up to 12 cm in size, that consist of an undulating sheet with radial grooves on its periphery and, in most specimens, a nodular or complexly folded central body (41) (**Figure 3*a*,*b***). Their undulating folds and carbon and sulfur isotope signatures are consistent with a macroscopic biological structure replaced by pyrite soon after death (37, 41, 42), although their similarity to nonbiogenic structures such as pyrite suns is cause for some caution in interpretation. If they are biological, their size indicates multicellular or syncytial organisms or colonies; evidence for cell-cell signaling and coordinated responses or a eukaryotic affiliation (41) is not obvious, however. Sinuous string-like structures, a few millimeters wide and up to 170 mm long, occur in the same unit and are also made of pyrite (43) (**Figure 3*c*,*d***). Though mostly parallel to bedding, some cut through and locally disturb laminae, which has been interpreted to suggest movement through the sediments by organisms analogous (but not related) to modern slime molds, which in response to starvation, formed syncytial or multicellular aggregates ("slugs") to search for food (43). As with the lobate forms, biogenic interpretation should be met with caution, and a eukaryotic affiliation is not obvious. But if in fact made by organisms, they would imply a grade of organization similar to that found among some modern eukaryotes [including protists (88)] that allowed motility at the macroscopic scale.

Paired, 0.5- to 1.0-mm-wide ridges preserved in sandstones of the 2,000–1,800 Ma Stirling Range Formation, Western Australia, have also been interpreted as evidence for macroscopic motility (10). The ridges run parallel for most of their length, joining at one end to form a loop, and have been suggested to represent strings or sheets made up of mucus and sediment formed by a macroscopic motile organism moving across the surface of a muddy seafloor (10).

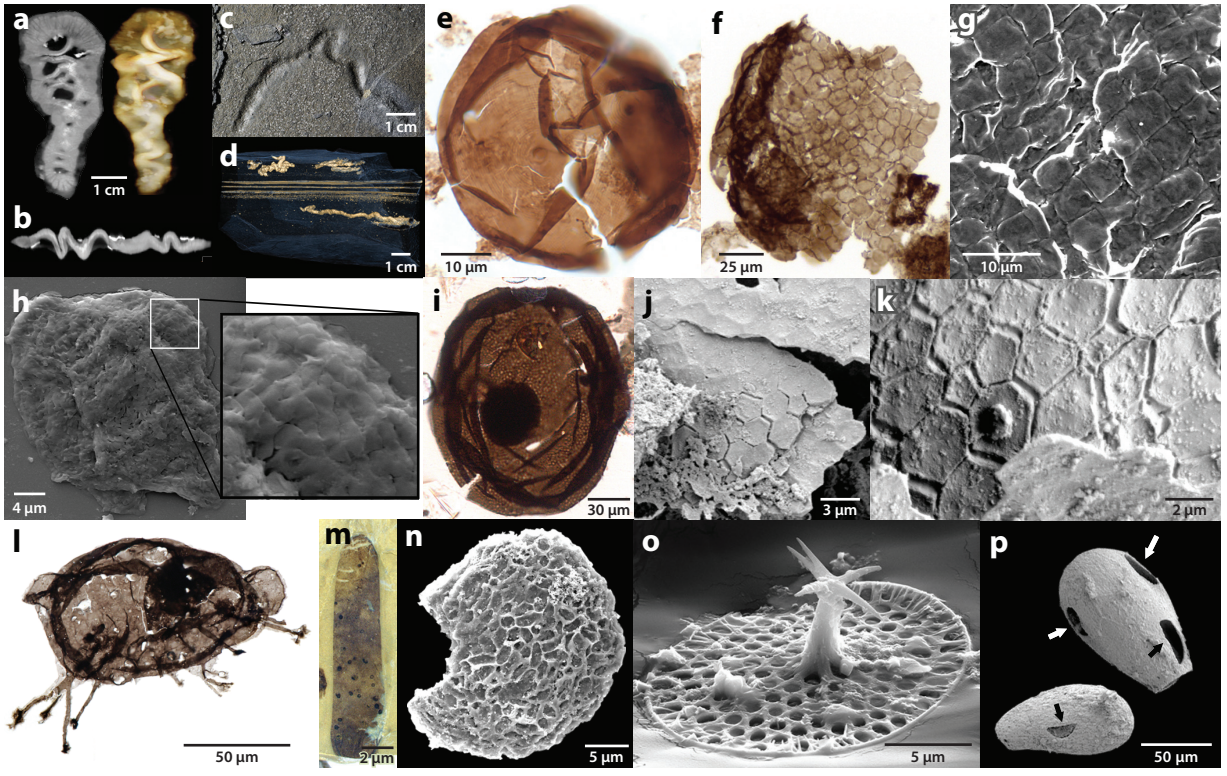

Figure 3

A selection of key fossils. (*a*) Micro-CT 2D (*left*) and 3D (*right*) reconstructions of a pyritized lobate form interpreted to be the remains of a colonial organism. Note radial grooves on the periphery and complexly folded central body. (*b*) Longitudinal cross section of panel *a* showing its undulating sheet-like nature. (*c*) Pyritized string-like structure interpreted to be a trail made by a macroscopic motile organism. (*d*) Micro-CT reconstructions of pyritized string-like structures; flat lines are pyritized, organic-rich layers interpreted to be the remains of microbial mats. Panels *a–d* from the 2.1-Ga Francevillian Series, Gabon. (*e*) *Valeria lophostriata*, characterized by concentric circular ridges on the vesicle's interior. Specimen from the late Tonian Chuar Group, United States. (*f*,*g*) *Satka favosa*, made of plates joined at their edges, from the mid-Mesoproterozoic Battle Creek Formation, Bullita Group, Birrindudu Basin, northern Australia. (*g*) Close-up view showing grooves on the outer vesicle surface where plates join together. (*h*) *Germinosphaera alveolata*. Note imbricated organic plates. From the 1,590- to 1,270-Ma Dismal Lakes Group, arctic Canada (81). (*i–k*) *Dictyosphaera macroreticulata* from the ~1,740- to 1,410-Ma Ruyang Group, China. (*i*) Vesicle with opaque circular internal body interpreted to be a contracted protoplast of the cell just before encystment (95). (*j*) Close-up showing the inner vesicle wall composed of polygons that in some places have broken apart, and, underneath, the outer vesicle wall with imprints of polygons. (*k*) Close-up showing the inner vesicle wall composed of polygons that fit together perfectly despite variations in shape and size, suggesting they formed in situ. (*l*) *Tappania plana* exhibiting two neck-like extensions and, arising from one hemisphere of the vesicle, multiple hollow, occasionally branching processes that flare at their ends. Specimen from the Greyson Formation, of the Belt Supergroup, Montana, United States. (*m*) *Tawuia* vesicle with several circular bodies interpreted to be ectobionts, from the ~1.0-Ga Liulaobei Formation, North China. (*n*) *Caelatimurus foveolatus*, characterized by numerous embossed pits on the vesicle surface, reminiscent of divots housing ectobionts found in modern eukaryotes, from the late Tonian Chuar Group, United States. (*o*) A phosphate-mineralized scale interpreted to have been part of an armor of such scales that surrounded a single protistan cell, from the Fifteenmile Group, Yukon, Canada. (*p*) Vase-shaped microfossils interpreted as the remains of amoebozoan testate amoebae, showing evidence of drill hole–like predation, including both circular holes (*white arrows*) and semicircular holes (*black arrows*). Abbreviation: CT, computed tomography. Panels *a* and *b* adapted with permission from Reference 41. Panels *c* and *d* adapted from Reference 43. Panels *e* and *n* adapted with permission from Reference 107. Panels *f* and *g* by L.A. Riedman. Panel *h* adapted with permission from Reference 81. Panel *i* adapted with permission from Reference 5. Panels *j* and *k* adapted with permission from Reference 4. Panel *l* adapted with permission from Reference 1. Panel *m* adapted with permission from Reference 126. Panel *o* adapted with permission from Reference 30. Panel *p* adapted with permission from Reference 103.

Finally, mid- to late Paleoproterozoic rocks (1,900 Ma) from the Lake Superior region of North America (118) preserve intriguing evidence for possible eukaryotes. *Grypania spiralis*, a carbonaceous filament ~1 mm in diameter and up to 90 mm in length, forms 5- to 30-mm-diameter coils (54) that could be aggregates of microscopic prokaryotes (though see 75, 115, 121) or unusually large, possibly coenocytic, single cells [cf. giant bacteria (129) or eukaryotes (54)].

The early FECA/LECA model could accommodate these fossils as early examples of eukaryotic life; in the late FECA/LECA model they are best regarded as unrelated prokaryotic lineages that independently evolved large cells, macroscopic organization, and macroscopic motility. It is perhaps notable that there is a cluster of appearances around 2,200–1,800 Ma, after the first significant rise in oxygen ~2,400–2,300 Ma (**Figure 2*b***), and that, at least in the case of the Gabon structures, these putative organisms lived in oxygenated environments. This broad correspondence is consistent with the view that oxygen levels were a gatekeeper on the size and complexity of life through time (e.g., 99), but given the spottiness of the rock record during this interval, the idea is speculative.

5. THE LATE PALEOPROTEROZOIC THROUGH LATE MESOPROTEROZOIC RECORD: FOSSILS OF TOTAL GROUP EUKARYOTES APPEAR

It is not until the late Paleoproterozoic that we start to get a relatively rich record of fossils that almost certainly belong to total Eukarya based on the presence of several characters diagnostic of modern eukaryotes (65). One of the oldest of these is *Valeria lophostriata*, whose 900-million-year stratigraphic range makes it the Methuselah of the fossil world (56) (**Figure 3*e***). This hollow, organic-walled vesicle, 16 to 450 μm in diameter, is characterized by evenly spaced concentric ridges on its inner surface, with circular foci at opposite poles (56). Pang et al. (96) proposed that these ridges formed during encystment as a result of a reaction-diffusion process (see also 56) in which two different types of small molecules diffuse (or are actively transported) from the polar axis of the cell and interact to generate multiple concentric cylindrical zones that, where they intersect with the wall, either inhibit or promote the formation of ridges (96, figure 5). In this model, the ridges function to concentrate tensile stress in the thinner-walled interridge zones and maximally so at the equator, creating a mechanism of programmed excystment whereby increased turgor pressure in response to environmental cues like decreasing salinity causes cyst rupture. If correct, *V. lophostriata* provides the oldest evidence for the programmed rupture of a cyst wall. Other fossils in the same rocks also show evidence of medial splits that may indicate programmed rupture [e.g., *Schizofusa sinica* (78, 90)], but the possible role of postmortem sedimentary compaction in forming these splits has not been critically considered. Similarly, holes reported in coeval taxa (132) and interpreted as possible pylomes (circular to subpolygonal vesicular excystment openings) (e.g., 4, 90) might simply be the results of postmortem breakage or degradation.

Several early eukaryote species possess walls composed of multiple repeated units—tessellated polygons, attached plates, or overlapping scales—interpreted to have been formed in intracellular vesicles and transported to the cell's periphery. Among the oldest of these is *Satka favosa* (**Figure 3*f*,*g***), which exhibits a wall composed of numerous polygonal organic plates, <1 μm thick and several micrometers across (e.g., 60, 63, 81). The plates are convex outward. Where they join, they form grooves on the outer vesicle surface as well as (reportedly) ridges on the interior surface (60). In specimens where plates are partly separated, it is apparent that the plates do not fit perfectly together (66, figure 3*b*) (**Figure 3*f***), suggesting that they were formed individually and subsequently joined. Some of the same rocks preserve another species, *Germinosphaera alveolata* (**Figure 3*b***), that possesses numerous <1-μm organic scales that form an irregular and densely imbricated layer (81, 90). Today, similar plates and scales occur in a phylogenetically diverse array

of eukaryotes, implying that they evolved convergently many times over, as either entirely organic or mineralized (e.g., siliceous, calcareous) structures. Nonetheless, in most cases their mode of formation is the same: They are secreted in the Golgi apparatus or in Golgi-derived vesicles and transported outside the plasma membrane [e.g., stramenopiles (39), amoebozoans (8, 110), rhizarians (9), Chloroplastida (89), haptophytes (133), centrohelids (9, 98), Cryptista (100)]. Thus, the plates of *S. favosa* and scales of *G. alveolata* provide compelling indirect evidence for the presence of intracellular vesicles, intracellular trafficking, and exocytosis (63, 75). Notably, these abilities may also suggest the cell had the capacity for phagocytosis (24), hypothesized to have been a prerequisite for eukaryotic multicellularity, the development of complex food webs, and, possibly, mitochondrial acquisition (91).

Less obvious is the significance of the complex walls found in *Dictyosphaera macroreticulata* (**Figure 3*i–k***) and *Shuiyousphaeridium macroreticulatum*, morphospecies thought to represent alternating asexual and sexual cysts in a bimodal, heteromorphic life cycle (4, 131). These multilayered walls consist of an inner layer of tessellated polygons that are variable in size and shape (but are mostly hexagonal and pentagonal) and an outer layer that also exhibits a polygonal texture, with raised rims marking the boundaries between polygonal fields (4, 5, 66) (**Figure 3*j***). Upon death and decay, whatever material kept the polygons in the interior layer together often broke down, and the polygons broke apart (4, 66) (**Figure 3*j*,*k***). Though the polygons have been hypothesized to have been formed by the Golgi apparatus and transported via the endoplasmic reticulum to the cell periphery (4, 63), aspects of their appearance suggest, on the contrary, that they were formed in place. In particular, the polygons fit together perfectly despite their irregular sizes and shapes, which seems unlikely if they were formed elsewhere and subsequently pieced together. In plan view, they are reminiscent of columnar basalts, which form from cooling, shrinkage, and efficient fracturing of igneous bodies (e.g., magma, lava, and hot ash); in cross-sectional view (66, figure 5*h*,*j*) they are reminiscent of mud cracks, which form when desiccation and shrinkage are greater near the top of the mud layer than lower down. This suggests that the tessellated polygons reflect physical properties of the wall and may have formed as a result of differential shrinkage of wall layers during life or after death. This is consistent with TEM evidence for a multilayered wall, and in particular, a relatively thin, electron-tenuous layer in the part of the wall that would have shrunk the most (the innermost layer in figure 5*h*,*i* of Reference 66).

One of the most fascinating fossils from this time interval is *Tappania plana* (**Figure 3*l***), an organic vesicle ~15–160 μm with hollow processes and bulbous, neck-like extensions that is found in rocks worldwide ~1,700–1,400 Ma (e.g., 1, 63, 84, 109). The most interesting aspect of *T. plana* is how variable the species is. Vesicles possess between 0 and 20 processes. These processes may be randomly distributed or restricted to one hemisphere, may be intermittently septate or not at all, and may or may not branch. If they do branch, they may do so into equally sized or thinner processes. The vesicles may have up to several neck-like extensions that appear randomly distributed relative to each other and to the processes (1, 63, 64). Some specimens exhibit an outer wall that is more translucent (and perhaps more delicate) than the primary vesicle and that can form its own processes that typically encompass less-well-developed processes on the inner wall. Some processes flare at their tips, suggesting they were attached either to some substrate or perhaps to a third, unpreserved outer envelope (1). This suggests that *T. plana* is the remains of a metabolically active cell—not a cyst—that adapted its shape in response to its external environment; it may have been phagotrophic, or it may have been an osmotroph whose variable shape reflects growth toward organic substrates (63, 76). What seems clear, however, is that it must have had a dynamic cytoskeleton, one that allowed the cell to change shape and form finger-like extensions, thereby creating hollow processes that interacted with the environment in some way (101).

Finally, a few of these total-group eukaryotes exhibit intriguing, opaque, organic bodies internal to the vesicle. While some are irregular in outline [e.g., those in *T. plana* (63, figure 6)], some appear almost perfectly circular (5, 95, 132) (**Figure 3*i***). Irregular internal bodies may simply reflect shrunken and degraded cytoplasm (cf. 74), but the circular bodies do not fit this model. Pang et al. (95) suggested those found in *D. macroreticulata* and *S. macroreticulatum* represent the early stages of cyst formation, wherein the cell's protoplast has condensed but the cyst wall has not yet formed. This seems like a reasonable interpretation that is supported by the observation of similar internal bodies in *Arctacellularia tetragonala*, a probable eukaryotic alga comprising a chain of vesicles, that correspond in shape to their enclosing vesicles and have nickel signatures suggestive of chlorophyll residues (120).

In the late FECA/LECA model, these fossils are members of stem eukaryotes. In the early FECA/LECA model, they may be either crown or stem eukaryotes: Though they possess several characters found in crown eukaryotes, they do not possess characters known to have evolved after the divergence of crown Eukarya. We do not know, for example, whether these organisms possessed mitochondria (92, 104, 114), whether—even without mitochondria—they were aerobic, whether they synthesized sterols similar to those found in modern eukaryotic organisms (e.g., 14, 94, 104), or whether they might have been phagotrophic or limited to osmotrophic lifestyles (40, 91).

Finally it is worth noting that the spotty record of putative macroscopic fossils that characterizes the early Paleoproterozoic record (see above) continues through the Mesoproterozoic. *Horodyskia moniliformis* from the Appekunny Formation [~1,600–1,450 Ma (13, 46)] in the Belt Supergroup of Montana is reminiscent of a string of beads comprising numerous ~1- to 3-mm-diameter, evenly spaced bodies connected by a filament (46, 57; see also 22, 50, and 51 for description of younger Mesoproterozoic specimens from Australia, and 35 for Ediacaran forms from China). Reports of correlated increases in bead size and spacing (50, 52, 57), which helped bolster the view that these were tissue-grade organisms (i.e., eukaryotes) showing regulated growth (46, 52), may be spurious and dependent on measurement techniques (35). *Katnia singhii*, from the ~1,600-Ma Rohtas Formation, Vindhyan Supergroup, India (121), is similar to *G. spiralis* in its size and coiled form but has closely spaced septa (six to eight per millimeter). It could be bacterial (121) or a multinucleate eukaryote (19). Finally, decimeter-scale, blade-shaped, organic compressions from the 1,560-Ma Gaoyuzhuang Formation, North China, have been interpreted as photosynthetic (or possibly osmotrophic) multicellular organisms that are either crown eukaryotes (stem red or green algae) or stem eukaryotes that independently evolved a photosynthetic habit (135), though given the absence of evidence for a stable, definable macroscopic form, they might instead be colonial prokaryotes (cf. 20). Depending on the model of early eukaryote evolution, these fossils may best be interpreted as (*a*) independent acquisitions of multicellularity in either a noneukaryote or a stem eukaryote lineage (late FECA/LECA model) or (*b*) independent acquisitions of multicellularity in a total eukaryote or crown eukaryote lineage (early FECA/LECA model). In any case, these fossils may signal an interval in the early Mesoproterozoic when conditions briefly supported the evolution of large size (53).

6. THE LATEST MESOPROTEROZOIC AND EARLY NEOPROTEROZOIC: FOSSILS OF CROWN EUKARYOTES APPEAR

In the late Mesoproterozoic to early Neoproterozoic, we start to see a number of fossils that are plausibly or convincingly attributed to various clades within the crown group, including *Bangiomorpha pubescens*, a total-group red alga (18) that occurs in rocks about 1,050 Ma in age (48); *Proterocladus antiquus*, a total-group green alga—and likely total-group chlorophyte—that is 1,050–950 Ma in age (127); a 950–900-Ma septate macroalga inferred to be a total-group green

alga (86); *Ourasphaira giraldae*, a possible total-group fungus from between 1,010 and 890 Ma (80a); *Bicellum brasieri*, a possible total-group holozoan from between 1,060 and 995 Ma (125); vermiform, possibly sponge, microstructures [(and thus total-group metazoans) (128)] in 850- to 900-Ma carbonates (49); and vase-shaped microfossils, probable amoebozoans from 790–730 Ma (106, 108, 113). However, most of the eukaryotic fossils from this time—the variety of organic-walled microfossils known from Mesoproterozoic and Neoproterozoic shales and cherts—are not easily allied with particular crown clades, and it is worth considering that they are stem eukaryotes that persisted alongside crown eukaryotes. *V. lophostriata*, for example, seems reasonably interpreted as a stem eukaryote (see Section 5); if so, its occurrence in mid-Neoproterozoic rocks (56, 107) shows that stem eukaryotes did persist well into the Neoproterozoic. Other fossils may represent crown eukaryotes that simply lack a sufficient number of informative characters (cf. 116).

Whatever their affinities, several Neoproterozoic fossils provide intriguing evidence about ecological interactions during this time. Tang et al. (126) report evidence for eukaryote ectosymbiosis ~1,000 Ma in the form of numerous dark bodies, a few hundred micrometers in size, attached to carbonaceous macrofossils *Tawuia* and *Sinosabellidites* (**Figure 3*m***). The fact that they are found only on the surface of these fossils, and not scattered on the bedding plane, suggests their association is not fortuitous, and the fact that they are not embedded in the larger fossils suggests they are not reproductive structures. Rather, their distinct Raman signatures and their denser distribution on what is thought to be the ontogenetically older part of the host vesicle support the idea that they are ectosymbionts. Other microfossils that appear around this time display surface sculptures that provide circumstantial evidence for ectosymbionts, including *Caelatimurus foveolatus*, which displays embossed pits on the vesicle surface (83, 112) (**Figure 3*n***); *Daedalosphaera digitisigna* (83), which displays multiple external grooves (83); and in mid-Tonian rocks, *Vidalopalla verrucata*, which displays a number of frequently and evenly distributed short knobs (112). These features have intriguing parallels in the divots, grooves, and holdfasts found across a variety of modern protists that serve as specialized attachment structures for bacterial, archaeal, or protistan epi- and endobionts (15, 26, 47, 58, 79, 111).

Finally, several discoveries suggest that predation was important in eukaryotic ecosystems by early to mid-Neoproterozoic time. One of the most remarkable of these is a diverse assemblage of intricate phosphate-mineralized scales (e.g., **Figure 3*o***) that presumably armored a single protistan cell, just as coccoliths form an outer overlapping cover of coccolithophorid cells today (6, 27). These are important not only because of their surprising mineralogy, which likely reflects higher levels of dissolved phosphate in early Neoproterozoic seas (31) (virtually no protists form phosphatic skeletons today), but also because they likely functioned in protecting the cell from predation (29, 102). Around the same time, the fossilized shells of amoebozoan amoebae appear (**Figure 3*p***), most of which are organic in composition, though there are reports of possible mineralized (93, 105) and agglutinated (108) forms. These tests might also have served as protection; in any case, the inhabitants themselves were, by analogy with their modern counterparts, presumably predators. In addition, circular, 0.1- to 1.0-μm holes with beveled edges found in a variety of organic-walled microfossils from this time period are strikingly similar to those made today by predatory vampire-like protists (82, 103). Much larger circular, half-moon-shaped holes in the tests of some vase-shaped microfossils (**Figure 3*p***) probably also reflect predation (103, 106).

7. DIFFERENT MODELS FOR THE RISE OF COMPLEX LIFE IN THE CONTEXT OF AN EVOLVING ENVIRONMENT

One of the great gifts of the fossil record is the ability to infer the process by which modern clades evolved; another is the ability to place that evolutionary history in the context of Earth's evolving physical and ecological environment. In the interval during which eukaryotes evolved,

atmospheric oxygen concentrations changed dramatically (84, 85), as did primary productivity (32, 55); the availability of bioessential elements was limited due to the prevalence of sulfidic and anoxic water masses (7); and microbial ecosystems first experienced the pressures of eukaryotic predation (82, 103). Depending on which model is followed (**Figure 2*a***), eukaryogenesis—the process by which the modern (crown) eukaryotic cell evolved—occurred in very different Earth environments. For example, if the early FECA/LECA model is followed, then eukaryogenesis spans the Great Oxidation Event, and thus its initial stages might have occurred in anoxic habitats, with much later adaptation to oxic environments, and presumably later acquisition of mitochondria. If the late FECA/LECA model is followed, however, then eukaryogenesis occurred in a world where oxygen, though limited compared to today, was freely available, and potentially at modern levels near cyanobacterial mats. A model somewhere between these two might suggest a close connection between the early stages of eukaryogenesis and the Great Oxidation Event.

Distinguishing among these models is not easy given the spottiness of the fossil record, but they do make different predictions that future discoveries might test. If the early FECA/LECA model is correct, Mesoproterozoic rocks should yield the remains of eukaryotic sterols (steranes), and at least some, if not most, Mesoproterozoic microfossils should be found in habitats that were oxygenated, since these eukaryotes will have already acquired mitochondria. We also might expect to discover Mesoproterozoic fossils that are recognizably members of crown eukaryotes. Going even further back in time, we might expect to discover late Archean and especially early to mid-Paleoproterozoic fossils that, like those in the late Paleoproterozoic, show convincing evidence of total eukaryote affinity. A late FECA/LECA model, on the other hand, would be supported if even our most-targeted searches for steranes in Mesoproterozoic rocks come up short; if Paleoproterozoic and Mesoproterozoic microfossils are restricted to anoxic habitats; and if future fossil discoveries fail to yield obvious crown eukaryotes from late Paleoproterozoic and early Mesoproterozoic rocks, and from older rocks any eukaryotes at all.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by the Moore–Simons Project on the Origin of the Eukaryotic Cell, Simons Foundation 735933LPI (**https://doi.org/10.46714/735933LPI**), and by the National Science Foundation (EAR-1855092). We thank Shuhai Xiao and Nick Butterfield for insightful feedback on an earlier version of this paper and Zach Adam, Heda Agić, Phoebe Cohen, Abderrazak El Albani, Corentin Loron, and Qing Tang for providing images.

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