# Eukaryogenesis, how special really?

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**Eukaryogenesis is widely viewed as an improbable evolutionary transition uniquely affecting the evolution of life on this planet. However, scientific and popular rhetoric extolling this event as a singularity lacks rigorous evidential and statistical support. Here, we question several of the usual claims about the specialness of eukaryogenesis, focusing on both eukaryogenesis as a process and its outcome, the eukaryotic cell. We argue in favor of four ideas. First, the criteria by which we judge eukaryogenesis to have required a genuinely unlikely series of events 2 billion years in the making are being eroded by discoveries that fill in the gaps of the prokaryote:eukaryote "discontinuity." Second, eukaryogenesis confronts evolutionary theory in ways not different from other evolutionary transitions in individuality; parallel systems can be found at several hierarchical levels. Third, identifying which of several complex cellular features confer on eukaryotes a putative richer evolutionary potential remains an area of speculation: various keys to success have been proposed and rejected over the five-decade history of research in this area. Fourth, and perhaps most importantly, it is difficult and may be impossible to eliminate eukaryocentric bias from the measures by which eukaryotes as a whole are judged to have achieved greater success than prokaryotes as a whole. Overall, we question whether premises of existing theories about the uniqueness of eukaryogenesis and the greater evolutionary potential of eukaryotes have been objectively formulated and whether, despite widespread acceptance that eukaryogenesis was "special," any such notion has more than rhetorical value.**

eukaryogenesis | endosymbiosis | evolutionary theory | major transitions

> The eukaryotic cell originated by the most complex set of evolutionary changes since life began: eukaryogenesis. Their complexity and mechanistic difficulty explain why eukaryotes evolved two billion years or more after prokaryotes. To understand these changes, we must consider the cell biology of all five major kinds of cells; determine their correct phylogenetic relationships; and explain the causes, steps, and detailed mechanisms of the radical transitions between them. [Cavalier-Smith (1)]
>
> The acquisition of mitochondria was the pivotal moment in the history of life. [Lane (2)]
>
> Compared to prokaryotes, eukaryotic cells represent not only an alternative mode of cellular organization but one endowed with far richer evolutionary potential: only among Eukarya do we find integrated multicellular creatures that bear embryos, respond to music, and reflect on their own nature. Whatever meaning one assigns to the history of life, the advent of eukaryotic cells marked a radical and fateful transition. [Harold (3)]

In the early 1960s, Stanier et al. famously wrote that the "basic divergence in cellular structure, which separates the bacteria and blue-green algae from all other cellular organisms, represents the greatest single evolutionary discontinuity to be found in the present-day world" (4). Nowadays, a tripartite division of the living world is more popular—prokaryotes comprising Bacteria (including "blue-green algae") and Archaea. However, the sentiment remains strong that the transition between prokaryotic and eukaryotic cell types, whether by symbiogenic or archezoan schemes (5), was the single most important step in cellular evolution. The founding endosymbiosis [or syntrophic merger a la the Hydrogen Hypothesis (6)] entailed unique cellular mechanisms and had uniquely important, radical consequences for both the subsequent evolution of organismal complexity and the articulation of evolutionary theory, it is believed. The biochemist Nick Lane has written, for instance,

> If the origin of the eukaryotic cell was not a bottleneck, then it was probably a genuinely unlikely sequence of events, for it happened only once. Speaking as a multicellular eukaryote, I might be biased, but I do not believe that bacteria will ever ascend the smooth ramp to sentience, or anywhere much beyond slime, here or anywhere else in the universe. No, the secret of complex life lies in the chimeric nature of the eukaryotic cell—a hopeful monster, born in an improbable merger 2000 million years ago, an event still frozen in our innermost constitution and dominating our lives today (2).

Writing for public audiences, and often even for themselves, biologists are not loath to make simplifying claims about uniqueness and importance that are rhetoric disguised as fact. Such generalizations serve purposes in the doing of science, but are often not themselves testable scientific claims and are subject to biases. Evolutionary biology may be especially vulnerable to hype, as suggested by the frequency with which revolutionary evolutionary claims in top-notch journals are debunked (7, 8). Additionally, anthropocentrism, as it grades into "zoocentrism" (9) and then "eukaryocentrism," surely remains a subtle distorter of objectivity.

Our aim here is to critique general claims about the uniqueness and special importance of eukaryogenesis, with an aim to making them more open to question and conceptual and empirical analysis. We ask (*i*) whether eukaryogenesis entailed such a "genuinely unlikely sequence of events" (2) as to justify belief in its uniqueness as a process, (*ii*) if, as is often claimed, eukaryogenesis has a problematic or unique theoretical status in evolutionary biology, (*iii*) what intrinsic features might have conferred on eukaryotes their presumed "richer evolutionary potential" (3), and (*iv*) if this greater potential might be just a presumption, an illusion reflecting eukaryocentric bias.

## Was Eukaryogenesis Unique as a Process?

It is often said that eukaryotes arose only once. What must really be meant by this is that there was but one last eukaryotic common ancestor (LECA) from which all contemporary living things that we call eukaryotes descend, and that if other lineages with similar features arose independently, they are now extinct or have not been found. Often, LECA is conceptualized as a single cell, but unless it was obligately asexual [which comparative genomics suggests that it was not (10)], single species seems a better bet. Also, unless eukaryogenesis was a once-in-a-universe cataclysmic miracle, in which all eukaryote-specific features

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appeared simultaneously and full-blown, there were very likely many contemporaneous lineages with all or some of those features. It need not be that eukaryogenesis was so onerous that it took 2 billion years to accomplish, as Cavalier-Smith (1) maintains. As likely, we think, is that only one of many near-eukaryotic lineages survived. Notwithstanding claims about its enormous evolutionary potential, perhaps that lineage was just lucky!

Along the lineage leading to LECA, what we might choose to designate as the first eukaryotic common ancestor (FECA) (11) depends (barring that miraculous cataclysm) on which eukaryote-defining feature we take as necessary and sufficient for eukaryoteness. Nowadays, many theorists would nominate the mitochondrion as that feature. Here again we should be thinking not only of multiple contemporaneous but also reticulating lineages leading from FECA to LECA. It seems certain, for instance, that endosymbiotic gene transfer (EGT) entails the physical destruction (lysis) of the specific donor endosymbiont. Therefore, reduced endosymbiont or organellar genomes must have had slightly, and could have had substantially, different evolutionary histories than any EGT-derived nuclear genes now contributing to mitochondrial function and maintenance. Additionally, if we, in principle, accept that interspecies lateral gene transfer (LGT) was involved in the assembly of such complex prokaryotic structures as the photosynthetic apparatus (12) or type III secretion systems (13), we must also allow that the true histories of many nonmitochondrial eukaryotic cellular features are complex and reticulated, only appearing linear because of extinction and as yet poor sampling of available diversity.

For much of the last half of the last century, leading theorists would have seen the development of phagocytosis (by whatever means) as the first and perhaps most crucial event in eukaryogenesis. For instance, Stanier wrote, in 1970, "that the progressive structural evolution of the eukaryotic cell received its initial impetus from the acquisition of a novel cellular property, the capacity to perform endocytosis" (14). Lacking such a capacity, prokaryotes would be unable, it was thought, to acquire intracellular symbionts. By rights then, there should have once been "archezoa," cellular organisms with many or all of the trappings of eukaryotes, except mitochondria (15). The fact that there seem not to be such creatures surviving to this day is one (albeit not a fatal) failing of the phagocytosis-first view.

Another is the recent discovery (16, 17) that the mealybug endosymbiotic β-proteobacterium *Tremblaya* harbors the γ-proteobacterial endosymbiont *Moranella*, on which it is absolutely dependent for complementation of many of its missing genes (it retains only 120!). This finding tells us that prokaryotes can acquire other prokaryotes as endosymbionts without first developing eukaryote-like phagotrophy. No doubt, exclusively prokaryotic endosymbiosis is a very rare occurrence, but since it is the very rarity (indeed, uniqueness) of eukaryogenesis that many seek to celebrate and rationalize, *Moranella* provides an ironically inverted sort of support for endosymbiosis-first (symbiogenic) scenarios.

In this century, it is probably the evolution of mechanisms for importing necessary proteins into organelles that is taken as the most difficult, eukaryogenesis-limiting step. It is such import machineries that make EGT functionally feasible, even inevitable, according to the "you are what you eat" ratchet described earlier by one of us (18). Without them, functions that must be performed within organelles would require that the cognate genes be retained within the organelles. By the same token, that such machineries seem to have unique origins is taken as the strongest proof that mitochondria and plastids arose only once each. Zimorski et al. write that,

> The unity of those import machineries among mitochondria and plastids, respectively, is thus widely regarded as the best evidence we have for the single origin of these organelles, as opposed to multiple independent origins in different lineages, even from endosymbionts so closely related as to be indistinguishable in phylogenies (19).

In just the last few years, however, at least one endosymbiont not genealogically related to mitochondria and plastids has been shown to (likely) have independently evolved protein transport machinery. Nowack and Grossman (20) reported that three genes encoding components of photosystem I that have been transferred to the nucleus of the (eukaryotic) amoeba *Paulinella chromatophora* produce cytosol-synthesized proteins that are imported back into chromatophores. These latter are cyanobacterial descendants with highly reduced genomes, which apparently first entered into endosymbiosis only some 60 Mya, long after and completely independently from (other) plastids. Whether there is some key difference between a highly dependent endosymbiont and an organelle is a definitional matter and not a scientific question answerable by experiment (21). Indeed, McCutcheon and Keeling, prefiguring our title, recently asked, "Is there really anything so special about organelles?" (22).

Less extensive and less extensively argued over but of increasing abundance and relevance are data bearing on the timing and order of the appearance among prokaryotes of precursors of structures or processes once thought to be exclusive to and universal among eukaryotes—endomembrane systems comprising the nucleus, endoplasmatic reticulum, and Golgi apparatus and a cytoskeleton involved in cellular motility, phagocytosis, and vesicular trafficking. Coincidentally, there is mounting evidence supporting something like James Lake's long discounted "eocyte hypothesis," in which the eukaryotic nucleocytoplasmic lineage emerged from within or near the Crenarchaeota rather than before any divergence within what we call Archaea (23). If this hypothesis holds up, then we might expect to find closer and closer homologs of components of the several eukaryote-specific cellular machineries [now called eukaryotic signature proteins (24)] as we explore inferred proteomes of such archaea [the group of phyla comprising Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota (TACK)].

Indeed, we do find such homologs, as Martijn and Ettema (24) and Koonin and Yutin (25) assert in recent reviews. Martijn and Ettema (24) are so bold as to formulate what they call the "phagocytosing archaeon theory" (phAT). Starting with an (imaginary) archaeon bearing all eukaryotic signature proteins so far found in one or another of the TACK archaea, such as actin, tubulin, the ubiquitin protein modifier machinery, and several transcription and translation proteins, they envision loss of the cell wall (which does occur) and duplication of the archaeal actin, facilitating shape changes and occasional engulfing of other prokaryotes, resulting in elevated rates of LGT. None of these capacities seem impossible, although such a multiply capable creature is not known to exist now. In part to accommodate this fact, Koonin and Yutin (25) propose, similar to the phAT,

> ... that the archaeal ancestor of eukaryotes was a complex form, rooted deeply within the TACK superphylum, that already possessed some quintessential eukaryotic features, in particular, a cytoskeleton, and perhaps was capable of a primitive form of phagocytosis that would facilitate the engulfment of potential symbionts. This putative group of Archaea could have existed for a relatively short time before going extinct or undergoing genome streamlining, resulting in the dispersion of the eukaryome. This scenario might explain the difficulty with the identification of the archaeal ancestor of eukaryotes despite the straightforward detection of apparent ancestors to many signature eukaryotic functional systems (25).

Additional genomic exploration among the still largely uncharacterized TACK and DPANN archaea (a newly recognized monophyletic superphylum) (26) cannot but further narrow the prokaryote–eukaryote gap. One might predict a future day in which no steps (inventions or mechanisms) entailed in whatever is then the prevailing theory of eukaryogenesis are not found as

homologs or reasonably matching analogs in one or another prokaryotic lineage. Still, we may never find all of them in a single surviving lineage: in any case, a patchy prokaryotic distribution of preeukaryotic features is what advocates of rampant LGT as a crucial evolutionary process should expect, as Martijn and Ettema (24) and Koonin and Yutin (25) note.

Evolutionary scenarios gain plausibility through elaboration, the filling in of steps. Elaboration directs comparative data collection, which is, for scenario building, the equivalent of experimentation in hypothesis testing. Good progress is currently being made on eukaryogenesis, as other papers in this collection report. In the end, we may still have what Lane (2) calls a "genuinely unlikely sequence of events," but there will be nothing unique or special about that: all evolutionary stories are genuinely unlikely. There is no unbiased calculus by which we can say that the appearance of subsection V cyanobacteria—which grow as filaments with differentiated heterocysts, produce cyst-like resting cells and differentiated motile trichomes (hormogonia), and show true branching—entailed fewer or simpler steps than the evolution of, say, marsupials. Given the larger population sizes of prokaryotes, their evolution stories might involve more fixations by natural selection than the stories of eukaryotes (27). Although fewer of the prokaryotic fixations would be visible or seem remarkable to multicellular eukaryotes like ourselves, cyanobacteria are possibly more highly derived (or "evolved") than marsupials. It is a matter of scale and perspective.

The combinatorial stringing together of all of the steps between LECA and *Macropus rufus* (the red kangaroo), *Trichomonas vaginalis*, or Mozart is, indeed, laughably improbable. Surely, if life's tape was rewound, none of these would recur (28). However, combinatorial improbability is how evolution works: that eukaryotes arose is not unique in its uniqueness. What might still be special about eukaryogenesis is that, whatever scenario is embraced, two evolutionary lineages enter and one emerges. Some have argued that this puts eukaryogenesis outside the purview of Darwinian theory.

## Does Eukaryogenesis Have a Problematic or Unique Theoretical Status in Evolutionary Biology?

Theoretical issues that have received attention from both biologists and philosophers of biology concern the individuality (or lack thereof) of the eukaryotic cell as well as the nature of the particular evolutionary processes that gave rise to it. Is the eukaryotic cell best seen as a kind of community composed of two or three taxonomically divergent entities or as a genuine individual that can be part of an evolving population of similar things? Should the answer have an impact on biological theory?

Margulis, a champion of endosymbiotic theory, argued for the community perspective on the eukaryotic condition. Symbiotic mergers similar to eukaryogenesis, she insisted, are ubiquitous in the history of life. As a result, our understanding of evolutionary processes requires the radical revision, perhaps abandonment, of many traditional neo-Darwinian ideas. Margulis takes the issue of individuality to be central to these critiques: "The fact that 'individuals'—as the countable unities of population genetics—do not exist wreaks havoc with 'cladistics'" (29). Moreover, "[f]ailure to acknowledge the composite nature of the organisms studied invalidates entire 'fields' of study" (29).

We agree that questions about the individuality of the eukaryotic cell are important and have ramifications for our theoretical understanding of eukaryogenesis as an evolutionary process. However, in contrast to Margulis, we think that contemporary evolutionary theorizing has the resources to helpfully illuminate eukaryogenesis in terms of both process and product. What also emerges is a picture in which symbiotic mergers similar in many respects to eukaryogenesis are not entirely uncommon and can be found at hierarchical levels above that of the cell.

The starting point is to understand eukaryogenesis as an instance of a "major transition in evolution" (30, 31). Major transitions can be characterized in several ways, often in terms of the evolution of complexity or new modes of intergenerational information transfer. For our purposes, however, the most important feature of transitions is that "entities capable of independent replication before the transition can replicate only as part of a larger whole afterwards" (30). Often, this "larger whole" is conceptualized as an emergent individual. Indeed, the literature on transitions has been closely linked with that on the evolution of individuality (32–34).

It is uncontroversial that eukaryogenesis involved the fusion of two taxonomically distinct units to form a new type of cell. Eukaryogenesis can thus be distinguished from evolutionary transitions based on the integration of closely related units (as in the evolution of multicellularity). Queller (35) has dubbed the former kind of transition "egalitarian" and the latter kind of transition "fraternal" (35). Egalitarian alliances are

> ... egalitarian in the sense that both partners reproduce (although not necessarily equally). The advantage of such alliances is the bringing together of two disparate units with distinct capabilities, a combination of function, which in the new entity becomes a division of labor. The greatest barrier to such alliances may be the potential for the two parties to exploit each other, but such conflicts can be limited if there is sufficient mutual interdependence (35).

Queller here makes two important points about transitions. First, the parts of a new whole may retain some independence with respect to their ability to reproduce. Second, interdependence of parts is emphasized as a threshold that must be crossed for a new type of individual to have truly evolved. Both are what we see in the case of the eukaryotic cell. Eukaryotic cells undoubtedly exhibit many of the hallmark features of biological individuality, including spatiotemporal boundedness, indivisibility, and the integration, cooperation, and interdependence of parts. Mitochondria divide by fission, much like their bacterial ancestors. Unlike that of their free-living ancestors, however, mitochondrial replication is not autonomous; most of the proteins required are encoded in the nucleus and imported into the organelle (36). Mitochondrial replication is an evolved outcome made possible by EGT. Precisely how EGT and the resulting loss of reproductive and metabolic autonomy by the bacterial symbiont occurred is a matter of debate, but the outcome is not. All extant eukaryotic cells reproduce but also contain parts that reproduce, albeit in a way regulated by the whole. Eukaryogenesis was thus a major transition in the sense that mitochondria and their host cells "can replicate only as part of a larger whole" (30).

Godfrey-Smith (37) speaks of "Darwinian individuals" as members of "Darwinian populations," collections of entities related by descent and showing sufficient heritability of fitness-affecting variation so as to evolve by natural selection. Two things are true about eukaryotic cells. First, they are Darwinian individuals [in Godfrey-Smith's sense (37)] arising through an egalitarian transition [in Queller's (35)]. Second, the partners involved in the transition retain sufficient reproductive autonomy so as to themselves sometimes be Darwinian individuals, capable of conflict as documented in cytoplasmic male sterility (38).

Vertical transmission of mitochondria during cell replication is particularly evolutionarily significant here and underwrites the first truth. In general, vertical transmission ensures that offspring always inherit the same lineage of symbiotic partners with which their parents associate. The result is a reproductive process, in which parts of several origins are transmitted together at the creation of a new generation of eukaryote, making eukaryotic cells Darwinian individuals (39) and not communities. Vertical transmission also opens the door to "partner fidelity feedback," in which a feedback effect involving intergenerational mutual

fitness-enhancing interactions between two entities can lead to the evolution of interdependence (40).

Margulis was right that eukaryogenesis poses a challenge for evolutionary theory but wrong that a suitable articulation of Darwinian individuality could not accommodate it. There is no theoretical barrier to conceptualizing and explaining the origin of a new kind of collective individual from the merging of two independent lineages. No wholesale rejection of Darwinian theorizing (broadly construed) is required, although analysis of how common reticulated patterns of ancestry and descent are at hierarchical levels above the gene may be in order.

Eukaryogenesis is not unique as an organismal merger of entities from taxonomically distinct lineages, although the tightness of integration between mitochondria and their host is probably the most derived currently known. [The mergers that are implied by Nelson-Sathi et al. (41) would, however, be even more derived or complete if these implications are born out]. Among studied exclusively prokaryotic multilineage consortia, varying levels of integration have been documented, although endosymbiosis is extremely rare (16). Nevertheless, syntrophic interactions between anaerobic methane-oxidizing archaea and sulfate-reducing bacteria have resulted in the evolution of aggregates with a measure of coordinated growth and reproduction between the partners (42). Even farther down the organismal path is the two-species bacterial consortium "Chlorochromatium aggregatum," which has been championed as a model of bacterial multilineage multicellularity (43). In this symbiosis, green sulfur bacterial epibionts (*Chlorobium chlorochromatii*) surround a motile β-proteobacterium (*Symbiobacter mobilis*). The central bacterium relies extensively on the photosynthetic products of its epibionts and has undergone extensive genome reduction (44), a mark of evolved dependence.

In eukaryotes, endosymbiosis is common, which other papers in this collection amply illustrate. *P. chromatophora*, as previously mentioned, harbors photosynthetic endosymbionts derived from free-living cyanobacteria. The chromatophores are vertically transmitted and partitioned to offspring equitably. There is also substantial genomic integration between host and symbiont as a result of EGT and the invention of protein import machinery. *Paulinella* and its symbionts are mutually interdependent, and neither can replicate independently, indicating that the symbiosis is the result of a transition similar in many respects to eukaryogenesis (21).

Intracellular endosymbiosis can also be found at the multicellular organismal level of the biological hierarchy. Bacteria frequently associate with insects in mutualistic symbioses (most commonly among insects with nutrient-poor diets). Bacterial associates provide nutritional supplementation. Pea aphids and their relationship with *Buchnera aphidocola* bacteria are a well-understood example of this kind of association. *Buchnera* reside in specialized bacteriocytes and are maternally transmitted to offspring: the aphid/Buchnera holobiont satisfies quite stringent reproductive criteria for Darwinian individuality (45). There are many other cases of insects associating with intracellular mutualistic bacteria for nutritional supplementation (46).

Broader definitions of endosymbiosis are available, moving beyond intracellular symbionts to include symbionts inside host tissue generally (46). Once this broader definition is taken on board, it becomes apparent that symbiotic alliances making up collective Darwinian individuals are also not infrequently formed between multicellular eukaryotic partners. Perhaps the most astounding case is that of the higher attine leaf cutting ants and their close association with Leucocoprinae fungi. Inside attine ant nests is a fungal garden, which constitutes the main source of nutrition for the workers of the colony. The two partners have been coevolving in a symbiotic relationship for an estimated 10 million years (47). Fertilized queens are the propagules that found new nests, and they always transport a small bit of the fungal cultivar from their nest of origin, a form of vertical transmission (48). Phylogenetic comparison between higher attines and their fungal cultivars reveals congruence in tree topology, suggesting long periods of sustained coevolution and partner fidelity feedback between specific cultivar strains and different species of ants (49).

Attine ant colonies and their associated fungal cultivars are mutually interdependent. Ant nests cannot persist without the nutritional supplementation that their members derive from the fungus. There is also evidence that the fungal strain associated with higher attines has been exclusively asexually propagated with the foundation of new ant nests (50), suggesting that the fungus requires ant participation in its own reproduction. Higher attine ants' nests, including their fungal component, are thus good candidates for Darwinian individuality at the colonial level. The relationships among the parts of these symbiotic collectives, such as endosymbiosis of fungus within the ant nest, vertical transmission of symbionts, mutual interdependence, and community-level reproduction, parallel those of the eukaryotic cell (51).

Thus, claims about both the process and product of eukaryogenesis as being unique or as challenging contemporary evolutionary theory should at least be treated with skepticism. Processes of evolution in which entities from different lineages can be bound together in the creation of new community-level reproductive individuals are not uncommon, and such symbiotic entities occur at hierarchical levels above that of the cell, sometimes among exclusively eukaryotic partners. Although there are some aspects of the eukaryotic condition that have not been documented in higher-level egalitarian alliances (e.g., gene transfer between partners), even these aspects are not unique to mitochondria and plastids, as both the *Paulinella* and "Chlorochromatium aggregatum" cases show (21, 44).

Following Wernegreen, we endorse a perspective according to which there are several variable "axes of integration" in endosymbiotic (or indeed, symbiotic) systems, including evolutionary stability, functional integration, cellular integration, and genomic integration, each of which can be instantiated to a greater or lesser degree (46). Eukaryogenesis is not necessarily different in kind from other symbiotic alliances that have occurred much more recently in evolutionary history, but it is a very ancient one, affected at a basic cellular level. Accordingly, it receives very high scores along each of the axes of integration. Some (but not all) of the other symbioses have lower scores along some axes. However, we suggest that there is no fundamental difference in kind between these various symbiotic alliances, just different evolutionary histories. Eukaryotes do not pose unique challenges to and do not require special treatment within evolutionary theory. What remains that might be considered special, and is the subject of the rest of this review, is eukaryotes' presumed richer evolutionary potential.

## Which, If Any, Intrinsic Feature(s) Conferred on Eukaryotes Their "Far Richer Evolutionary Potential" (3)?

Once on the scene, eukaryotes are imagined to have radically altered evolution's tempo and mode. With cells structurally more complex—but at the same time, more self-similar at the cellular level—than their prokaryotic ancestors, eukaryotes radiated into a great diversity of organismal types, including complex multicellular forms with the transcendent potential to "bear embryos, respond to music, and reflect on their own nature" (3). Although few admit to frank essentialism, most would imagine that it is some (possibly single) intrinsic feature of eukaryotes (as a cell type and not a clade) that confers all of these benefits. Here, we consider some proposals as to what that intrinsic feature might be and some connections between them.

**Phagocytosis and Cellular Organization.** Phagotrophic predation as an early eukaryotic or late archaeal invention [by Martijn and

Ettema's phAT (24), or earlier such schemes] has several entailments, which might be taken as defining eukaryotic features. The need to maintain genes enabling the use of a variety of substrates and oxidants is reduced when metabolites are present in prey. Thus, although eukaryotes are behaviorally and anatomically diverse, prokaryotes house most of the world's repertoire of catabolic and anabolic enzymes and energy-yielding pathways. Cell motility and structural compartmentalization facilitate phagotrophic digestion and may have driven the evolution of further cellular complexity, as Cavalier-Smith (52) has exhaustively detailed. Also, as predators, larger protists will, of necessity, have smaller population sizes than their prey, with implications for fixation of additional, possibly maladaptive, complexities.

**Small Populations.** Lynch (27, 53) has repeatedly emphasized that drift, triumphing over selection in small populations, can account for many of the genomic peculiarities (and large genome sizes) of eukaryotes vis à vis prokaryotes and explain other eukaryotic evolutionary "advancements". He radically questions the evolutionary benefit or selective advantage of such features.

> But where is the direct supportive evidence for the assumption that complexity is rooted in adaptive processes? No existing observations support such a claim, and given the massive global dominance of unicellular species over multicellular eukaryotes, both in terms of species richness and numbers of individuals, if there is an advantage of organismal complexity, one can only marvel at the inability of natural selection to promote it. (53)

That mildly deleterious traits, some contributing to genomic complexity, can be more readily fixed in smaller populations is a prediction of population genetic theory (54). Moreover, Lynch and his colleagues, as well as one of us, have described ratchet-like processes that can create elaborate cellular machinery without improving organismal fitness (55). Whether the default interpretation of complex features is that they are adaptations and whether complexity (however defined) is itself adaptive are long-standing contentious issues in biology in general (56) that need to be readdressed in the case of eukaryogenesis.

**Introns.** Spliceosomal introns might well be one of the genomic feature that eukaryotes must put up with because of small population sizes (55). When these genic interruptions (found in all eukaryotes but, as yet, not in prokaryotes) were first discovered, Gilbert (57) ventured that their presence held the key to the enhanced evolutionary potential of eukaryotes. By permitting the reassortment of exonic information, they facilitate both the production of multiple and possibly differently functional proteins from a single gene ("alternative splicing") and the evolution of novel genes by recombination of exons from different genes ("exon shuffling"). Both processes have respectable evidential support, and alternative splicing is widely held to be an answer to the riddle of how humans can be (supposedly) more evolutionarily "advanced" than nematodes or fruit flies, say, while boasting hardly any more genes (58, 59). Alternative splicing seems less common in unicells (58), and therefore, it might be seen as an accidental eukaryotic "preadaptation" available to more complex multicellular descendants.

**Energy.** The current favorite candidate for the intrinsic eukaryotic advantage is probably energy metabolism. In a series of papers and a popular book, Lane (2) [sometimes with Martin (60)] has asserted that the enormous energy production differential that cells with mitochondria have over even the most actively respiring aerobic bacteria is what makes all of the difference. By transferring most genes other than those needed for rapidly responsive respiration to the nucleus and amplifying those few many times over (many mitochondrial genomes per organelle and many organelles per cell), eukaryotes have vastly increased the average energy available per gene expressed, protein synthesis being the cell's greatest expense. Respiring prokaryotes could have in principle done the same by amplifying a membrane-associated plasmid bearing only the relevant genes, Lane and Martin (60) admit. However, "in practice such plasmids are not found" (60), and the alternative for bacteria (whole-genome amplification) would oblige them to replicate and express hundreds of other genes superfluous to energy production. Thus,

> Put another way, a eukaryotic gene commands some 200,000 times more energy than a prokaryotic gene, or at a similar energy per gene, the eukaryote could in principle support a genome 200,000 times larger. The implications for complexity can hardly be overstated. Whereas prokaryotes frequently make a start towards eukaryotic complexity, they rarely exhibit more than one complex eukaryotic trait at a time. This is because each trait has energy costs in terms of evolving and expressing novel protein families, and unless these costs can be met generously, complexity is counter-selected for energetic reasons. (60)

This argument is appealing but flawed in several ways. First, a 200,000-fold larger genome is extravagantly in excess of requirements. We humans have, at most, five times as many genes as *Escherichia coli*, and the eukaryotic and prokaryotic versions of slime molds (with, coincidentally, amazingly similar complex phenotypes) differ only by about 70% in gene number (12,500 for *Dictyostelium discoideum* and 7,316 for *Myxococcus xanthus*). Makarova et al. (11) calculate only a twofold increase in paralogs (gene duplication being the major route to gene number increase) on the path from FECA to LECA. Moreover, any claim that eukaryotes now need hundreds or thousands of times as much DNA, simply to control that fraction that is protein coding with greater flexibility than prokaryotes can muster, must confront the C-value paradox (the fact that comparably complex eukaryotes exhibit enormous ranges in genome size) (61).

Second, an argument for why eukaryotes can, in principle, accomplish complex evolutionary feats that prokaryotes cannot should not rest on prokaryotic failures to do so in practice. There must be a compelling and articulable reason: that something has not happened is not an explanation of why it has not. Indeed, we think that conflating what has not (or has) happened with what cannot (or must) happen is one of the flaws of many eukaryogenesis stories. Pursuing the laudable goal of determining how some contingent event played out, we persuade ourselves that it was inevitable. An analogy might be the contrast between winners at poker and roulette. Although both may celebrate, only the former has the right to boast that his success involved some intrinsic skill. We do not actually know, despite much theorizing and rhetoric, whether it is luck or skill that has given eukaryotes the advantages that we perceive them to have.

Third, and perhaps most telling with respect to the ideas of Lane (2) about energy, there are many full-fledged anaerobic eukaryotes that do not derive energy from mitochondrial respiration. The genome of the protist *T. vaginalis* bears an improbably many 60,000 genes (62), and *Trichomonas* cells show most hallmark eukaryotic features (except oxygen-respiring mitochondria). Therefore, high energy production is not, in principle, necessary to be a DNA-heavy and gene-rich eukaryote. We may find it difficult to imagine how such a cell could have evolved, incremental Darwinian step by incremental Darwinian step, from anaerobic prokaryotic antecedents, but invoking a transient aerobic intermediate does not reduce the difficulty. There is no reason to believe that evolving a new trait ["expressing novel protein families" (60)] takes more cellular energy than maintaining it.

**Unique Combination of Factors.** If, indeed, eukaryotes have an intrinsic evolutionary advantage, we suspect that there is no one single feature responsible. Conferral of such advantage should have led, almost by definition, to an adaptive radiation into

multiple ecological niches and increased the likelihood that diverse lineages, some lacking the full panoply of eukaryotic traits, would survive to today. The abiding mystery of LECA is that it seems to have already possessed most defining eukaryote characteristics (63). Therefore, either the adaptive radiation-generating addition was the most recent to be acquired or that advantage, if any, requires the full eukaryotic cellular phenotype. Arguments to the effect that full-fledged eukaryotes were so much more efficient at exploiting the niches that their ancestors occupied that they drove them (and other near-eukaryotic sister lineages) to extinction seem, in the context of extensive knowledge about the fine-scale mapping of microorganisms to microniches, ecologically naive.

Unquestionably, eukaryotes evolve differently in tempo and mode than do prokaryotes, if we assess this by uniform measures of structural and functional innovation at the levels of genes and their products or cellular phenotypes. However, cyanobacteria will also surely evolve differently from mycoplasmas or methanogens. What are our principled metrics for degrees of difference, and how are we sure that these are free of the sorts of eukaryocentric bias that we discuss in the next section? What is our metric for "richer evolutionary potential" (3)?

An instructive comparison, alluded to previously, is between the bacterial and protistan slime molds *Myxococcus* and *Dictyostelium*. In both genera, amino acid starvation causes individual motile cells to aggregate (in response to analogous signaling mechanisms) into fruiting bodies that differentiate, with some cells sacrificing their evolutionary futures so that their sisters (not always identical) might form spores. Fruiting bodies are structurally diverse among species and, without a microscope, prokaryotic and eukaryotic forms would be hard to tell apart on the grounds of structural complexity alone. In either, cheaters less inclined to self-sacrifice can arise (64). It is not clear that the ∼12,500 genes of *Dictyostelium discoideum* or any of its eukaryote-specific cell structures give it an advantage over *Myxococcus xanthus*, with ∼7,500 genes (and no eukaryotic cell structures). Although some might demur that *Myxococcus* is an unusually sophisticated (and large-genomed) prokaryote, the point is not that prokaryotes routinely evolve eukaryote-level complexity but that they sometimes can. To the objection that eukaryotic slime molds still do not "respond to music, or reflect on their own natures" (3), we would counter that *D. discoideum* and its congeners are, nevertheless, likely well above the eukaryotic average in complexity of form and behavior. Most eukaryotes live unicellular lives.

## Is the Evolutionary Advantage of Eukaryotes over Prokaryotes in Fact Illusory?

Perhaps before the question, "What feature(s) confer on eukaryotes their evolutionary advantage?" should have been the question, "Is there such an advantage?" Although Lane may have been facetious when he wrote, "Speaking as a multicellular eukaryote, I might be biased …" (2), we take the possibility of eukaryocentrism seriously. Despite the efforts of Gould (9, 28) and others, biologists may still be unconsciously predisposed to a sort of biological progressivism. We may see eukaryotes as possessing, to a greater degree than prokaryotes, "advanced" traits that we value—in particular, structural complexity and diversity—and then frame how we think about such very difficult-to-define traits from a eukaryotic perspective. Although differently motivated and of less social consequence, eukaryocentrism is comparable with that eurocentrism in which the preeminence of the West is interpreted as reflecting some intrinsic racial or cultural superiority rather than mere historical contingency coupled with metrics of success that inevitably make Europe look good. In this section, we name and explore potential eukaryocentric biases.

**Statistical Eukaryocentrism.** Of multicellularity, widely hailed as an achievement enabled by eukaryogenesis, Lynch writes,

> Complex multicellularity has only arisen twice, once in animals and once in vascular plants. One might add fungi to the list, although the number of fungal cell types is not large, and there is some question as to whether multicellularity was ancestral to the phylogenetic group that contains animals, fungi, and slime molds. In any event, the probability that two or three origins of multicellularity simply arose by chance within eukaryotes as opposed to prokaryotes is somewhere on the order of 1/4–1/2, well below the general standards of statistical validity. Of course, many other eukaryotes are capable of producing a few different cell types, but the same is true for prokaryotes, some of which produce radically different cell morphologies. (53)

Many will, of course, disagree on how often multicellularity or "complex multicellularity" (53) has evolved, how either should be defined, and indeed, whether prokaryotes have not, in fact, evolved simpler forms of multicellularity more often (65). There is no comparability in definitions of taxa at any rank (66) that would allow quantitative comparisons of frequency. An even more ambitious comparative analysis of "complexity" or realized "evolutionary potential" designed to show that eukaryotes fall far outside any normal distribution would surely be even more problematic given unavoidable biases at both organismal and genomic levels. That truly complex multicellularity is an exclusively eukaryotic achievement is unquestionably true. That it could not have been otherwise is speculation bolstered by the false belief that what has happened must have happened.

**Organismal Eukaryocentrism.** Of course, a human is in many ways more complex an organism than a single *E. coli* cell, and to understand the former is, by far, the more daunting task. However, perhaps this comparison is not the right one. Shapiro (67) has long held that it is the microbial colony and not the solitary cell on which biologists should focus their attention. A more inclusive—multispecies—organized entity would be the biofilm (68). Indeed, some current thinking in microbiomics would have us look at microbial communities with their complex patterns of shared metabolites and (over a longer time frame) shared genes as semiorganized and extraordinarily complex biological entities (69), forming, in some cases, holobionts with their hosts. In such a perspective, prokaryotes collectively will, of course, seem different from those few large multicellular eukaryotes that have preoccupied biologists and philosophers of biology as long as there have been either. However, it is not easy to say which is the more advanced or "endowed with far richer evolutionary potential" (3). Where is the commensurability between metabolic and morphological innovation?

**Genomic Eukaryocentrism.** Maynard Smith and Szathmáry (30), not unlike others, entertained a genomic measure of complexity, noting in its favor a general increase in DNA content from prokaryotes to protists to multicellular animals and plants. Although there is some roughly similar correlation in the number of protein coding sequences, most differences in genome size reflect transposable elements and noncoding intergenic spacers. Accepting (for argument only) that this material is regulatory in nature, we might still ask whether its possession is an evolutionary advancement, enabling greater phenotypic complexity. Some theorists have argued that the eukaryotic nucleus is the more primitive, burdened with inefficiencies left over from a rather messy origin of cellular life (70). Others, as we noted earlier, see it as replete with maladaptive genomic accumulations, which may or may not affect regulation of gene expression (27, 53, 61).

There is no reason to believe that unicellular protists with large genomes regulate the expression of their genes more efficiently or with greater flexibility than do prokaryotes with much less DNA. In fact, because of their more rapid generation times and larger population sizes, we might expect prokaryotes to have more exquisitely refined regulatory systems, more selection per base pair as it were—a sort of historical complexity, alluded to

above in our cyanobacteria vs. marsupial comparison. We are left then with the evolutionary potential of excess eukaryotic DNA and its ability to be co-opted into function, which might well impart a different tempo and mode to eukaryotic evolution. However, we would surely be hard pressed to show that co-optation is a more effective route to evolutionary innovation than LGT. Indeed, if LGT is fully integrated into our understanding of genomic complexity, then it is perhaps the full *E. coli* pangenome, soon to be if not already exceeding in number of coding sequences the human genome, that should be compared with the latter. Conceivably, its pangenome (especially if including rarer interspecific transfers) gives this single bacterial "species" a far greater range of metabolic innovation than that shown by all eukaryotes, a defensible alternative metric of evolutionary potential.

A common move in philosophy of biology is into pluralism, which in this context would be the admission that there are several ways of conceptualizing eukaryogenesis, each with its advantages but none having an exclusive claim on truth. From our perspectives as large multicellular eukaryotes able to reflect on our own natures, it is not wrong to see eukaryogenesis as a signal event. However, this is not the only justifiable perspective. Sentiments such as those quoted at the beginning of this essay, if taken as objective, mislead us into thinking that it is.

## Conclusion

It is easy to problematize any scientific statement by asking, "What do you mean by X?", with X being one of its terms. Mere semantic quibbling is the sort of thing that can give philosophy a bad name, and we hope to have accomplished more than that here. The eukaryogenesis story as usually told (and exemplified in our epigraphs) is a sort of metascientific narrative, invaluable in motivating and positioning new research activities of all sorts and organizing existing bodies of knowledge in our minds and our textbooks. However, it is as much rhetoric as fact, and the public, not to mention biologists themselves, may often conflate the two. Our analysis has had four arguments.

*i*) The criteria by which we judge eukaryogenesis to have required a "genuinely unlikely series of events" (2) 2 billion years in the making are gradually being eroded by discoveries (of other symbioses and archaeal features) that fill in the gaps of the prokaryote:eukaryote "discontinuity" (4). Scenarios embracing LGT and invoking transient populations of archezoa-like ancestors are increasingly appealing.

*ii*) Eukaryogenesis confronts evolutionary theory in a way not different from other egalitarian transitions; parallel systems can be found at several hierarchical levels. In eukaryogenesis, the product of fusion and integration is, in the end, almost back to its starting point as a cellular reproducer, because one partner has nearly completely lost its Darwinian individuality, as discussed by Godfrey-Smith (71) elsewhere in this collection. Mitochondria are still reproducers, albeit heavily scaffolded—but that fact does not make them different from transposable elements, which are not usually considered to be coeffectors of a major transition. It is thus at least possible to deflate the "majorness" of eukaryogenesis as a transition.

*iii*) Identifying which of several complex cellular features confers on eukaryotes a "far richer evolutionary potential" (3) remains an area of unbridled speculation, in which various keys to success have been proposed and rejected over the five-decade history of research in this area. Several prokaryotic lineages have arguably achieved an organizational complexity greater than that of the average eukaryotic lineage without benefit of these particular features (for which a selective benefit has not been shown).

*iv*) The fact that eukaryotes have, indeed, the "far richer evolutionary potential" (3) is perhaps the most celebrated element of eukaryogenesis stories but the most easily criticized. It is difficult and may be impossible to eliminate eukaryocentric bias from the measures by which eukaryotes as a whole are judged to have achieved greater success than prokaryotes as a whole. The ways in which we define complexity, organisms, and genomes all privilege eukaryotes. Also, if eukaryotes have indeed triumphed by some purely objective measure, we have not eliminated the null hypothesis that this was caused by chance.

There are many useful ways of understanding evolution, and their articulations can be intellectually valuable and experimentally fruitful. We advocate a more self-conscious pluralism that would require not that we stop telling eukaryogenesis stories but that we do recognize them for what they are. We do not claim to know whether there is any best story, any theory by which the apparent differential success of eukaryotes can be objectively probed and causally rationalized. What we have questioned here is whether premises of existing theories have been objectively formulated and whether, despite widespread acceptance that eukaryogenesis was "special," any such notion has more than rhetorical value.

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