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Review article

Symbiogenesis, gradualism, and mitochondrial energy in eukaryote evolution

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Abstract

The origin of eukaryotes is one of the big questions in evolution. Many different ideas about eukaryote origin currently coexist in the literature that weight the evolutionary significance of mitochondria differently. Gradualist theories depict the origin of eukaryotic cell complexity and the origin of mitochondria as independent, unrelated processes that occurred in series. Symbiogenic theories depict the origin of eukaryotic cell complexity as emerging from the symbiosis that gave rise to mitochondria. Since it was introduced over 100 years ago, the idea of symbiogenesis has been controversial. It posits that rarely in Earth history, and only in microbes, a mechanism of evolution has operated in nature that forges new taxa at highest ranks via the endosymbiotic combination of two cells into one. The role of symbiogenesis in evolution versus the gradualist paradigm of constant and incremental mutation accumulation is still debated. A perceived problem with symbiogenesis is that it operates discontinuously and rarely during evolution. Moreover when it does operate, major evolutionary transitions, such as the origin of eukaryotes, plants, and algal groups are the outcome. Here I briefly contrast current symbiogenic and gradualist views on eukaryote origin regarding phagocytosis, the host for mitochondria, eukaryote anaerobes, the eukaryote endomembrane system, gene transfers from organelles, lateral gene transfer, and the number of endosymbiotic partners in eukaryote history. Special attention is given to energy conservation in mitochondria, which fostered eukaryotic complexity by lifting energetic constraints on protein synthesis in mitochondrion bearing cells relative to their prokaryotic ancestors.

INTRODUCTION

The term „symbiogenesis“ made its debut in the literature in the title of Konstantin Mereschkowsky's four parted 1910 series (*1*), which followed his 1905 exposition that the plastids of plants and algae descend from cyanobacteria via symbiosis (*2*). The idea of symbiogenesis got off to a rocky start. Mereschkowsky gave the term symbiogenesis (origin via living together) a specific meaning: some higher taxa arise via combination of preexisting cells, one living inside the other. The word placed De Bary's 1878 term symbiosis (*3*), in Darwin's context of natural variation and cladogenesis. Mereschkowsky pioneered the idea that plants and algae arose symbiogenically, and that eukaryotes arose via symbiogenesis, but he paradoxically rejected the notion that mitochondria were descended from endosymbionts, his symbiogenic origin of eukaryotes has the nucleus being an endosymbiont (*1*). The symbiogenic origin of mitochondria (but not of eukaryotes) was argued by Portier (*4*) in French and by Wallin (*5*) in English, but not, as often claimed, by Altmann (*6*) in German, who mentions neither

symbiosis nor mitochondria in his book and whose colonies of 'bioblasts' refer to aggregations (colonies) between organizational states of matter between the level of molecules and cells (bioblasts). Altmann likened his bioblasts to crystals, not to bacteria or to cells. Curiously, Wallin (*5*) thought that Altmann was talking about mitochondria when he wrote of bioblasts, but having read and reread Altmann (*6*) neither Höxtermann nor I (*7*, *8*) have a clue how Wallin came to that conclusion. Today we know that mitochondria and chloroplasts really did arise via endosymbiosis. We probably know that just as surely as we know that Darwin's basic idea about evolution was correct or that chromosomes are made of DNA. Today people still disagree however whether endosymbiosis was significant as a mechanism of evolution, or whether it just occurred but without evolutionary impact. The debate is not new. Since its inception, the concept of symbiogenesis has polarized biologists and caused debate. Current debate on symbiogenesis has focused on the role of mitochondria at the origin of eukaryotes. Proponents of symbiogenesis, or endosymbiotic theory, have it that symbiosis is a rare but crucial mechanism of evolution (*7*, *8*, *9*, *10*, *11*, *12*, *13*, *14*). Proponents of the opposing gradualist view admit that endosymbiosis occurred when mitochondria arose, but hold that it had no impact on the evolutionary process (*15*, *16*, *17*). Even in 2017, there are staunch opponents of symbiogenesis who argue that mitochondria did not even arise from endosymbionts in the first place (*18*). Clearly, current views concerning the evolutionary significance of mitochondria in today's literature are divergent.

Diverging views about mitochondrial and eukaryote origin have been summarized in many recent special issues devoted to the topic. The February 2014 special issue of *Cold Spring Harbor Perspectives in Biology* published a series of reviews on eukaryote origin, as did a 2015 issue of *The Philosophical Transactions of the Royal Society of London* (vol. 370, no. 1678). A 2015 special issue of *The Proceedings of the National Academy of Sciences of the USA* (vol. 112, no. 33) published a collection of papers on endosymbiosis and eukaryotes, and in 2017 a special issue of *The Journal of Theoretical Biology* (still in press at time of submission) was specifically devoted to the topic of endosymbiosis in eukaryote evolution as a summary of what has happened since Lynn Margulis (then under her married name Sagan) published her 1967 paper rekindling and modifying endosymbiotic theory (*19*). Those special issues underscore broad interest in the question. Other reviews and papers dedicated to the topic have also appeared recently (*10, 11, 14, 20*, *21*, *22*, *23*, *24*). Why is so much being written on eukaryote origins? Arguably because the origin of eukaryotes was a very important event in evolution, marking the singular transition in Earth and life history from simply organized cells (prokaryotes) to the complex organized cells (eukaryotes) that make up all complex life. Why yet one more paper? Because we have evidently still not gotten to the heart of the matter.

The main controversy on symbiogenesis concerns eukaryote origin. The debate is not about hopeful monsters (*25*), saltational evolution (*26*), or punctuated equilibrium (*27*), where the conceptual focus gravitates towards the possible evolutionary significance of mutations in one or a few genes that have very large morphological effects. It has more in common with the debate about hybridization in plant speciation, where one rare hybridization event can produce new species with fertile progeny having stark differences in morphology and chromosomes that dwarf, in terms of evolutionary effects per unit time, the workings of standard allele combinations in populations (*28*). The big difference between hybridization at the origin of new species vs. symbiogenesis at the origin of eukaryotes is that in the former, the partners are interfertile conspecifics or congeners whose progeny have more chromosomes, whereas in the symbiogenic origin of eukaryotes the partners were an archaeon and a bacterium separated by over a billion years of distinct evolutionary history whose progeny have new organelles (mitochondria) and energy metabolism. The debate between gradualism and symbiogenesis at eukaryote origin concerns the nature of evolutionary forces that helped microbes cross the deepest divide in the living world at the level of cell organization: the prokaryote eukaryote boundary.

Serious debates over symbiogenesis have never been about the origin of *new species*, though Margulis in some of her later writings (*29*) made wild claims to that effect. From its first appearance in the library over 100 years ago until today, symbiogenesis has been about the origin of *two higher taxa of unicellular life forms* having novel cell organization relative to their predecessors: eukaryotes and plants (*7*). This paper is about symbiogenesis at the origin of eukaryotes. Each of the following eight sections briefly highlights diametrically opposed gradualist and symbiogenic views (Table 1) regarding the nature of the evolutionary process underlying the prokaryotic roots (the mitochondrial symbiont and its host) of eukaryotic cells.

I. Phagocytosis

Traditional views have it that the host that acquired the mitochondrion obtained it by phagocytosing another cell. In that view, which one might call „phagocytosis first", the cellular machinery required for phagocytosis (uptake of bacterium-sized particles as food) and for gleaning energy from the chemical breakdown of ingested food particles was already present in the host cell that acquired the mitochondrion. In phagocytosis first models, the mitochondrion was the result of a fortuitous meal, the fateful food was not digested and somehow became useful. This view has been around since Margulis (as Sagan) repopularized endosymbiotic theory (*19*, *30*, *31*, *32*, *33*, *34*, *35*) and has many modern incarnations (*36*, *37*, *38*, *39*) which envisage the host starting out in evolution organized as a prokaryote, with the evolutionary origin of phagocytosis

being gradually selected via point mutation and gene duplication before the origin of mitochondria, with phagocytosis being key to acquiring the mitochondrion.

The symbiogenic view posits that the mitochondrion was not acquired by phagocytosis, it entered the host cell by some other mechanism, with phagocytosis arising after the mitochondrion, as reviewed elsewhere (*7*). Phagocytosis is a feeding habit. For 50 years, people have assumed that phagocytosis was somehow advantageous (*35)* relative to feeding habits among heterotrophic prokaryotes, which digest their substrates extracellularly and import small molecules. Nobody ever checked to see whether that assumption is true for a prokaryote, until recently (*23*). We recently did the calculations and found, surprisingly perhaps, that if a prokaryote were to evolve phagocytosis before it acquires mitochondria it would have a much poorer energy yield from substrates than a non-phagocytotic prokaryote (*23*). That is, ATP yield and energy flux for a hypothetical prokaryote trying to become phagocytic *without* mitochondria significantly decreases relative to normal prokaryotic heterotrophic energy metabolism, which usually entails chemiosmotic harnessing. This is mainly because a phagocyte cannot maintain chemiosmotic energy conservation in its plasma membrane (*23*), for which reason a hypothetical prokaryotic phagocyte would have to ingest 34–170 times its weight in 'food bacteria' in order to energetically finance one cell division (*23*). Phagocytes live from amino acid breakdown, because cells are made mostly out of protein, about 50–60% by weight, the most common sugar in the cell being ribose, about 8% by weight (*40*). In the *presence of mitochondria*, a phagocytotic feeding style could be extremely beneficial for the cell, but the energetic prospects for the kind of phagocytosing host for the *origin of mitochondria* that people have had in mind for 50 years are bleak, at best (*23*). In other words, if cell already had mitochondria, acquired by any of the phagocytosis-independent mechanisms of symbiotic association that we know among modern prokaryotes (*23*) such as anaerobic syntrophy (*41*), then the subsequent origin of phagocytosis could be beneficial (*23*). Phagocytosis rather clearly appears to be an invention of a cell that had mitochondria, not *vice versa* (*23*).

II. The nature of host that acquired mitochondria

Related, but not identical, to the proposition that the host for the origin of mitochondria was a phagocyte is the proposition that the host for the origin of mitochondria was a eukaryote, having eukaryotic cell organization, a nucleus, endomembrane system, mitosis, meiosis and so forth, before acquiring the mitochondrion. That view is also about 50 years old (*30*, *31*, *32*, *33*, *34*, *35*, *37*, *39*) and is still popular among some specialists (*42*). It relates to the idea of archezoa (*43*, *44*), that is, it entails the existence of primitively amitochondriate eukaryotes, as reviewed elsewhere (*45*). In essence, the eukaryote host model proposes that eukaryote cell complexity arose before the origin of mitochondria and that — as with phagocytosis first — mitochondria came in after the host had already attained eukaryote cell complexity by standard gradualist mechanisms (mutation, gene duplication, selection).

In symbiogenic models, eukaryotic complexity arose after the origin of mitochondria. Since the late 1970's the archaeal nature of eukaryotic ribosomes (*46, 47*) has been evident, hence the minimalist premise for symbiogenetic models in the modern era (in the times since we knew about archaea) is that the host was an archaeon. Several archaeal host models have been suggested, based on sulfur cycling (*48*), ATP exchange (*49*) or H_2-based syntrophy (*41*). Sulfur does play an important role today in mitochondrial metabolism (*50*, *51*, *52*). Clearly, ATP export from the mitochondrion also did come into play at some point in mitochondrial evolution (*49*, *53*, *54*). Yet from the physiological perspective, the proposition of H_2-based syntrophy as the initial benefit linking mitochondrion and host (*41*) is of particular interest because of the widespread occurrence of H_2-dependence among modern archaeal lineages that appear related to the host (*55*, *56*), the widespread existence of anaerobic syntrophy among modern prokaryotes (*57*) and especially because of the widespread occurrence of hydrogenosomes and H_2-producing mitochondria among eukaryotic anaerobes (*41*, *58*).

As current metagenomic studies uncover new archaeal lineages that seem, based on trees of concatenated ribosomal proteins, to be more closely related to the host than cultivated archaeal lineages (*24*), most observers interpret this as supporting the archaeal host (symbiogenic) concept. That the host should turn out to be an archaeon is precisely the prediction made by some archaeal host models for the origin of mitochondria (*7, 41, 55, 56*). But in the same breath one should say that concerns have recently come to the fore about the data underlying the phylogenies of the new archaeal lineages (*59*), in that one of the proteins in the archaeal metagenomic data set might be eukaryotic, not archaeal (*59*) and might be influencing the phylogenetic signal. It would not be the first time that metagenomic data used for generating concatenated alignments contained small amounts of contaminating signal that could drive the phylogenetic result in a particular direction, as the contrasting results of Rinke et al. (*60*) and Williams and Embley (*61*) exemplify. This can happen if the phylogenetic signals of the individual proteins in a concatenated alignment are weak and mildly conflicting (*62*) in which case one protein with a strong signal out of dozens with weak signals can determine the phylogenetic result in every bootstrap sample of the concatenated data (*63*). At the same time, the new archaeal lineages that appear to be related to the host *are* archaea, as archaeal host models predicted (*41*), and some trees obtain an archaeal host using rRNA sequences alone (*20*).

III. Hydrogenosomes and eukaryotic anaerobes

As recently explained elsewhere (*64*), neither Margulis's explicit version of endosymbiotic theory (*19*) nor less explicit versions descended from it (*65*) acknowledged the existence of eukaryotic anaerobes. Eukaryotic anaerobes simply did not fit into Margulis's versions of endosymbiotic theory, which were built entirely on oxygen (*64*). An after the fact remedy to that gap was the archezoa hypothesis, which argued that eukaryotic anaerobes were deep branching lineages that never possessed mitochondria (*43*). Early trees of rRNA seemed to support that view (*66*, *67*). Two main ideas are currently discussed for the origin of eukaryotic anaerobes, one gradualist, one symbiogenic. The gradualist view is that eukaryotes started out their evolutionary history as obligate aerobes, that the ancestral mitochondrion was an obligate aerobe specialist like Rickettsia (*68*), and that the ability to survive in anaerobic environments was gradually acquired during evolution in various eukaryotic lineages via lateral gene transfer (LGT), either from prokaryotes or from eukaryotes that had themselves become anaerobic via earlier gradual LGTs (*69*, *70*, *71*, *72*, *73*). There are several major problems with the origin of eukaryotic anaerobes via LGT, as discussed at length elsewhere (*74*), in addition to minor problems, such as the fact that the gradualist LGT origin for eukaryote anaerobes hinges wholly upon Lamarckian inheritance (traits being impressed from the environment into eukaryotic inheritance).

Symbiogenesis has it that eukaryotes started out their evolutionary history as facultative anaerobes that were able to respire oxygen like rat liver mitochondria, but were also able to survive under anaerobic conditions with H_2-producing anaerobic fermentations using the oxygen sensitive enzymes germane to hydrogenosomes, anaerobic forms of mitochondria found among all major eukaryotic lineages (*41*, *58*, *75*). In that view, the ancestor of mitochondria was a normal generalist facultative anaerobe, like most modern proteobacteria are (*76*, *77*), able to respire oxygen when it was present, but able to switch to anaerobic ATP synthesis and H_2-producing fermentations when needed. The symbiogenic view for eukaryote anaerobe origin posits that in addition to the roughly 1000 genes required to make a mitochondrion heritably functional, a dozen or so genes for anaerobic fermentations also entered the eukaryotic lineage at mitochondrial origin, such that aerobic respiration, anaerobic respiration and hydrogen-producing fermentations, in addition to heterotrophy in general, entered the eukaryotic lineage at mitochondrial origin, as a single inheritance from the facultatively anaerobic metabolism of the mitochondrial endosymbiont, followed by ecological specialization differential loss in independent mitochondrion bearing eukaryotic lineages (*41*, *58*).

Another important aspect of eukaryotic anaerobes concerns their connections to Earth history. Eukaryotes arose more than 1.5 billion years ago (*78*), whereby modern views of ocean geochemistry have it that the Earth's oceans were anoxic up until about 580 million years ago (*79*). That is a major change relative to Margulis's day (*19*), when it was thought that the oceans became oxic 2.3 billion years ago. That also means that eukaryotes arose and diversified into their major lineages at a time in geological history when anaerobic conditions dominated the Earth's ocean habitats. The gradualist theory that eukaryotes were ancestrally obligate aerobes is not easily integrated into the modern view of Earth ocean chemistry and would require a number of corollaries that its proponents have so far not explicated because they generally make no attempt to integrate the view of anaerobes late into geological accounts of Earth history. The symbiogenic view that eukaryotes were facultative anaerobes from the outset fits very naturally with modern versions of Earth ocean chemistry without additional corollaries (*58*, *75*).

IV. The origin of the eukaryotic endomembrane system

Gradualist depictions have it that the eukaryotic endomembrane system — the endoplasmic reticulum, the Golgi, and its associated vesicle flux — arose from invaginations of the plasma membrane in a prokaryotic cell (*35*, *42*, *80*, *81*, *82*). That view is often tightly coupled to the proposition that phagocytosis preceded mitochondria, as outlined in section (I) above, that the host was a eukaryote, as outlined in section (II) above, and with the proposition, usually implicit, that the ER lumen is homologous to the environment surrounding the host that acquired the mitochondrion (*35*, *42*, *80*, *81*, *82*).

The symbiogenic version has it that the ancestor of mitochondria, when it took up residence as an endosymbiont in its archaeal host, secreted outer membrane vesicles (OMVs), like modern bacteria do (*22*). In the symbiogenic context, these OMVs, vesicles of bacterial lipids, accumulated in the archaeal cytosol, providing the seeds of an endomembrane system with ancestrally outward flux upon which the host's archaeal Sec pathway machinery for the insertion of proteins into membranes could operate (*22*). These vesicles, ancestrally stemming from the mitochondrion, ultimately gave rise to a primitive endomembrane system comprising the ER, from which the nuclear membrane ultimately arose. This would fit with the observation that the nucleus arises from the ER during the cell cycle (*83*), with the observation that lipid synthesis in eukaryotes is today associated with the mitochondrion and the ER (not with the plasma membrane as in prokaryotes) (*22*), and with the observation that mitochondria still today actively secrete membrane vesicles into the cytosol (*22*), they are called mitochondrion derived vesicles, or MDVs (*84*). Peroxisome biogenesis, long a point of heated debate in the context of eukaryotic endomembrane system origin (*30*, *85*), is now increasingly understood as a result of mitochondrial origin (*86*, *87*) and was even recently

shown to require the participation of MDVs (*88*, *89*). A physical origin of the eukaryotic endomembrane system from OMVs of the mitochondrial endosymbiont has it that the archaeal lipids of the host were replaced by bacterial lipids through the secretion of bacterial OMVs produced by the mitochondrion, vesicles which today are homologous to MDVs (*22*, *23*), and to the proposition that the ER lumen is homologous to the periplasmic space of the mitochondrial symbiont (*22*, *23*). This would explain why lipid synthesis in eukaryotes is localized on the mitochondrion and ER while it is localized on the plasma membrane in prokaryotes (*22*). It would also explain why protein glycosylation occurs at the ER of eukaryotes (*90*) and at the cytoplasmic membrane of prokaryotes (*91*, *92*). In the symbiogenic view, archaeal mechanisms of membrane protein targeting, including the Sec pathway, and dolichol phosphate glycosylation became targeted to cytoplasmic OMVs derived from the mitochondrial endosymbiont, giving rise to a primitive endomembrane system in the nascent eukaryote — an archeaon that harboured a mitochondrial ancestor that secreted vesicles of bacterial lipids into the archaeal cytosol, generating ancestrally outward endomembrane flux (*22*).

V. A mechanistic role for mitochondria and their ATP synthesis at eukaryote origin

For as long as it has been known that mitochondria make ATP via oxidative phosphorylation, it has been clear that ATP production itself cannot have been the *initial* benefit that associated mitochondria to their host (*41*, *53*), which is why other initial benefits of host symbiont association such as H_2-production in anaerobic syntrophy have to be considered (*14*, *41*). However, it is also clear that the ADP-ATP translocase of the inner mitochondrial membrane was present in the eukaryote common ancestor (*93*), as was the import machinery of the mitochondrial inner and outer membrane (*94*, *95*) that allows mitochondria to import the cytosolic products of genes that it had outsourced to the nucleus (*96*). From that it follows that in the last eukaryote common ancestor, the mitochondrion was no longer an endosymbiotic bacterium, it had already been converted into an organelle that imported proteins from the cytosol and exported ATP in exchange for ADP before the major eukaryotic lineages has started to diverge.

That is important information to hold onto in thinking about eukaryote origin. The ensuing questions remain simple but the answers are harder. Were mitochondria just an afterthought in the origin of eukaryotes, as the gradualist view would have it? Or did mitochondria actually *do something* that affected the course of eukaryote origin that would help to explain why the only cells that became eukaryotic derive from an ancestor that possessed mitochondria, as proponents of symbiogenic eukaryote origins contend (*22*, *23*, *41*, *83*, *97*).

The recognition that the origin of mitochondria changed the bioenergetic configuration of the eukaryotic cell in such a way as to provide eukaryotes with more energy per gene relative to prokaryotes added impetus to the case for symbiogenesis (*54*) and helped to underscore the importance of energy in evolution more generally (*98*). Improved energetics in eukaryotes as a result of mitochondrial origin (the ability of eukaryotes to perform ATP synthesis on internalized bioenergetic organelles as opposed to prokaryotic ATP synthesis at the plasma membrane) goes a long way to explaining why the only cells in nature that are genuinely complex trace to a common ancestor that had mitochondria. Importantly, the energetic difference between prokaryotes and eukaryotes is not oxygen respiration, because facultatively anaerobic but oxygen respiring prokaryotes like *E. coli* never became complex, only the cells that have mitochondria did. The reason is that O_2 respiration makes a difference of about 6-fold in ATP yield per glucose — 30 ATP per glucose with O_2 *vs.* 4–5 ATP without O_2 (*58*) — but O_2 makes the *same* difference both in prokaryotes and eukaryotes; by contrast, the presence of mitochondria conferred upon their host several orders of magnitude improvement in terms of energy per gene (*54*). The connection between energy and complexity is that eukaryotes have additional energy at their disposal for the synthesis of proteins that are not required for core energy metabolic and housekeeping needs, affording them freedom to explore the expression of new proteins, the ones that make the difference between prokaryotes and eukaryotes (*54*).

The energetic and symbiogenic argument that mitochondria really made a difference at eukaryote origin (*54*) prompted Booth and Doolittle (*15*) to argue from a philosophical standpoint that mitochondria had no impact on evolution, to which an exchange (*99*, *100*) was published. It also prompted Lynch and Marinov to argue from a population genetic standpoint that mitochondria had no impact on eukaryote evolution and that „*variation in the power of random genetic drift has played a central role in the historical diversification of genome and possibly cellular architecture across the tree of life*“ (*16*), to which a separate exchange (*101*, *102*) was published.

Lynch and Marinov (*17*) did some more counting in the meantime and concluded once again that „*there is no reason to think membrane bioenergetics played a direct, causal role in the transition from prokaryotes to eukaryotes*“ (*17*). Lynch has previously argued, independent of energetics, that gradual mutational processes and effective population size, N_e, are the sole determinant of the prokaryote to eukaryote transition, hence the papers with Marinov are nothing new. Lynch and Conery (*103*) stated: „*If the theory that we present is correct, and should free-living prokaryotes with sufficiently small long-term N_e be found, we predict that they will harbor many of the same genomic changes that we have described here for eukaryotes*“. That view that is reasserted by Lynch and Marinov (*17*):

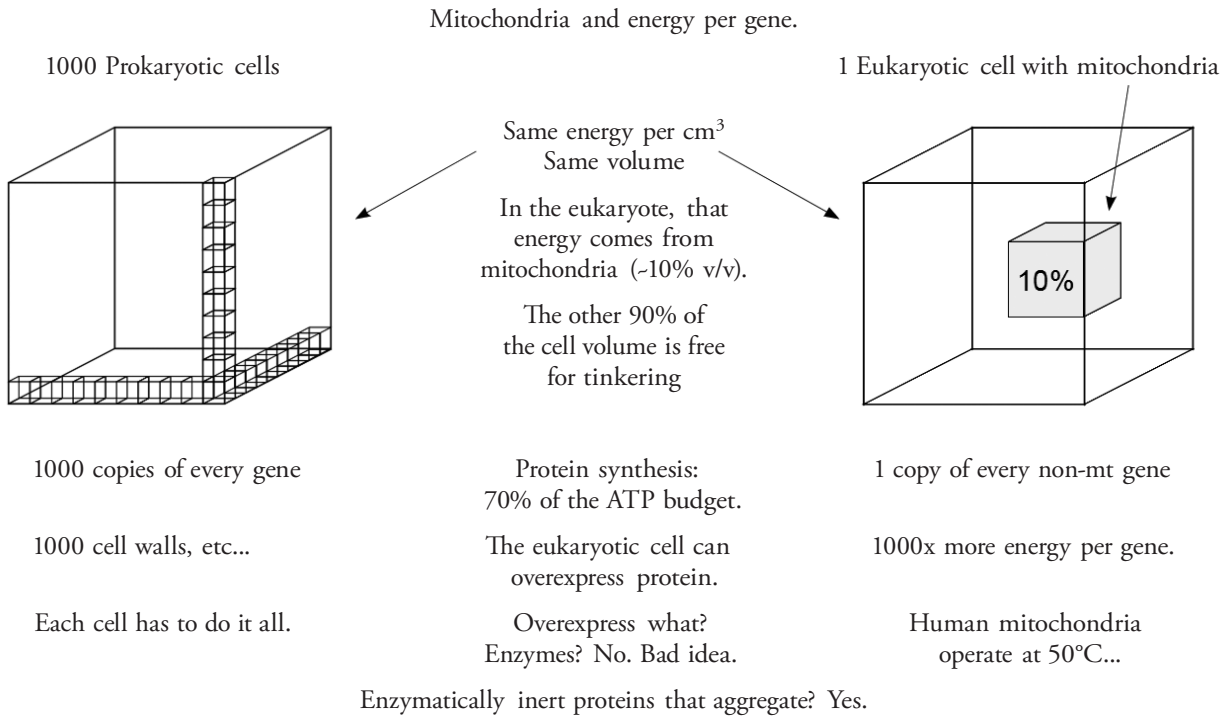

Figure 1. *Schematic depiction of the energetic difference between prokaryotes and eukaryotes. Prokaryotes respire at the plasma membrane, eukaryotes respire in mitochondria. The energetic configuration in eukaryotes has substantial consequences with regard to the energetic capacity of the cell to explore evolutionary tinkering with proteins (see text, see also ref. (54)).*

„*A plausible scenario is that the full eukaryotic cell plan emerged at least in part by initially nonadaptive processes made possible by a very strong and prolonged population bottleneck*". Lynch usually argues that nothing other than nonadaptive processes or N_e impacted any aspect of the evolutionary process, in any organism group (*104*).

For the sake of those critics (*15, 16, 17, 100, 102*), Figure 1 summarizes once more the bioenergetic difference between prokaryotes and eukaryotes. As summarized from literature values compiled in ref (*54*), eukaryotes and prokaryotes transduce just as much energy per gram of cells, measured in terms of respiratory rates. No one is debating that. But a gram of prokaryotes contains at least 1000 times more cells than a gram of eukaryotes, if we assume that the prokaryote is about 1 µm in diameter and that the eukaryote about 10 µm in diameter, whereby eukaryotic cells typically tend to be much larger (*17*), such that the factor 1000 is conservative. No one is debating that either. That mitochondria comprise about 10% of the cell volume in a typical heterotrophic eukaryotic cell (*105*) is also uncontroversial. Thus, in a cell that obtains 2 ATP per glucose from glycolysis and 28 ATP from mitochondrial respiration (*106*), 10% of the volume of the cell provides 93% of the energy. Energy production in eukaryotes is spatially more confined in eukaryotes than it is in prokaryotes. That is because it takes place in mitochondria (eukaryotes), rather than at the plasma membrane (prokaryotes). That spatial confinement is also part of the reason why mammalian mitochondria, for example, operate at about 50°C (*107*). There is a lot of energy flowing through mitochondria.

What is the evolutionary significance of mitochondrial energy? It is this. Eukaryotes and prokaryotes have roughly the same elemental composition (*108*) and are mostly made of protein, about 50% by weight (*109*, *110*), with protein synthesis accounting for about 75% of the cell's energy budget (*109*). Using the same energy per volume, the 1000 prokaryotes (Figure 1 left panel) have to make 1000 cell walls, 1000 genome copies, 1000 cell division machineries and everything else required to make 1000 energy converting self-replicating systems. The eukaryote (Figure 1 left panel) has solved the energy problem with 10% of the cell volume, and does not need to synthesize 1000 cell walls, 1000 host genomes, 1000 plasma membranes or 1000 lipopolysaccharide layers. The eukaryote has typically one or two non-mitochondrial genome copies, though ribosomal RNA operons are typically amplified in eukaryotes, either as middle repetitive DNA or on plasmids (*111*, *112*, *113*). The function of 1000 genome copies are replaced by mRNA copies stemming from a smaller number of genes. The eukaryote has three orders of magnitude more energy per gene (Figure 1) than the prokaryote (*54*).

Moreover, the eukaryote does not need to express 1000 times the amount of glycolytic enzymes in order to maintain glycolytic flux, because enzyme levels in cells are al-

most never rate limiting. Enzymes typically have vast activity reserves to permit regulation and physiological response. A rare exception is RuBisCO, which is a notoriously poor enzyme (*114*), probably because it arose to do a different job (*40*), and compensates for poor catalytic rate by comprising about 50% of leaf protein (*115*). The eukaryote has solved its carbon and energy problems with $1/10^{th}$ of the cell volume. Relative to the prokaryote, the eukaryote has the same amount of energy per volume in the remaining 90% of the cell, the cytosol, but with $1/1000^{th}$ the number of transcriptionally active genes. The eukaryote has three orders of magnitude more energy per gene (*54*) to embark upon the evolutionary tinkering of expressing, overexpressing proteins in the cytosol. It does not have to but thanks to mitochondria it *can* express cytosolic proteins in large amounts because it has an uncommitted allocation in its energy budget that allows it to do so. That surplus in the eukaryotes energy budget can cover the cost of selectively unconstrained protein expression, an option that the 1000 prokaryotes do not have. In the currency of proteins, a standard currency of cell evolution, tinkering costs energy. The eukaryote can energetically *afford* to tinker, because mitochondria liberate resources in the cell's energy budget. If evolution works like a tinkerer, evolution with mitochondria works like a corps of engineers (*54*).

The eukaryote can experiment with overexpressed proteins without sacrificing energy required for other vital functions. It has energy to spare. Because cytosol always ends up being about 400 mg/ml protein in all cells, that energy *can* end up being sunk into protein synthesis (where 75% of the energy in the cell goes anyway). None of the 1000 prokaryotes (Figure 1, left) have the option of tinkering with and overexpressing new proteins to an extent anywhere approaching that in eukaryotes (Figure 1 right), because prokaryotes do not have the energetic benefit of mitochondria.

What can the eukaryote do in an evolutionary sense with novel overexpressed proteins? Improve energy metabolism? Hardly, its energy metabolism is not in need of improvement, it is already off the scale relative to anything that ever lived before it. Overexpress enzymes? Not likely, enzymes when overexpressed tend to interfere with metabolism with detrimental effects for the cells, as most scientists who have ever tried to overexpress enzymes in genetically engineered systems know first hand. Overexpress inert proteins without enzymatic activity? That would appear to be a possible option, at least one that is not immediately deadly, and it is the option that lineage leading to eukaryotes apparently took (*116*). It is a surprisingly common latent natural property of soluble proteins that they tend to aggregate into linear filaments with only minimal mutational perturbation (*117*). Eukaryotic cytoskeletal and structural proteins do just that: they spontaneously aggregate in a reversible manner that can be regulated.

In terms of structural cellular complexity, what sets eukaryotes apart from prokaryotes is i) the eukaryotic network of interacting structural proteins in the cytosol, proteins that spontaneously aggregate and undergo active disaggregation at ATP expense and ii) the eukaryotic endomembrane system. From my standpoint (symbiogenesis), it is plainly evident that both pillars of eukaryote cytological complexity — a dynamic proteinaceous cytoskeleton (*116*) and a dynamic endomembrane system (*22*) — are properties that emerge from the endosymbiotic origin of mitochondria (*23*), the former as an energetic consequence, the latter as physical entities. The mitochondrial localization of ATP synthesis permits modern eukaryotes, and permitted the first eukaryotes, to explore the opportunity of protein overexpression that mitochondria afforded, because the intracellular concentration of ATP synthesis in mitochondria provided a cytosol with an increased space and a sufficient energy supply for protein tinkering. My proposition is thus that the mitochondrion arose in a prokaryotic host (*41*), that the mitochondrial energy configuration permitted that cell to become complex (a eukaryote) (*54*, *116*), and that a small population size was conducive to the traversal of that evolutionary transition (*118*).

Lynch and Marinov's (*16*, *17*, *102*) proposition is that mitochondria are irrelevant to eukaryote origin and that nothing more than point mutations and the power of population bottlenecks are needed to transform a prokaryote into a eukaryote. Such a proposition appears somewhat narrowly focused. If population bottlenecks can make prokaryotes complex, then with all the bottlenecks that prokaryotes underwent in evolution, for example since they started wandering around the land in the guts of land animals over the last 400 million years, we should have seen some bottleneck-generated eukaryote-like complex prokaryotes emerging by now. How many cells might that have been? If we assume a conservative one-week generation time, 400 million years converts to roughly $2{\cdot}10^{11}$ generations. For how many cells? Sender et al. (*119*) estimate $4{\cdot}10^{13}$ cells per human gut microbiome. If we assume that all land animals, including invertebrates, had a total biomass equal to that of 6 billion humans during the last 400 million years, all with a similar microbiome/bodyweight ratio, we arrive at roughly $5{\cdot}10^{34}$ prokaryotic cells that have constantly been going through prolonged bottlenecks over the last 400 million years. Eukaryote-like prokaryotes have not been reported to be emerging from those gut microbiome bottlenecks yet. And what about the singularity of eukaryotes? Are we to believe that there only was one prokaryotic population size effect during the last 4 billion years that was strong enough to make a prokaryote turn into a mitochondriate eukaryote? Lynch and Marinov (*16*, *17*, *102*) appear, in my view, to be overestimating the power of bottlenecks to create eukaryotes, organelles, or eukaryote like cells.

Though Booth and Doolittle (*15*) phrase it differently, their message is the same: „*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", and „*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!*" Their message is that mitochondria made no difference. In a different paper, Doolittle (*120*) uses the principle of luck to account for the persistence of mitochondria and plastids „*Following this logic, it seems that the 'specialness' of organelles comes not from any complex mechanism, or singularity of events, it is just that they are old and have managed not to go extinct. They are the lucky ones*." From reading that students might get the impression that chemiosmotic ATP synthesis occurs in mitochondria for no particular reason, it is complete coincidence and it is not only irrelevant for evolution, it could just as easily be taking place in the ER. From my standpoint (physiology), viewing life as an energy releasing chemical reaction and evolution as a process of the maintenance of that exergonic reaction from one generation to the next across nearly 4 billion years (*121*), the proposition that mitochondria have persisted over 1.5 billion years of eukaryote evolution for no reason other than luck is like saying that vertebrates retained eyes and teeth for 400 million years by pure coincidence, for no evolutionary reason. I find it far more reasonable to think that eukaryotes retained mitochondria and plastids not because of luck, but because they perform the core chemical reactions that run the physiology of the cell (carbon and energy metabolism). In the real world, one cannot replace food and energy with a bit of luck.

Using basic numbers provided by Whitman et al. (*122*) that roughly 10^{30} prokaryotes are alive today, one can estimate that roughly 10^{40} prokaryotes have lived on Earth during the last 2 billion years (*23*), during which time all eukaryotes alive today (the only ones scientists have to account for) arose once. During that same time, the plant lineage arose once. I find that those two transitions are more likely attributable to symbiogenesis (*54*) than to bottlenecks or luck (*15, 16, 17*).

It is undeniable that the advent of plastids *corresponds* to the origin of all plants we know today, and the advent of mitochondria *corresponds* to the origin of all eukaryotes we know today. Doolittle and Lynch argue that such correspondence is pure coincidence, that presence of the organelle and the origin of the clade are completely unrelated phenomena, Doolittle adding that the evolutionary persistence of the organelles has no functional basis, it is luck. Their propositions are neither compelling, nor do they make physiological sense. This section was long because I find it important to underscore how fundamentally different gradualist and symbiogenic views are on the role of mitochondria at eukaryote origin. In contrast to gradualist claims, there are well-founded biochemical and physiological reasons to think that mitochondria played an energetic role at eukaryote origin.

VI. Gene transfers from organelles

Small population sizes allow mutations to become fixed more rapidly than in large populations (*123*). But it is mutation, not N_e, that provides the substance with which evolution produces change (*123*). No mutation, no evolution, regardless of population size. Gene transfers from endosymbiotic organelles to their host are a mechanism of mutation that is both specific to symbiogenesis and specific to the eukaryotic lineage (*124*, *125*, *126*). Eukaryotes are genetic chimaeras, with more bacterial genes than archaeal genes (*127*, *128*, *129*, *130*), and gene transfers from mitochondria and chloroplasts continue to permeate eukaryotic chromosomes via known mechanisms — non homologous end joining (*126*) — that can be observed in the laboratory (*131*). Gene transfers from the mitochondrial symbiont to host chromosomes were important at eukaryote origin (*41*). Given what we have learned about the abundance of plastid and mitochondrial DNA in eukaryotic genome sequences (*124*, *125*, *126*, *132*, *133*), that set of propositions would seem far less controversial than it did 20 years ago, one would think.

Proponents of the gradualist case now even challenge the idea that gene transfers from organelles to the nucleus took place. One recent suggestion by Keeling and McCutcheon has it that maybe there really weren't any transfers from organelles after all (*134*). They make that case by extrapolating from bacterial endosymbionts of insects under the highly questionable premise, which they do not articulate, that such symbioses (between bacteria and insects) serve as a relevant biological model that might shed light on symbioses between bacteria and archaea that occurred at the origin of mitochondria. Insects are not archaea. The endosymbionts of insects are exceedingly common and the symbiotic associations occur recurrently in individual insect lineages (*135*), which is in stark contrast to the singular origin of plastids in the plant lineage or the singular origin of mitochondria. In arguing the case that gene transfers from organelles might not occur, Keeling and McCutcheon overlook repeatedly and independently confirmed evidence that nuclear fragments of mtDNA really do permeate all genomes of eukaryotes that possess DNA in their mitochondria, that humans are polymorphic for mtDNA insertions at 141 loci and that at least five mtDNA insertions cause human disease (*133*). We can see clear and concrete evidence for the workings of gene transfers from organelles today. The principle of continuity would have it that such transfers were taking place in the past as well.

Clearly, there is diversity of views regarding gene transfers from mitochondria and chloroplasts, and diversity of views is not uncommon in the field of evolutionary biology. At any rate, the 580 kb of recently acquired mitochondrial DNA in our own human nuclear genome (*126*, *133*) in addition to the complete 660 kb mitochondrial genome on chromosome 2 of *Arabidopsis* and the 130 kb complete plastid genome on chromosome 10 of rice (*136*)

are best explained by accepting the proposition that gene transfers from mitochondria and plastids to the nucleus really do occur during evolution (*137*).

VII. Gradual lateral gene transfer vs. gene transfers from organelles

The role of lateral gene transfer in eukaryote evolution has been more thoroughly addressed elsewhere (*74*). In brief, most claims for eukaryote LGT are probably untrue (*74*). Here it is only important to stress that there are gradualist and symbiogenetic views on *LGT as it specifically relates to eukaryote origin*. The gradualist view has it that gene transfer between prokaryotes and eukaryotes is just as common in evolution as gene transfer among prokaryotes (*138*, *139*, *140*). Specific formulations of LGT at eukaryote origin even have it that gradual LGT into the eukaryotic lineage is what made eukaryotes eukaryotic (*141*). Indeed, Pittis and Gabaldon (*141*) are saying that eukaryotes gradually acquired, from prokaryotes, the genes that make eukaryotes eukaryotic, deriving a mitochondrion lacking eukaryote (an archezoon) that acquires the mitochondrion to terminate the process of eukaryogenesis.

There are three very fundamental flaws with the gradualist LGT suggestion of Pittis and Gabaldon (*141*) and its content. First, its conclusions derive entirely from multiple and severe data analysis artefacts (*142*). Second, the paper suggests that the stem eukaryotic lineage acquired *from prokaryotes* the genes for the traits that make eukaryotes eukaryotic (a fully fledged endomembrane system, vesicle flux, nucleus, a true cytoskeleton, cell cycle, mitosis, meiosis, syngamy, karyogamy, sex, alternation of generations, etc.), but prokaryotes *do not have those traits*, only eukaryotes do (*23, 116, 143*). How can eukaryotes acquire genes from donors that lack the traits to be acquired? Third, the popularity of eukaryote LGT (*69*, *70*, *71*, *72*, *140*, *144*, *145*) should not obscure the fact that it is Lamarckian in tooth and claw. Eukaryote LGT proponents are saying that the genes required for survival in anaerobic environments (*72*), or the genes required for the origin of eukaryote complexity (*141*) are first acquired from outside the eukaryotic lineage, then inherited within eukaryotes to fulfill some purpose, for example adaptation to anaerobic environments or origins of complexity. How are such views *not* Lamarckian, and does anybody notice or care? Should we teach our students that eukaryotes, including animals (*146*), evolve in a Lamarckian manner? There is something fundamentally wrong with eukaryote LGT claims (*74*).

At the other end of the spectrum is the symbiogenic proposition that eukaryotes do not acquire genes via outright LGT either from other eukaryotes or from prokaryotes except at endosymbiotic events (the origin of mitochondria, the origin of primary plastids, the origin of secondary plastids) (*21*, *83*, *130*, *147*). We can see cumulative effects of LGT in prokaryotic genomes (*148*). We can also see evidence for mass transfers in prokaryotic genomes (*149*, *150*). But when we look for cumulative effects of LGT in eukaryotes, there is no evidence to be found (*21*, *147*). What do I even mean by cumulative effects? Cumulative effects are Darwinian effects. If changes occur over time along a lineage, they accumulate, and cumulative effects become visible. Darwin saw that for morphology. The Modern Synthesis saw it for mutations. Since Dayhoff's day (*151*) we see it in proteins as accumulated substitutions alias sequence divergence. LGT has clear cumulative effects in prokaryotes (*148*) but not in eukaryotes (*21*, *147*). The symbiogenic version of eukaryote gene transfer is that eukaryotes acquired genes at the origins of mitochondria and chloroplasts and with the help of that genetic starting material plus its ensuing mutations (and mitochondrial energy), selection and differential loss set in to allow eukaryotes to evolve in a very normal Darwinian manner, at least one they had evolved meiosis (*116*), without the inheritance of acquired traits.

VIII. Gradualist versions of symbiogenesis

There are also gradualist versions of symbiogenesis. Gradualistic symbiogenesis — how can that be, one might ask. Ideas about the role of symbiogenesis can be distributed along a scale from zero to one, as sketched in Figure 2. At the one extreme is the pure gradualist view that symbiogenesis had no role at all in eukaryote origin, that eukaryotes arose without the participation of symbiogenesis or mitochondria. There are versions of nonsymbiogenic eukaryote origins starting from archaea (*47*) (Fig. 2a) and versions starting from bacteria (*35*) (Fig. 2b), models in which mitochondria are added to a eukaryotic cell that arose gradually. More modern versions of nonsymbiogenic eukaryote origins tend to focus on gradualist mechanisms while largely disregarding the issue of „origin from which kinds of prokaryote" (*15*, *16*, *17*, *102*, *120*). By contrast, older gradualist hypotheses of Van Valen and Maiorana (*47*) and Cavalier-Smith (*35*) are more courageously explicit on phylogenetic relationships.

At the other extreme is the undiluted version of symbiogenesis (Fig. 2f), namely that the host for the origin of mitochondria was a prokaryote, an archaeon, and that eukaryote specific traits arose as a consequence of that symbiosis (*22*, *23*, *41*, *54*, *118*). There are several versions of the idea that the host was an archaeon (reviewed in (*45*, *51*)), though only one of those suggestions accounted for eukaryotic anaerobes (*41*), having been developed into a fuller theory that makes an earnest attempt to account for the origin of the nucleus (*7*), the eukaryotic endomembrane system (*22*), meiosis mitosis and sex (*116*).

In the middle of the autogeny-symbiogenesis scale are very many published versions of symbiogenesis that posit the participation of additional symbioses that took place before the origin of mitochondria in the process of eu-

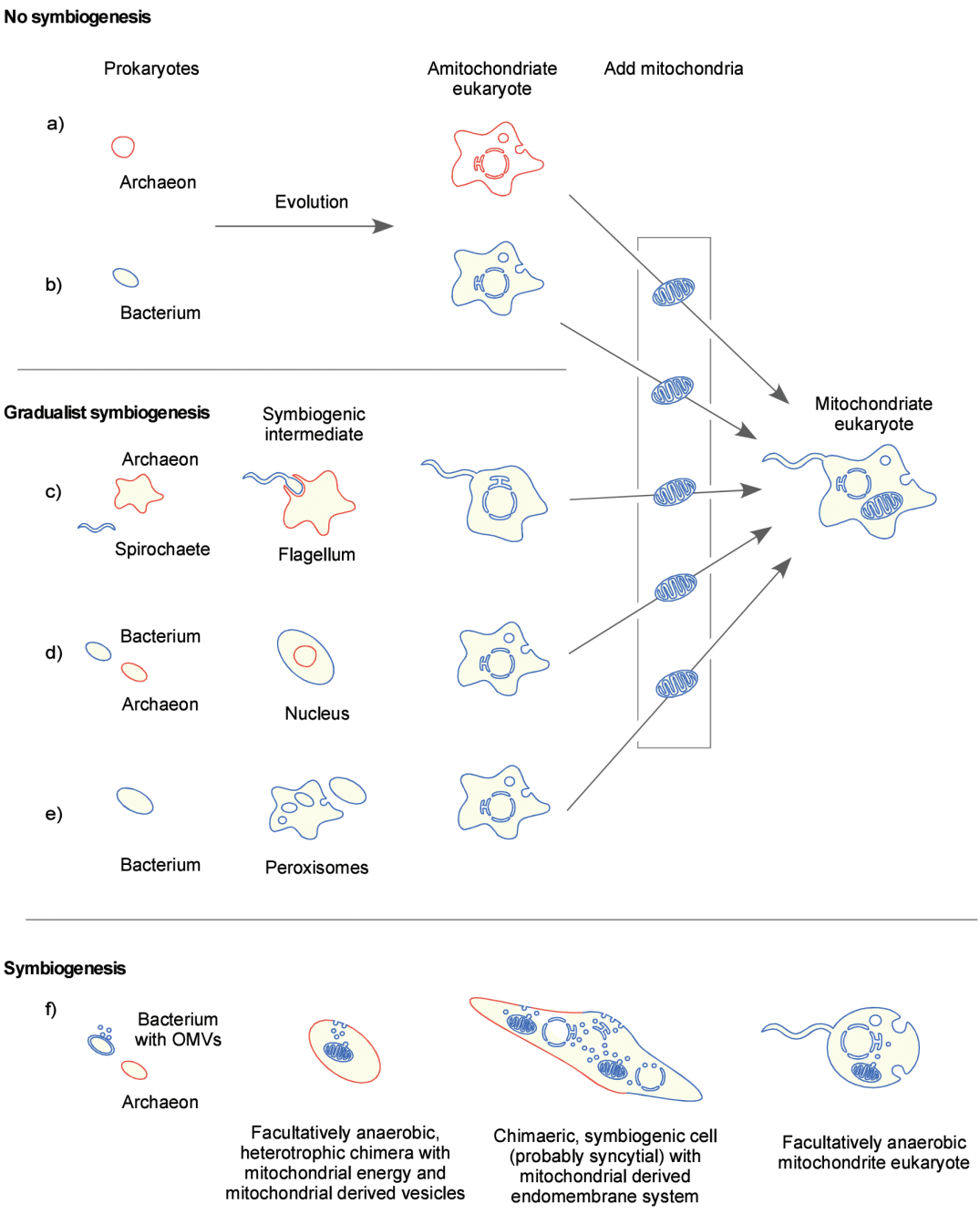

Figure 2. *Schematic comparison of selected theories for eukaryote origin. For a more complete overview see (7). In the top panel, two autogenous models are shown. a) Origin from an archaeon (47), b) origin from a bacterium (35), mitochondria coming later. In the middle panel, three gradualist symbiogenic models are shown in which a symbiosis takes place to generate a primitively amitochondriate eukaryote (an archezoon), which then acquires mitochondria. c) An amitochondriate eukaryote arises via a symbiosis involving a spirochaete (159). d) An amitochondriate eukaryote arises via a symbiosis involving the origin of the nucleus (152, 153). e) An amitochondriate eukaryote arises via a symbiosis involving the origin of peroxisomes (30, 85, 154). f) A eukaryote having mitochondria, a nucleus, an endomembrane system, meiosis, and sex arises from a symbiotic association of an archeaon (the host) and a bacterium (the common ancestor of mitochondria and hydrogenosomes) without additional symbiotic partners (7, 10, 22, 23, 41, 54, 55, 58, 116). In f) a syncytial intermediate is sketched, because such an intermediate would decouple the origin of nuclear division, chromosome partitioning onto progeny and cell division, which would help to account for the origin of sex and meiosis in the eukaryote ancestor (see text, see also ref. (116)).*

karyogenesis. These additional symbioses give rise to a cell that does not have mitochondria but is more complex than a prokaryote. There are many such suggestions in the literature (reviewed in (*7*)). The best known examples are the idea that an additional symbiont gave rise to the flagellum (Fig 2c) (*19*), the idea that the nucleus was an endo-

symbiont (Fig. 2d) (*152*, *153*) and the idea that peroxisomes were endosymbiotic bacteria (Fig. 2e) (*30, 85, 154*). What gradualist versions of symbiogenesis do is simple, but incorrect in my view: They take the principle of endosymbiosis, which we know to be correct for plastids and mitochondria, and they apply it once more to cell evolution in order to *make the host for mitochondrial origin more complex than a simple prokaryote*. They make the symbiogenic origin of eukaryotes more gradual. They tend to associate the additional symbiosis with the origin of a particular cell structure (flagellum, nucleus, peroxisome), giving rise to a eukaryotic like cell that lacks mitochondria, then they add the mitochondrion. Curiously, I have never come across a paper where someone suggests endosymbiosis for the origin of flagellum, and for the nucleus, and for peroxisomes (or any combination of thereof). It is always either *one* endosymbiosis preceding the mitochondrion, or *none*. Taylor (*80*) called such successive symbioses *serial* endosymbiotic theory, or SET, a term I have never used because the serial part of it (Margulis's spirochaete flagellae) has always been obviously flawed (*64*), while the mitochondrial and plastid part has been undeniable. Perhaps there is something about the principle of symbiogenesis (endosymbiosis) that tells biologists to use it sparingly. One can entertain more than two prokaryotic endosymbionts in eukaryote evolution (mitochondria and plastids), but not fewer.

There is also a gradualist symbiogenic version for the origin of plastids. That idea involves a chlamydial symbiont that helped the cyanobacterium become a plastid. The „chlamydioplast" notion was construed entirely from genome data; for a critical review of the idea explaining its a flaws in context, see (*155*). Importantly, the phylogenies that are claimed as support for the chlamydia story do not support it at all (*155, 156*).

The minimalist and most radical version of symbiogenesis is this: i) A symbiosis between an archaeal host and a bacterial endosymbiont gave rise to a mitochondrion and nucleus bearing cell, the first eukaryote (*41*), which was probably syncytial as meiosis was arising (*116*) and from which all eukaryotes descend (Figure 2). ii) One more symbiogenic association gave rise to the first cell with plastids (*7, 9, 10, 11*), from which all plants (now called archaeplastida) descend. iii) Secondary symbioses occurred among eukaryotes to give rise to algae with secondary plastids (*83*). That assumes the rock bottom minimum number of symbioses at the origin for groups *whose cell biology harbours unequivocal evidence in the form of bioenergetic organelles — mitochondria, primary plastids and secondary plastids — that symbioses occurred*. It does not assume, entail or require the existence of any additional endosymbionts for which there is no evidence in the currency of organelles. Gradualist versions of symbiogenesis strive to make the steps at the origin of eukaryotes less steep by adding additional symbiotic partners (Fig 2c–e) to create intermediate forms unknown among modern cells. In Fig 2f, the bottom panel, one can add additional symbionts that do not give rise to a eukaryote with mitochondria, but one cannot get by with fewer. That is, symbiogenic models can only get more complicated, they cannot get simpler. I see that as a strength of the theory.

WHY ALL THE CONTROVERSY, WHAT IS THE PROBLEM?

On the bottom line, the gradualist and symbiogenic theories differ over a simple but profound question: Did mitochondria *terminate* the process of eukaryogenesis as gradualists say, or did mitochondria *initiate* the process of eukaryogenesis, as symbiogenesis would have it (Table 1). Many observers will no doubt opt for a compromise solution and say „*Well, the answer is somewhere in between*". The literature has plenty of compromise solutions in supply. One compromise solution involves archezoa or „phagocytosing archaea" as intermediates in the prokaryote eukaryote transition (*39*, *157*). The problem with the notion of phagocytosing archaea is that the idea will not work, for physiological and energetic reasons (*23*). Another compromise solution involves gradualist versions of symbiogenesis that conjure additional symbionts to make the host for the origin of mitochondria more complex than a prokaryote (Figure 2 c–e). There are two main problems with such additional symbiont theories. First, they derive an archezoon, usually a phagocytosing one, as the host for mitochondria (Figure 2 c–e), which does not work for many reasons (*7, 23, 45*). Second they have been specifically tested with extensive genome sequence data with the

Table 1. *Differing views about eukaryote origin*

Aspect	Gradualist view	Symbiogenic view
1. Phagocytosis	Arose before mitochondria	Arose after mitochondria
2. The host for mitochondria	Was a eukaryote	Was a prokaryote (an archaeon)
3. Eukaryote anaerobes	Arose late through LGT	Facultatively anaerobic ancestral state
4. Endomembrane system	Arose before mitochondria	Arose from mitochondrial vesicles
5. Mitochondrial ATP synthesis	Irrelevant, of no significance	Permits increased protein expression
6. Gene transfers from organelles	Not frequent, not important	Frequent, important, transformative
7. LGT to and among eukaryotes	A mechanism of evolution	Lamarckian artefact of data analysis
8. Endosymbiotic partners	Host, mitochondrion, plus others	Host and mitochondrion only
9. The role of mitochondria	Terminated eukaryote origin	Initiated eukaryote origin

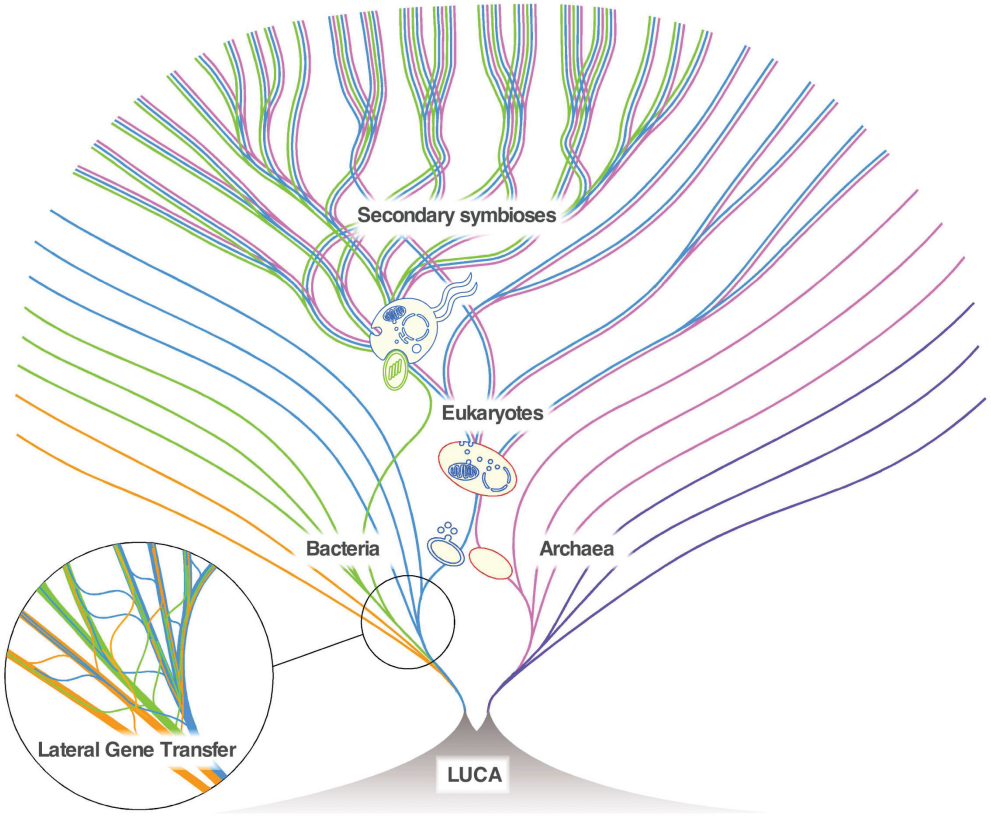

Figure 3. *Symbiogenesis in eukaryote evolution. The figure is slightly modified from reference (162). The symbiogenic origin of eukaryotes (mitochondria), plants (plastids) and lineages with secondary plastids is shown. For further details see (7, 10, 22, 23). In the figure, four independent secondary symbiotic events are depicted, one each leading to euglenids and chlorarachniophytes (both green algal symbionts) and two more involving red algal endosymbionts, although at present, information about protein targeting into secondary plastids suggests that only one secondary symbiosis involving a red algal symbiont took place, for further details see (163). LUCA designates the last universal common ancestor of all cells, see reference (164).*

sobering result that genomes harbour no evidence for such ideas (*21*). Yet another compromise solution is to draw pictures of eukaryogenesis as involving extra symbiotic partners in addition to the mitochondrion and the host, but without specifying what those symbiotic partners are or what they were doing in a physiological sense (*158*). Such propostions are untestable.

Tests are an issue with which endosymbiotic theory traditionally has had a problem. Margulis held on to her spirochaete origin of flagella for 40 years (*159*) despite the idea failing all molecular tests. The kind of evidence that virtually all scientists accepted as supporting the idea that plastids and mitochondria are descended from endosymbionts, namely molecular sequence comparisons and molecular evolution (*160*), rejects the idea that endosymbionts other than the plastid and the mitochondrion participated in eukaryote evolution (*21*). Some scientists reject the idea the mitochondria were endosymbionts at all (*18*). Others argue that the role of symbiosis in the origin of new species of termites or lichens constitutes a test of the idea that mitochondria played a role at eukaryote origin (*161*). Margulis (*29*) made exactly the same mistake by trying to sell symbiogenesis as a *replacement* for gradualist evolutionary mechanisms as opposed to incorporating it into the broader fabric of evolutionary biology as a rare (extremely rare, but indispensable) *exception* to the gradualist rule. As I see it, the central problem surrounding acceptance of symbiogenic mechanisms is that they only operate very rarely in evolution. That makes them interesting, but controversial, as summarized in Figure 3.

Current views on the role of symbiogenesis in evolution diverge to extremes. Physicists and chemist find such dissent unfathomable. Why is biology so different? Maybe it is simple: Everyone agrees that the laws governing physical and chemical processes have been constant over the last 4 billion years. The same level of agreement does not exist in biology, specifically in evolution, where scientists are still vigorously debating whether gradualist mechanisms will suffice to account for all of evolutionary history. Here I have made a case that symbiogenesis is not only *required* to explain the origin of cells with mitochondria and plastids during the last 4 billion years, but it actually *does a better job* than gradualist mechanisms at the origin of eukaryotes and plants. Alas, gradualists will not change their views on

the nature of mechanisms that operated in early evolution. That's fine. But *why* do biologists not agree on such fundamental issues, how can such divergent interpretations of the same body of literature coexist? A main factor is that symbiogenesis pertains to the study of early evolution.

I have observed that scientists (*n.b.*) tend to make up their mind very early in their careers about the course of early evolution — that is, the origin of life and the origin of eukaryotes —, not seldom by absorbing and clinging to the first halfway convincing thing they ever heard or read on the topic. We have a tendency to hang onto those initial views regardless of what comes along over the years in terms of new evidence, because that initially imprinted view of early evolution is somehow incorporated into the fabric of our thinking as a kind of internal scaffold. As our careers progress, we attach new information onto that scaffold while we construct our own private view of life history. To be sure, there is nothing wrong with that, moreover I consider that to be a non-negotiable component of our scientific freedom, or a God given right, as one would prefer to express it.

We hang new observations or new information from the literature on that scaffold as we move forward through our careers, constructing our own increasingly detailed, private narrative about life history in the process. If new information comes along that conflicts with our private narrative, our tendency is to dismiss it, ignore it, hope that it will somehow go away, or inspect the robustness of the new information rather than modify the scaffold. Such inertia is not entirely bad, because the literature on early evolution is very diverse, and we are certainly ill-advised to believe everything we read, in which case the scaffold would collapse in conflict. On the contrary, we need to be critical and stay critical in the face of the many strange claims that constantly appear in the literature about early evolutionary history. Modifying our scaffold entails time, effort, and internal conflict (decisions) as we rebuild and reconstruct our private life history narrative. We cannot incorporate and reconcile all views that are out there, anyway, because they conflict in basic substance. We have to select. But we do have to update and modify our scaffold from time to time, otherwise it is not part of science. That is too much effort for most of us, especially if early evolution is not our main research focus. In essence, no amount of data will cause some of us to change our minds about early evolution. That is certainly understandable, though not always compatible with of the scientific process. Many heated debates about early evolution are argued from the fabric of our inner scaffolds and hence end without agreement. Scientists who do not care about early evolution do not debate it.

Be that as it may, taxpayers expect scientists to adjust their views on early evolution as new information becomes available. The difference between science and religion is that in religion the truth stays the same over millennia while in science the truth (our premises, the things we believe to be true for the sake of scientific inquiry) is supposed to change over time. It is called progress. Ideally we are supposed to be able to see views progress during our own lifetime. That is the nature of what we do. In evolution there are no facts, there are only observations and their interpretation. That is what makes it such a rich and exciting field.

CONCLUSION

The term symbiogenesis entered the literature at a time when Spiridion Brusina (1845–1909) was still active (*165*). He founded the learned academic society that publishes this distinguished journal, which has been continuously in print since 1886. Brusina was a prominent biologist and leading malacologist of his day. The title of his 1904 contribution (*165*) translates to „On saving our molluscan fauna". Today we would think that a paper with such a title would be about saving endangered species. Brusina was not warning that the species themselves were endangered. Rather he was explaining how his unfinished monographs on the species of European molluscs were becoming endangered because of administrative duties, because of competitors at better funded institutions, and because of the tedious burden of having to straighten out a complete mess in the literature that had arisen through very problematic recent publications that were impeding his work and holding back progress in general. Brusina was ahead of his time, he was also right: European molluscs harbour fascinating biology. Some molluscs even sequester plastids from algae, for reasons relating to feeding physiology, however (*166*), not symbiogenesis. Were Spiridion Brusina still alive today, it is my hope that he would have enjoyed this paper.

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