Lynn Margulis and the endosymbiont hypothesis: 50 years later

Michael W. Gray*
Department of Biochemistry and Molecular Biology and Centre for Comparative Genomics and Evolutionary Bioinformatics, Dalhousie University, Halifax, NS B3H 4R2, Canada

ABSTRACT **The 1967 article "On the Origin of Mitosing Cells" in the *Journal of Theoretical Biology* by Lynn Margulis (then Lynn Sagan) is widely regarded as stimulating renewed interest in the long-dormant endosymbiont hypothesis of organelle origins. In her article, not only did Margulis champion an endosymbiotic origin of mitochondria and plastids from bacterial ancestors, but she also posited that the eukaryotic flagellum (undulipodium in her usage) and mitotic apparatus originated from an endosymbiotic, spirochete-like organism. In essence, she presented a comprehensive symbiotic view of eukaryotic cell evolution (eukaryogenesis). Not all of the ideas in her article have been accepted, for want of compelling evidence, but her vigorous promotion of the role of symbiosis in cell evolution unquestionably had a major influence on how subsequent investigators have viewed the origin and evolution of mitochondria and plastids and the eukaryotic cell per se.**

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In 1967, Lynn Margulis (then Lynn Sagan) published an article entitled "On the Origin of Mitosing Cells" in the *Journal of Theoretical Biology* (Sagan, 1967). This publication did not have an auspicious beginning, reportedly having been rejected by more than a dozen journals before eventually finding a home (Archibald, 2014). Now, it is widely regarded as marking the modern renaissance of the endosymbiotic theory of organelle origins.

In her article, Margulis hypothesized that "three fundamental organelles: the mitochondria, the photosynthetic plastids and the (9 + 2) basal bodies of flagella were once themselves free-living (prokaryotic) cells." That mitochondria and plastids might have originated endosymbiotically from prokaryotic progenitors was not at the time a new idea, having first emerged in various forms in the late 19th and early 20th centuries before fading from mainstream biological view (Sapp, 1994). Margulis' article was notable, however, in that it laid out an all-encompassing view of (endo)symbiosis as the end-all and be-all of the eukaryotic cell: it was perhaps the first unified theory of eukaryogenesis. The article included the novel proposition that a third subcellular structure, the eukaryotic flagellum ("undulipodium" in her usage), originated from "ingestion of certain motile prokaryotes," "perhaps spirochaete-like," which eventually "became symbiotic in their hosts." This overall scenario was later dubbed the serial endosymbiosis theory (Taylor, 1974).

Although a discussion of the origin of mitosis that Margulis outlined comprises a substantial portion of her article, there is no evidence supporting it, in contrast to the proposed endosymbiotic origin of mitochondria and plastids. The reason is simple: no genome has been associated with the eukaryotic flagellar apparatus despite efforts to find one (Johnson and Rosenbaum, 1991), and it is through the genomes contained in the mitochondrion and the plastid—the genes they harbor and how they are arranged and expressed—that we know with a high degree of certainty from whence these organelles originated: the bacterial clades α-Proteobacteria and Cyanobacteria, respectively (Gray and Doolittle, 1982; Gray, 1992).

Margulis' vigorous promotion of the role of symbiosis in eukaryotic cell evolution (Margulis, 1970) sparked a spirited debate throughout the 1970s and into the 1980s between proponents of autogenous origin ("origin from within") and xenogenous origin ("origin from without") theories of organelle evolution. Although various authors rejected an endosymbiont scenario for both mitochondria and plastids (Uzzell and Spolsky, 1974), controversy during this period focused especially on the mitochondrion (Raff and Mahler, 1972). A particularly troubling issue, noted early by Mahler (1981), was the fact that "the mitochondrial genetic system exhibits unmistakable signs of great inter- and intra-species diversity," suggesting that "this system is unique and that its features are distinct from both its prokaryotic and eukaryotic counterparts." Subsequent comparative analysis of mitochondrial

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*Address correspondence to: Michael W. Gray (m.w.gray@dal.ca).

Abbreviations used: LECA, last eukaryotic common ancestor; PhAT, phagocytosing archaeon theory.

genomes and their expression only reinforced the view that in mitochondria, anything goes (Burger *et al.*, 2003). Nevertheless, a confluence of data—biochemical, molecular, and cell biological, coupled with the characterization in a group of eukaryotic microbes (the jakobid flagellates) of a gene-rich mitochondrial genome that strongly resembles a shrunken bacterial genome (Burger *et al.*, 2013)—now provides a compelling case for a single, endosymbiotic, α-proteobacterial origin of mitochondria (Gray *et al.*, 1999; Gray, 2012).

A compelling case for an endosymbiotic origin has always been easier to make for the plastid than for the mitochondrion. For one thing, the plastid is evolutionarily younger than the mitochondrion: whereas the last eukaryotic common ancestor (LECA) already had a functional mitochondrion approximating its modern counterpart (Koumandou *et al.*, 2013), several major eukaryotic lineages (e.g., that containing animals and fungi) are clearly primitively aplastidic, descending from ancestors that never had plastids. In consequence, in most (although not all) plastid-bearing eukaryotes, the resemblance between plastid and cyanobacterial structure and biochemistry is considerably more pronounced than in the mitochondrion/α-proteobacteria comparison. In addition, plastid genomes generally contain substantially more genes on which to base such a comparison than do mitochondrial genomes, and the plastid translation system displays decidedly more bacterial character than does its counterpart in most mitochondrial systems (Gray, 1992).

Margulis' treatment of the plastid in her 1967 article is remarkably brief: she simply asserted (p. 244) that "eukaryotic plant cells did not evolve oxygen-eliminating photosynthesis"; instead, "they acquired it by symbiosis" (from blue-green algae, i.e., cyanobacteria). She further suggested that "different photosynthetic eukaryotes (protoplastids) were ingested by heterotrophic protozoans at various times" during evolution, becoming "obligately symbiotic plastids, retaining their characteristic photosynthetic pigments and pathways." This theme of multiple plastid origins was later taken up by others (e.g., Raven, 1970). The current consensus, however, is a single, separate, endosymbiotic origin of mitochondrion and plastid, with a primary origin of the latter (from an endosymbiotic cyanobacterium) occurring in an ancestor of Archaeplastida, the eukaryotic lineage containing land plants and green, red, and cyanophyte algae. Plastids subsequently entered other algal clades via a process of secondary symbiosis in which a eukaryotic host takes up a eukaryotic symbiont (a green or red alga) (Archibald and Keeling, 2002).

In spite of a general acceptance that mitochondria and chloroplasts are descended from free-living bacterial ancestors through a process of endosymbiosis, how this symbiogenesis actually happened is still incompletely understood. Particularly in the case of the mitochondrion, questions still being debated include when the initiating event occurred (both within the overall timeline of biological evolution and relative to the origin of the eukaryotic cell per se), how long the process of converting bacterial endosymbiont to fully integrated organelle took, what this conversion process involved, and by what evolutionary mechanisms it occurred. Indeed, a host of symbiogenesis models that invoke different hosts and processes have been proposed over the five decades since the publication of Margulis' 1967 article (see, e.g., Martin *et al.*, 2015).

A particularly contentious issue is the nature of the host. Textbook descriptions of endosymbiosis often depict the host as a primitive (i.e., amitochondriate) eukaryote ingesting a prokaryotic symbiont through phagotrophy—the process of capturing and internalizing other organisms (phagocytosis). Other depictions show the host as a prokaryote ingesting another prokaryote by what looks like phagocytosis, even though phagotrophy is unknown so far in free-living prokaryotes.

In her 1967 article, Margulis suggested that "the first step in the origin of eukaryotes from prokaryotes was related to survival in the new oxygen-containing atmosphere: an aerobic prokaryotic microbe (i.e., the protomitochondrion) was ingested into the cytoplasm of a heterotrophic anaerobe. This endosymbiosis became obligate and resulted in the evolution of the first aerobic amitotic amoeboid organisms." It is not certain from this description whether the proposed host was itself a prokaryote or something more "advanced": Margulis is not explicit on this point. The allusion to "amoeboid" and "ingestion" does suggest a type of protoeukaryote, albeit without many of the defining features of the contemporary eukaryotic cell, in particular a nucleus and mitotic apparatus. Later, however, Margulis (1981) made it clear that she favored a prokaryotic host, stating, "it is likely that protomitochondria invaded their hosts just as modern predatory bacteria *Bdellovibrio* invade prey bacteria": "an amazing example of prokaryote-prokaryote 'emboîtement' without phagocytosis." Mind you, given that *Bdellovibrio* very effectively destroys its "host" bacterium in the process of invading it, this type of scenario does offer a particularly promising route to a stable prokaryote–prokaryote symbiosis.

The nature of the host is, in fact, central to widely differing symbiogenesis models of mitochondrial origin and evolution, which fall into roughly two broad categories: mitochondria early (mito-early, or mito-first) and mitochondria late (mito-late, or mito-last), differing on timing–within the transition from first eukaryotic common ancestor to LECA–and having different implications for the overall origin of the eukaryotic cell (Poole and Gribaldo, 2014). Comparative genomics and other analyses emphasize that the LECA was already a complex organism with a fully functioning mitochondrion (Koumandou *et al.*, 2013) and that all supposedly amitochondrial eukaryotic lineages (with one recently described exception; Karnkowska *et al.*, 2016) contain mitochondrion-related organelles and descend from mitochondria-containing ancestors. Thus, initial acquisition of a bacterial symbiont destined to become the mitochondrion could not have occurred very close to the emergence of the LECA, given the numerous and complex changes that obviously had to occur in the symbiont-to-organelle transition, although evidence for a late acquisition of the mitochondrion has recently been published (Pittis and Gabaldón, 2016).

Perhaps the best-known mito-early model is the hydrogen hypothesis (Martin and Müller, 1998), in which the host, an anaerobic, hydrogen-dependent archaeon, takes up an α-proteobacterium that is able to respire but that generates molecular hydrogen as a waste product of anaerobic heterotrophic metabolism. Here selection for endosymbiosis is driven by metabolic syntrophy between the two partners: the waste product (hydrogen) of one is used as an essential metabolic resource by the other. In this scenario, the origin of the mitochondrion and the origin of the eukaryotic cell are contemporaneous, with the subsequent emergence of the defining subcellular features of the latter directly dependent on a pronounced increase in cellular energy provided by the latter.

In contrast, in mito-late models, the underlying mechanism of symbiogenesis is phagotrophy, a hallmark of eukaryotic cells and widespread within the eukaryotic domain. Phagotrophy involves endocytosis, by which the bounding membrane of one organism (the host) surrounds another organism (symbiont), internalizing it in a membrane-bound phagosome. In some mito-late models, the host is effectively an amitochondriate eukaryote capable of phagocytosis (Cavalier-Smith, 1987).

Recent evidence supports the idea that eukaryotes are specifically related to a newly described clade of Archaea, the Asgard superphylum (Zaremba-Niedzwiedzka *et al.*, 2017). This archaeal group encodes a

number of proteins whose homologues had previously been found only in eukaryotes, suggesting that an archaeal lineage that had already developed features characteristic of eukaryotes, including possibly phagocytosis, might have been the host for the mitochondrial endosymbiosis. These observations provide the basis for the phagocytosing archaeon theory (PhAT) model of eukaryogenesis, with the mitochondrial endosymbiont having been acquired by a transiently complex phagocytosing archaeon (Martijn and Ettema, 2013).

My purpose here is not to enumerate and critically evaluate all of the different symbiogenetic models of organelle evolution, but rather to illustrate by a few examples the divergent (and changing) opinions on this subject. The symbiont-to-organelle transition evidently involved many steps: loss of the bacterial cell wall; early acquisition by the symbiont of essential metabolite transporters; massive (and variable) reduction of the symbiont genome through loss of genes or their transfer to the nucleus; functional activation of transferred genes in the nucleus and retargeting of their cytoplasmically synthesized protein products back into the evolving organelle—via specific organellar targeting sequences—or elsewhere in the cell; and wholesale recruitment of many additional organellar proteins, the origin of which is obscure. We infer this remodeling process by comparing contemporary organelles with their closest bacterial relatives and conclude that it was a gradual process occurring over a long period of time.

Although we have learned much about the origin of mitochondria and plastids in the five decades since the publication of Margulis' 1967 article and about their role in the overall process of eukaryogenesis, there is clearly much more to be discerned. A particularly challenging problem is to decipher the origin of the bulk of the mitochondrial and plastid proteomes, which appear not to have been of α-proteobacterial or cyanobacterial origin, respectively. We accept that the mitochondrion and the plastid are the direct evolutionary products of bacterial endosymbiosis, but at the same time we recognize that these are mosaic organelles whose components and functions have more than one origin (Cavalier-Smith, 1987). As Margulis asserted throughout her career, symbiosis has played a crucial role in organelle origins and overall eukaryogenesis, although perhaps not the all-pervasive and all-encompassing role that she initially envisaged.

REFERENCES

Archibald JM (2014). One Plus One Equals One: Symbiosis and the Evolution of Complex Life, New York: Oxford University Press.

Archibald JM, Keeling PJ (2002). Recycled plastids: a 'green movement' in eukaryotic evolution. Trends Genet 18, 577–584.

Burger G, Gray MW, Lang BF (2003). Mitochondrial genomes: anything goes. Trends Genet 19, 709–716.

Burger G, Gray MW, Forget L, Lang BF (2013). Strikingly bacteria-like and gene-rich mitochondrial genomes throughout jakobid protists. Genome Biol Evol 5, 418–438.

Cavalier-Smith T (1987). The simultaneous symbiotic origin of mitochondria, chloroplasts, and microbodies. Ann NY Acad Sci 503, 55–71.

Gray MW (1992). The endosymbiont hypothesis revisited. Int Rev Cytol 141, 233–357.

Gray MW (2012). Mitochondrial evolution. Cold Spring Harb Perspect Biol 4, a011403.

Gray MW, Burger G, Lang BF (1999). Mitochondrial evolution. Science 283, 1476–1481.

Gray MW, Doolittle WF (1982). Has the endosymbiont hypothesis been proven? Microbiol Rev 46, 1–42.

Johnson KA, Rosenbaum JL (1991). Basal bodies and DNA. Trends Cell Biol 1, 145–149.

Karnkowska A, Vacek V, Zubáčová Z, Treitli SC, Petrželková R, Eme L, Novák L, Žárský V, Barlow LD, Herman EK, *et al.* (2016). A eukaryote without a mitochondrial organelle. Curr Biol 26, 1274–1284.

Koumandou VL, Wickstead B, Ginger ML, van der Giezen M, Dacks JB, Field MC (2013). Molecular paleontology and complexity in the last eukaryotic common ancestor. Crit Rev Biochem Mol Biol 48, 373–396.

Mahler HR (1981). Mitochondrial evolution: organization and regulation of mitochondrial genes. Ann NY Acad Sci 361, 53–75.

Margulis L (1970). Origin of Eukaryotic Cells, New Haven, CT: Yale University Press.

Margulis L (1981). Symbiosis in Cell Evolution, New York: W. H. Freeman.

Martijn J, Ettema TJ (2013). From archaeon to eukaryote: the evolutionary dark ages of the eukaryotic cell. Biochem Soc Trans 41, 451–457.

Martin W, Müller M (1998). The hydrogen hypothesis for the first eukaryote. Nature 392, 37–41.

Martin WF, Garg S, Zimorski V (2015). Endosymbiotic theories for eukaryote origin. Philos Trans R Soc Lond B Biol Sci 370, 20140330.

Pittis AA, Gabaldón T (2016). Late acquisition of mitochondria by a host with chimaeric prokaryotic ancestry. Nature 531, 101–104.

Poole AM, Gribaldo S (2014). Eukaryotic origins: how and when was the mitochondrion acquired? Cold Spring Harb Perspect Biol 6, a015990.

Raff RA, Mahler HR (1972). The non symbiotic origin of mitochondria. Science 177, 575–582.

Raven PH (1970). A multiple origin for plastids and mitochondria. Science 169, 641–646.

Sagan L (1967). On the origin of mitosing cells. J Theor Biol 14, 225–274.

Sapp J (1994). Evolution by Association. A History of Symbiosis, New York: Oxford University Press.

Taylor FJR (1974). Implications and extensions of the serial endosymbiosis theory of the origin of eukaryotes. Taxon 23, 229–258.

Uzzell T, Spolsky C (1974). Mitochondria and plastids as endosymbionts: a revival of Special Creation? Am Sci 62, 334–343.

Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Bäckström D, Juzokaite L, Vancaester E, Seitz KW, Anantharaman K, Starnawski P, Kjeldsen KU, *et al.* (2017). Asgard archaea illuminate the origin of eukaryotic cellular complexity. Nature 541, 353–358.