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Serial endosymbiosis or singular event at the origin of eukaryotes?

Nick Lane

Department of Genetics, Evolution and Environment, University College London, Darwin Building, Gower Street, London WC1E 6BT, United Kingdom

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ABSTRACT

'On the Origin of Mitosing Cells' heralded a new way of seeing cellular evolution, with symbiosis at its heart. Lynn Margulis (then Sagan) marshalled an impressive array of evidence for endosymbiosis, from cell biology to atmospheric chemistry and Earth history. Despite her emphasis on symbiosis, she saw plenty of evidence for gradualism in eukaryotic evolution, with multiple origins of mitosis and sex, repeated acquisitions of plastids, and putative evolutionary intermediates throughout the microbial world. Later on, Margulis maintained her view of multiple endosymbioses giving rise to other organelles such as hydrogenosomes, in keeping with the polyphyletic assumptions of the serial endosymbiosis theory. She stood at the threshold of the phylogenetic era, and anticipated its potential. Yet while predicting that the nucleotide sequences of genes would enable a detailed reconstruction of eukaryotic evolution, Margulis did not, and could not, imagine the radically different story that would eventually emerge from comparative genomics. The last eukaryotic common ancestor now seems to have been essentially a modern eukaryotic cell that had already evolved mitosis, meiotic sex, organelles and endomembrane systems. The long search for missing evolutionary intermediates has failed to turn up a single example, and those discussed by Margulis turn out to have evolved reductively from more complex ancestors. Strikingly, Margulis argued that all eukaryotes had mitochondria in her 1967 paper (a conclusion that she later disavowed). But she developed her ideas in the context of atmospheric oxygen and aerobic respiration, neither of which is consistent with more recent geological and phylogenetic findings. Instead, a modern synthesis of genomics and bioenergetics points to the endosymbiotic restructuring of eukaryotic genomes in relation to bioenergetic membranes as the singular event that permitted the evolution of morphological complexity.

1. The landscape of endosymbiosis in 1967

There can be no doubt that Lynn Margulis's 1967 paper 'On the Origin of Mitosing Cells' (Sagan, 1967) was a seminal, punctuating statement in a century of biology. Little that she wrote was actually new, in that many of the ideas she outlined reached back much earlier in the century. Indeed, reading the paper today, one is struck by how much her cell biology was indebted to the detailed findings of the great cell biologists of the early 20th century, notably Edmund Beecher Wilson (Wilson, 1925) and Clifford Dobell (Dobell, 1914), as well as Ivan Wallin on the endosymbiotic origin of mitochondria (Wallin, 1927). Wilson, of course, had written a famously withering put-down of early work on endosymbiosis (Wilson, 1925 p. 739); I couldn't help wondering whether Margulis cited him so often deliberately, ironically using his own cell biology to build a compelling contrary case for endosymbiosis. If by 1967 polite biological society was not yet ready to embrace the centrality of endosymbiosis to eukaryotic evolution, after Margulis's paper serious biologists could no longer afford to ignore it. While many aspects of her paper have been debated or contradicted over the ensuing half century, the explanatory power of her main thesis still hits the reader with real force today. And in some respects, Margulis's argument in 1967 was closer to the modern view than her later modifications. Having said that, as this volume will attest, the 'modern view' is by no means unified and uncontested, even if few would any longer support Margulis's case that both mitosis and motility arose from the endosymbiotic acquisition of spirochaetes bacteria (Sagan, 1967).

1.1. Phylogenetic and geological context

Perhaps the most striking and important aspect of her paper was its orchestration of multiple lines of evidence from very different disciplines. Margulis went beyond her own expertise in cell biology to discuss the latest evidence from earth sciences, atmospheric chemistry and genetics, and pointed to the possibilities of phylogenetics. Though written a decade before Carl Woese's revolutionary ribosomal RNA phylogenies were published (Woese and Fox, 1977), she seems to have been aware of (if not citing)

E-mail address: nick.lane@ucl.ac.uk

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Francis Crick's remarks in the late 1950s (Crick, 1958) on the hidden wealth of phenotypic information available from amino acid sequences, and the pioneering work in the early 1960s by Emile Zuckerkandl and Linus Pauling on molecular clocks, which compared the amino acid sequences of hemoglobin chains from different mammals (Zuckerkandl and Pauling, 1965). Margulis writes, for example: "in determining the relationship of two microbes—that is, the amount of time elapsed since they diverged from a common ancestor—we may ask: how many homologous base pair sequences in DNA do they share? The number of mutational steps which occurred to produce one from the other is related to the number of generations elapsed since the two populations diverged" (Sagan, 1967 p. 249). On the other hand, her estimates of the number of genes and amino acid changes required were startlingly inaccurate. She suggested that the chloroplasts in *Euglena* have "at least 15 different kinds of enzymes" with each one containing about 100 amino acid residues (Sagan, 1967 p. 250, footnote). The chloroplast proteome actually contains as many as 3000 proteins (Qiu et al., 2013), often assembled into giant enzyme complexes, each containing thousands of amino acid residues (Zouni et al., 2001). I find it fascinating the degree to which Margulis and her contemporaries underestimated the molecular complexity of the microbial world, and the multi-subunit protein machines that make it up. This is not a criticism of Margulis, merely a reflection of how much more we know now about protein structures.

But beyond signaling her awareness of the potential, it was too early for phylogenetics to impinge on Margulis's thinking, and later on she distrusted or even rejected the gene-centered view. In 2006, for example, she wrote: "Especially dogmatic are those molecular modelers of the 'tree of life' who, ignorant of alternative topologies (such as webs), don't study ancestors. Victims of a Whiteheadian 'fallacy of misplaced concreteness,' they correlate computer code with names given by 'authorities' to organisms they never see!" (Margulis, 2006). While there may be more than a grain of truth in this, her repudiation of phylogenetics was equally dogmatic, and in stark contrast to her early vision of its possibilities. The fact was that the phylogenetic tree did not correspond well with Margulis's conception of the microbial world, so she preferred to dismiss it altogether in favor of the 'god in the details' of cell biology. Where these two worlds meet, rather than collide, remains a knotty problem which I will explore later.

In contrast, Margulis was arguably decades ahead of her time in considering the detailed geological context of eukaryotic evolution. Preston Cloud, whom she cites extensively, was then reinterpreting the geological record to trace the composition of the atmosphere and oceans from the oxidation of iron and other metals in sedimentary rocks, in relation to fossils of early life (Cloud, 1965). Margulis accordingly split Earth history into a prolonged primordial anaerobic phase, during which oxygenic photosynthesis arose in cyanobacteria (ending in the Great Oxidation Event around 2.4 billion years ago), followed by a long oxygenated phase, during which eukaryotes arose through a succession of endosymbioses. In the 1967 paper, Margulis had the first of these endosymbioses taking place in this oxygenated environment between an unspecified heterotrophic anaerobe and an aerobic bacterial endosymbiont that eventually became integrated as mitochondria in all eukaryotes. Margulis accordingly argued that eukaryotes are fundamentally aerobic, developing their tolerance of oxygen early on through the acquisition of mitochondria (Sagan, 1967).

She was explicit about the basis of the symbiosis, as well as the roles of the two partners involved: "The anaerobic breakdown of glucose to pyruvate along the Embden–Meyerhof pathway occurred in the soluble cytoplasm under the direction of the host genome. Further oxidation of glucose using molecular oxygen via the Krebs cycle… occurred only in the symbiotic mitochondrion under the direction of its own genes" (Sagan, 1967 p. 229). Margulis did not anticipate the level of integration that actually occurs, and seems to have assumed that the mitochondria retained a fully functional genome of their own (capable of controlling replication), as did the host cell. The idea that many mitochondrial genes would eventually be transferred to the nucleus, and that the great respiratory complexes would be composed of proteins encoded by both host and endosymbiont genomes was not easy to predict. Nor was it consistent with an old and hopeful prediction (Wallin, 1927) that Margulis shared, that mitochondria could be cultured: "If these organelles did indeed originate as free-living microbes, our advancing technology should eventually allow us to supply all growth factors requisite for in vivo replication… the *coup de grace* to genetic autonomy" (Sagan, 1967 p. 270). We now know that those 'growth factors' would need to include the protein products of 1500 genes that are located physically in the nucleus (Vafai and Mootha, 2012).

1.2. Oxygen, UV radiation and extinction

Margulis displayed both an unusual breadth of thinking and a curious blind spot in her discussion of atmospheric chemistry. I can only imagine what stimulating conversations she and her cosmologist husband Carl Sagan must have enjoyed over dinner; but it was certainly unusual for biologists to take such a cosmic view of life. Her discussion of prebiotic chemistry is reminiscent of the Miller-Urey experiment (Miller, 1953) in that she called upon a reducing atmosphere containing hydrogen and methane (but trace CO_2); and in some respects she is strikingly modern, invoking cyanide and UV radiation as substrate and driving force. I am not persuaded by the concept of a cyanosulfidic protometabolism driven by UV radiation (Patel et al., 2015), but others do find this approach to the origins of life appealing. Margulis's details, however, lack credibility. She talks about ultraviolet radiation in the upper atmosphere, for example, somehow conjuring ATP (and nucleotides) into existence. In her Table 1, she even refers to 'precellular replicating polynucleotides'. What exactly she had in mind is not clear, but this is close to an RNA world in conception, a hypothesis that was first raised around the same time by Carl Woese (Woese, 1967) and Francis Crick (Crick, 1968). Plainly the idea was in the air. I am struck by how much of the 1967 paper was in harmony with the newest thinking at the time; while Margulis was laying out a radical conception in cell evolution, her thinking clearly resonated with other leading pioneers of the time. That was not always true later in her life.

At the same time, Margulis seemed oblivious of the link between radiation and oxygen toxicity, first pointed out by Rebeca Gerschman in an emblematic *Science* paper, 'Oxygen poisoning and X-irradiation: a mechanism in common?' (Gerschman et al., 1954). Gerschman's central point was that radiation (including UV radiation) can split water to generate reactive oxygen free radicals, which damage organic molecules including DNA, RNA and proteins. Ground-state oxygen is not particularly reactive or toxic, despite being a free radical itself, as it can only accept single electrons from relatively willing donors, such as Fe^{2+}. On accepting single electrons, the same reactive oxygen species are formed that are produced by irradiation of water—superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$). Only the hydroxyl radical is aggressively reactive; and that is more likely to be formed directly by a single-electron oxidation of water than the three-electron reduction of oxygen (Lane, 2002). So it is ironic that Margulis credits UV radiation as the driving force behind prebiotic chemistry, and yet considered oxygen to be "lethal to early self-replicating systems" (Sagan, 1967 p.258).

Over evolutionary time, Margulis plainly saw oxygen as a kind of a binary geological switch, whereby global conditions were either anoxic or aerobic (with limited anaerobic refugia), leading to

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