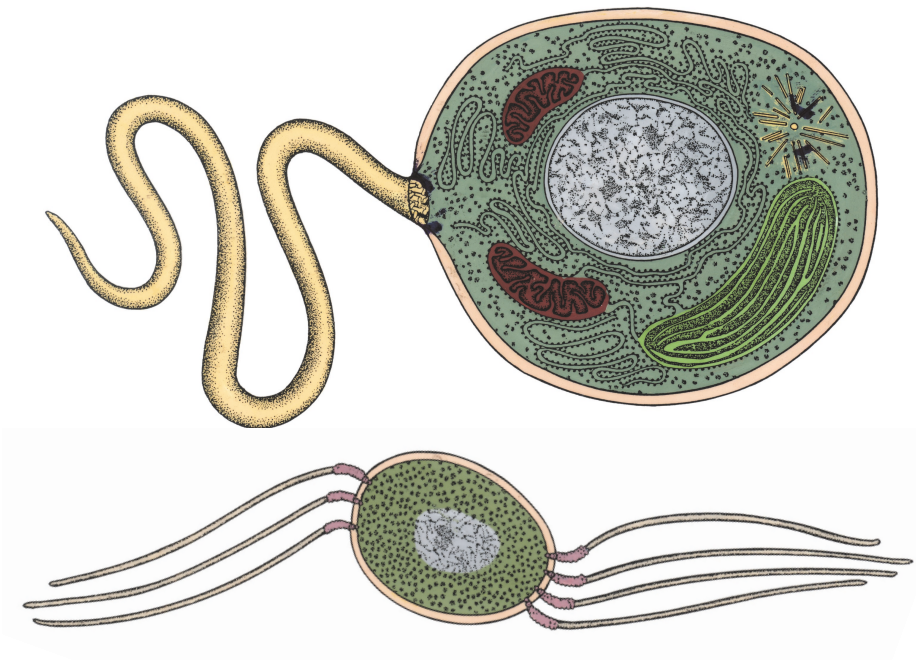
Serial endosymbiotic theory (SET) and composite individuality

Transition from bacterial to eukaryotic genomes

Lynn Margulis



Distinguished evolution expert and writer Lynn Margulis discusses the serial endosymbiotic theory of the transition from bacteria to eukaryotes.

ABOVE:
Fig. 1. Comparison of the two types of cells. *Top*: eukaryote cell with membrane-bounded nucleus that contains chromatin, undulipodium, centriole-kinetosomes, mitochondria, plastid, large ribosomes and intracellular motility. *Bottom*: prokaryote cell with rotary motor flagella, nucleoid, small ribosomes and peptidoglycan cell walls.
DRAWINGS BY CHRISTIE LYONS

OPPOSITE PAGE, TOP:
Fig. 2. A walled oxygenic photosynthetic cell (top) compared with an intracellular organelle from a chlorophyte (green) alga (bottom). Both contain chlorophylls *a* and *b* in a ratio of 1 : 3.
SEE MARGULIS (1993), PP. 116 & 337

The living world's highest taxa: bacteria and eukarya

The Russian–American *Drosophila* geneticist Theodosius Dobzhansky wrote that '*nothing in biology makes sense except in the light of evolution*'. I paraphrase him by suggesting that today nothing in molecular biology makes sense except in the light of the evolutionary history of organisms in specific paleoenvironments. As Darwin noted, our classification systems should become genealogies. If our taxa classify, identify and name life accurately, our grouping will reflect evolution; this is possible because strong inferences concerning the past are embodied in the living. The contribution to evolution of microbiology (*sensu lato*, the study of both bacteria and their protist descendants) has only recently begun to be appreciated. The cells of microbes are the units of life, hence the recognition of their importance in their own evolution and evolution of larger life forms is bound to increase.

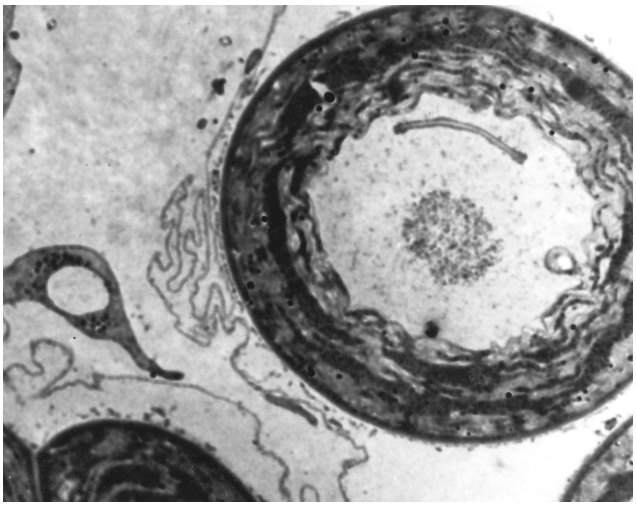
The living world unambiguously is divided into two definitive never-overlapping categories: prokaryotes and eukaryotes (Fig. 1). In spite of the immensely useful 'three-domain' 16S rRNA classification scheme proposed by Carl Woese, only two fundamentally different kinds of life exist on Earth. No evidence from either the fossil record or the living world can be mustered for any 'progenote' or other deviation from the prokaryote–eukaryote rule. This prokaryote–eukaryote divide (=Prokarya–Eukarya) remains the largest discontinuity in the living world. First recognized by Edouard Chatton and first analysed by the Delft School of Microbiology (e.g. A.J. Kluyver, Cornelius van Niel and Roger Stanier) the list of differences between Archaebacteria, Eubacteria and Eukarya unequivocally shows that the two prokaryote groups are far more closely related to each other than each of them is to any eukaryote. The cell, whether bacterial [where its genome is chromonemal (see below) and unbounded by membrane] or mitotic (nucleated, where a lipoprotein membrane bounds the proteinaceous chromosome) is the unit of all life.

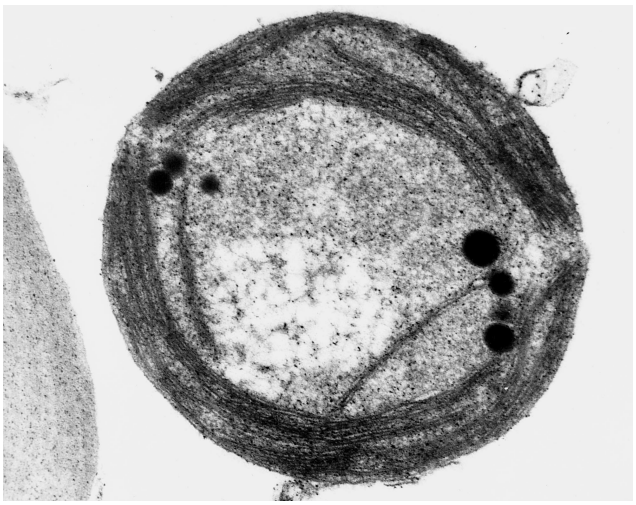
No system of matter and energy flow less complex than a cell is alive. The presence of the nucleus is the only feature that uniquely defines the eukaryotes and distinguishes them from bacteria. The origin of the bacterial cell is the origin of life itself, whereas Serial Endosymbiotic Theory (SET) describes the subsequent origin of the nucleated cell by symbiogenesis.

To proceed we need to explain how the ecological concept of 'symbiosis' differs from the evolutionary term 'symbiogenesis'. Symbiosis refers to the living together of organisms of different species. Endosymbiosis, a topological condition, is a kind of symbiosis where one partner lives inside of another. Symbioses usually, if not always, have environmentally contingent outcomes. Symbiosis, not an evolutionary process *per se*, refers to physiological, temporal or topological associations with environmentally determined fates. Symbiogenesis, however, implies the appearance of new tissues, new organs, physiologies or other new features that result from protracted symbiotic association. Two great classes of eukaryotic cell organelles, plastids and mitochondria, evolved symbiogenetically. Oxygen-respiring, heterotrophic α-proteobacteria were probably phagocytosed by anaerobic motile protists (like today's mastigamoebae). Genetic and metabolic redundancies were selected against as once free-living eubacteria evolved into the organelles we recognize as mitochondria. The descendants of this merger include most heterotrophic protists such as most amoebae, cryptomonads, chilomonads and chytrids,

Definition of *chromonemal*

The term chromosome does not apply to bacteria, even though it is often used. Chromosomes (*chromo*-coloured, *soma*-body) are the staining bodies, approximately 40 % DNA by weight and 60 % histone protein, that are segregated by the mitotic apparatus to the opposite ends of nucleated cells. The unit fibre is 100 Å diameter in chromosomes. Nothing resembling chromosomal mitosis has ever been found in any prokaryote, although it was claimed to be present in cyanobacteria. The much smaller unit fibre of bacteria, about 25 Å and nearly entirely composed of DNA does not take up Fuelgen and other cytological stains; it is properly referred to as 'chromoneme' and is the typical organization of nearly all bacterial nucleoids. The nucleoid refers to the electron microscopical appearance of the chromonemal DNA organization, if visible, in prokaryotic cells.





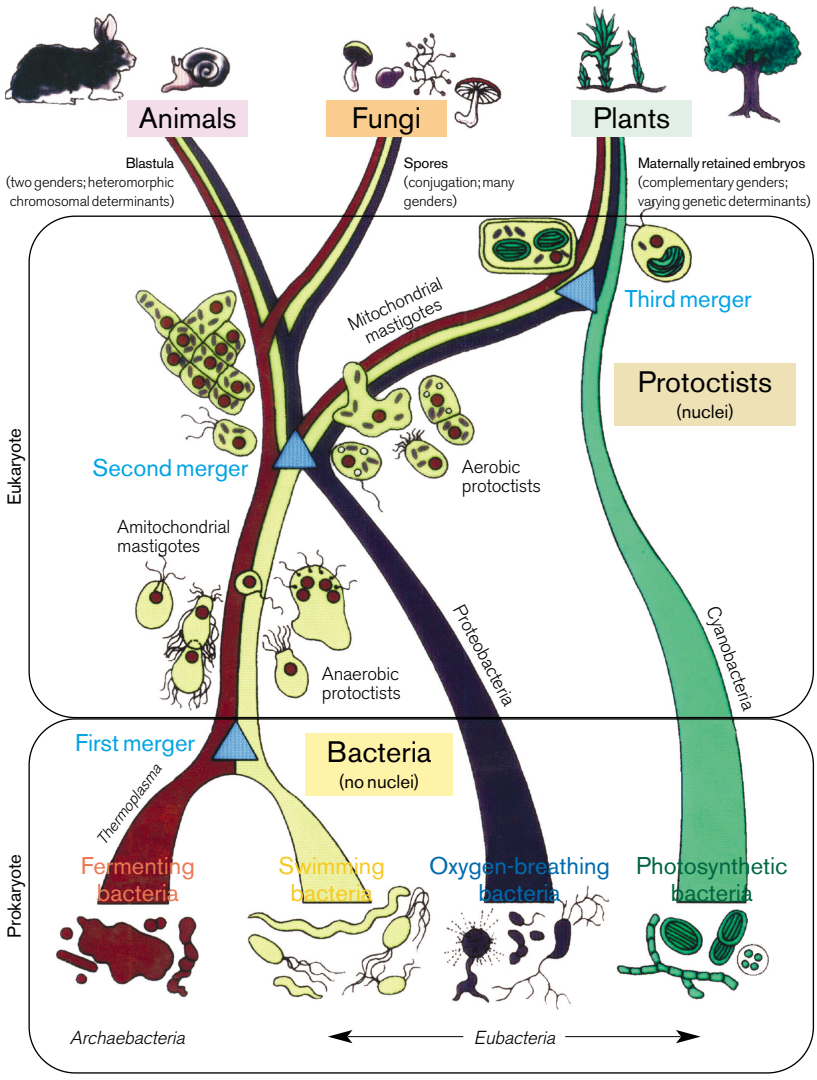
oomycotes (like *Phytopthora infestans*, the potato blight organism). No doubt some motile protists ingested, but failed to digest, food – cyanobacterial cells – that eventually became symbionts. The retention of undigested cyanobacteria in well-lit waters led to permanent unions in which, once again, natural selection favoured the reduction of genetic and metabolic redundancy. In this way algae, eukaryotic organisms that bear photosynthetic organelles in their cytoplasm, evolved and some became, eventually, the ancestors to the land plants. The Apicomplexa (a phylum which *Plasmodium*, the genus to which the malarial parasite is assigned) apparently evolved from one lineage of such algae. The members of this phylum, including *Toxoplasma* have retained a residuum plastid with its DNA, but they are no longer capable of photosynthesis. The principle of 'use it or lose it' can be invoked. Natural selection does not plan ahead; the unused plastids that began as cyanobacteria were severely reduced as they evolved. The striking resemblance of some free-living bacteria (such as cyanobacteria) to certain intracellular organelles (such as green algal chloroplasts) bolsters the concept that certain bacteria have been trapped inside other cells for millennia (Fig. 2).

With respect to the acquisition of mitochondria from free-living α-proteobacteria and that of plastids from free-living cyanobacteria, no one any longer doubts that the oxygen respiratory and photosynthesizing organelles evolved by symbiogenesis (Fig. 3).

Modern symbioses, both intra- and extracellular, that can be subjected to experimental analysis are of extraordinary importance for understanding evolution. How cells merge and how redundancy is reduced is especially relevant to the appearance of the first eukaryotes (which, by definition, were the first protists). Ironically, although most disease conditions are variations on the general theme of cyclical symbioses, few protistologists and microbiologists are familiar with the insightful, burgeoning literature that analyses these nearly ubiquitous associations.

No 'missing link' exists in our hypothetical evolutionary scenario on the origin of the complex individuality of Eukarya; every hypothetical event can be observed in extant microbes. This is why we have been able to make the 17 minute video entitled *Eukaryosis* (see www.sciencewriters.org). The study of genomics and proteomics will confirm or falsify this historical reconstruction that was made primarily based on observations of live organisms. Organismic biology coupled with direct knowledge of the fossil record are indispensable to evolutionary reconstruction. The techniques of molecular biology and sequence analysis by themselves are inadequate to the creation of testable evolutionary hypotheses.

BELOW:
Fig. 3. The minimal four prokaryotes of the plant cell. Swimming eubacteria (1) merged with sulfidogenic archaebacteria (2) and formed archaeprotists (amitochondriate mastigotes). O_2-respiring eubacteria (3) in the second merger produced ancestors to eukaryotic heterotrophs. Some acquired cyanobacteria (4) as undigested food and became algae with the third merger. All eukaryotes evolved from symbiotic mergers, whereas prokaryotes did not. Past (at bottom) to present (at top) is represented by the Archean Eon dominated by prokaryotes, the Proterozoic Eon of protoctists and the upper level Phanerozoic Eon, marked by the abundance of plants, animals and fungi.
DRAWING BY KATHY DELISLE



Further reading

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Complex individuality and the evolution of the tethered nucleus

How did the distinctive nucleus evolve? What was the first eukaryote? In the past decade, since the publication of the second edition of my book *Symbiosis in Cell Evolution*, with close colleagues (Drs Michael Dolan and Dennis Searcy) we have further developed the SET. New sets of data from three sources have permitted us to make good progress toward understanding the crucial step of the origin of the nucleus. We reconstruct the transition from the earliest prokaryotic (bacterial level of organization) during the Archean Eon (3500–2500 million years ago) to the complex individuality of the first eukaryotes. The Proterozoic Eon (2500–541 million years ago) was the backdrop for the appearance of cells at the protist level of organization. All eukaryotes, in the SET, are products of symbiogenesis whereas no prokaryote cell evolved by merger of whole-cell predecessors.

Sulfur syntrophy, we hypothesize, united thermoplasmic archaebacteria (such as *Thermoplasma acidophilum*) with motile *Spirochaeta*-like eubacteria in the evolution of the karyomastigont organellar system of swimming protists. The first eukaryotes were composed of at least two integrated bacterial genomes with a tethered nucleus (nuclear connector or rhizoplast, kinetosome-axoneme). This organellar system called the 'karyomastigont' has been known to protozoologists since it was first described by C. Janicki in the 1930s. Think of it as the nucleus attached by fibres to the kinetosome-centrioles of the undulipodia: what those who know nothing about bacteria would call 'the nucleus attached to basal bodies and their flagella, usually two or four'. We interpret this organellar system [the karyomastigont of the so-called flagellates (which should be called mastigotes), zoospores of water molds and slime molds, many motile algal cells, etc.] as a legacy of that first genomic integration of these bacteria. The evolution of mitosis with its histone-coated, nucleosome-studded chromatin occurred under anoxic, acidic, organic-rich, and probably muddy conditions prior to the symbiotic acquisition of oxygen-respiring α-proteobacteria that became the mitochondria.

New biochemical data on the role of sulfur oxidation and reduction in nucleated cells and on free-living sulfur consortia, as well as geological information on the prevailing conditions of aquatic environments during the Proterozoic Eon make our evolutionary scenario plausible. We continue our studies of contemporary archaeprotists (amitochondriate, often multinucleate single-celled organisms) that we interpret to be relicts of stages in the evolution of the earliest motile eukaryotes. Much of this work has been published or is in press. It permits us to present the karyomastigont model summarized here. The karyomastigont concept of mastigont multiplicity brilliantly developed by Harold Kirby, who was chair of the Zoology department of the University of California, Berkeley, when he died in 1952, refers to the organellar system known to be present, although often inconspicuous in many kinds of nucleated cells. By definition, the karyomastigont has at least these three components: the nucleus, the nuclear connector and the kinetosome/centriole-axonemes. (In certain protists other components of the karyomastigont organellar system are routinely present, such as axostyles, peltas and Golgi apparatus, the latter known as the parabasal body.) We argue that the earliest nucleus was in the form of the minimal karyomastigont and that this organellar system was a response to selection pressure. The nucleus with the combined genomes of at least two different prokaryotes evolved to assure genetic continuity of the now integrated archae- and eubacterial symbionts. The nucleus itself began in the karyomastigont as the integrated symbionts, in an act homologous to conjugation between very different bacteria, fused to form the first eukaryote. The untethering of the nucleus in many lineages led to the free nuclei. Free nuclei seen today in animals, plants and fungi we interpret as the derived state. Tethered nuclei evolved simultaneously with the first protist. No missing links need to be hypothesized. Certain amitochondriate eukaryotes always were confined to anoxic environments and never had mitochondria. The nucleus, in this scenario preceded both the mitochondria and the plastids. Indeed, in the bowels of xylophagous insects (wood-ingesting roaches and termites) and in anoxic muds all 'intermediate stages' that we envision as steps in the origin of nucleated cells are still found today.

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