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The origin of life –

or how single-cell organisms learned to cooperate

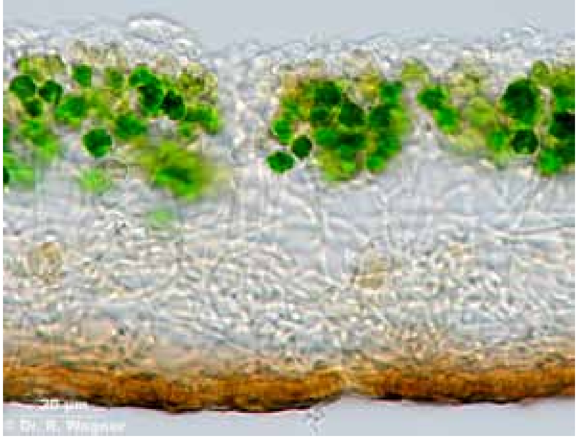
"Evolution must have been extremely satisfied with its accomplishments. So satisfied that it seems to have rested on its laurels for three billion years. Perhaps it just looked proudly at its work without feeling any compulsion to strive for higher things. Sure, the membrane pouch with that supermolecule at its core turned out to be a master stroke that could conceivably lead to greater things. But seriously, nothing but unicellular organisms for three and a half billion years?"* On this flippant but observant note, Frank Schätzing delves into the history of evolution in his book News from an Unknown Universe. *"A tiny sack that could only drift in the open water but still had, neatly assembled, everything needed to maintain a viable cell [...] Thus the basic building block of all complex beings was invented. A small bag packed with genetic information. A practical bag. The handbag of evolution."

This "membrane pouch" emerged several times, with various results. Archaebacteria and eubacteria, the "real" bacteria emerged. Together they form the group of **prokaryotes**. Karyon is the Greek word for "nucleus", so a prokaryote is a cell before the invention of the nucleus. In fact, archaebacteria and eubacteria contain no internal membrane system, and their DNA is present as a molecule floating freely in the cell's cytoplasm. Or as Schätzing writes: "Everything in the handbag still slid around wildly." The eukaryotes encompass all other living creatures. Above all, they differ from prokaryotes in that they possess a true cellular nucleus as well as membrane-enclosed organelles, some of which contain their own genes.

NOVEL LIFE FORMS THROUGH SYMBIOSIS

But what exactly happened for prokaryotes to evolve into eukaryotes, cells believed to be the ancestors of the three great kingdoms: fungi, plants and animals? As early as 1867, the Swiss botanist Simon Schwendener realized that **lichens** are made up of paired organisms – plant and fungus. They consist of one or more fungi, known as mycobionts, and one or more photosynthetic partners, known as photobionts, usually green algae or cyanobacteria. The fungus almost always forms the actual vegetative body, a network of fungal threads (hyphae) enclosing a population of photobionts **(Abb. A)**. The benefits of symbiosis are strongly on the side of the mycobiont. The photobiont, the alga, supplies it with nutrients which it synthesizes by photosynthesis. Schwendener writes about the "enslavement" of the captured alga by the fungus. Researchers today are more likely to speak of "controlled parasitism."

Fig. A: Cross-section of a lichen



At the top is a cortex of densely matted fungal mycelium. Below that is a layer containing the symbiotic green alga Trebouxia. Situated below the algal layer is a loose mesh of fungal mycelium, and finally below that a brown cortex formed of densely matted hyphae.

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The characteristics of lichens are very different from those of the organisms that make them up. For example, the typical lichen growth forms only in the symbiotic form, and the mycobionts synthesize the characteristic lichen acids only in symbiosis with a photobiont. For the Russian naturalist Konstantin Mereschkowski, lichens therefore provided a first indication that new forms of life can arise through the combination of individual organisms. In 1905 he published a first theoretical work, *The Nature and Origins of Chromatophores in the Plant Kingdom*, which is still considered the seminal work on **endosymbiosis** theory. It would prove revolutionary for our understanding of the origin of eukaryotic life.

A COMMUNITY WITH GRAVE CONSEQUENCES

But the idea was not new. Other biologists, including Andreas Schimper, had already given the matter consideration in the 1880s. Mereschkowski, however, was the first to suggest that **chloroplasts** – the organelles in which photosynthesis takes place, i.e. the synthesis of glucose from carbon dioxide and water in sunlight – were once living prokaryotes that were engulfed by alien eukaryotic host cells but had not been digested. Instead, they entered into a stable form of partnership with the host cells. *"That moment marked the invention of communal living, scientifically called symbiosis, in in which a symbiotic organism lives within the body of its partner. Commune 1, so to speak,"* Schätzing writes.

An important indication for Mereschkowski was the fact that chloroplasts always emerge from fellow chloroplasts by division, rather than being regenerated in the cycle of cell division, as would be expected for cellular constituents. In addition, the cyan-colored plastids bore striking physiological and morphological similarities to photosynthetically active **cyanobacteria**. Although it was far from easy at the time to observe these extremely tiny organisms under the microscope, Mereschkowski was convinced that cyanobacteria possessed neither a nucleus nor chloroplasts; the cyanobacterium as a whole acted as a single chloroplast. Well-known cases of symbiosis also supported his claim that chloroplasts are actually cyanobacteria. Mereschkowski pointed to algae (zoochlorellae and zooxanthellae) that live symbiotically in protozoa, freshwater sponges, hydra and some flatworms. Symbiotic algae, he argued, could be found in almost every class of lower invertebrates.

A WEALTH OF EVIDENCE

Modern molecular biological methods revealed what Mereschkowski could not yet know at the time. If you observe the structure of plastids (and, incidentally, mitochondria), it is obvious that they are separated from the cytoplasm by two enveloping membranes as a result of **phagocytosis**, the incorporation of one cell into another. The outer membrane is typically eucytic, while the inner membrane has protocytic, i.e. bacterial characteristics. Chloroplasts have their own circular DNA, and DNA replication and protein production are similar to those in bacteria. For example, chloroplast DNA has bacterium-like promoters, sequences that regulate gene reading. And unlike eukaryotic cells, chloroplasts have 70S ribosomes, which are also characteristic of bacteria. And their genes show a high degree of concordance with cyanobacterial genes.

There is thus a wealth of evidence to support the endosymbiotic theory. But that does not mean that all the questions have been answered, specifically how, how often and exactly when the various stages of endosymbiosis occurred. Much remains unknown. With regard to the question of how many times chloroplasts evolved, scientists can say that all chloroplasts (even the complex ones) of (monocellular and multicellular) algae and land plants are of monophyletic origin, i.e. they are the outcome of a single endosymbiotic event. As to when that occurred, however, disagreement reigned for many decades. Estimations of the time when the common ancestor of all eukaryotes existed varied widely: between 1.5 and 2.8 billion years ago.

A NEW TIMELINE OF EVOLUTION

The gap of more than a billion years resulted from discrepancies between fossil finds and chemical traces. To understand the emergence of higher organisms, scientists have analyzed fatty molecules known as steroids, which are contained in the cell walls of eukaryotic organisms. Steroid molecules can be preserved as steranes in old sediments, i.e. the fossilized floor of primeval bodies of water. Some scientists identified such molecular traces in sediment samples 2.5 to 2.8 billion years old and concluded that eukaryotic algae must have emerged by then. On the other hand, the oldest fossil microalgae, which are undisputedly regarded as remnants of eukaryotes, are found in rocks in northern Australia dating back approximately 1.5 billion years. Could the chemical samples have been contaminated? In 2015 scientists from the Max Planck Institute for Biogeochemistry in Jena, together with US colleagues, developed a new extremely elegant method to analyze 2.7-billion-year-old rocks that were classified as steroid-containing. The highly sensitive mass spectrometers of the various laboratories were unable to detect even picogram levels of eukaryotic steroids. "All the organic material in these samples had been altered by pressure and temperature over the course of millions of years. No biomarker molecules could have survived," says Max Planck researcher Christian Hallmann. Thus, the presumably 2.7-billion-year-old steroid molecules could no longer be held up as proof that eukaryotes originated much earlier than the fossil finds.

HOW OXYGEN CHANGED THE WORLD

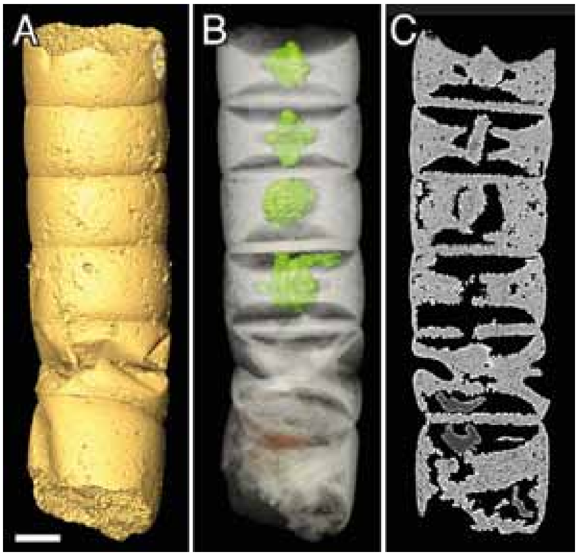
In any case, the chemical data to date have been a conundrum for researchers. Since all eukaryotes require oxygen, the evolution of oxygen-producing photosynthesis must have preceded the evolutionary transition to eukaryotes. This biochemical innovation, known as the "oxygen crisis", altered the entire planet and is unambiguously dated to 2.5 to 2.4 billion years ago. It has so far been difficult to explain how eukaryotes could have emerged several hundred million years before that when they absolutely required oxygen to survive.

SPECTACULAR FOSSIL FINDS IN INDIA

Meanwhile, there have been further discoveries: In 2017 Swedish researchers discovered in central India what may be the oldest fossils of eukaryotic cells to date. They found what they were looking for in the approximately 1.6-billion-year-old Chitrakoot formation. This sediment formed in shallow coastal waters which harboured colonies of filamentous cyanobacteria whose characteristic tube-shaped relicts have been preserved as stromatolites in the rock. However, between the fossilized cyanobacteria the researchers discovered several tubes that were significantly larger at up to two millimetres in length and had an unusual internal structure, as revealed by micro-CAT scans **(Abb. B)**. The researchers surmise that these intracellular structures represent an early form of plastids. If confirmed, these 1.6-billion-year-old microfossils would be among the oldest, if not the oldest, evidence of eukaryotic cells.

What began as a "loose community" about a billion and a half years ago led to the **co-evolution** of the symbionts, during which they lost their autonomy and were transformed into organelles. In the process, parts of the symbiont DNA were integrated into the nuclear genome of the host cell **(Abb. C)**. Researchers believe that endosymbiotic cyanobacteria and proteobacteria (precursors of mitochondria) have transferred up to 90 percent of their genome into the nucleus of the host cell. However, such functional **gene transfer** requires that the genes be properly inserted into the nuclear genome in order for them to be read. Since the transfer of thousands of genes from the organelles into the nucleus occurred over vast evolutionary periods and could therefore never be observed, the answer has remained elusive. "Only new technologies that allow chloroplast genomes of higher plants to be genetically modified have made it possible in recent years to elucidate major steps of this evolutionary process in the laboratory – in fast motion, as it were – and to understand the molecular basis of gene transfer between organelles and nuclear genomes," explains Ralph Bock, Director at the Max Planck Institute of Molecular Plant Physiology.

Fig. B: Ancestor of eukaryotes discovered?



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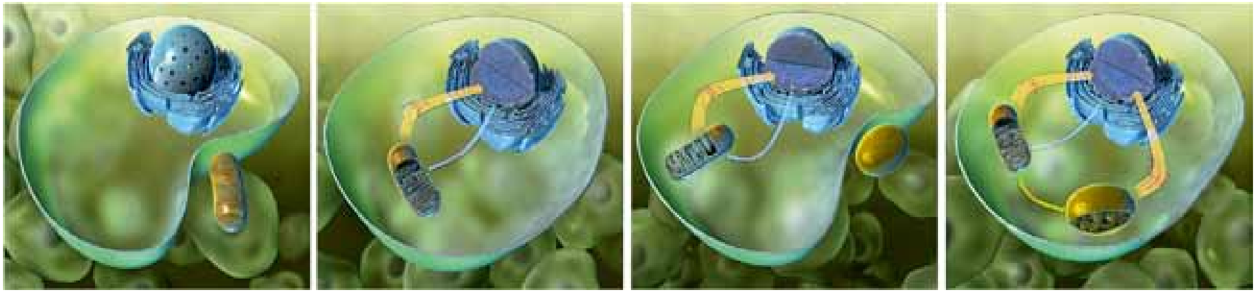
CAT scan of fossil cellular tubes *(Rafatazmia chitrakootensis)*, bar = 50 µm: (A) surface (B) internal layer with rhombic structures, stained (C) virtual longitudinal section

GENE TRANSFER IN FAST MOTION

The Max Planck researcher and his team introduced an extra gene into the chloroplasts of tobacco plants. This gene confers resistance to the antibiotic kanamycin, but only if it is in the nuclear genome. Consequently, the genetically modified plant cells could only be resistant to kanamycin if the gene had migrated from the chloroplasts into the nucleus and had successfully taken up home in the nuclear genome. To test this, the researchers transferred the plant cells to a tissue culture and placed them on a kanamycin-supplemented nutrient medium. Cells that survived must have transferred the resistance gene from the plastid genome to the nuclear genome. Such cells can eventually give rise to entire plants that are resistant to the antibiotic. "The frequency at which such gene transfers occur exceeded all our expectations," says Ralph Bock: "In about one in five million cells, the gene had taken up residence in the cellular nucleus." The significance of this becomes clear when you consider that a single leaf is made up of substantially more than five million cells. Now, the transfer of a gene from the chloroplasts into the nucleus does not automatically result in a functional nuclear gene. This is because prokaryotic, i.e. bacterial organelle genes, and eukaryotic nuclear genes are structurally different. In the experiment described above, the researchers circumvented this problem by inserting eukaryotic control elements (promoter, terminator) into the gene that mediates kanamycin resistance. Consequently, the gene was active immediately after migrating into the nuclear genome. However, this is not what happens in the case of evolutionary gene transfer: although the transferred gene is incorporated into the cellular nucleus, it cannot usually

Fig. C: The plant cell and its DNA-containing organelles



The precursors of organelles were independent bacteria that were engulfed by a primordial cell. In this way, proteobacteria gave rise to mitochondria and cyanobacteria to chloroplasts. The arrows show the direction and extent of gene transfer.

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be read there at first – unless, in a second step, a eukaryotic promoter is inserted in front of the gene.

RANDOMNESS PLAYS A ROLE

To test whether such an event occurs, the researchers introduced another gene – this time, however, with a bacterial gene structure – into the chloroplast genome that confers resistance to the antibiotic spectinomycin. In the course of the experiment, plants emerged in which the cell nucleus contained both a functioning kanamycin resistance gene and an inactive (because bacterial) spectinomycin resistance gene. Consequently, those plants should be resistant to kanamycin but susceptible to spectinomycin. In fact, though, resistance to spectinomycin also appeared in the cultivation experiments in eight selected plant lines, meaning that the relevant gene must have become active. "It turned out that in each of these cases, the deletion of a small DNA segment placed an active promoter in front of the gene," Bock explains. This molecular restructuring was sufficient to activate the spectinomycin resistance gene. For the first time, it was possible to track processes in fast motion that otherwise take place over geological periods of time and to elucidate the underlying mechanisms. It is therefore not surprising that a number of endosymbionts were able to transfer and activate a good part of their genome into the host nucleus within a few million years.

DIVISION OF WORK, A CLEVER IDEA

And how did the story continue? *"Although unicellular organisms are tiny, they're not in fact all that tiny. It has been calculated that unrestrained propagation would have literally covered the Earth with unicellular organisms – without gaps – within a few days! The early creation of a eukaryotic organism would have been the cause of its own suffocation. […] Maybe the idea with the handbag wasn't so ingenious?"* And that, Schätzing says, leads us to *"Miss Evolution's third stroke of genius"*: *"Her plan was based on specialization. […] The great secret of multicellular organisms is that they are not just aggregates of microbes; their cells cooperate to share some of the work."* So, Miss Evolution also made sure that only very specific cells were capable of reproduction. And that leads us to the matter of sexual reproduction. But that's another story."

Keywords

prokaryotes, eukaryotes, lichens, symbiosis, endosymbiosis theory, chloroplasts, cyanobacteria, phagocytosis, co-evolution, gene transfer

Reading tip

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- Zellorganellen – die Endosymbiontentheorie
  > www.youtube.com/watch?v=9LTMDLDsL98

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