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EVOLUTION

Organelle Genomes: Going, Going, Gone!

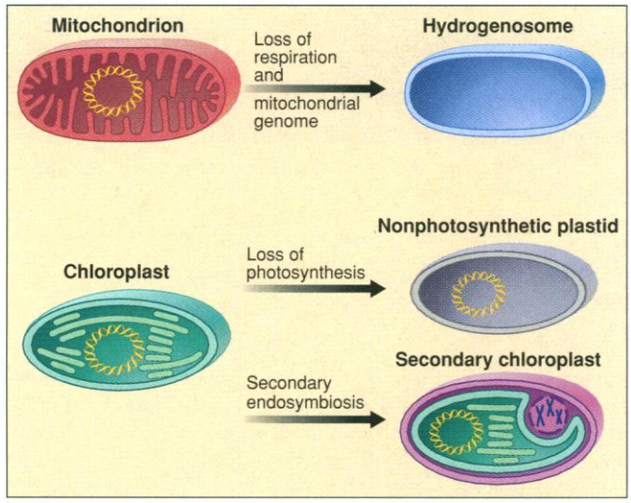
Jeffrey D. Palmer

An organism without a genome? Inconceivable, at least for free-living creatures. But what about an obligate, long-term endosymbiont—such as the mitochondrion and chloroplast—"living" within another organism? These organelles have persisted within eukaryotic cells for a long time—about 1 to 2 billion years—and have lost or passed to the nucleus most of their genes. Yet all chloroplasts and respiring mitochondria retain a functional genome of at least five genes.

Now, however, a eukaryotic organelle—the hydrogenosome—has been identified as endosymbiotic in origin, yet it lacks a genome and is entirely dependent on the nucleus for its genetic livelihood (*1*).

The hydrogenosome occurs widely, but its history has been elucidated clearly only in trichomonads—largely parasitic, flagellated protists. These air-tolerating anaerobes lack classical mitochondria (and also peroxisomes) and instead possess unusual energy-generating hydrogenosomes (*2*). Recent studies from four groups show that the hydrogenosome of *Trichomonas vaginalis* is actually a highly derived mitochondrion (*3*–*6*). Like mitochondria, trichomonad hydrogenosomes have a double-membrane envelope, divide autonomously by fission, import proteins posttranslationally, and produce ATP by substrate-level phosphorylation (*2*, *7*). However, they differ from mitochondria in that they lack a genome, cytochromes, the tricarboxylic acid cycle, and oxidative phosphorylation; they use enzymes (pyruvate:ferredoxin oxidoreductase and hydrogenase) typically restricted to anaerobes; and they produce large quantities of hydrogen.

Two main hypotheses have been advanced for the origin of the trichomonad hydrogenosome: It is either the product of an independent endosymbiosis of an anaerobic eubacterium (*8*) or a highly modified mitochondrion adapted to an anaerobic lifestyle (*9*). The latter hypothesis is now strongly supported by the demonstration that the *Trichomonas* nucleus carries genes for one (*4*–*6*) or all three (*3*) of the mitochondrial heat-shock proteins Hsp10, Hsp60, and Hsp70. These Hsps are among the most reliable tracers of the eubacterial ancestry of both the mitochondrion and chloroplast, and all three *Trichomonas* Hsps ally firmly with mitochondrial Hsps in phylogenetic analyses (*3*–*6*). Immunological studies (*3*, *10*) verify that the Hsp60 and Hsp70 proteins reside in the *Trichomonas* hydrogenosome. Although other interpretations cannot be ruled out (*5*), these data indicate a common origin for the mitochondrion and hydrogenosome; most likely, the hydrogenosome is a highly derived mitochondrion (*3*–*6*).



Origins of organelles. The secondary chloroplast shown still contains a residual nucleus; other secondary chloroplasts have lost their nuclei [see text and (*22*)].

Hydrogenosomes are a spectacular example of the repeated evolution of biochemically similar organelles, as adaptations to life with little or no oxygen. Hydrogenosomes are present not only in trichomonads, but in a wide variety of otherwise unrelated anaerobic or microaerobic eukaryotes, virtually all of which lack "mitochondria." These include several phylogenetically disparate lineages of ciliates (*11*), both free-living and rumen-dwelling; certain rumen fungi; and some percolozoan protists (*2*, *7*). The hunt will now be on to find molecular phylogenetic clues to the ancestry of these independently derived hydrogenosomes. Ultrastructural affinities to mitochondria are observed only for hydrogenosomes of free-living ciliates (*12*), and hydrogenosomes of the rumen fungus *Neocallimastix* are claimed to be of either mitochondrial or peroxisomal origin (*13*).

These findings should also prompt renewed inquiry into the early evolution of the eukaryotic cell and of eukaryotic phylogeny in general. The prevailing view for the past 10 years has been that the mitochondrion is not an ancestral feature of the eukaryotic cell (*14*, *15*). This follows from ribosomal RNA (rRNA) phylogenies, which generally place three amitochondrial groups—trichomonads, microsporidians, and diplomonads—at the base of the eukaryotic tree (*15*). But it is now clear that trichomonads do contain "mitochondria" (*3*–*6*). There is increasing evidence that microsporidians are misplaced in rRNA trees and are actually highly derived fungi that have lost mitochondria and most other organelles in the course of becoming obligate intracellular parasites (*16*); and there is suggestive immunological evidence (as yet unconfirmed by

The author is in the Department of Biology, Indiana University, Bloomington, IN 47405, USA. E-mail: jpalmer@bio.indiana.edu

gene isolation) that diplomonads may also contain an Hsp60 of mitochondrial origin (*17*). These considerations led to the proposition (6) that the common ancestor of all extant eukaryotes contained mitochondria (and peroxisomes) and was thus aerobic, and that subsequent evolution has been reductive, not acquisitive (with the exception of the chloroplast).

One endeavor these findings should not stimulate is a renewed search for a genome in hydrogenosomes. All characterized mitochondrial genomes are geared solely toward the hallmark function of the organelle—respiration (*18*). Since loss of respiration is a defining feature of hydrogenosomes, one would also expect rapid loss of their mitochondrial genome, making such a search pointless. In contrast, plastid gene products, although geared largely toward photosynthesis—the hallmark function of the chloroplast—are involved in other important metabolic processes as well. Consequently, a residual, functional plastid genome persists in the plants, algae, and other protists that have lost photosynthesis (*19*) (see figure).

Why organelle genomes at all? The present notion is that plastids and respiring mitochondria have kept their genomes because some of the key integral membrane proteins of respiration and photosynthesis are intensely hydrophobic and are therefore unimportable across the organellar outer membranes (*20*). Mitochondrial genome retention may ultimately be driven by the only two proteins (both highly hydrophobic) encoded by all examined mitochondrial genomes (*18, 20*), whereas photosynthetic plastids encode about 10 times as many putatively unimportable proteins (*20, 21*). Unfortunately, this theory fails to account for genome retention in three disparate lineages of nonphotosynthetic plastids, which do not encode any hydrophobic proteins (*19*). Nor does it explain the surprising diversity of organelle genomes in both number and kind of gene products (*18, 21*).

Genomic extinction has also happened repeatedly for the nucleus in many cases of secondary plastid endosymbiosis, the process whereby a protist engulfs a eukaryotic alga and permanently retains part of its prey as a degenerate endosymbiont (*22*). In chlorarchniophytes and cryptomonads, a remnant of the algal endosymbiont's nucleus, termed the nucleomorph (and bearing a tiny genome of 380 to 660 kilobases), persists between the inner and outer pair of membranes surrounding its chloroplast (see figure). But in all other cases, the endosymbiotic nucleus (or nucleomorph) has vanished (*22*). This means that hundreds of ancestrally plastid genes must have been transferred twice during evolution, first from the plastid to the nucleus (then nucleomorph) after primary plastid endosymbiosis, and then again from the nucleomorph to the host nucleus after each of many independent secondary symbioses. All in all, then, the host nucleus seems to be a tremendous magnet, both for organellar genes and for endosymbiotic nuclear genes.

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BIOTECHNOLOGY

DNA Ordering on a Lipid Membrane

Mark S. Spector and Joel M. Schnur

Recent advances in understanding the structure of bioassemblies have suggested their potential use for industrial as well as medical applications (*1*). This has been made possible by the development of tools such as atomic force microscopy (AFM) and near-field optical microscopy, improved synchrotron x-ray sources, and new techniques that permit selective modification of lipids, proteins, and DNA. Theoreticians working with molecular biologists are now using these techniques to gain a much better picture of biological function on the molecular scale. The state of experimental art in this area can been seen on p. 810, where Rädler *et al.* (*2*) present the structure of a bioassembly of DNA and lipid membrane.

Such bioassemblies might be used for the encapsulation and cellular delivery of intact genetic material. Liposomes, micrometer-size hollow spheres, remain the most common lipid-based drug carrier (*3*). Traditional preparations can lead to DNA degradation and low encapsulation efficiency. The use of charged systems has led to improved efficiency in cellular uptake. In 1987, Felgner *et al.* developed a cationic lipid that can be formed into liposomes before the addition of nucleic acids (*4*). Subsequent addition of negatively charged DNA leads to electrostatic binding to the liposome surface, for a 10-fold or greater improvement in cellular uptake (*5*).

The structure and function of these ionic complexes have been the subject of many recent studies. Mixing polyanionic DNA with cationic lipids leads to aggregation of the liposomes and ordering of the DNA (*6*). Similar transformations have long been known to occur in simpler lipid systems when multivalent cations are added to anionic liposomes, causing the bilayers to roll up like a jelly-roll into structures called cochleated cylinders (*7*). However, the structure of the aggregates in the DNA–cationic lipid system has remained unclear.

Using high-resolution x-ray scattering, Rädler *et al.* investigated the structure of these DNA-lipid aggregates (*2*). When DNA is added to small cationic liposomes (less than 0.1 μm in diameter), they collapse into micrometer-sized multilamellar globules. Sur-

The authors are at the Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, DC 20375, USA. E-mail: mspector@cbmse.nrl.navy.mil and jschnur@cbmse.nrl.navy.mil