GEOSCIENCE

Subterranean Life

William C. Ghiorse

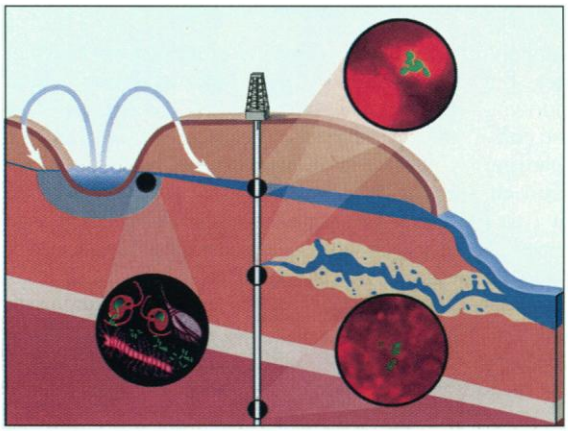
The topic of subterranean life traditionally conjures up images of shadowy creatures in a netherworld beyond our ken. A new vision, however, is of sparse but resilient and reliable microbes distributed throughout Earth's crust, extending to the depths of the temperature-limited biosphere (*1–3*). During the past few decades, subsurface microbiologists (*4–6*) and groundwater ecologists (*7*) have documented the activities of a surprising array of subterranean microorganisms in a wide variety of geological formations. At last we can see the range and extent of subterranean life and begin to appreciate its importance in maintaining life at the surface. Subsurface microbiologists from around the world recently met to discuss their progress (*8*).

Subsurface microbiology has its roots in at least three older disciplines: microbiology, geology, and hydrology. The members of this young field are still trying to define its boundaries. Most of them focus on the microbial ecology and biogeochemistry of microorganisms in continental aquifer sediments, fractured and porous rocks, near-surface unsaturated zones (*1*, *4*), deep ocean sediments (*9*), and arctic permafrost (*10*).

Groundwater ecology is an older field with a broader ecosystem perspective stemming from its roots in ecology and limnology (*7*). Its major focus is the aquatic ecology of groundwater systems, including the entire range of dark-adapted groundwater organisms from the microbes in aquifer sediments to pigment-less crustaceans in gravelly riverbank aquifers and blind salamanders and fish in karstic (limestone) cave ecosystems.

Research in subsurface microbiology accelerated a decade ago because of the urgent need to understand microbial processes in polluted aquifers (*11*). A critical first step was to develop specialized microbiological drilling and sampling techniques and tracer technologies (*5*, *12*). This allowed subsurface samples to be monitored for microbiological contamination. At the same time, sensitive techniques were developed to measure microbial biomass and activity in the ultra-low nutrient content subsurface material (*13*). These technical achievements boosted our confidence in sample integrity and our life detection ability. Now they provide a solid foundation for our present and future work.

Among the exciting novel concepts and achievements are (i) new hypotheses on the origins and long-term survival of microbial life in deep crustal rocks (*1*, *2*); (ii) recognition of subsurface ecosystems supported by chemosynthetic primary production (*14*) and the possibility of subsurface life on Mars (*15*); (iii) new sources of industrially important organisms (*1*); (iv) low-cost, in situ bioremediation of polluted groundwater (*5*); and (v) improved methods for microbially enhanced oil (*13*) and mineral (*3*) recovery. We are also better able to understand the transport and fate of microbial pathogens and toxic chemicals in groundwater systems (*13*) and to assess the impacts of agricultural practices on groundwater quality (*16*).



Hydrologic, geologic, and biological features of subterranean habitats. The circles contain magnified images of some of the microorganisms (mostly bacteria) that dominate the subterrahean world. Most of our knowledge of this subterranean life is derived from drilling and sampling, which is costly and has limited value for increasing our knowledge of in situ microbial activity.

The accumulated body of knowledge strongly reaffirms a basic tenet of microbial ecology known as Beijerinck's Principle (*17*): "Everything is everywhere, the environment selects." Microorganisms, particularly bacteria, inhabit all allowable habitats of the biosphere including subterranean ones. Subsurface habitats are dark, generally low in organic nutrients, relatively constant in temperature, and relatively large in mineral surface area. Subsurface geochemical processes can be expected to proceed at a very slow pace owing to the generally low energy status of the habitat. These and other habitat-specific chemical and physical factors select and regulate the microbial communities in subsurface habitats as they do in all other habitats on Earth.

Methods to directly measure subsurface microbial processes in situ are severely limited. Geochemical and hydrologic models (*5*, *7*) can help us to understand how subterranean microbiological and hydrogeochemical processes can combine to effect changes in groundwater chemistry, but we are still very uncertain how microbial communities function in situ. Current approaches are inadequate. Drilling and sampling are costly: They introduce many uncertainties into the results and alter the original habitat drastically. Methods are needed to assess microbial activity directly in undisturbed subsurface habitats. How can we move to direct measurement of real subsurface processes in real time? How can we do it at a reasonable cost?

One obvious answer is to adapt some of the methods that are used successfully in aquatic microbial ecology for in situ measurements, especially in the deep sea (*18*). Another is to work with geophysicists who are developing high-resolution, minimally invasive methods to "see" into the shallow subsurface (*19*). A combination of the minimally invasive geophysical methods with limited sampling and the in situ methods may allow us to better assess subsurface microbial activities, at a fraction of the cost of a present-day microbiological drilling and sampling program. This effort would be a new endeavor for both the microbiological and geophysical communities. Such a plan, of course, would require us to work toward a much deeper understanding between these communities than exists today. If we can make that effort, then the future of studies on subterranean life looks very bright indeed.

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EVOLUTION

Organelle Genomes: Going, Going, Gone!

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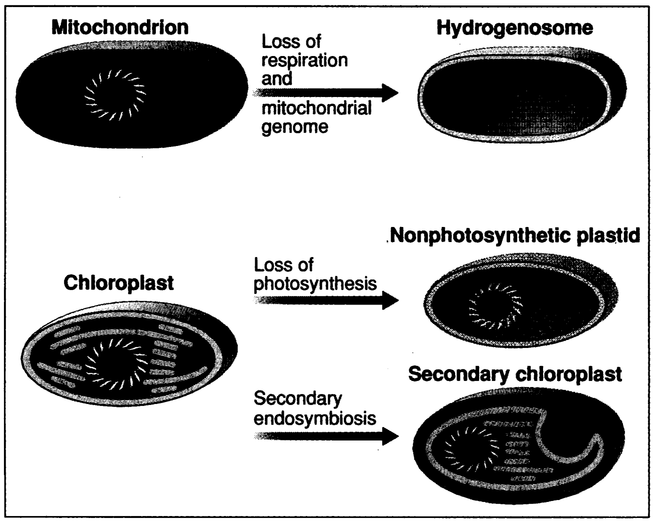
An organism without a genome? Inconceivable, at least for free-living creatures. But what about an obligate, long-term endosymbiont—such as the mitochondrion and chloroplast—"living" within another organism? These organelles have persisted within eukaryotic cells for a long time—about 1 to 2 billion 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

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