

The Evolution of Life without Oxygen

Organisms in oxygen-free habitats engage in unique symbiotic relationships that provide clues to the evolution of the first eukaryotic cells

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In various nooks and crannies on the Earth, whole communities of microscopic organisms go about their daily lives in the absence of oxygen. It is a form of existence utterly different from the aerobic lives we know. Yet these minute, anaerobic organisms do offer some valuable insights into the existence of oxygen breathers. Indeed, the first living cells—ancestors of all life on the planet—were themselves anaerobic organisms.

The story of the link between those who would and those who would not use oxygen in their lives begins with the ancestral anaerobes some three to four billion years ago. These ancient relatives were prokaryotes: single-celled organisms that lacked the ensemble of intracellular organelles (most notably a nucleus) found in their descendants, the eukaryotes (including human beings). The ancestral prokaryotes and the first eukaryotes were anaerobes by necessity; the atmosphere of the early earth bore only the smallest traces of oxygen.

Anaerobic organisms flourished on the surface of the planet for more than

500 million years before oxygen began to play a role in the evolution of life. Somewhere along the way, a group of prokaryotes, the cyanobacteria, developed the means to use the sun's energy in the process of photosynthesis. This event had the consequence of loading the atmosphere with oxygen, since cyanobacteria released the gas as waste. (Some of the oxygen molecules we breathe today were undoubtedly excreted by a cyanobacterium about two billion years ago.)

The advent of an oxygen-filled atmosphere opened the way for the evolution of organisms (prokaryotes and eukaryotes) that could use the gaseous molecule in their energy metabolism. It was an extremely successful event in evolution. For one thing, it permitted a tremendous increase in the efficiency of the organism's energy metabolism. Although many prokaryotic organisms do use oxygen in their metabolism, it is largely the eukaryotes that have evolved and diversified as oxygen-dependent organisms.

Nonetheless, many eukaryotes, such as human beings, still show signs of their anaerobic ancestry. This is evident in the biochemical significance of free oxygen. It is largely restricted to only two roles: energy metabolism and toxicity. All aerobic organisms must produce enzymes that detoxify oxygen radicals. Although aerobic organisms depend on oxygen for energy metabolism, with only a few exceptions, they do not use elemental oxygen in synthetic pathways. It is as if free oxygen began to have an impact on the history of life only after most biochemistry had been "invented."

Anaerobic organisms, on the other hand, go about their lives studiously

avoiding oxygen because they lack protective enzymes. Although most species of anaerobes are prokaryotes, modern anaerobic eukaryotes do exist. These organisms include direct descendants of the earliest eukaryotes as well as forms that have secondarily adapted to life without oxygen. Most of them are protozoans, the smallest of all animals (less than one-tenth of a millimeter in length), and all are unicellular.

During the last five years we have studied the biology and ecology of protozoa living in oxygen-free habitats. We have been particularly engaged by their strange and specialized symbiotic relationships with bacteria. These relationships are intriguing because they provide a contemporary analogy for the symbiotic origin of mitochondria, the organelles responsible for energy metabolism. They also shed light on the behavior of biological communities as they might have functioned three billion years ago, before the widespread distribution of oxygen. Our story ultimately suggests why oxygen was necessary for the diversification of eukaryotes and the evolution of multicellular animals.

Anoxic Habitats

Although oxygen has been generated by photosynthetic organisms for the last three-and-one-half billion years, anaerobic habitats have existed continuously throughout the earth's history. Such habitats can arise whenever dead organic material accumulates from the surrounding aerobic regions. In the process of converting organic carbon to form carbon dioxide (mineralization), anaerobic organisms consume (and deplete) the local oxygen. The worldwide distribution of black shales, a conse-

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Figure 1. Ciliated protozoa of the species *Metopus contortus* live in an intimate metabolic union with at least two different species of symbiotic bacteria. The ciliate host provides organic molecules for the bacterial symbionts, and the bacteria improve the energy metabolism of the ciliate by removing certain metabolites. The symbiotic relationship offers an analogy for the types of interactions present in the earliest oxygen-free habitats about two billion years ago. (All photographs courtesy of the authors.)

quence of anoxic conditions, suggests that during some periods in earth's history, vast shallow seas were anoxic at certain depths. To this day there are a number of places where anoxic conditions persist.

The extent and distribution of anoxic habitats are often surprising to those who do not normally consider these niches. Marine sediments, for example, are often anoxic a few millimeters below the surface. Remarkably, they constitute an enormous, globally continuous anoxic environment. The ecological significance of such a habitat has yet to be fully explored or understood.

Sediments and detrital deposits of lakes also provide an oxygen-free environment. Here organic particles—dead algal cells, fecal pellets of planktonic animals and other debris—sink to the lake bottom. In the sediment, oxygen is supplied only by molecular diffu-

sion. This process is so slow over distances exceeding a few millimeters that it cannot meet the demand of aerobic organisms living beneath the sediment surface.

Even in shallow-water sediments where there is sufficient light to allow the growth of oxygen-producing microalgae, oxygen diffuses only as far as the upper five to eight millimeters. Digging in the sediment reveals the oxygen-free zone, distinguished by the contrast between its grey-black color (and its smell of hydrogen sulfide) and the yellowish oxidized zone. The black color comes from iron sulfides, compounds that are not stable in the presence of oxygen.

In biologically productive shallow waters, the anoxic zone may reach almost to the surface. When this happens a white veil of sulfur bacteria is often seen. These organisms make their liv-

ing by oxidizing hydrogen sulfide, which diffuses up from the anaerobic sediment. The presence of purple sulfur bacteria (which use hydrogen sulfide in a form of photosynthesis that does not produce oxygen) reveals that anaerobic conditions exist right up to the surface of the sediments.

Lakes are often thermally stratified so that the upper warm layer does not mix with deeper, colder water. As a result the deeper water is effectively isolated from contact with the atmosphere, and becomes anoxic. A similar phenomenon is often seen in marine fjords that have a sill at the entrance, and in bays with deeper basins. The Black Sea is the largest anoxic body of water in the world; oxygen is undetectable below a depth of about 150 meters. The deep waters of certain basins in the Baltic Sea and the Cariaco Trench in the Caribbean Sea also represent large anoxic marine basins.



Figure 2. Communities of purple sulfur bacteria cover a shallow inlet in Nivå Bay, north of Copenhagen. Purple sulfur bacteria use hydrogen sulfide (H_2S) in a form of photosynthesis that does not release oxygen. The presence of these anaerobic bacteria indicates that the sediments in the inlet are essentially free of molecular oxygen right up to the surface. The sediments consist of decaying seagrass leaves that form a layer about half a meter deep. Such habitats are a rich source of the anaerobic organisms described by the authors.

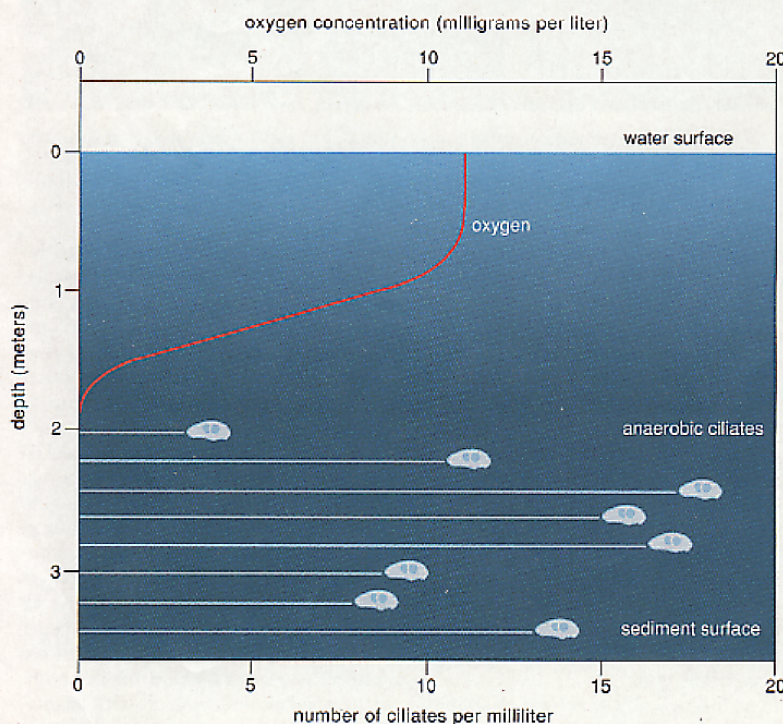


Figure 3. Concentration of dissolved oxygen (red) must fall to zero before anaerobic organisms can survive in the depths of a freshwater pond in summer. In this instance the anaerobic organisms first appear at a depth of about two meters below the surface of the pond.

There are other, less romantic, anoxic habitats. These range from anaerobic sewage-treatment plants, to fermenters that produce useful methane gas from organic refuse, to landfills packed with domestic waste. The intestinal tracts of herbivorous animals also constitute an important class of anoxic (or nearly anoxic) habitats. Many herbivores depend for food on the metabolites of fermenting microorganisms that degrade plant structures such as straw and wood. The rumens of cows and sheep and the hindguts of termites have been extensively studied, but similar systems have evolved independently in other herbivores, including several groups of mammals, green turtles and sea-urchins.

It should be clear that anaerobic habitats are diverse and widely distributed. Even so, the microorganisms that live within them share a number of common features, including their interactions with each other.

Anaerobic Community Structure

Most anaerobic ecological systems are fueled almost entirely by dead organic material, typically plant debris, imported from the aerobic surroundings. Under anaerobic conditions, the material is decomposed primarily by fermenting bacteria, which break down large organic molecules into smaller sugars and amino acids. The dissolved compounds are then transported into the cells where they are fermented, providing energy for the bacteria.

The bacteria's fermentative pathways produce, among other things, acetate (which is made up of carbon, hydrogen and oxygen) and molecular hydrogen. Oxidizing a substrate by hydrogen production yields about twice as much energy as does glycolysis alone (leading to lactic acid or to ethanol and carbon dioxide). This is nevertheless a relatively modest yield of energy compared to aerobic respiration. Only about 10 percent of the substrate is incorporated into new cell matter (in microorganisms most of the energy is used for the synthesis of new macromolecules required for cell growth and renewal). The remaining material is released, primarily as acetate and hydrogen.

The excreted metabolites of fermenting bacteria are used by other types of bacteria. In environments where oxygen-supplying sulfate ions are in low concentrations (lakes and sewage di-

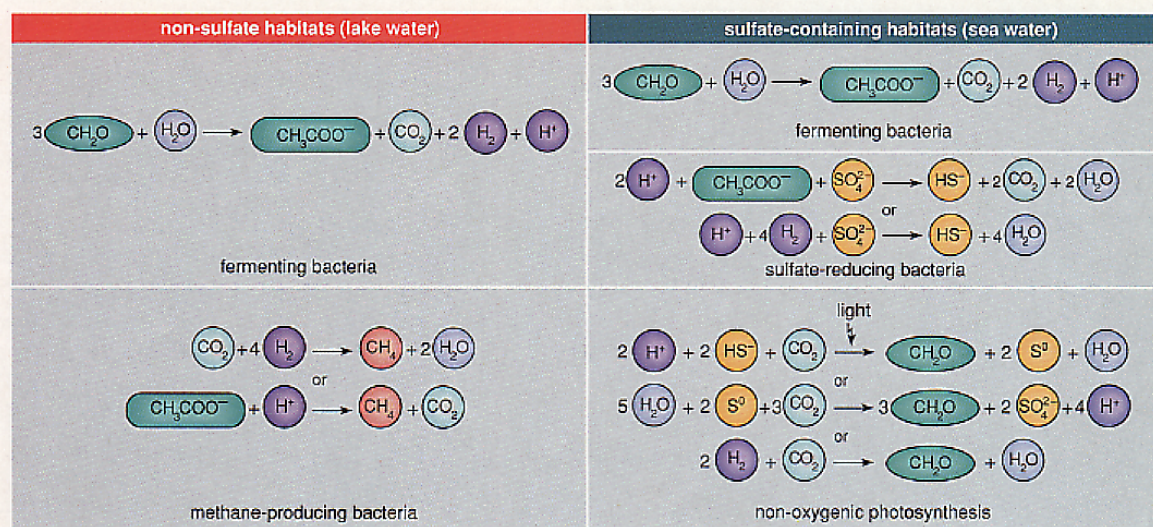


Figure 4. Metabolic processes in bacteria differ when sulfate is present or absent in oxygen-free environments. In either type of habitat, organic molecules (here represented by CH_2O) are fermented into acetate (CH_3COO^-), carbon dioxide and hydrogen (top). In non-sulfate habitats these molecules are then transformed into methane (CH_4), water and carbon dioxide by methanogenic bacteria (bottom left). In sulfate-containing habitats, sulfate-reducing bacteria oxidize the products of fermentation with sulfate (SO_4^{2-}) (middle right). Photosynthetic bacteria in anoxic environments use the reduced sulfide (HS^-) as an electron donor to produce organic compounds and elemental sulfur or sulfate (bottom right). These products can be recycled again by the sulfate-reducing bacteria. Because sulfate-reducing metabolism is energetically more efficient than the methanogenic system, it predominates in anaerobic communities where sulfate is present. These processes are quantitatively the most important. There are, however, other forms of fermentation, and some bacteria use electron acceptors other than sulfate.

gesters), methane-producing bacteria are responsible for the terminal mineralization process. The methanogens are capable of obtaining energy for growth by converting carbon dioxide (CO_2) and molecular hydrogen (H_2) into methane (CH_4) and water. Some methanogenic bacteria are also capable of transforming acetate into methane and carbon dioxide. When lake sediments are stirred, the bubbles of methane that rise to the surface are evidence of the activity of these bacteria.

Other types of bacteria predominate in seawater, which contains large amounts of sulfate (SO_4^{2-}) compounds. These anaerobes—the “sulfate reducers”—oxidize volatile fatty acids and hydrogen using sulfate rather than oxygen. In turn they produce hydrogen sulfide (H_2S) rather than water (H_2O) as their principal metabolite. Since sulfate respiration provides more energy than does methanogenesis, sulfate reducers are competitively superior to methanogens in regions where sulfate is available. Even so, a low level of methanogenesis is usually detectable in marine anaerobic environments. The presence of sulfate reducers or methanogens in the habitat means that organic material can be completely mineralized without oxygen. In anaerobic

conditions this is accomplished by diverse types of bacteria, whereas in an aerobic habitat it can be accomplished by a single organism.

Hydrogen plays a key role as a substrate and a metabolite in anaerobic communities. Hydrogen produced by fermenting bacteria is rapidly metabolized by the methanogens and the sulfate reducers. This results in low hydrogen pressure, which is essential for the function of the whole ecological system. This is because fermentation that involves the release of hydrogen is thermodynamically feasible only if the ambient hydrogen pressure is very low. In a sense, fermenting bacteria and hydrogen-scavenging bacteria are engaged in a mutualistic relationship. Fermenting bacteria produce the substrates for methanogens and sulfate reducers, and the consumers maintain a favorable habitat for the producers by removing hydrogen. This interspecies hydrogen transfer is an example of a syntrophic interaction. It is crucial to the interaction of anaerobic microbial communities.

Where light is present, photosynthetic bacteria will re-oxidize the end products of mineralization. Purple and green sulfur bacteria use light to chemically reduce carbon dioxide to make

organic material. In this instance, hydrogen sulfide (H_2S) is used as an electron donor instead of water (H_2O) as in green plants. Consequently, sulfur bacteria produce elemental sulfur or sulfate rather than molecular oxygen. Other photosynthetic bacteria use molecular hydrogen or organic compounds as electron donors. Photosynthetic systems that do not rely on oxygen are mechanistically simpler than those that do. It seems likely that oxygenic photosynthesis appeared later in evolutionary history.

In principle anoxygenic photosynthesis allows an anaerobic community to operate as a closed system fueled by light alone. The system has a complete cycle in which sulfur atoms function as electron acceptors in respiration and as electron donors in photosynthesis. In turn, the cycle produces substrates for fermentors and sulfate reducers. Such anaerobic communities may have dominated the early history of life.

Anaerobic Eukaryotes

Prokaryotes (bacteria) do not eat each other. They make a living from dissolved substances only. Eukaryotes, on the other hand, routinely ingest other organisms. Such predation, a type of interspecies interaction, is immensely important in aer-

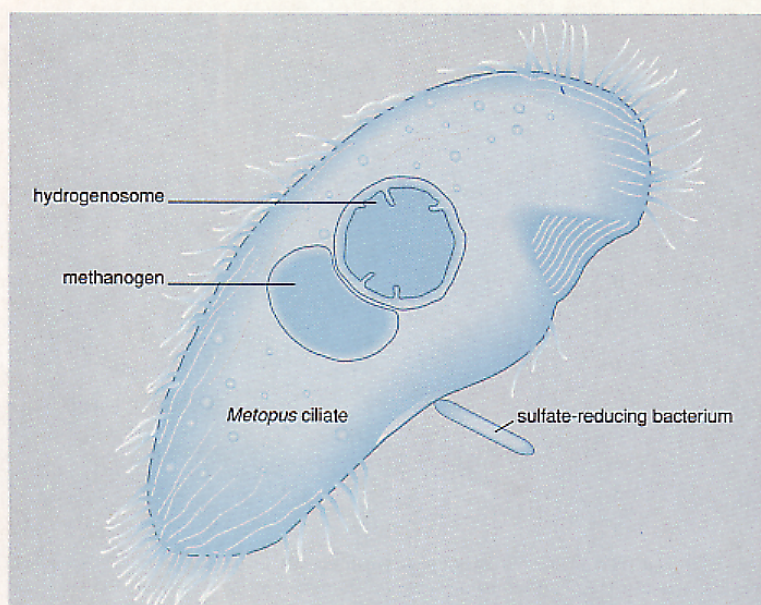


Figure 5. A single-celled anaerobic eukaryote, *Metopus*, harbors at least two types of symbiotic bacteria. Each *Metopus* cell contains about 7,000 methane-producing bacteria (methanogens) that live endosymbiotically within the cell, and 4,000 sulfate-reducing bacteria that live ectosymbiotically on the surface of the cell. Hydrogenosomes are intracellular organelles, closely related to mitochondria, which are thought to be highly modified endosymbiotic bacteria.

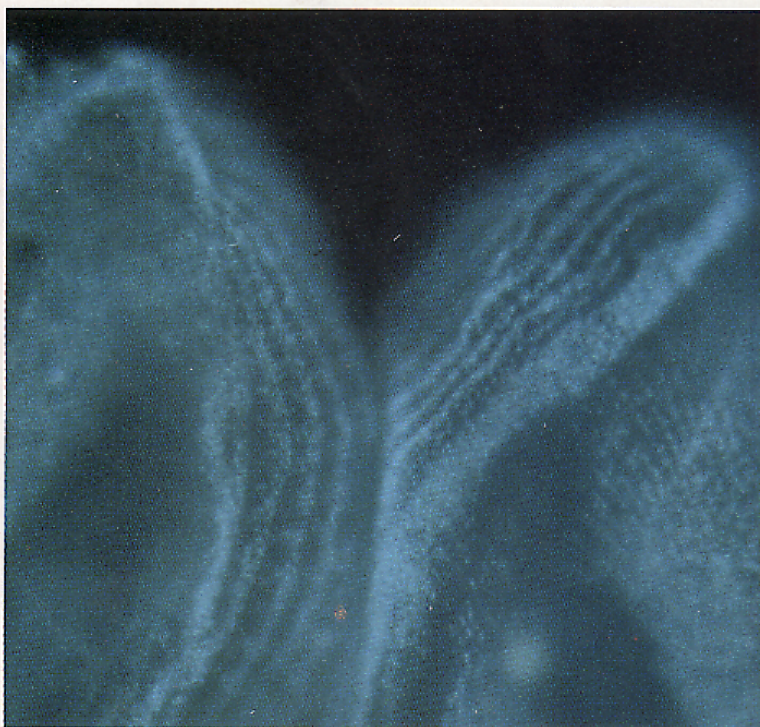


Figure 6. Methanogenic bacteria, inside two *Metopus* cells, are recognized by the characteristic emission of a bright blue fluorescence when illuminated by violet light. It has recently been discovered that nearly all anaerobic ciliates living in fresh water, and about half of those living in marine habitats, contain methanogens.

obic ecosystems, but it also occurs in anaerobic habitats. In this case, the predators are unicellular eukaryotes that engulf other cells. These predatory eukaryotes are the protozoa.

Anaerobic protozoa belong to two distinct groups. One group probably evolved directly from the ancient ancestral anaerobes; they are devoid of any mitochondria-like structures. The second group of protozoa consists of organisms that have secondarily adapted to anaerobic habitats.

Diplomonad flagellates (single-celled organisms with two nuclei) belong to the first group. They are best known as occasional inhabitants of animal intestines. *Giardia intestinalis*, for example, has been studied very closely because it can cause diarrhea in human beings. Other free-living species of diplomonads that feed on bacteria are also known. Because these organisms lack mitochondria, their energy metabolism is based on fermentation that does not involve hydrogen production.

Mitchell Sogin and his colleagues at the Marine Biological Laboratory at Woods Hole, Massachusetts, have recently studied the nucleotide sequence of the diplomonad's ribosomal RNA molecule. A comparison with the ribosomal RNA of other single-celled organisms suggests that the diplomonads branched off as an independent group during a very early stage in the evolution of eukaryotes. Some speculate that these organisms are the direct descendants of an anaerobic eukaryote that had not yet acquired mitochondria. It is possible, however, that the diplomonads lost their mitochondria during a later stage in evolution.

Other groups of anaerobic protozoa—certain flagellated amoeboid organisms—also lack mitochondria. In particular, species of the genera *Mastigella* and *Pelomyxa* (the giant freshwater "amoeba") may also be living fossils from the dawn of eukaryotic life. These organisms lack certain other organelles that are characteristic of most eukaryotic cells, including the Golgi apparatus, the endoplasmic reticulum and the microbodies.

Most anaerobic protozoa, however, belong to the second group, organisms that have secondarily adapted to life without oxygen. The ciliated protozoa, or infusoria, are the most common. Although there are probably more than 100 species of anaerobic infusoria, they

all belong to taxonomic groups that include aerobic species. Anaerobic ciliates evolved independently within six different lineages. These organisms, which have secondarily adapted to anaerobic conditions, have been the focus of our own studies. In particular, we have studied the biology of five species, which we keep in controlled laboratory cultures.

The five species share a special sensitivity to oxygen. One species is killed within an hour of exposure to only about two percent of the atmospheric oxygen pressure. Other species seem capable of growth under the same conditions and may even recover from temporary exposure to atmospheric pressures of oxygen if they are returned to anoxic conditions. These organisms are sensitive to oxygen partly because they lack (or have low levels of) the enzymes that detoxify oxygen. They also lack respiratory enzymes (cytochromes), so they are unable to use oxygen in their energy metabolism.

Although they do not have respiratory enzymes, anaerobic ciliates have organelles that are almost identical to the mitochondria of aerobic ciliates. These organelles, known as hydrogenosomes, were originally discovered in 1973 by Donald G. Lindmark and Miklós Müller of Rockefeller University. Hydrogenosomes contain two enzymes that were previously found only in anaerobic bacteria. One of these enzymes, hydrogenase, catalyzes the oxidation of molecular hydrogen, yielding two protons and two electrons ($H_2 \rightarrow 2H^+ + 2e^-$). Because of their structural similarities, it seems likely that the hydrogenosomes of anaerobic ciliates are modified mitochondria. Ultimately, the nucleotide sequence of the DNA in this organelle may provide a conclusive answer.

About 15 years ago one of us (Fenchel) observed that anaerobic ciliates often harbor symbiotic bacteria. In some instances, the bacteria were living as endosymbionts within the cytoplasm of the ciliate. In other cases, the bacteria formed a dense, fur-like cover on the surface of the ciliate. Some ciliate species (such as *Metopus contortus*) harbor both types of bacteria.

A few years later, Claudius Stumm and his colleagues at the University of Nijmegen, in the Netherlands, discovered that the endosymbionts are methane-producing bacteria. Methanogens can be recognized in the light

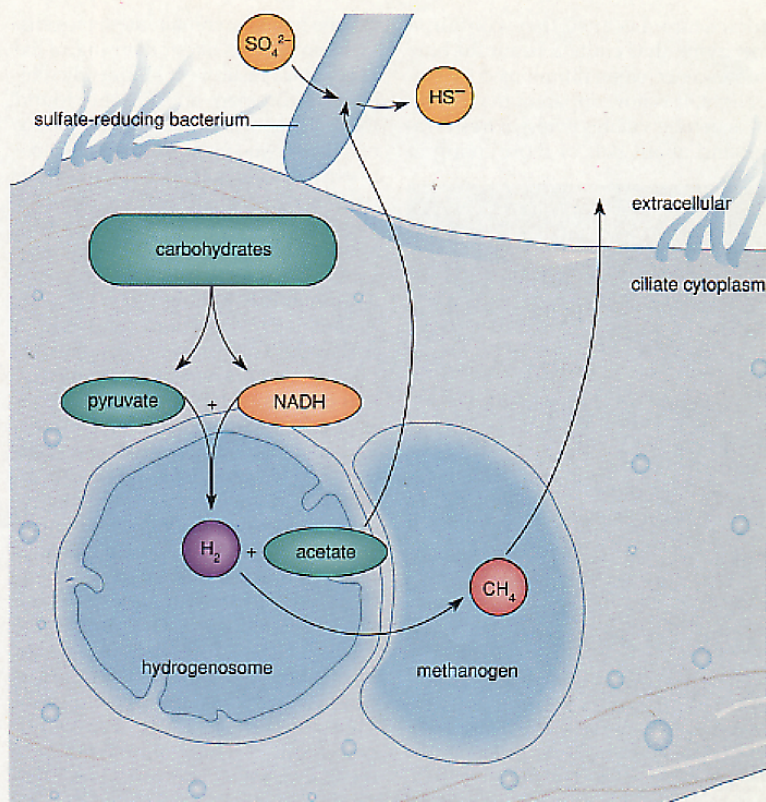


Figure 7. A chain of molecular events characterizes the symbiotic interactions between the *Metopus* ciliate and its attendant bacteria. The symbiotic community's energy metabolism begins with the ingestion of bacteria, which are hydrolyzed into smaller molecules, such as carbohydrates. These small molecules are converted by fermentation into pyruvate and the reduced form of nicotinamide-adenine dinucleotide (NADH). Hydrogenosomes oxidize pyruvate and the reduction equivalent of NADH (malate) to produce molecular hydrogen (H_2) and acetate. Methanogens oxidize the hydrogen with carbon dioxide (CO_2) and produce methane (CH_4) and water, which are released from the cell. Sulfate-reducing bacteria on the cell's surface oxidize metabolites such as acetate that diffuse out of the ciliate. Ultimately the symbiotic bacteria act to remove hydrogen from the ciliate, which increases the metabolic efficiency of the host cell.



Figure 8. Hydrogenosomes (large, dense structures) and methanogens (large, light structures) are found relatively close to each other within the cytoplasm of a *Metopus* cell. The proximity of the structures facilitates the transfer of hydrogen from the hydrogenosome to the methanogen. Each of these structures measures about one micrometer in diameter.

microscope because they contain a coenzyme that emits a beautiful blue fluorescence when illuminated by violet light. It is now apparent that nearly all anaerobic ciliates living in fresh water, and about half of those living in

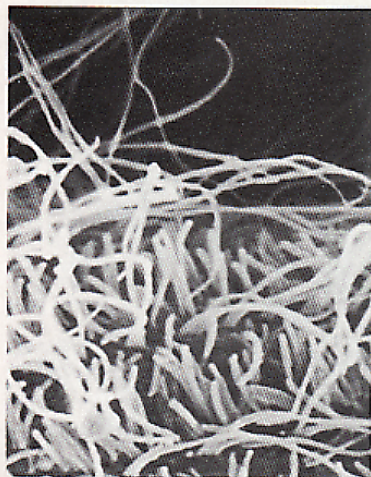


Figure 9. Putative sulfate-reducing bacteria (short bristles) are densely packed among the much longer cilia on the surface of the anaerobic ciliate *Parablepharisma pellitum*. The bacteria are about 2 to 3 micrometers long.

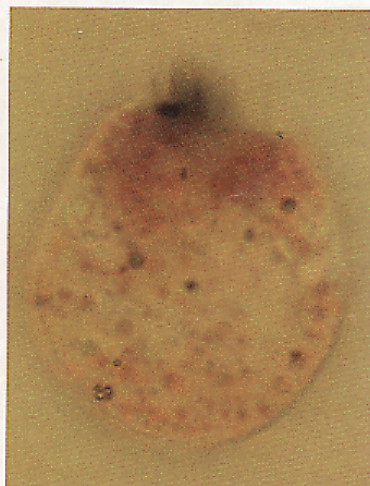


Figure 10. The ciliate *Strombidium purpureum* harbors 200 to 700 endosymbiotic purple bacteria that offer an analogy to the origin of mitochondria in eukaryotic cells from photosynthetic bacteria. The host ciliate is an anaerobic eukaryote that can tolerate low levels of oxygen in the dark because the photosynthetic endosymbionts remove the molecule through respiration. The symbiotic relationship shows how an anaerobic host can be gradually converted to an aerobic life-style.

marine habitats, contain methanogens.

Recently Niels Birger Ramsing, of the Max Planck Institute of Marine Microbiology in Bremen, and Fenchel found that the organisms living on the surface of at least two species of anaerobic marine ciliates are sulfate-reducing bacteria. The observation might explain the absence of such ectosymbionts where sulfate is nearly absent.

A Community of One

A symbiotic consortium consisting of a ciliate and one or two types of bacteria represents an anaerobic community that can completely mineralize ingested food. A *Metopus* cell, for example, harbors about 7,000 methanogenic endosymbionts and about 4,000 sulfate-reducing ectosymbionts. A brief overview of the process reveals the economic elegance of the community. The ciliate's food (mainly bacteria) is first hydrolyzed into small molecules. These are fermented into pyruvate and the reduced form of nicotinamide-adenine dinucleotide (NADH). These molecules are then oxidized in hydrogenosomes to produce acetate and hydrogen. Methanogenic endosymbionts then oxidize the hydrogen with CO_2 to produce methane and water. The ectosymbiotic sulfate-reducers oxidize the acetate (and any other metabolites such as lactate or ethanol) that diffuse out of the ciliate.

The symbiotic bacteria obviously gain from the association by receiving substrates from the ciliate host. But is there any advantage for the host? It is possible to test this possibility with some relatively simple experiments. Although ciliates always harbor methanogens in nature, they can be freed of the endosymbiotic bacteria by the addition of a compound (bromoethanesulfonic acid) that specifically inhibits the growth of methanogenic bacteria. In this way the ciliates can be maintained free of methanogens in laboratory cultures. Indeed, we have not been able to re-infect the ciliates with methanogens.

In two relatively large species of ciliates, the growth rate and metabolic efficiency of the eukaryotes decreased by about 25 percent when the methanogens were inactivated. (One smaller species appeared to be almost unaffected.) We considered the possibility that the methanogens excrete substances that are used by the ciliate host, but simple calculations show that this can-

not account for the large advantage of hosting the symbionts. A clue to the methanogens' significance came when we found that the symbiont-free ciliate host does not produce enough hydrogen to account for the production of methane in the normal symbiotic union. The observation suggests that the methanogens maintain low levels of hydrogen within the ciliate, making hydrogen production thermodynamically more favorable for the host.

At first it seemed unlikely that a ciliate could maintain high levels of hydrogen given the short diffusion path to the cell's surface and the very low levels of hydrogen outside of the cell. A simple theoretical model (assuming a spherical ciliate and a realistic rate of hydrogen production) suggests that hydrogen concentration would increase by 1,000-fold at the cell's surface in the absence of the methanogens. This would make hydrogen production considerably less favorable, especially for the re-oxidation of NADH. This effect may explain the adaptive significance of harboring methanogenic bacteria.

T. Martin Embley of the British Natural History Museum in London and Finlay have compared the ribosomal RNA sequences of some of the methanogen symbionts with the sequences of free-living methanogens maintained in laboratory cultures. The sequences of the symbionts are host-specific; they resemble (but are not identical to) sequences from free-living species. Distantly related anaerobic ciliates can have symbionts that are closely related to each other. Conversely, some ciliates that are closely related to each other (different species within the same genus) have very different types of methanogens as their symbionts. These latter symbiotic interactions must have been established after the speciation of the hosts.

The associations may have started when ingested methanogens avoided digestion and survived by using the host's metabolites in their own digestion. Perhaps such a primitive stage in the evolution of symbiosis may still be discovered. In the cases we have studied the methanogens are no longer symbionts, but organelles. They are found only in their specific hosts, they are transmitted by cytoplasmic inheritance and, most importantly, the association between the host and the symbionts has become a unit of natural

selection. It has little meaning to ask about the adaptive significance for one or the other component. Their mutual interaction is no longer symbiosis but rather the physiology of a single, complex cell.

In some ways the symbiotic relationship between methanogens and anaerobic ciliates presents an analogy to the endosymbiont theory for the origin of mitochondria, which holds that mitochondria originated as endosymbiotic bacteria about one to two billion years ago. An anaerobic ciliate such as *Metopus* is really a composite organism representing all three kingdoms of organisms. Eukaryotes are represented by the ciliate host, the prokaryotic eubacteria are represented by the hydrogenosomes (related to mitochondria) and the prokaryotic archaeobacteria are represented by the methanogens. The sulfate-reducing ectosymbionts are also members of this complex. Remarkably, a *Metopus* cell and its symbiotic bacteria fulfill nearly all the roles of an entire anoxic microbial community.

Recently, Catherine Bernard and Fenchel discovered a new type of endosymbiosis in anaerobic protozoa. It consists of a phototrophic non-sulfur bacterium living inside the ciliate *Strombidium purpureum*. In many respects this symbiotic relationship is a closer analogy and a more useful model of the origin of mitochondria. The bacterium belongs to the alpha group of purple bacteria, which includes the ancestors of mitochondria. The particular type of purple bacteria to which the endosymbionts belong uses molecular hydrogen or low-molecular-weight organic compounds as electron donors in photosynthesis. In the absence of light, however, these bacteria become microaerobes capable of oxygen respiration.

The ciliates, on the other hand, avoid even minute traces of oxygen when they are in the light. The ciliates also tend to gravitate toward light, especially to wavelengths corresponding to the absorption spectrum of the photosynthetic pigments of the symbionts. In the dark, the ciliates prefer conditions that correspond to the oxygen requirements of the symbionts. Access to some oxygen improves their survival in the dark. In this instance the symbiont not only uses the metabolites of its anaerobic host, but it also transforms the ciliate into an oxygen-consuming organism in the dark.

Aerobes vs. Anaerobes

It remains to be explained why so few eukaryotes inhabit anaerobic environments and why those that do are so small. Consider for a moment the classic description of a "who-eats-who" food chain in an aerobic community. Pelagic algae are eaten by copepods, which are eaten by small fish, which are eaten by larger fish and so on. Ultimately the great white shark at the top of the chain has enough to eat because aerobic organisms are extremely efficient at converting food into energy.

Aerobic organisms are efficient because oxygen respiration is efficient. Although growth efficiencies vary somewhat, a realistic figure is 40 percent. So, if a copepod eats 100 units of algae, 40 units of copepods are produced. The 40 copepods translate into about 16 units of small fishes, which translate into 6.4 units of large fishes, which support the production of 2.5 units of sharks.

This scenario cannot take place in an anaerobic habitat. The low energy yield of anaerobic processes translates into a low growth efficiency, about 10 percent on average. This means that 100 units of organic matter will produce 10 units of fermenting bacteria, which support only one unit of anaerobic ciliates. Such a low yield is insufficient for any hypothetical anaerobic predator of the ciliates. In anaerobic communities the biomass production is simply too low to sustain more than a two-level food chain—there are no niches for larger animals.

In this light, it seems reasonable to propose that eukaryotes evolved into a diverse group of large and complex organisms mainly to swallow other organisms or to avoid being swallowed themselves. The evolution of such predatory interactions and complex food chains is dependent on a high level of bioenergetic efficiency. In earth's history this followed the evolution of oxygenic photosynthesis and aerobic respiration. Had this not taken place, the diversity of life would still resemble the foul-smelling sites where we collect our samples.

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