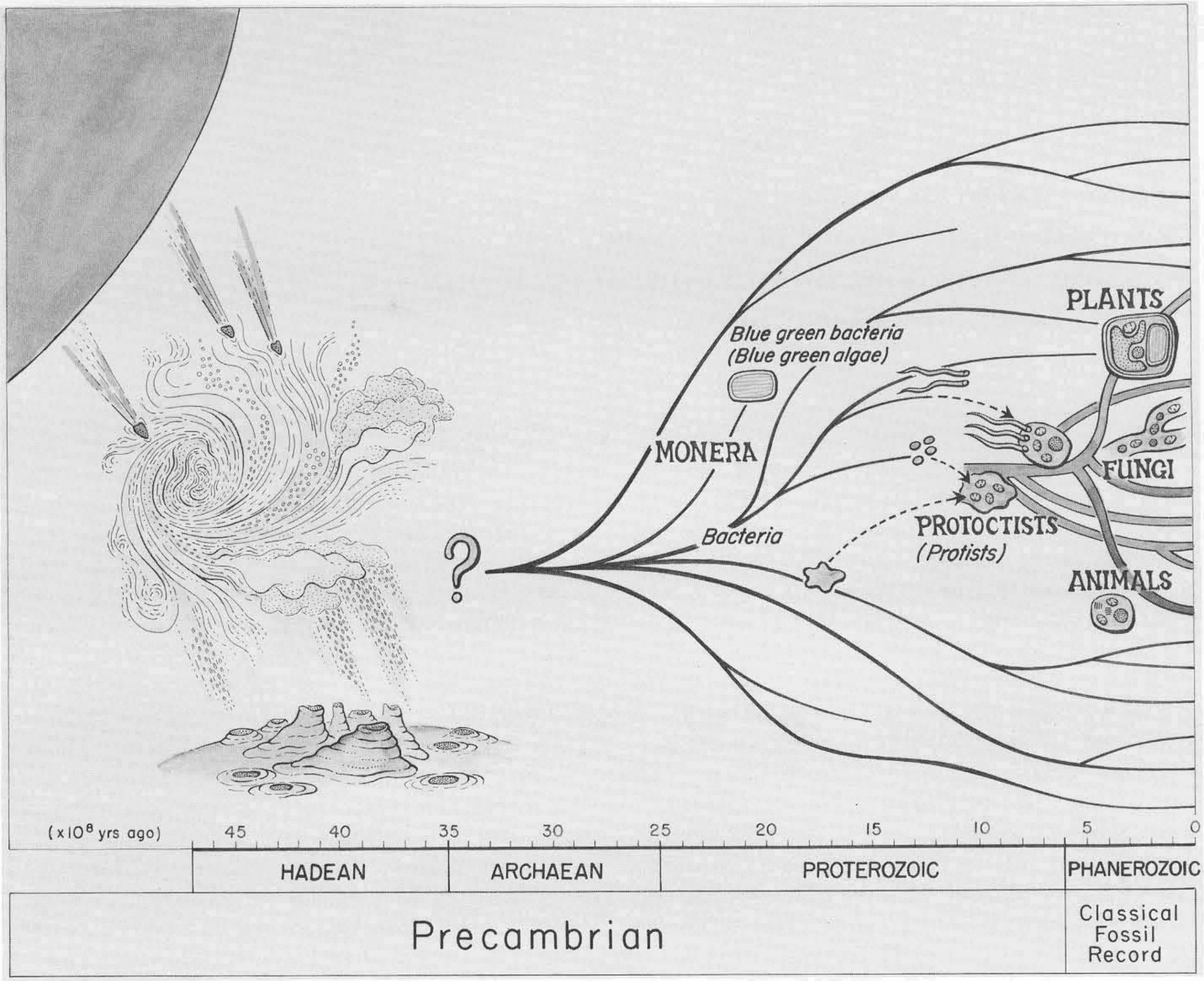


A most unusual geological time chart puts into proper proportion the time scale on which life probably evolved. Reading from left to right — the earth was formed something like 4500 million years ago; by 3500 million years ago a primitive form of life had appeared, probably a microorganism that then developed, combined, and became more complex through all that vast stretch of time called Precambrian. It was not until a brief 600 million years ago that plants, animals, and fungi finally evolved and began to leave behind them the traces that we call the classical fossil record. Man's time on earth is too short to be shown on a chart of this scale.

Life on the Early Earth

by LYNN MARGULIS

SCIENTISTS who look for evidence of life in the past have been mainly preoccupied with the last 600 million years, the period known as the classical fossil record. But what was happening for the 3000 million years before that? Many different theories have been offered, and there are now discoveries from several fields that give us an idea of what was happening.

Life did not originate with animals 600 million years ago. The earliest fossils are remains of microorganisms greater than 3000 million years old. They went through the same sort of evolution that the human line and the fish line and all the other lines of more conspicuous animals and plants did. Their effects are very large and their numbers prodigious, even though their bodies are very small. The period of time before the appearance of animals, the Precambrian, was a time of an enormous amount of evolution, mainly biochemical evolution.

The period of time before the appearance of animals was one of an enormous amount of evolution — mainly biochemical evolution

All the major evolution was on the micro-level — the level of tiny cells which had profound effects on the environment. These statements can be strongly supported by recent evidence.

All organisms that are around today are very good at what they are doing, or they wouldn't be here. These enormously successful kinds of adaptations to environment are what is behind evolution, even at the microbial level. Evolution works, very briefly, like this: A pair of fish will have 10,000 to 50,000 eggs in a season. If left unchecked, each of those eggs would grow up, pair off, and lay more eggs; and those eggs would develop into fish laying more eggs, and so on until very soon the mass of the earth would be all fish. That doesn't happen because, out of those 50,000 eggs, only one or two survive to become parents of the next generation. And, like it or not, that is the rule of evolution — that only a tiny fraction of potential numbers of organisms actually survive into adulthood. This is the rule for microbes just as it is for animals. Microbes, too, evolve; the ones best adapted to their environment leave the most offspring. That has been the rule since life originated on the planet something like 3500 million years ago, and it will be the same from here on.

In many places around the world there are large outcrops, surface rocks that have been dated from very ancient times. Much of southern Africa, for example, is very ancient. Large areas of West Africa, South America, and Canada consist of rocks that date from before the emergence of animals. These rocks have been an enigma for a long time, because they seem to have been subject to normal temperatures and atmospheric conditions. Many seem to be remains of sediments deposited at ocean and lake margins; yet they do not have any animal and plant fossils in them. Why?

The breakthrough to an answer came when Elso Barghoorn of Harvard hypothesized that macroscopic life was preceded by microscopic forms that probably left a detectable record. A diamond saw was used to cut certain rock samples so thin that light passed through them. The rock slivers were looked at through a microscope. Their secret was revealed. Many kinds of well-preserved microorganisms were seen. Since then we have figured out what kinds of microorganisms they were because they are similar to organisms now living in environments like those in which the rocks were made.

Compare live microorganisms with some 1000 million years old, and you will find in some cases that they are really very similar, not only in appearance, but in the kinds of communities they form. Most scientists find it difficult to preserve and stain microorganisms as beautifully as these rocks have done. Of course, the abundant microbes cannot be seen unless the right tools and techniques are used. On the other hand, without using microscopic techniques one could probably tell from as far away as Mars that the earth has been inhabited for several thousand million years because these microorganisms have had profound effects on the earth's surface and atmosphere.

Take, as one example, the production of oxygen. The oxygen we breathe is produced by photosynthetic microorganisms very similar to those found in rocks as old as 2000 million years. Probably before 3500 million years ago there was no free oxygen because the microbes that give off oxygen had not yet evolved. But 1000 million years later the world was teeming with life — not dinosaurs or forests, but life nevertheless. The earth's atmosphere was composed of about 20 percent oxygen by then, a gas that on chemical grounds alone would not be a prevalent component of a planet's atmosphere. Evidence for this oxygen-producing life has not only been found by microfossil studies but deduced from the widespread appearance of ancient layered rocks called stromatolites. Stromatolites are actually made by communities of microorganisms, many of which excrete oxygen gas — the same oxygen we breathe in. In fact, without the organisms that are responsible for the appearance of stromatolites we wouldn't have the oxygen we breathe, nor would we

Communities of microorganisms were producing layered rocks like these stromatolites more than *3000 million years ago. Once* animal life was made possible by the oxygen they emitted, however, stromatolite-production was reduced. From the beginning, animals have liked to eat the blue-green algae microorganisms that made the stromatolites.

— genus *Homo sapiens* — ever have evolved.

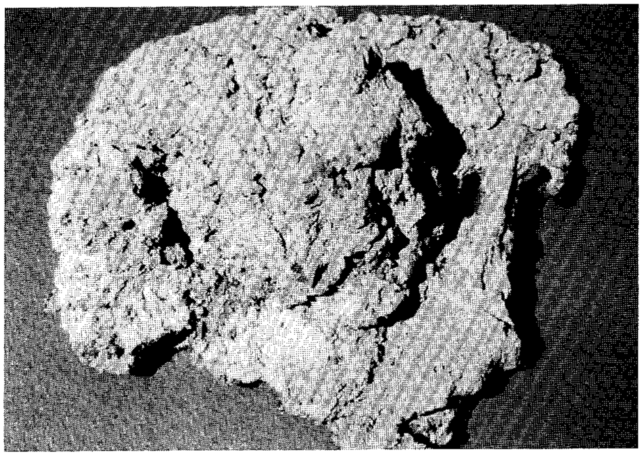
Stromatolites were extremely abundant before there were animals. They are also found in the fossil record after the appearance of animals. But many marine animals eat the organisms that make the stromatolites, and so their frequency and diversity went down — and now stromatolite-formation is restricted to only a few regions of the world.

Often what looks like a clump of mud will be a potential stromatolite. Cohesive clumps of mud often really are "microbial mats." The underside of such muddy stuff may be blackish green. Under a microscope this turns out to be intertwined filaments of microorganisms. The kind of microorganisms that trap and bind the mud, sand, and rock particles have been living in this way at the seashore, in sunny warm zones over the world, for the last 3000 million years. These ancient oxygen-producing photosynthetic organisms are called blue-green algae. You are familiar with them too, because they often appear in sinks, toilets, and on shower curtains as a greenish scum. Some don't mind soap — unlike most other organisms. And they love running water and sunlight. Soon after the Lascaux caves were lit and opened to the public, the blue-green algae started to grow on the paintings; eventually the caves had to be closed. Blue-green algae are abundant and prevalent wherever they are not out-competed; they have been on this earth for several thousand million years.

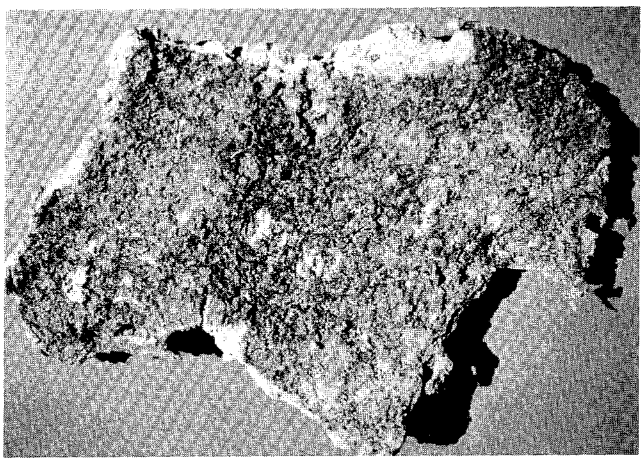
In tropical western Australia today there are stromatolites in the process of forming. Like their ancient ancestors, these layered rocks with a living coat of blue-green algae on top give off oxygen, and trap and bind the sediment in the process of rock formation. The detailed structure of these rocks is practically identical to that of rocks in northwest Canada dated older than 2300 million years. The big difference is that, no matter how hard you look at the ancient Canadian stromatolites, there is no evidence of animal or plant fossils in them. On the other hand, if you look at the modern stromatolites, you find an occasional crayfish-type or clam-type animal in them. This is one way we deduce that animals were simply not on the earth 2300 million years ago. The question is — Why not?

Until very recently, the biological world was divided into animals and plants. This classification is the same in almost all cultures. The Aztec civilization used it, as recorded in the Florentine codex. But it turns out that with the use of modern techniques the traditional plant-animal dichotomy is much less profound than it seems. In all cellular details animals and plants are very much alike when compared to dissimilar organisms like the blue-green algae. Most biologists now recognize a very different dichotomy: They first classify all organisms into simple (prokaryotic) or complex (eukaryotic) on the basis of their cells. The first group includes the blue-green algae and the bacteria, while the animals and plants go together with other forms like fungi into the second group.

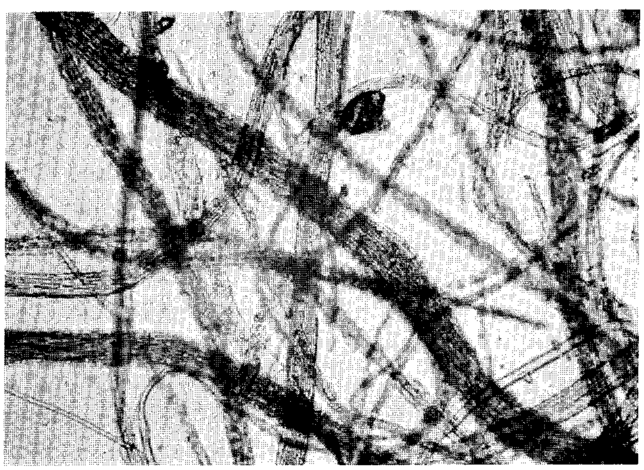
All organisms on earth are made of cells, but of these



From the top it looks like just another clump of mud, but the mud, sand, and rock particles of which it is composed are trapped and bound together by a living microorganism.



The binding agent — blue-green algae — shows up on the underside of the stromatolite.



Magnified several hundred times, the thread-like structure of this "microbial mat" becomes evident.

two fundamentally distinct kinds. Like all animal cells, all plant cells have a nucleus with a membrane. All green plant and animal cells have little bodies in them called mitochondria, which allow the cell to respire — to take oxygen from the air and "burn" food. Animal and plant cells often have flagella or cilia, like small whips, that have a very characteristic substructure. (When you smoke, if you do, you may rip your throat cilia off. But it doesn't matter too much; as long as they have their basal granules, they'll grow in again.) The whipping part of the cilium is exactly like the tail of a sperm. This cell structure, the flagellum — or its shorter analog, the cilium — is completely absent in all blue-green algae and bacteria. It is one of the characteristics that distinguishes simple bacterial cells from the complicated cells of plants and animals.

Furthermore, plant cells contain chloroplasts, or green bodies, that allow them to use sunlight directly. Plants make their food by using their sunlight-to-chemical-conversion factories, the sites of photosynthetic activity.

The punch line is that those microorganisms that dominated the earth for its first 2300-3000 million years were the bacteria and their relatives the blue-green algae-organisms of simple cell construction. They did not have any mitochondria, they did not have any cilia, they did not have nuclei, and they lacked the photosynthetic ability packaged into chloroplasts. Before there could have been animals and plants, the complex sort of cell from which they are made had to evolve.

I don't think anyone would disagree with anything I've said yet. They would only disagree with this: It is my opinion, and I think many people now agree with at least part of this, that the complex cells that make up our bodies (and those of all other animals and the green plants) — become complex through a series of what we call cell symbioses. These cells, which contain nuclei, cilia, and mitochondria packages for oxygen utilization, evolved as a result of populations of simple bacterial-type cells setting up partnerships. Partnerships in some cases did better than individual cells. The partners came from microorganisms of quite distinct ancestry, that had evolved with different sorts of selection pressures on them. Not until simple cells formed stable partnerships could the potential to grow large be realized.

Symbiosis, by definition, is when an organism of one type, one species, lives in a regular association with an organism of quite a different species. For example, certain vitamins in our digestive tract are provided by bacteria that live symbiotically within us, never hurting us. We hurt *them,* in fact, when we take too much penicillin; antibiotic-induced diarrhea sets in when our microbial symbioses get out of balance. Our symbiotic partners must be in there in proper numbers and proportions to maintain normal digestive processes.

Thus, we are involved in symbioses, and it's pretty obvious in this case who is the dominant partner. But many symbioses have been established between much more equal-sized partners, partnerships on the cell level. Study of cell symbioses may be useful in providing models, analogies to what happened to give us the complex animal, plant, and fungal cells that have been so conspicuous all over the world in the last 600 million years. For example, the British Soldier lichen, one of the most common lichens in the northeast, is a symbiosis of fungi and algae. Lichens are never made by either partner alone; all lichens are partnerships. The British Soldier lichen is one of the few symbioses that has been disentangled experimentally; the green partner that uses the sunlight, the photosynthetic partner, has been separated from the protective fungus partner.

It is possible that just this kind of partnership was involved in the origin and evolution of all the cells in your body.

Here is another example of a symbiosis. On the beaches of Brittany and the Channel Islands, people who go swimming are sometimes annoyed at the seaweed that covers the beach, interfering with their enjoyment. If you get close enough to this seaweed, you will see that it's not seaweed at all; it's worms. When these marine worms hatch, they eat certain special photosynthetic microorganisms — flagellates. Their skins are so thin that the sunlight penetrates them, and the flagellates multiply and photosynthesize in the worm tissues and provide their hosts with all of the nutrients they require. The worms may never eat again. They have developed a way of life as a seaweed — but use their muscles to swim down through the sand when the tides are out, protecting themselves and their symbionts from being swept out to sea. So something that looks and lives like seaweed is really the product of symbiosis between worms and photosynthetic flagellates.

There are many examples of symbioses involving a moving partner teamed up with a stationary photosynthesizing partner. For example, some of the snail-like animals called mollusks are products of symbiosis.

The coast of Brittany at low tide is littered with "seaweed" that on closer inspection turns out to be living worms. Whenever the tide is out, the worms come out of the sand so that the algae living in their tissues can be exposed to light and produce the food on which the worms live.

One, *Elysia*, is a green sea snail that lives like a plant. It takes in CO_2 from the air and makes food using sunlight. It comes out in the sun, it goes back among the rocks in the dark. Unlike most photosynthetic animals, such as corals, it lacks algae in its tissues. *Elysia* doesn't really want the algae. It wants the photosynthesizing machinery of the seaweeds. At a young age it sucks out the chloroplasts from its seaweed food — that is, it eats only the parts of the cells responsible for photosynthesis. It eats, but does not digest, these chloroplasts. It moves them to special places in its digestive tract and uses them for the rest of its life for its own photosynthesis. The snail does not feed any longer; as an adult it only basks in the sun. This is a symbiosis between an animal and parts of seaweed cells.

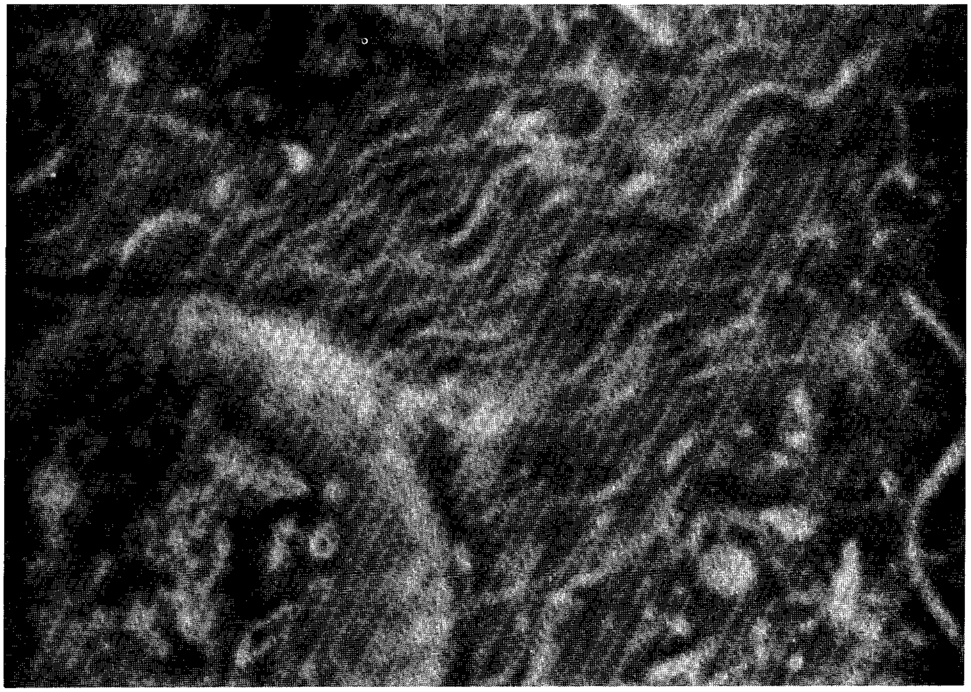
Symbiosis is indeed very common. However, I am aware of only one documented case in the modern biological literature where a microorganism association that began as a lethal disease ended up as a required partnership. This occurred in some amoebae grown by Kwang Jeon of the University of Tennessee. Over a period of time he noticed his laboratory amoebae were becoming ill. He became worried as many died. At first he didn't know what was killing them. Being very persevering, he looked at the amoebae very carefully. First he saw dots, and then discovered they were tiny microorganisms in the bodies of the dying amoebae. These dots were bacteria; his sick amoebae were dying from a bacterial infection. So he carefully isolated these bacteria-filled amoebae and by gentle care and feeding succeeded in saving some. These survivor amoebae had over 100,000 bacteria each.

It happens that Jeon is one of the best microsurgeons in the world; he can transplant a nucleus — that is, the genes — from one amoeba and stick it into another, often without any loss of life. Jeon showed that five years after the infection the few amoebae that survived had completely recovered their health. Their growth rates had become normal again. However, these recovered amoebae were still loaded with bacteria. They could no longer live without their previous so-called disease. By careful nuclear transplantation experiments Jeon showed, in other words, that the nuclei of the host amoebae had become completely dependent on something that was produced by its original bacterial enemies. Disease organisms had become, by all criteria, integral parts of the amoebae cells. Something starting as a bacterial infection went through a period where it was an indifferent symbiont, and by five years later it was necessary to the life of the host amoeba cell. It is evident that new symbioses still are forming; it does not necessarily take millions of years to establish such cell associations.

Another fascinating symbiotic organism is a little protozoan called *Mixotricha paradoxa*. Actually, we are not permitted to work on it directly because it lives only in a certain termite species in Australia, one of the most devastating termites known to man. People in the Outback say it takes two years for these termites that harbor *Mixotricha* to take an old ranch and convert it to dust. (Obviously, the U.S. Department of Agriculture has a vested interest in not letting those termites into southern California.) The termite host is primitive, but the flagellated *Mixotricha* symbionts living in the termite gut are very complex organisms. They have nuclei and complex flagella, as we do, but they do not use these normal flagella to move; they only use their flagella as rudders to change the direction of their movement. The *Mixotricha* move steadily forward in the fluid of the hind gut of the termite by means of the action not of flagella but of other hair-like surface structures that on close inspection turn out to be surface spirochetes. Spirochetes are a type of highly motile bacteria. There are half a million of these spirochete "hairs" per *Mixotricha*, and when they wave together, they move the *Mixotricha*.

I think there's a lesson in this story. The *Mixotricha* has normal flagella, basically indistinguishable from sperm tails and cilia in the human body, but it is moved by its symbionts. The 500,000 surface spirochetes, the special moving-bacteria, live and grow permanently on the surface of *Mixotricha* protozoan — conferring motility on their host.

Why do I tell you about this bizarre organism? Spirochetes, unfortunately, are notorious because they are the causative agent in syphilis. Of course, *these* spirochetes have nothing to do with syphilis — they simply move like syphilis spirochetes and look superficially like them. Though I disagree with most biologists in this, I personally believe that the cilia/flagella system that is so universal in complex organisms has an ancestry that once was free-living. At one point some spirochetes nosed up around cells, liked the materials that were leaking out from the membrane, and used these as food. Some populations of spirochetes then "decided" not to let go of a good thing. So they hooked on (the way they're hooked on to the *Mixotricha*), and they duplicated on the surface of the host organism, just as the spirochetes of *Mixotricha* duplicate on the surface of their hosts. With time, these spirochetes became the familiar, integrated complex flagella of our ancestral cells. Subsequently at various occasions, in several different groups of organisms, some flagella (which



The wavy "hairs" are really spirochetes living symbiotically on the surface of a protozoan (lower left). The movement of the spirochetes propels the host protozoan.

had once been spirochetes) crawled inside their host cells — permanently — and made available to them all kinds of movement inside.

The reconstruction of the possible origin of the cilia/flagella system is a long story. It has to do with the origin of mitosis and eventually meiosis. In short, all the organisms made of complex cells — green plants, animals, and fungi — may have a collection of different microbial ancestors. Our most recent common ancestor was probably a flagellated microbe that possibly had become flagellated by acquiring surface spirochetes. (We are testing the spirochete hypothesis now, here at Caltech.) Some of the flagellated forms adopted photosynthesis and some of them didn't, just as some of the mollusks and some of the worms acquired photosynthesis and most didn't. Those that did found it was a good thing to stay in the same place and photosynthesize. Eventually these evolved into complex plants. Some that didn't acquire photosynthesis went on to evolve into animals. Basically, if you remove the photosynthetic plastids from plant cells, what is left over is very similar to animal cells. Both types have evolved from flagellated ancestors.

So we started with extremely simple bacterial microorganisms. They evolved in many, many ways. But they were always tiny. They formed communities, such as those dominated by bacteria and by the blue-green algae that first "polluted" the atmosphere with oxygen. These tiny microbes can sense each other. They are sensitive to light, to chemicals. In that sense they can smell and see. They have formed many kinds of very complicated partnerships; formation of microbial communities led to great laminated rock structures that geologists can find today. But the individual organisms stayed small. Their activities are represented in the microbial fossil record during the huge Precambrian stretch of time — from 3500 to 600 million years ago. Subsequent to their origin and diversification, after there was the production of quantities of breathable oxygen, a really different kind of cell evolved. The complex cells probably resulted from partnerships among different bacterial types of ancestors. Once complex cells evolved, they provided a unit component from which even more structurally complex organisms could be formed — namely, the fungi, animals, and plants.

When one looks back into the fossil record, it may seem that there was a time when there weren't any organisms, but now we know that though there weren't any animals and plants — large organisms — there were all kinds of microorganisms. Fossil evidence for microbial activities extends back, as far as we know, to some of the oldest sedimentary rocks that have not been subsequently heated or broken up.

The origin of life is an extremely mysterious event; it probably happened early on the surface of the earth — between 4500 and 3500 million years ago. But that early origin was followed by much evolutionary diversification on the microbial level. Microbial evolution, including the formation of partnerships by symbiosis, must have preceded the origins of animals and plants by at least 2000 million years. □