Bacterial Bedfellows

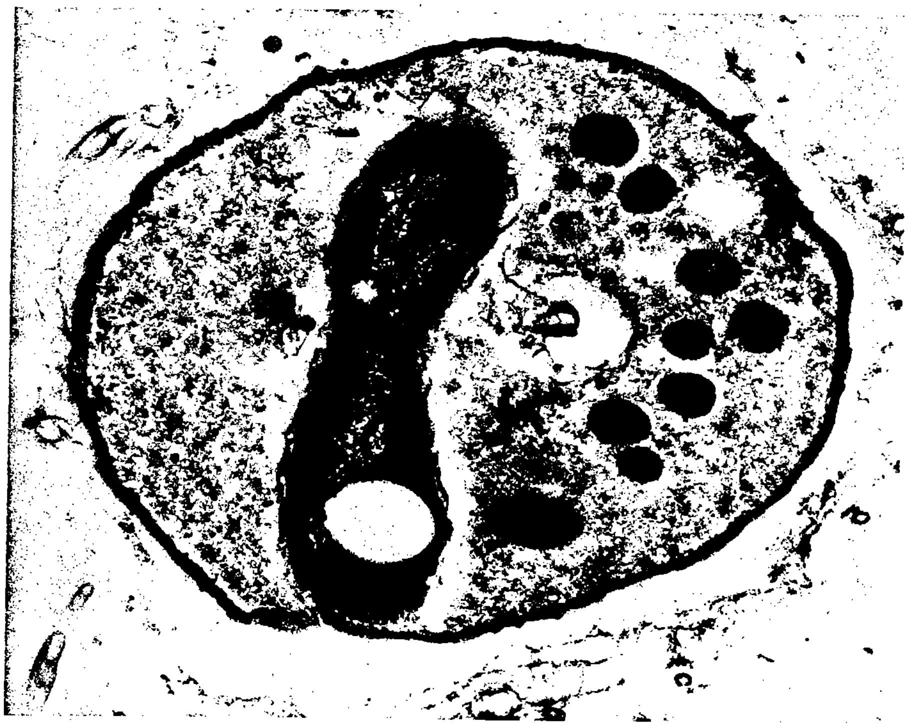
A microscopic ménage à trois *may be responsible for a major step in evolution*

by Dorion Sagan and Lynn Margulis

Named for its flowing, ever-changing form, *Amoeba proteus*, a one-celled microbe with a nucleus, represents in its tiny body a whole modern tale of transmutation. A writhing, jellylike mass, such an organism probably inspired that genre of low-budget horror films exemplified by *The Blob*. In real life, amoebas are usually predictable creatures. So it came as some surprise to Prof. Kwang Jeon of the University of Tennessee when, upon looking through his microscope, he realized his amoeba collection was going through a major epidemic. Approximately 150,000 dots were seen in each amoeba, and each dot was a perfectly normal-looking bacterium. These bacteria had not been there before. Now they were growing inside the amoebas and killing off Jeon's collection. He picked out the least sick amoebas and kept a record of their progress over the next several months. Those that were apparently more resistant to the invaders returned to health and began growing by division at almost their former rates. Jeon examined these survivors and found that each still contained the foreign bacteria but far fewer of them—some 40,000 in each amoeba. Had *A. proteus* somehow transmuted, incorporating the invaders into its own system? Had infector and infected merged?

To the question, "Can the nucleus of the amoeba cell now live without the formerly pathogenic bacteria?" the answer turned out to be "No." When Jeon transplanted the nuclei of infected amoebas to amoebas lacking the bacteria, the hybrid ameobas died in about four days. Yet if at the very last moment he reinfected these hybrids with the once-lethal "parasites," the amoebas revived and grew. Today, the mutual beings are alive and well and living in Knoxville, Tennessee.

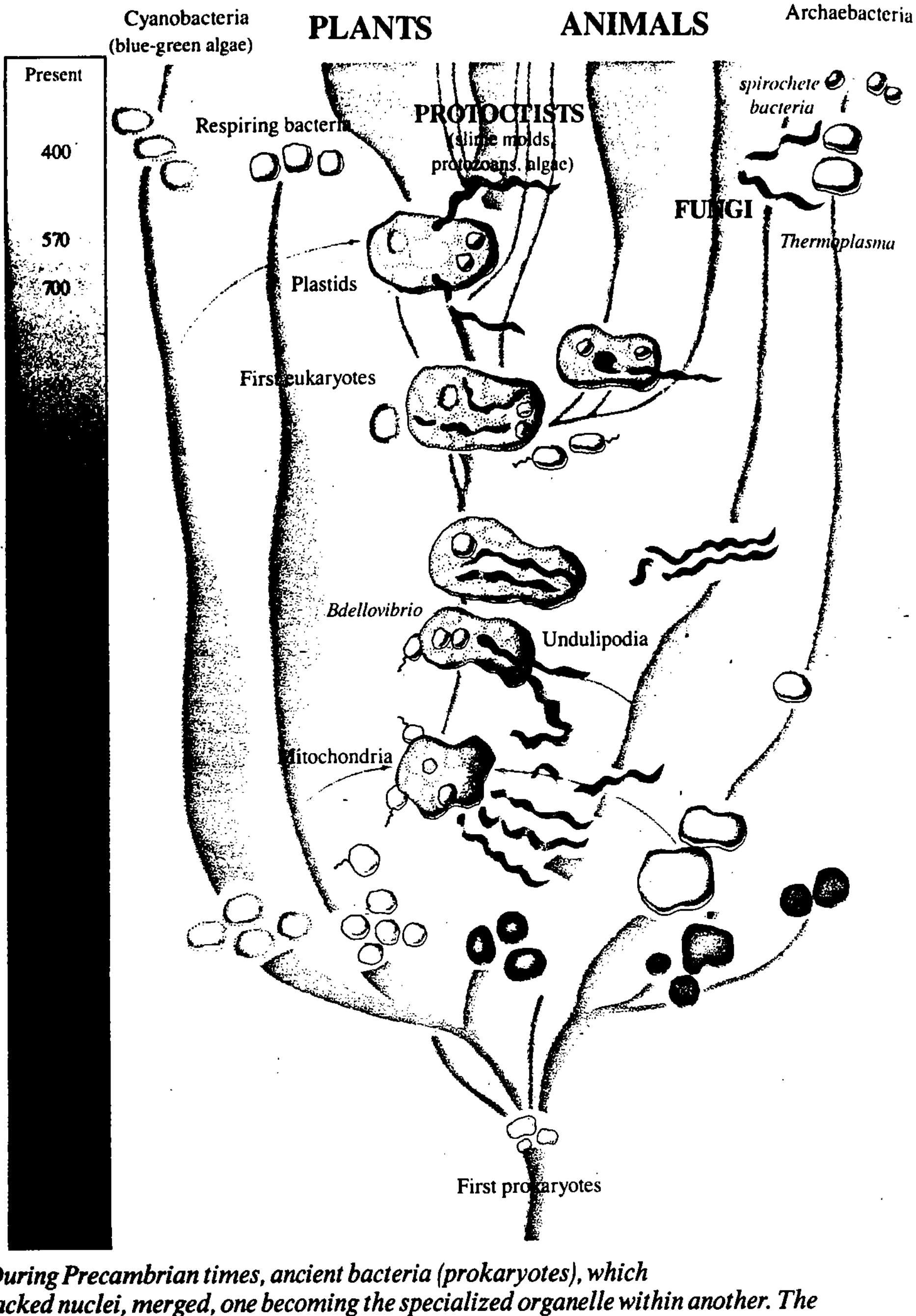
Jeon caught evolution in the act. What is more, the evolution of a new organism occurred by symbiosis, not by an accumulation of mutations. Furthermore, the new amoebas evolved not over millions of years but in eighteen months, which geologically speaking is instantaneous. Natu-



A newly discovered predatory bacterium called Daptobacter, *above, penetrates a* Chromatium *bacterial cell. Once inside, right, the invader begins to divide. Is this the way the threadlike bodies known as mitochondria, essential to the evolution of plants and animals, were incorporated into ancient cells?*

Photomicrographs by Ricardo Guerrero and Isabel Esteve ral selection eliminated not competitors but competition itself. After the smoke had cleared, only symbionts—bacteria and amoebas that could work and live together—survived. (We should not be surprised. The deadliest parasites destroy not only their hosts and habitats but also their own chances for continued survival.)

Jeon's tale of two microbes hints at the answer to a major evolutionary puzzle. Of all the missing links in evolution none is more profound than the gap between eukaryotes, cells with nuclei, and all bacteria, which lack nuclei. The difference between bacteria and any nucleated cells makes the difference between people and apes look negligible. Plant and animal cells have far more in common than do bacteria and nucleated cells. Cells with nuclei contain up to a thousand times more genetic material than their smaller relatives. This material is tightly coiled into chromosomes that are contained in a membrane-bounded nucleus.



During Precambrian times, ancient bacteria (prokaryotes), which lacked nuclei, merged, one becoming the specialized organelle within another. The products of such mergers were eukaryotic cells, which have nuclei and from which arose all living things other than bacteria. Respiring bacteria, such as Bdellovibrio *and* Daptobacter, *penetrated ancestral* Thermoplasma, *becoming the oxygen-breathing mitochondria of the new cells. Undulipodia (cilia, flagella) may have been formed when fast-moving spirochetes invaded the new cells and made them motile. Cyanobacteria were eaten by the new cells and formed plastids, which perform photosynthesis within plant cells.*

Joe Le Monnier

Nucleated cells divide by a complex "dance of the chromosomes," during which the chromosomes pull the hereditary material to opposite ends of the cell and then divide. Bacterial cells simply split apart: they don't form chromosomes. They indulge in a wide range of metabolic variations, consume nitrogen and sulfur, produce methane, precipitate iron and manganese while breathing, and grow in boiling water and brine. Bacteria obtain their food and energy by using every sort of plant fiber and animal waste. If they did not, we would be living in a mounting heap of garbage.

A microscopic look at the waters of the earth of the Proterozoic eon 2,500 million years ago would have revealed flotillas of bobbing purple, blue-green, red, and yellow spheres: colonies of organisms crowding on rocks, gliding on water, or darting about with whipping tails. Shoals of bacterial cells waved with the currents, coating pebbles with brilliant hues. Bacterial spores blown by breezes showered the muddy terrain. Their genetic material, DNA and RNA, was not bound up; their genes were not packed into chromosomes wrapped by a nuclear membrane. They reproduced asexually by growing to twice their size, replicating their single strand of DNA, and then dividing, with one copy of the DNA going to each offspring cell. Or a small cell containing a complete set of genetic material budded on the parent and then broke off. They also encased their DNA in spores that survived long periods of dryness, waiting to come alive when conditions became wetter or more generally favorable. By 1,500 million years ago, the earth's modern surface and atmosphere were largely established and the bacteria flourished. Microbial life permeated the air, soil, and water, recycling gases and other compounds as they do today.

From this low-lying milieu came new forms of life. A new kind of cell formed, larger and more complex than bacteria. This cell had circuitous channels of internal membranes, including one enveloping the nucleus. It had parts called mitochondria: dark bodies providing the cell surrounding them with energy derived from oxygen. Some would soon have plastids, chlorophyll-bearing packets capable of photosynthesis, suspended in their cytoplasm.

What brought about this new cell? As with other evolutionary puzzles, the solution to the mystery of the origin of the nucleated cell lies first in the circumstantial

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evidence. History must be reconstructed from clues. If the ancestors of mitochondria were themselves bacteria without nuclei that raided and reproduced inside their hosts without killing them—in a fashion similar to Kwang Jeon's "dots"—an ancestral line of complex cells could have become established. There would be no record of transitional forms because the new entity would have evolved rapidly, the result of interspecies merger.

Imagine the ancestor of the mitochondria: a bacterial attacker, capable of breathing oxygen or even doing without it when necessary. Such microscopic predators still exist. *Bdellovibrio* (the Greek *bdello* means leech; *vibrio* refers to their vibrating comma shape) burst asunder bacterial prey, eating them from the inside out. *Daptobacter* (the "gnawing bacterium") enters both the inner and the outer membranes of its victim's cell walls. Then it divides, again and again. The mitochondrial ancestor's original prey may have been a larger bacterium like modern-day *Thermoplasma*. The DNA of *Thermoplasma* is unlike that of other bacteria and similar to that of eukaryotes. This rugged bacterium can survive very hot and acidic water such as that found in the hot springs of Yellowstone National Park.

When they were first invaded, occupied hosts like *Thermoplasma* probably couldn't survive, and when they died, they took the invaders with them. Eventually, some of the prey evolved a tolerance for their aerobic predators, which then remained alive and well in the food-rich interior of their hosts. As they reproduced inside the invaded cells without causing harm, the predators gave up their independence and moved in for good. The two organisms thrived on internal leftovers—the products of each other's metabolism. Invaded victims and tamed mitochondria recovered from the attack and have lived ever since, for 1,000 million years, in dynamic alliance. Because of the mitochondria, all earthly beings made of nucleated cells—which includes fungi, plants, animals, humans, and all organisms except bacteria—have remarkably similar metabolisms.

The presence of DNA in mitochondria helped tip off scientists to the possibility that these cells used to be free-living bacteria. When this DNA was examined, it was found to resemble the DNA in certain free-living bacteria far more than it resembled the DNA in the nucleus of the cell from which the mitochondria had come. Mitochondria have their own genes, their own reproductive timetable, and they often divide out of step with the rest of the cell. The bacteria that became mitochondria in our cells can be thought of as raiders that took over their hosts and formed cells with nuclei—cells ancestral to every plant and animal on the planet.

If we know where to go and how to look, we can see that these kinds of mergers are still occurring today.

In a scene from a beautifully colorful silent film called *Intimate Strangers*, Oxford University botanist David C. Smith stands on a beach on the Brittany coast of France. Beneath his feet is what appears to be seaweed. But as Smith begins stepping on the spinachy green matter, it squirms straight down into the sand. Very soon all that's left is a cleared patch of beach.

Where did it go? Actually, "it" is "they." *Convoluta roscoffensis* are flatworms within whose translucent bodies live grass-green algae. Annoyances to bathers, they long baffled biologists. The flatworms and algae have merged into a composite creature. They lie in dense green masses on the shore, and instead of eating, make their own food from sunlight and air. They resemble plants until bothered by pounding surf or a predator, at which point they burrow for cover. The algae not only live inside the tissues of the flatworm and produce food for it but also recycle the worm's waste products, such as uric acid, into additional food. Due to this symbiotic relationship, adult worms do not have to eat and their mouths remain permanently closed.

Symbiosis—the living together in intimate association of different kinds of organisms—is more than an occasional oddity. It is a basic mechanism of evolutionary change. Some plants and animals would long ago have become extinct were it not for the help of their partners: blind shrimps are led around by sighted fish, flowering plants need to be pollinated by specific insects, cows and other ruminants cannot digest grasses without the aid of gut bacteria. Humans also need live bacteria in their intestines. We have trillions of animal cells—and ten times as many bacterial cells.

Although many plant and animal symbionts are known, symbiosis and its fundamental role in evolution really become conspicuous in the microcosm.

Perhaps a hundred million years after mitochondria had become established, a new type of organism joined them in the cytoplasm of certain cells. But the genesis of the union was not through infection but ingestion. Like Jonah swallowed by the whale, the forebears of the photosynthetic parts of nucleated cells were engulfed by larger bacteria but, far from being destroyed, found shelter within, resisted being digested, and kept their valuable light-trapping pigments alive. Today, locked inside every plant, these organelles, or plastids, make food from water and sunlight. Chloroplasts are green plastids and are even larger and more like bacteria than are the mitochondria. Plants turn toward sunlight because without it the plastids within would die.

Plastids provide the biosphere with food and oxygen. From a planetary point of view, the major role of mammals may be as fertilizers of plants and carriers of mitochondria. But if all mammals were to die in one instant, insects, birds, and other organisms would carry mitochondria and fertilize plants. If plants with their plastids were to suddenly disappear, the output of food on the planet would be so severely hampered that all mammals would certainly die. A cell that didn't exist before would soon become indispensable to future generations. The new evolving cell now had mitochondria for oxygen metabolism and plastids to provide food. Both were the products of bacterial mergers. The question that remains is whether the cell's ability to move—even within its own cell wall—is the product of yet another symbiotic merger.

If you look at a living eukaryotic cell under the microscope, you may be startled by the vigorous movement within it. In sharp contrast to a bacterial cell, whose contents are motionless or drift passively about, the interior of eukaryotic cells is swarming like a city. The cytoplasm streams. Many cells rhythmically expand and contract. For example, in a chameleon that is changing color, particles of pigment are carried from the surface to the interior of cells when the animal's skin becomes lighter.

We believe that cellular motion by nucleated cells may be the result of a symbiotic merger between still other kinds of bacteria: rapid, whiplashing bacteria called spirochetes. Close study of the tiny cell whips on many kinds of cells with nuclei shows an amazing uniformity. These filaments have traditionally been called flagella if they are long and few like sperm tails, or cilia if they are short and numerous like hairs. Since there is no basic difference between them, they are all called undulipodia. Nearly all algae and ciliates—the earliest organisms with nucleated cells to have evolved—have them. We are currently exploring the idea that undulipodia come from spirochetes, among the tiniest, fastest, most mobile members of the microcosm.

Shaped like corkscrews or bits of fusillini pasta, spirochetes thrive everywhere, from garden soil to people's gums. Some use oxygen; others are poisoned by it. They tend to attach to things, living or not. They form a major part of the microbial community that lives inside the swollen intestines of termites. There they can be seen attached to and feeding at the surfaces of larger organisms.

When, 2,000 million years ago, an organism with spirochetes propelling it found more food and reproduced more often, natural selection would have favored the alliance. A certain modern amoeba, for example, that draws in its whiptail and gorges itself when food is plentiful, grows a tail when food is scarce in order to swim in search of a meal. The advent of spirochete alliances would have altered the microcosm, leading to the first animal cells—a sort of symbiotic *ménage à trois* formed of *Thermoplasma*, mitochondria, and spirochetes. Plant cells may also be multispecies assemblies, composed of these plus plastids.

Proving the spirochete connection is difficult. As bacteria merge, promiscuous genes ultimately blend, and it becomes very difficult to sort out the original partners. The integrity of individual partners is sacrificed to the formation of a new cell. As Smith puts it, what remains after the living merger, after billions of years of life within a supporting living habitat, is only the smile of Lewis Carrol's Cheshire cat: "the organism progressively loses pieces of itself, slowly blending into the general background, its former existence betrayed by some relic."

New techniques of molecular genetics confirm that parts of organisms dwindle within the life support system of other living cells. Bacteria can donate and receive varying numbers of genes, not only from each other but also from viruses and cells with nuclei. The free transfer of parts and pieces of living things from one area of a cell to another may explain how symbiotic organisms became streamlined into mere semblances of their former selves.

The malleability of microbial life is exploited by genetic engineers who identify proteins they want to produce in large quantity, such as human insulin, and put the genes for them inside bacteria capable of rapid and prodigious reproduction. Not to belittle the human effort, it is worth pointing out that bacteria have been using "genetic engineering" techniques—transferring genes among themselves for their own purposes—for billions of years.

In the traditional view of a cutthroat Darwinian world, merged life forms have always seemed a bit odd, aberrations from the law of the jungle that the poet Tennyson characterized as "red in tooth and claw." Yet it now seems plants and animals never would have evolved at all were it not for attacks and defenses followed by symbiosis and reciprocity. Uneasy alliances are at the core of our very many different beings. Individuality, independence—these are illusions. We live on a flowing pointillist landscape where each dot of paint is also alive. Earth itself is a living habitat, a merger of organisms that have come together, forming new emergent organisms, entirely new kinds of "individuals" such as green hydras and luminous fish. Without a life-support system none of us can survive. It is in this light that we are beginning to see the biosphere not only as a continual struggle favoring the most vicious organisms but also as an endless dance of diversifying life forms, where partners triumph.

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