



Uprooting the Tree of Life

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Charles Darwin contended more than a century ago that all modern species diverged from a more limited set of ancestral groups, which themselves evolved from still fewer progenitors and so on back to the beginning of life. In principle, then, the relationships among all living and extinct organisms could be represented as a single genealogical tree.

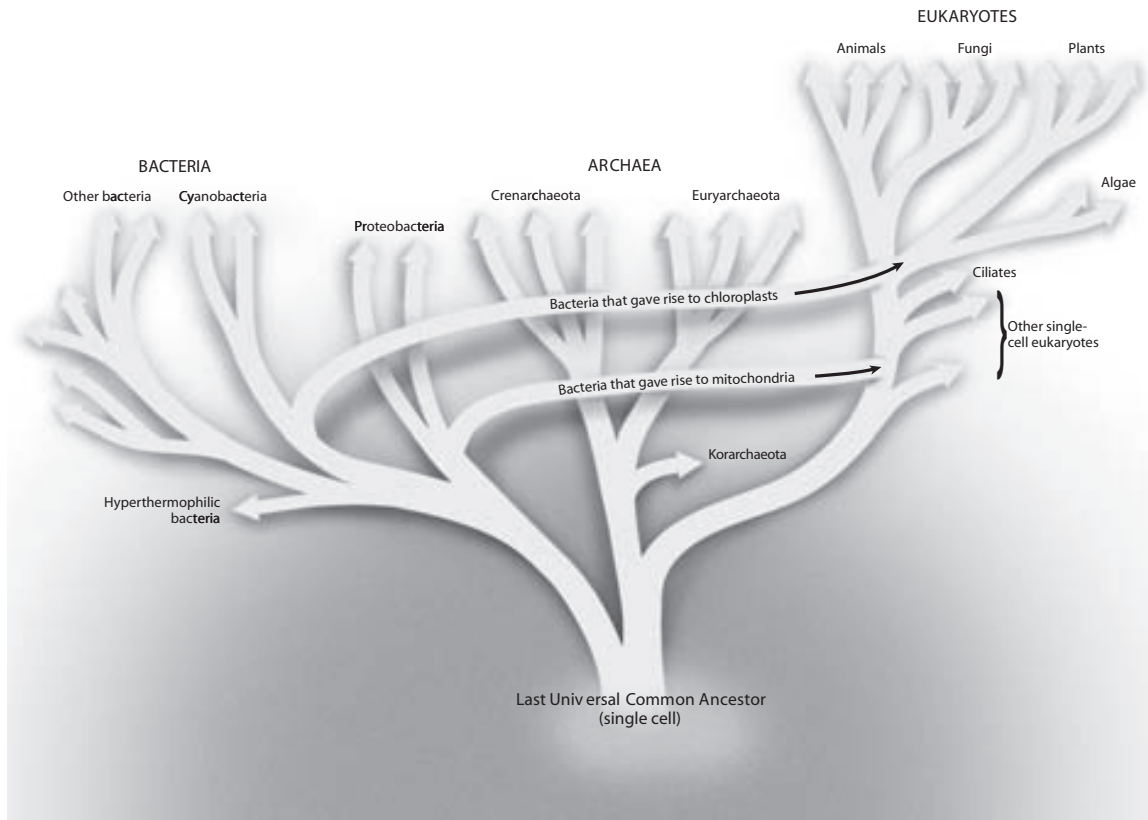
Most contemporary researchers agree. Many would even argue that the general features of this tree are already known, all the way down to the root—a solitary cell, termed life’s last universal common ancestor, that lived roughly 3.5 to 3.8 billion years ago. The consensus view did not come easily but has been widely accepted for more than a decade.

Yet ill winds are blowing. To everyone’s surprise, discoveries made in the past few years have begun to cast serious doubt on some aspects of the tree, especially on the depiction of the relationships near the root.

THE FIRST SKETCHES

Scientists could not even begin to contemplate constructing a universal tree until about 35 years ago. From the time of Aristotle to the 1960s, researchers deduced the relatedness of organisms by comparing their anatomy or physiology, or both. For complex organisms, they were frequently able to draw reasonable genealogical inferences in this way. Detailed analyses of innumerable traits suggested, for instance, that hominids shared a common ancestor with apes, that this common ancestor shared an earlier one with monkeys, and that *that* precursor shared an even earlier forebear with prosimians, and so forth.

Microscopic single-celled organisms, however, often provided too little information for defining relationships. That paucity was disturbing because microbes were the only inhabitants of the earth for the first half to two thirds of the planet’s history; the absence of a clear phylogeny (family tree) for microorganisms left scientists unsure about the sequence in



Consensus view of the universal tree of life holds that the early descendants of life's last universal common ancestor—a small cell with no nucleus—divided into two prokaryotic (nonnucleated) groups: the bacteria and the archaea. Later, the archaea gave rise to organisms having complex cells containing a nucleus: the eukaryotes. Eukaryotes gained valuable energy-generating organelles—mitochondria and, in the case of plants, chloroplasts—by taking up, and retaining, certain bacteria.

which some of the most radical innovations in cellular structure and function occurred. For example, between the birth of the first cell and the appearance of multicellular fungi, plants and animals, cells grew bigger and more complex, gained a nucleus and a cytoskeleton (internal scaffolding), and found a way to eat other cells.

In the mid-1960s Emile Zuckerkandl and Linus Pauling of the California Institute of Technology conceived of a revolutionary strategy that could supply the missing information. Instead of looking just at anatomy or physiology, they asked, why not base family trees on differences in the order of the building blocks in selected genes or proteins?

Their approach, known as molecular phylogeny, is eminently logical. Individual genes, composed of unique sequences of nucleotides, typically serve as the blueprints for making specific proteins, which consist of particular strings of amino acids. All genes, however, mutate (change in

sequence), sometimes altering the encoded protein. Genetic mutations that have no effect on protein function or that improve it will inevitably accumulate over time. Thus, as two species diverge from an ancestor, the sequences of the genes they share will also diverge. And as time passes, the genetic divergence will increase. Investigators can therefore reconstruct the evolutionary past of living species—can construct their phylogenetic trees—by assessing the sequence divergence of genes or proteins isolated from those organisms.

Thirty-five years ago scientists were just becoming proficient at identifying the order of amino acids in proteins and could not yet sequence genes. Protein studies completed in the 1960s and 1970s demonstrated the general utility of molecular phylogeny by confirming and then extending the family trees of well-studied groups such as the vertebrates. They also lent support to some hypotheses about the links among certain bacteria—showing, for instance, that bacteria capable of producing oxygen during photosynthesis form a group of their own (cyanobacteria).

As this protein work was progressing, Carl R. Woese of the University of Illinois was turning his attention to a powerful new yardstick of evolutionary distances: small subunit ribosomal RNA (SSU rRNA). This genetically specified molecule is a key constituent of ribosomes, the “factories” that construct proteins in cells, and cells throughout time have needed it to survive. These features suggested to Woese in the late 1960s that variations in SSU rRNA (or more precisely in the genes encoding it) would reliably indicate the relatedness among any life-forms, from the plainest bacteria to the most complex animals. Small subunit ribosomal RNA could thus serve, in Woese’s words, as a “universal molecular chronometer.”

Initially the methods available for the project were indirect and laborious. By the late 1970s, though, Woese had enough data to draw some important inferences. Since then, phylogeneticists studying microbial evolution, as well as investigators concerned with higher sections of the universal tree, have based many of their branching patterns on sequence analyses of SSU rRNA genes. This accumulation of rRNA data helped greatly to foster consensus about the universal tree in the late 1980s. Today investigators have rRNA sequences for several thousands of species.

From the start, the rRNA results corroborated some already accepted ideas, but they also produced an astonishing surprise. By the 1960s microscopists had determined that the world of living things could be divided into two separate groups, eukaryotes and prokaryotes, depending on the structure of the cells that composed them. Eukaryotic organisms (animals,

plants, fungi and many unicellular life-forms) were defined as those composed of cells that contained a true nucleus—a membrane-bound organelle housing the chromosomes. Eukaryotic cells also displayed other prominent features, among them a cytoskeleton, an intricate system of internal membranes and, usually, mitochondria (organelles that perform respiration, using oxygen to extract energy from nutrients). In the case of algae and higher plants, the cells also contained chloroplasts (photosynthetic organelles).

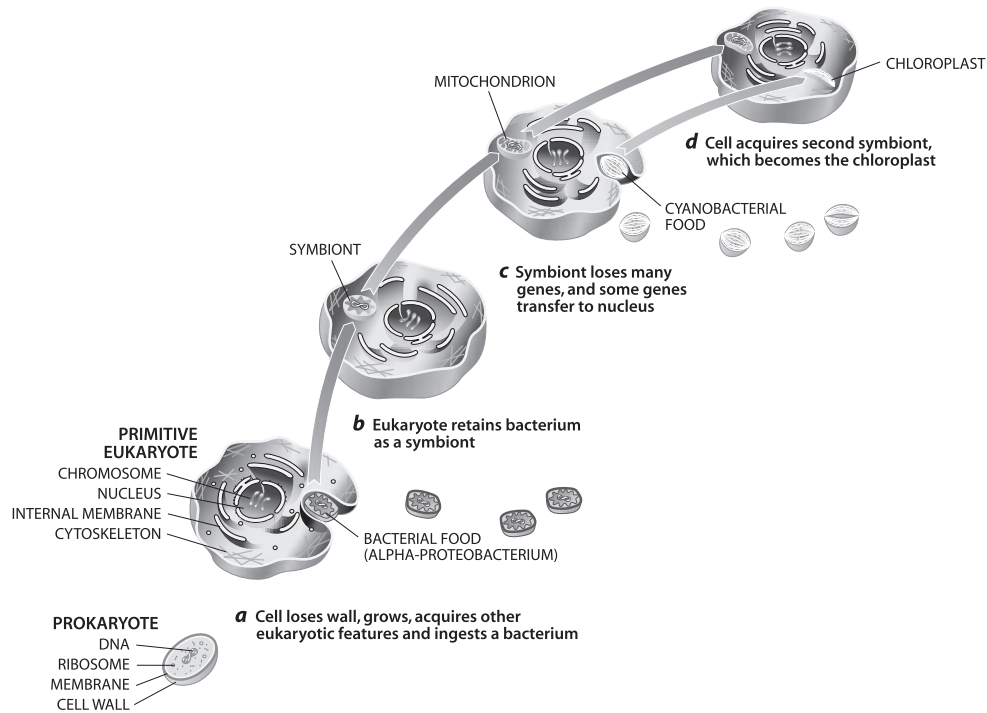
Prokaryotes, thought at the time to be synonymous with bacteria, were noted to consist of smaller and simpler nonnucleated cells. They are usually enclosed by both a membrane and a rigid outer wall.

Woese's early data supported the distinction between prokaryotes and eukaryotes, by establishing that the SSU rRNAs in typical bacteria were more similar in sequence to one another than to the rRNA of eukaryotes. The initial rRNA findings also lent credence to one of the most interesting notions in evolutionary cell biology: the endosymbiont hypothesis. This conception aims to explain how eukaryotic cells first came to possess mitochondria and chloroplasts [see "The Birth of Complex Cells," by Christian de Duve, this volume].

On the way to becoming a eukaryote, the hypothesis proposes, some ancient anaerobic prokaryote (unable to use oxygen for energy) lost its cell wall. The more flexible membrane underneath then began to grow and fold in on itself. This change, in turn, led to formation of a nucleus and other internal membranes and also enabled the cell to engulf and digest neighboring prokaryotes, instead of gaining nourishment entirely by absorbing small molecules from its environment.

At some point, one of the descendants of this primitive eukaryote took up bacterial cells of the type known as alpha-proteobacteria, which are proficient at respiration. But instead of digesting this "food," the eukaryote settled into a mutually beneficial (symbiotic) relationship with it. The eukaryote sheltered the internalized cells, and the "endosymbionts" provided extra energy to the host through respiration. Finally, the endosymbionts lost the genes they formerly used for independent growth and transferred others to the host's nucleus—becoming mitochondria in the process. Likewise, chloroplasts derive from cyanobacteria that an early, mitochondria-bearing eukaryote took up and kept.

Mitochondria and chloroplasts in modern eukaryotes still retain a small number of genes, including those that encode SSU rRNA. Hence, once the right tools became available in the mid-1970s, investigators decided to see if those RNA genes were inherited from alpha-proteobacteria and



Endosymbiont hypothesis proposes that mitochondria formed after a prokaryote that had evolved into an early eukaryote engulfed (a) and then kept (b) one or more alpha-proteobacteria cells. Eventually, the bacterium gave up its ability to live on its own and transferred some of its genes to the nucleus of the host (c), becoming a mitochondrion. Later, some mitochondrion-bearing eukaryote ingested a cyanobacterium that became the chloroplast (d).

cyanobacteria, respectively—as the endosymbiont hypothesis would predict. They were.

One deduction, however, introduced a discordant note into all this harmony. In the late 1970s Woese asserted that the two-domain view of life, dividing the world into bacteria and eukaryotes, was no longer tenable; a three-domain construct had to take its place.

Certain prokaryotes classified as bacteria might look like bacteria but, he insisted, were genetically much different. In fact, their rRNA supported an early separation. Many of these species had already been noted for displaying unusual behavior, such as favoring extreme environments, but no one had disputed their status as bacteria. Now Woese claimed that they formed a third primary group—the archaea—as different from bacteria as bacteria are from eukaryotes.

ACRIMONY, THEN CONSENSUS

At first, the claim met enormous resistance. Yet eventually most scientists became convinced, in part because the overall structures of certain

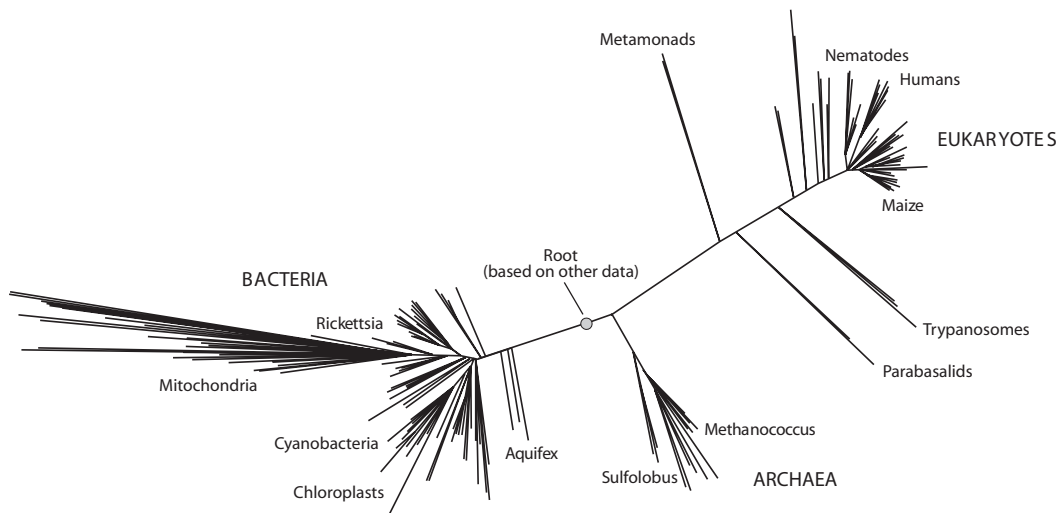
molecules in archaeal species corroborated the three-group arrangement. For instance, the cell membranes of all archaea are made up of unique lipids (fatty substances) that are quite distinct—in their physical properties, chemical constituents and linkages—from the lipids of bacteria.

Similarly, the archaeal proteins responsible for several crucial cellular processes have a distinct structure from the proteins that perform the same tasks in bacteria. Gene transcription and translation are two of those processes. To make a protein, a cell first copies, or transcribes, the corresponding gene into a strand of messenger RNA. Then ribosomes translate the messenger RNA codes into a specific string of amino acids. Biochemists found that archaeal RNA polymerase, the enzyme that carries out gene transcription, more resembles its eukaryotic than its bacterial counterparts in complexity and in the nature of its interactions with DNA. The protein components of the ribosomes that translate archaeal messenger RNAs are also more like the ones in eukaryotes than those in bacteria.

Once scientists accepted the idea of three domains of life instead of two, they naturally wanted to know which of the two structurally primitive groups—bacteria or archaea—gave rise to the first eukaryotic cell. The studies that showed a kinship between the transcription and translation machinery in archaea and eukaryotes implied that eukaryotes diverged from the archaeans.

This deduction gained added credibility in 1989, when groups led by J. Peter Gogarten of the University of Connecticut and Takashi Miyata, then at Kyushu University in Japan, used sequence information from genes for other cellular components to “root” the universal tree. Comparisons of SSU rRNA can indicate which organisms are closely related to one another but, for technical reasons, cannot by themselves indicate which groups are oldest and therefore closest to the root of the tree. The DNA sequences encoding two essential cellular proteins agreed that the last common ancestor spawned both the bacteria and the archaea; then the eukaryotes branched from the archaea.

Since 1989 a host of discoveries have supported that depiction. In the past five years, sequences of the full genome (the total complement of genes) in half a dozen archaea and more than 15 bacteria have become available. Comparisons of such genomes confirm earlier suggestions that many genes involved in transcription and translation are much the same in eukaryotes and archaea and that these processes are performed very similarly in the two domains. Further, although archaea do not have nuclei, under certain experimental conditions their chromosomes resemble those of eukaryotes: the DNA appears to be associated with eukaryote-type



Relationships among ribosomal RNAs (rRNAs) from almost 600 species are depicted. A single line represents the rRNA sequence in one species or a group; many of the lines reflect rRNAs encoded by nuclear genes, but others reflect rRNAs encoded by chloroplast or mitochondrial genes. The mitochondrial lines are relatively long because mitochondrial genes evolve rapidly. Trees derived from rRNA data are rootless; other data put the root at the dot, corresponding to the lowest part of the tree shown on page 88.

proteins called histones, and the chromosomes can adopt a eukaryotic “beads-on-a-string” structure. These chromosomes are replicated by a suite of proteins, most of which are found in some form in eukaryotes but not in bacteria.

NEVERTHELESS, DOUBTS

The accumulation of all these wonderfully consistent data was gratifying and gave rise to the now accepted arrangement of the universal genealogical tree. This phylogeny indicates that life diverged first into bacteria and archaea. Eukaryotes then evolved from an archaealike precursor. Subsequently, eukaryotes took up genes from bacteria twice, obtaining mitochondria from alpha-proteobacteria and chloroplasts from cyanobacteria.

Still, as DNA sequences of complete genomes have become increasingly available, my group and others have noted patterns that are disturbingly at odds with the prevailing beliefs. If the consensus tree were correct, researchers would expect the only bacterial genes in eukaryotes to be those in mitochondrial or chloroplast DNA or to be those that were transferred to the nucleus from the alpha-proteobacterial or cyanobacterial precursors of these organelles. The transferred genes, moreover, would be ones involved in respiration or photosynthesis, not in cellular processes that would already be handled by genes inherited from the ancestral archaean.

Those expectations have been violated. Nuclear genes in eukaryotes often derive from bacteria, not solely from archaea. A good number of those bacterial genes serve nonrespiratory and nonphotosynthetic processes that are arguably as critical to cell survival as are transcription and translation.

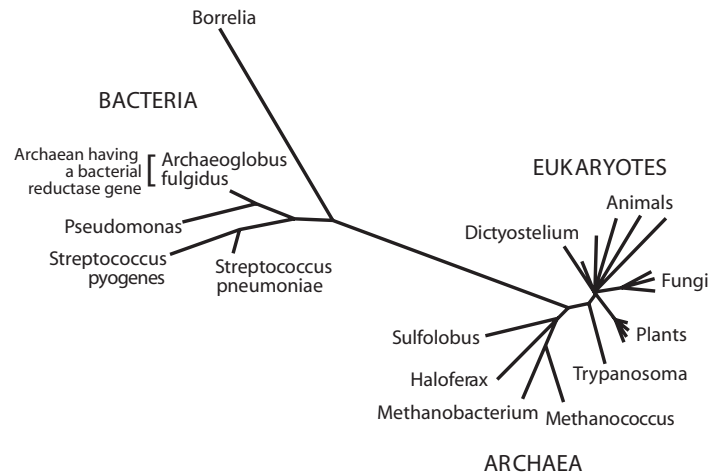
The classic tree also indicates that bacterial genes migrated only to a eukaryote, not to any archaea. Yet we are seeing signs that many archaea possess a substantial store of bacterial genes. One example among many is *Archaeoglobus fulgidus*. This organism meets all the criteria for an archaean (it has all the proper lipids in its cell membrane and the right transcriptional and translational machinery), but it uses a bacterial form of the enzyme HMGCoA reductase for synthesizing membrane lipids. It also has numerous bacterial genes that help it to gain energy and nutrients in one of its favorite habitats: undersea oil wells.

The most reasonable explanation for these various contrarian results is that the pattern of evolution is not as linear and treelike as Darwin imagined it. Although genes are passed vertically from generation to generation, this vertical inheritance is not the only important process that has affected the evolution of cells. Rampant operation of a different process—lateral, or horizontal, gene transfer—has also affected the course of that evolution profoundly. Such transfer involves the delivery of single genes, or whole suites of them, not from a parent cell to its offspring but across species barriers.

Lateral gene transfer would explain how eukaryotes that supposedly evolved from an archaeal cell obtained so many bacterial genes important to metabolism: the eukaryotes picked up the genes from bacteria and kept those that proved useful. It would likewise explain how various archaea came to possess genes usually found in bacteria.

Some molecular phylogenetic theorists—among them, Mitchell L. Sogin of the Marine Biological Laboratory in Woods Hole, Mass., and Russell F. Doolittle (my very distant relative) of the University of California at San Diego—have also invoked lateral gene transfer to explain a long-standing mystery. Many eukaryotic genes turn out to be unlike those of any known archaea or bacteria; they seem to have come from nowhere. Notable in this regard are the genes for the components of two defining eukaryotic features, the cytoskeleton and the system of internal membranes. Sogin and Doolittle suppose that some fourth domain of organisms, now extinct, slipped those surprising genes into the eukaryotic nuclear genome horizontally.

In truth, microbiologists have long known that bacteria exchange genes horizontally. Gene swapping is clearly how some disease-causing bacteria



Mini phylogenetic tree groups species according to differences in a gene coding for the enzyme HMGCoA reductase. It shows that the reductase gene in *Archaeoglobus fulgidus*, a definite archaean, came from a bacterium, not from an archaean ancestor. This finding is part of growing evidence indicating that the evolution of unicellular life has long been influenced profoundly by lateral gene transfer [occurring between contemporaries]. The consensus universal tree does not take that influence into account.

give the gift of antibiotic resistance to other species of infectious bacteria. But few researchers suspected that genes essential to the very survival of cells traded hands frequently or that lateral transfer exerted great influence on the early history of microbial life. Apparently, we were mistaken.

CAN THE TREE SURVIVE?

What do the new findings say about the structure of the universal tree of life? One lesson is that the neat progression from archaea to eukaryote in the consensus tree is oversimplified or wrong. Plausibly, eukaryotes emerged not from an archaean but from some precursor cell that was the product of any number of horizontal gene transfers—events that left it part bacterial and part archaean and maybe part other things.

The weight of evidence still supports the likelihood that mitochondria in eukaryotes derived from alpha-proteobacterial cells and that chloroplasts came from ingested cyanobacteria, but it is no longer safe to assume that those were the only lateral gene transfers that occurred after the first eukaryotes arose. Only in later, multicellular eukaryotes do we know of definite restrictions on horizontal gene exchange, such as the advent of separated (and protected) germ cells.

The standard depiction of the relationships within the prokaryotes seems too pat as well. A host of genes and biochemical features do unite the prokaryotes that biologists now call archaea and distinguish those

organisms from the prokaryotes we call bacteria, but bacteria and archaea (as well as species within each group) have clearly engaged in extensive gene swapping.

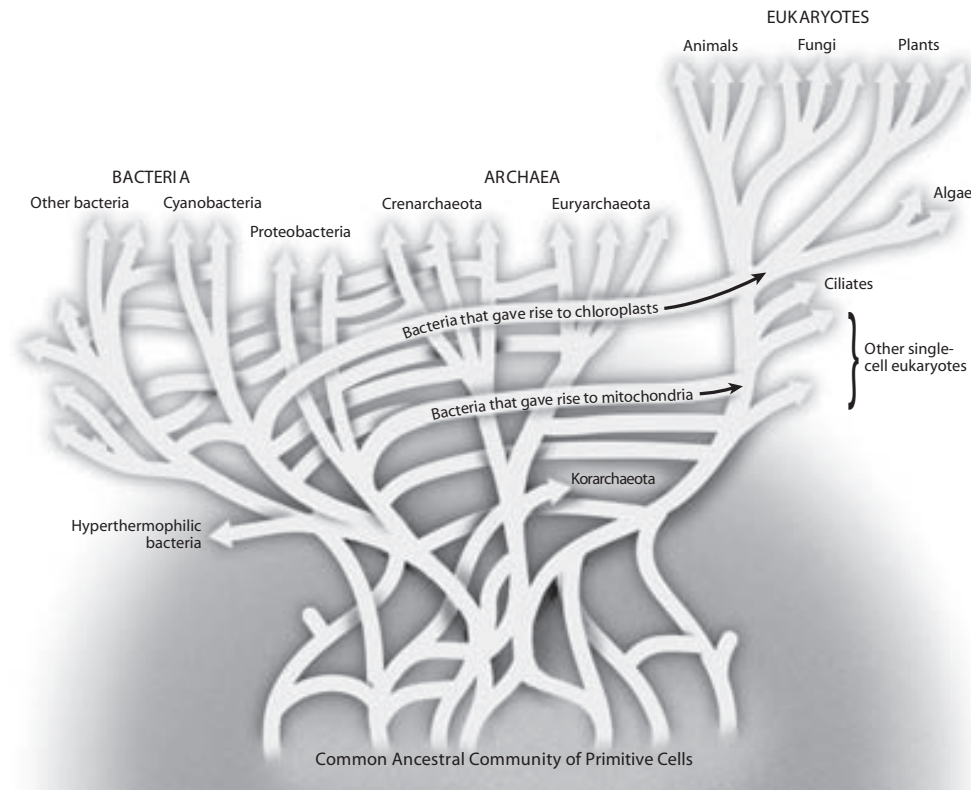
Researchers might choose to define evolutionary relationships within the prokaryotes on the basis of genes that seem least likely to be transferred. Indeed, many investigators still assume that genes for SSU rRNA and the proteins involved in transcription and translation are unlikely to be moveable and that the phylogenetic tree based on them thus remains valid. But this nontransferability is largely an untested assumption, and in any case, we must now admit that any tree is at best a description of the evolutionary history of only part of an organism's genome. The consensus tree is an overly simplified depiction.

What would a truer model look like? At the top, treelike branching would continue to be apt for multicellular animals, plants and fungi. And gene transfers involved in the formation of bacteria-derived mitochondria and chloroplasts in eukaryotes would still appear as fusions of major branches. Below these transfer points (and continuing up into the modern bacterial and archaeal domains), we would, however, see a great many additional branch fusions. Deep in the realm of the prokaryotes and perhaps at the base of the eukaryotic domain, designation of any trunk as the main one would be arbitrary.

Though complicated, even this revised picture would actually be misleadingly simple, a sort of shorthand cartoon, because the fusing of branches usually would not represent the joining of whole genomes, only the transfers of single or multiple genes. The full picture would have to display simultaneously the superimposed genealogical patterns of thousands of different families of genes (the rRNA genes form just one such family).

If there had never been any lateral transfer, all these individual gene trees would have the same topology (the same branching order), and the ancestral genes at the root of each tree would have all been present in the genome of the universal last common ancestor, a single ancient cell. But extensive transfer means that neither is the case: gene trees will differ (although many will have regions of similar topology), *and* there would never have been a single cell that could be called the last universal common ancestor.

As Woese has written, "The ancestor cannot have been a particular organism, a single organismal lineage. It was communal, a loosely knit, diverse conglomeration of primitive cells that evolved as a unit, and it eventually developed to a stage where it broke into several distinct



Revised “tree” of life retains a treelike structure at the top of the eukaryotic domain and acknowledges that eukaryotes obtained mitochondria and chloroplasts from bacteria. But it also includes an extensive network of untrelke links between branches. Those links have been inserted somewhat randomly to symbolize the rampant lateral gene transfer of single or multiple genes that has always occurred between unicellular organisms. This “tree” also lacks a single cell at the root; the three major domains of life probably arose from a population of primitive cells that differed in their genes.

communities, which in their turn become the three primary lines of descent [bacteria, archaea and eukaryotes].” In other words, early cells, each having relatively few genes, differed in many ways. By swapping genes freely, they shared various of their talents with their contemporaries. Eventually this collection of eclectic and changeable cells coalesced into the three basic domains known today. These domains remain recognizable because much (though by no means all) of the gene transfer that occurs these days goes on within domains.

Some biologists find these notions confusing and discouraging. It is as if we have failed at the task that Darwin set for us: delineating the unique structure of the tree of life. But in fact, our science is working just as it should. An attractive hypothesis or model (the single tree) suggested experiments, in this case the collection of gene sequences and their analysis with the methods of molecular phylogeny. The data show the model to be

too simple. Now new hypotheses, having final forms we cannot yet guess, are called for.

FURTHER READING

- Carl Woese. "The Universal Ancestor" in the *Proceedings of the National Academy of Sciences*, Vol. 95, No. 12, pages 6854–6859; June 9, 1998.
- W. Ford Doolittle. "You Are What You Eat: A Gene Transfer Ratchet Could Account for Bacterial Genes in Eukaryotic Nuclear Genomes" in *Trends in Genetics*, Vol. 14, No. 8, pages 307–311; August 1998.
- W. Ford Doolittle. "Phylogenetic Classification and the Universal Tree" in *Science*, Vol. 284, pages 2124–2128; June 25, 1999.



The Birth of Complex Cells

CHRISTIAN DE DUVE

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About 3.7 billion years ago the first living organisms appeared on the earth. They were small, single-celled microbes not very different from some present-day bacteria. Cells of this kind are classified as prokaryotes because they lack a nucleus (*karyon* in Greek), a distinct compartment for their genetic machinery. Prokaryotes turned out to be enormously successful. Thanks to their remarkable ability to evolve and adapt, they spawned a wide variety of species and invaded every habitat the world had to offer.

The living mantle of our planet would still be made exclusively of prokaryotes but for an extraordinary development that gave rise to a very different kind of cell, called a eukaryote because it possesses a true nucleus. (The prefix *eu* is derived from the Greek word meaning “good.”) The consequences of this event were truly epoch-making. Today all multicellular organisms consist of eukaryotic cells, which are vastly more complex than prokaryotes. Without the emergence of eukaryotic cells, the whole variegated pageantry of plant and animal life would not exist, and no human would be around to enjoy that diversity and to penetrate its secrets.

Eukaryotic cells most likely evolved from prokaryotic ancestors. But how? That question has been difficult to address because no intermediates of this momentous transition have survived or left fossils to provide direct clues. One can view only the final eukaryotic product, something strikingly different from any prokaryotic cell. Yet the problem is no longer insoluble. With the tools of modern biology, researchers have uncovered revealing kinships among a number of eukaryotic and prokaryotic features, thus throwing light on the manner in which the former may have been derived from the latter.

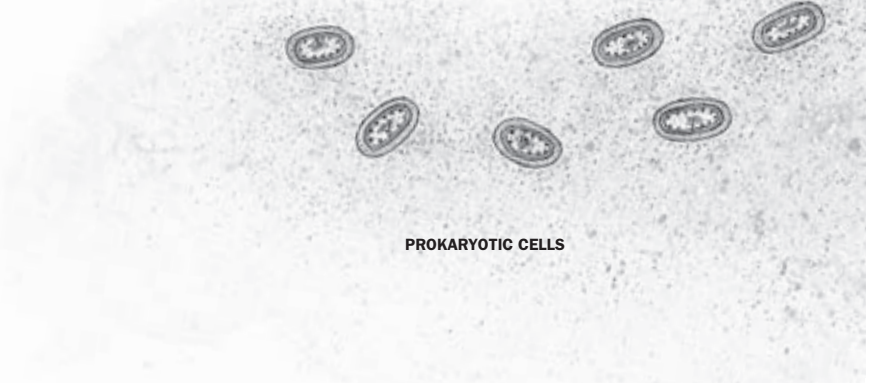
Appreciation of this astonishing evolutionary journey requires a basic understanding of how the two fundamental cell types differ. Eukaryotic cells are much larger than prokaryotes (typically some 10,000 times in volume), and their repository of genetic information is far more organized.

In prokaryotes the entire genetic archive consists of a single chromosome made of a circular string of DNA that is in direct contact with the rest of the cell. In eukaryotes, most DNA is contained in more highly structured chromosomes that are grouped within a well-defined central enclosure, the nucleus. The region surrounding the nucleus (the cytoplasm) is partitioned by membranes into an elaborate network of compartments that fulfill a host of functions. Skeletal elements within the cytoplasm provide eukaryotic cells with internal structural support. With the help of tiny molecular motors, these elements also enable the cells to shuffle their contents and to propel themselves from place to place.

Most eukaryotic cells further distinguish themselves from prokaryotes by having in their cytoplasm up to several thousand specialized structures, or organelles, about the size of a prokaryotic cell. The most important of such organelles are peroxisomes (which serve assorted metabolic functions), mitochondria (the power factories of cells) and, in algae and plant cells, plastids (the sites of photosynthesis). Indeed, with their many organelles and intricate internal structures, even single-celled eukaryotes, such as yeasts or amoebas, prove to be immensely complex organisms.

The organization of prokaryotic cells is much more rudimentary. Yet prokaryotes and eukaryotes are undeniably related. That much is clear from their many genetic similarities. It has even been possible to establish the approximate time when the eukaryotic branch of life's evolutionary tree began to detach from the prokaryotic trunk. This divergence started in the remote past, probably before three billion years ago. Subsequent events in the development of eukaryotes, which may have taken as long as one billion years or more, would still be shrouded in mystery were it

Prokaryotic and eukaryotic cells differ in size and complexity. Prokaryotic cells are normally about one micron across, whereas eukaryotic cells typically range from 10 to 30 microns. The latter, here represented by a hypothetical green alga, house a wide array of specialized structures—including an encapsulated nucleus containing the cell's main genetic stores.

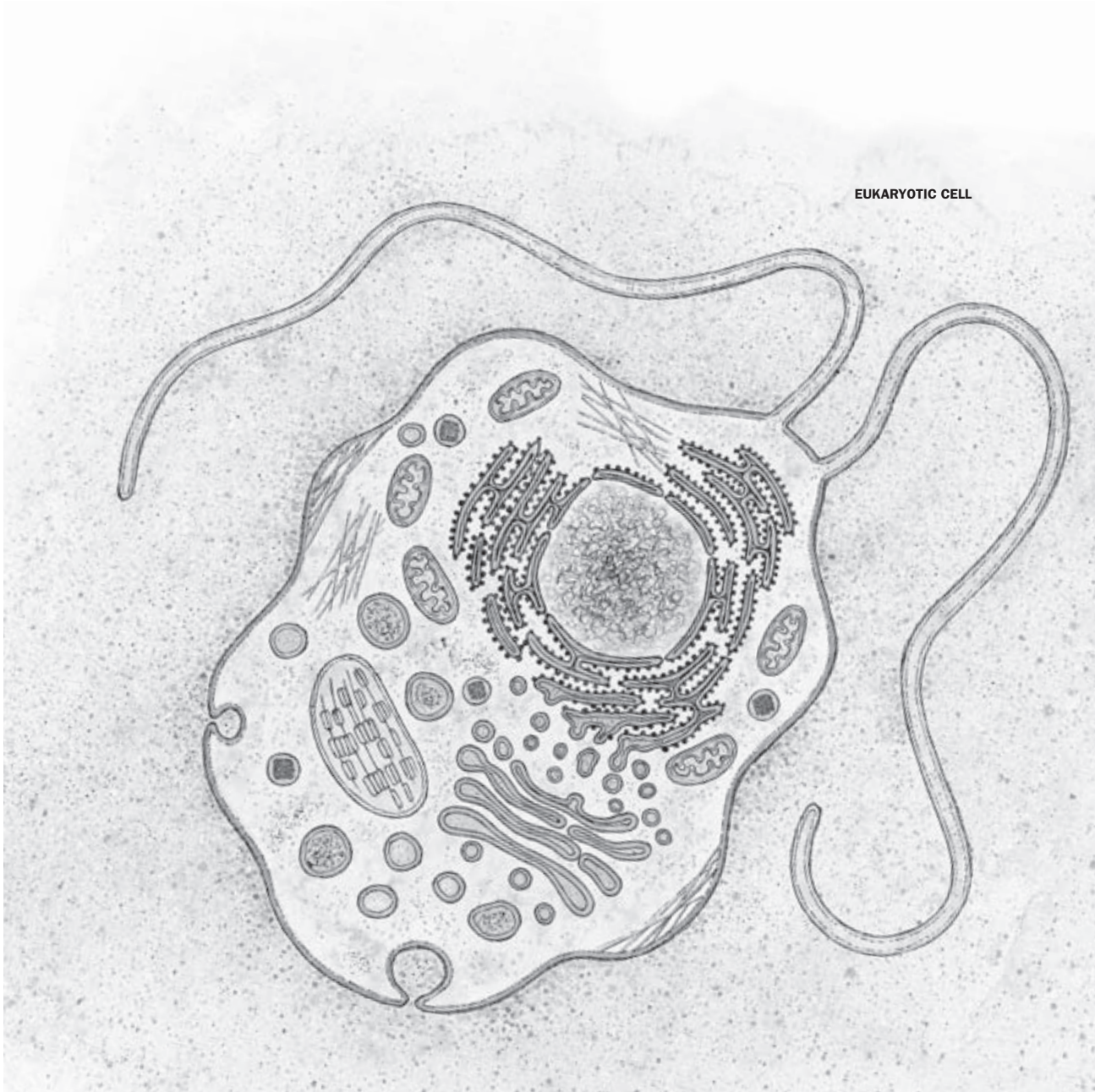


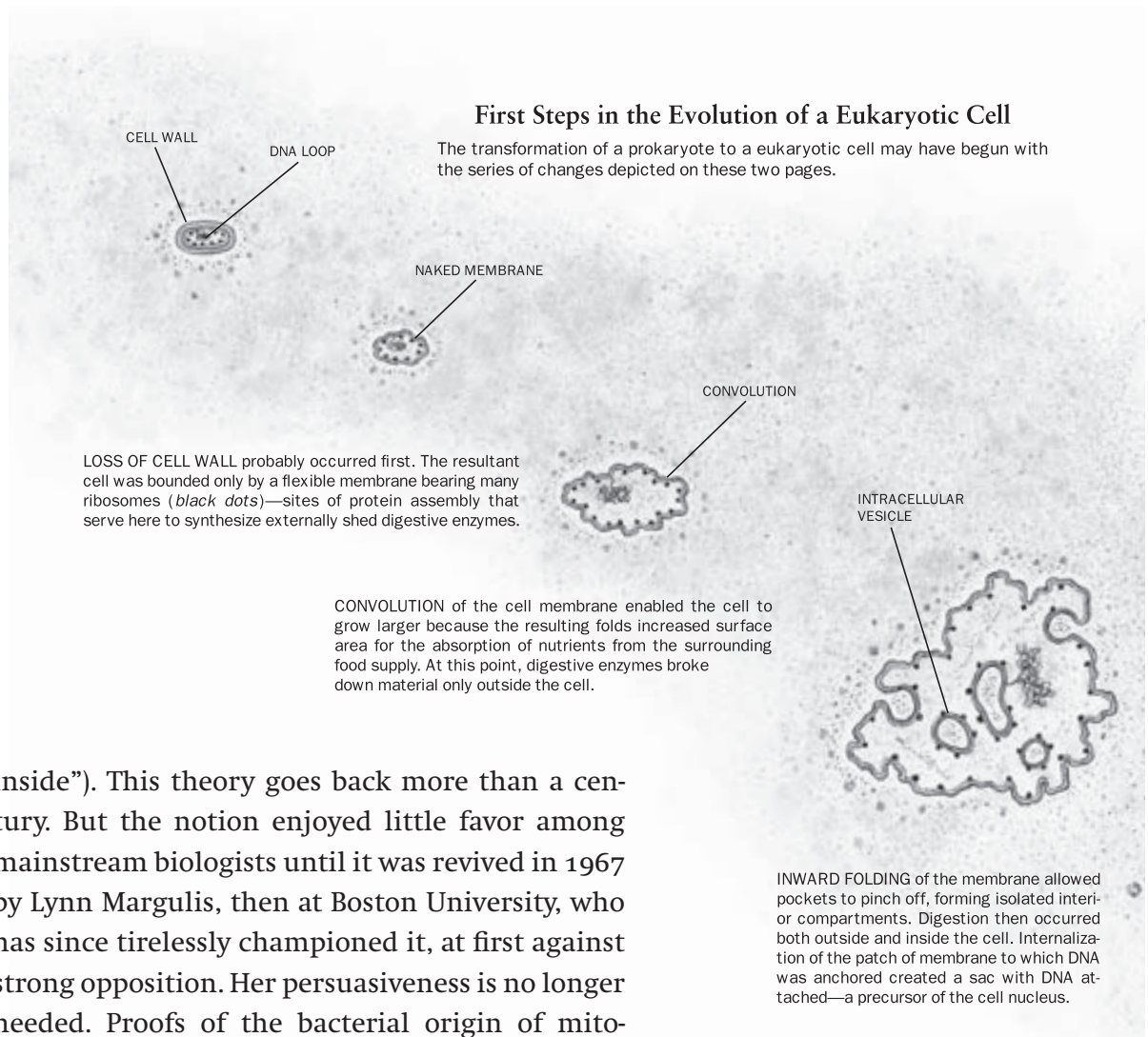
not for an illuminating clue that has come from the analysis of the numerous organelles that reside in the cytoplasm.

A FATEFUL MEAL

Biologists have long suspected that mitochondria and plastids descend from bacteria that were adopted by some ancestral host cell as endosymbionts (a word derived from Greek roots that means “living together

EUKARYOTIC CELL



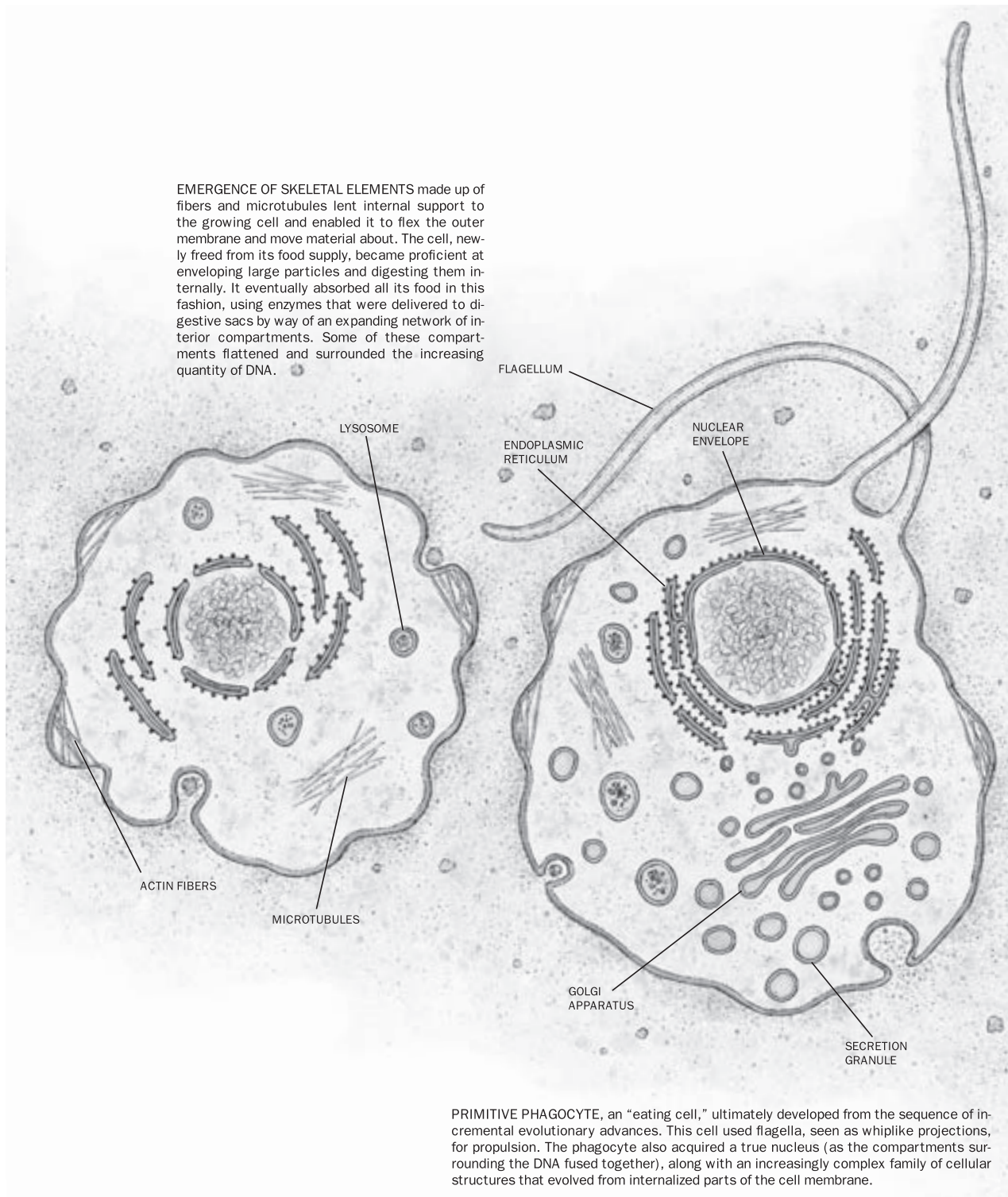


inside”). This theory goes back more than a century. But the notion enjoyed little favor among mainstream biologists until it was revived in 1967 by Lynn Margulis, then at Boston University, who has since tirelessly championed it, at first against strong opposition. Her persuasiveness is no longer needed. Proofs of the bacterial origin of mitochondria and plastids are overwhelming.

The most convincing evidence is the presence within these organelles of a vestigial—but still functional—genetic system. That system includes DNA-based genes, the means to replicate this DNA, and all the molecular tools needed to construct protein molecules from their DNA-encoded blueprints. A number of properties clearly characterize this genetic apparatus as prokaryotelike and distinguish it from the main eukaryotic genetic system.

Endosymbiont adoption is often presented as resulting from some kind of encounter—aggressive predation, peaceful invasion, mutually beneficial association or merger—between two typical prokaryotes. But these descriptions are troubling because modern bacteria do not exhibit such behavior. Moreover, the joining of simple prokaryotes would leave many other characteristics of eukaryotic cells unaccounted for. There is

EMERGENCE OF SKELETAL ELEMENTS made up of fibers and microtubules lent internal support to the growing cell and enabled it to flex the outer membrane and move material about. The cell, newly freed from its food supply, became proficient at enveloping large particles and digesting them internally. It eventually absorbed all its food in this fashion, using enzymes that were delivered to digestive sacs by way of an expanding network of interior compartments. Some of these compartments flattened and surrounded the increasing quantity of DNA.



PRIMITIVE PHAGOCYTE, an "eating cell," ultimately developed from the sequence of incremental evolutionary advances. This cell used flagella, seen as whiplike projections, for propulsion. The phagocyte also acquired a true nucleus (as the compartments surrounding the DNA fused together), along with an increasingly complex family of cellular structures that evolved from internalized parts of the cell membrane.

a more straightforward explanation, which is directly suggested by nature itself—namely, that endosymbionts were originally taken up in the course of feeding by an unusually large host cell that had already acquired many properties now associated with eukaryotic cells.

Many modern eukaryotic cells—white blood cells, for example—entrap prokaryotes. As a rule, the ingested microorganisms are killed and broken down. Sometimes they escape destruction and go on to maim or kill their captors. On a rare occasion, both captor and victim survive in a state of mutual tolerance that can later turn into mutual assistance and, eventually, dependency. Mitochondria and plastids thus may have been a host cell's permanent guests.

If this surmise is true, it reveals a great deal about the earlier evolution of the host. The adoption of endosymbionts must have followed after some prokaryotic ancestor to eukaryotes evolved into a primitive phagocyte (from the Greek for “eating cell”), a cell capable of engulfing voluminous bodies, such as bacteria. And if this ancient cell was anything like modern phagocytes, it must have been much larger than its prey and surrounded by a flexible membrane able to envelop bulky extracellular objects. The pioneering phagocyte must also have had an internal network of compartments connected with the outer membrane and specialized in the processing of ingested materials. It would also have had an internal skeleton of sorts to provide it with structural support, and it probably contained the molecular machinery to flex the outer membrane and to move internal contents about.

The development of such cellular structures represents the essence of the prokaryote-eukaryote transition. The chief problem, then, is to devise a plausible explanation for the progressive construction of these features in a manner that can be accounted for by the operation of natural selection. Each small change in the cell must have improved its chance of surviving and reproducing (offered a selective advantage) so that the new trait would become increasingly widespread in the population.

GENESIS OF AN EATING CELL

What forces might drive a primitive prokaryote to evolve in the direction of a modern eukaryotic cell? To address this question, I will make a few assumptions. First, I shall take it that the ancestral cell fed on the debris and discharges of other organisms; it was what biologists label a heterotroph. It therefore lived in surroundings that provided it with food. An interesting possibility is that it resided in mixed prokaryotic colonies of

the kind that have fossilized into layered rocks called stromatolites. Living stromatolite colonies still exist; they are formed of layers of heterotrophs topped by photosynthetic organisms that multiply with the help of sunlight and supply the lower layers with food. The fossil record indicates that such colonies already existed more than 3.5 billion years ago.

A second hypothesis, a corollary of the first, is that the ancestral organism had to digest its food. I shall assume that it did so (like most modern heterotrophic prokaryotes) by means of secreted enzymes that degraded food outside the cell. That is, digestion occurred before ingestion.

A final supposition is that the organism had lost the ability to manufacture a cell wall, the rigid shell that surrounds most prokaryotes and provides them with structural support and protection against injury. Notwithstanding their fragility, free-living naked forms of this kind exist today, even in unfavorable surroundings. In the case under consideration, the stromatolite colony would have provided the ancient organism with excellent shelter.

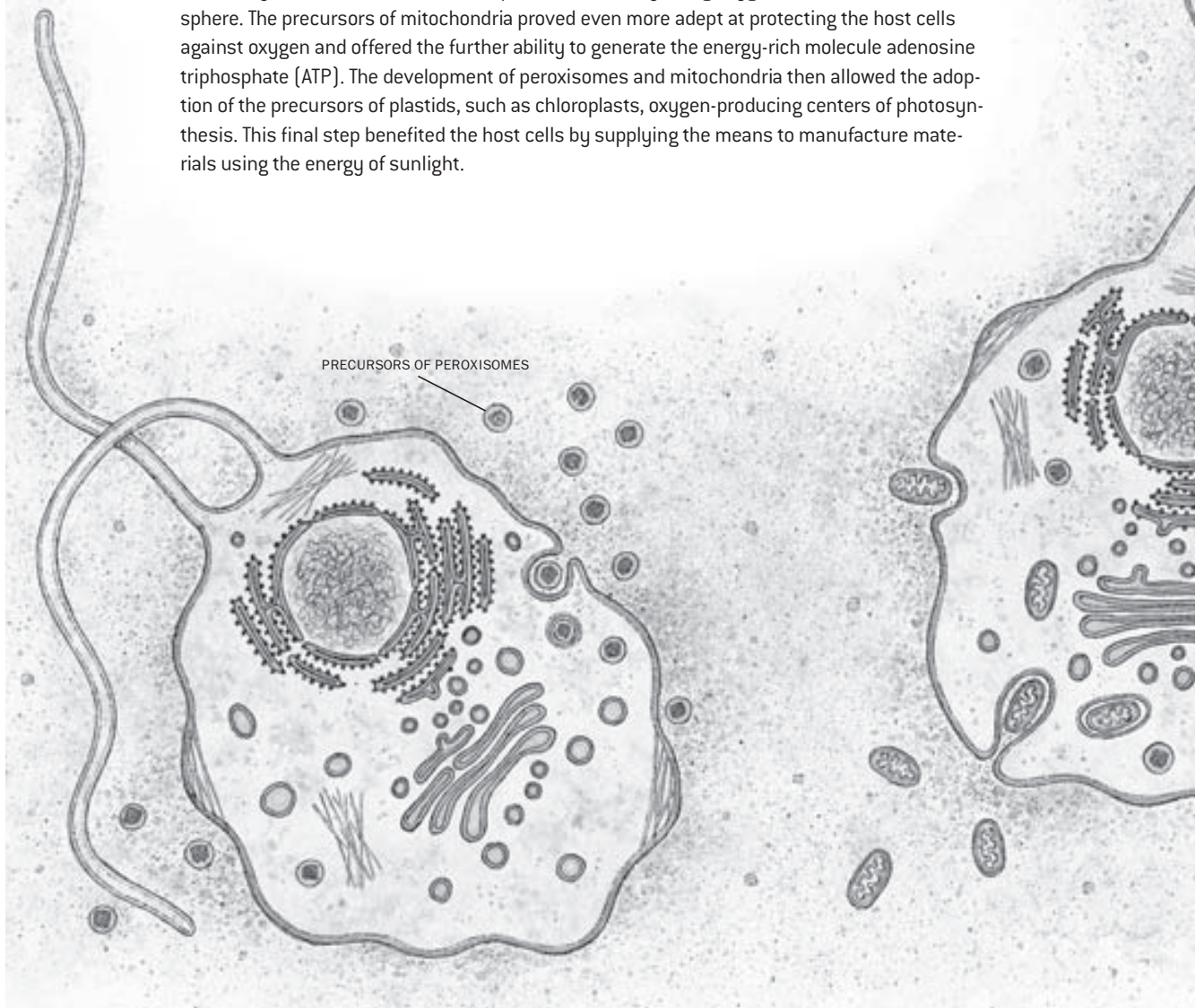
Accepting these three assumptions, one can now visualize the ancestral organism as a flattened, flexible blob—almost protean in its ability to change shape—in intimate contact with its food. Such a cell would thrive and grow faster than its walled-in relatives. It need not, however, automatically respond to growth by dividing, as do most cells. An alternative behavior would be expansion and folding of the surrounding membrane, thus increasing the surface available for the intake of nutrients and the excretion of waste—limiting factors on the growth of any cell. The ability to create an extensively folded surface would allow the organism to expand far beyond the size of ordinary prokaryotes. Indeed, giant prokaryotes living today have a highly convoluted outer membrane, probably a prerequisite of their enormous girth. Thus, one eukaryotic property—large size—can be accounted for simply enough.

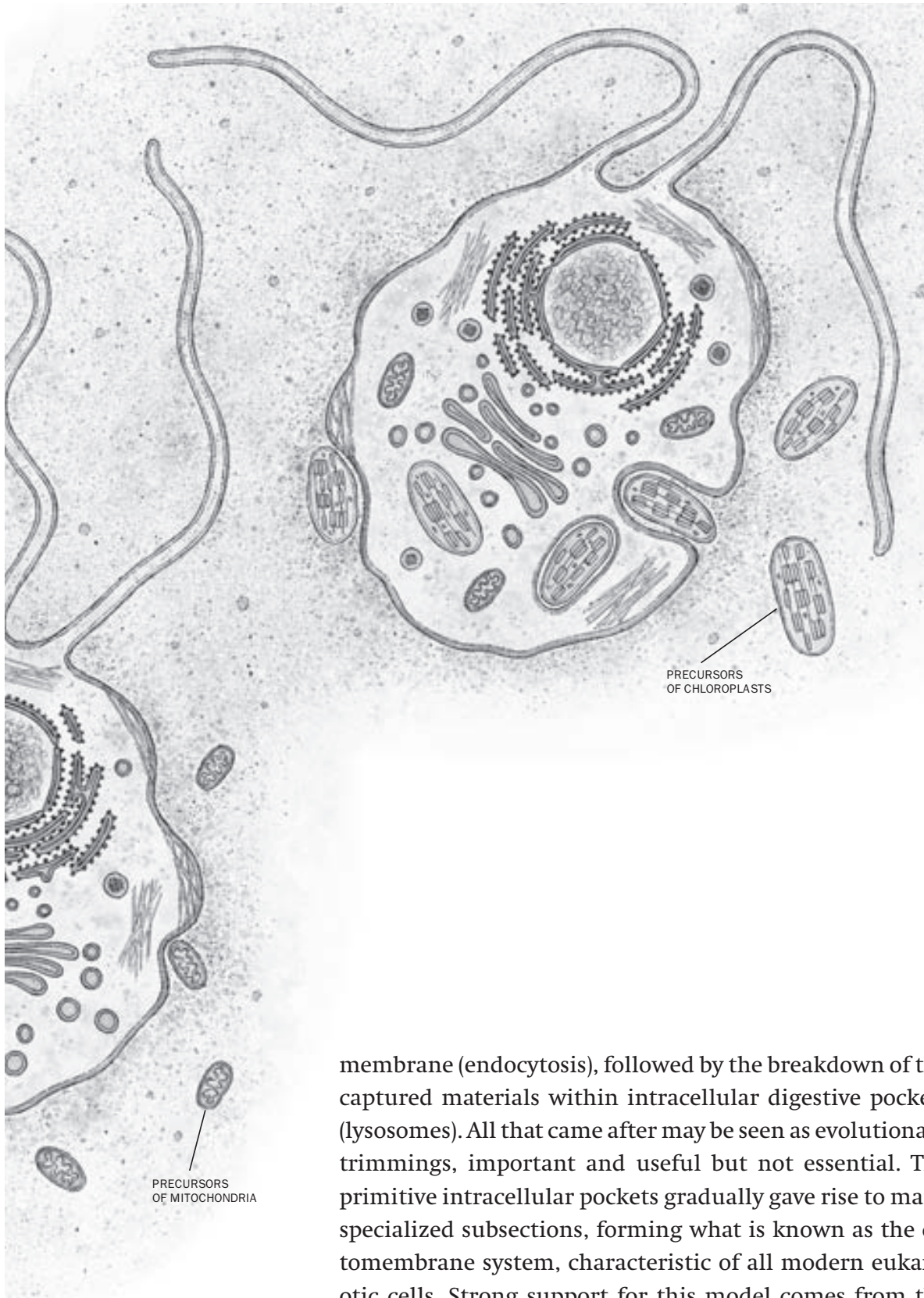
Natural selection is likely to favor expansion over division because deep folds would increase the cell's ability to obtain food by creating partially confined areas—narrow inlets along the rugged cellular coast—within which high concentrations of digestive enzymes would break down food more efficiently. Here is where a crucial development could have taken place: given the self-sealing propensity of biological membranes (which are like soap bubbles in this respect), no great leap of imagination is required to see how folds could split off to form intracellular sacs. Once such a process was initiated, as a more or less random side effect of membrane expansion, any genetic change that would promote its further development would be greatly favored by natural selection. The inlets would have turned

into confined inland ponds, within which food would now be trapped together with the enzymes that digest it. From being extracellular, digestion would have become intracellular.

Cells capable of catching and processing food in this way would have gained enormously in their ability to exploit their environment, and the resulting boost to survival and reproductive potential would have been gigantic. Such cells would have acquired the fundamental features of phagocytosis: engulfment of extracellular objects by infoldings of the cell

Final Steps in the Evolution of a Eukaryotic Cell. Adoption of prokaryotes as permanent guests within larger phagocytes marked the final phase in the evolution of eukaryotic cells. The precursors to peroxisomes may have been the first prokaryotes to develop into eukaryotic organelles. They detoxified destructive compounds created by rising oxygen levels in the atmosphere. The precursors of mitochondria proved even more adept at protecting the host cells against oxygen and offered the further ability to generate the energy-rich molecule adenosine triphosphate (ATP). The development of peroxisomes and mitochondria then allowed the adoption of the precursors of plastids, such as chloroplasts, oxygen-producing centers of photosynthesis. This final step benefited the host cells by supplying the means to manufacture materials using the energy of sunlight.





membrane (endocytosis), followed by the breakdown of the captured materials within intracellular digestive pockets (lysosomes). All that came after may be seen as evolutionary trimmings, important and useful but not essential. The primitive intracellular pockets gradually gave rise to many specialized subsections, forming what is known as the cytomembrane system, characteristic of all modern eukaryotic cells. Strong support for this model comes from the

observation that many systems present in the cell membrane of prokaryotes are found in various parts of the eukaryotic cytomembrane system.

Interestingly, the genesis of the nucleus—the hallmark of eukaryotic cells—can also be accounted for, at least schematically, as resulting from the internalization of some of the cell's outer membrane. In prokaryotes the circular DNA chromosome is attached to the cell membrane. Infolding of this particular patch of cell membrane could create an intracellular sac bearing the chromosome on its surface. That structure could have been the seed of the eukaryotic nucleus, which is surrounded by a double membrane formed from flattened parts of the intracellular membrane system that fuse into a spherical envelope.

The proposed scenario explains how a small prokaryote could have evolved into a giant cell displaying some of the main properties of eukaryotic cells, including a fenced-off nucleus, a vast network of internal membranes and the ability to catch food and digest it internally. Such progress could have taken place by a very large number of almost imperceptible steps, each of which enhanced the cell's autonomy and provided a selective advantage. But there was a condition. Having lost the support of a rigid outer wall, the cell needed inner props for its enlarging bulk.

Modern eukaryotic cells are reinforced by fibrous and tubular structures, often associated with tiny motor systems, that allow the cells to move around and power their internal traffic. No counterpart of the many proteins that make up these systems is found in prokaryotes. Thus, the development of the cytoskeletal system must have required a large number of authentic innovations. Nothing is known about these key evolutionary events, except that they most likely went together with cell enlargement and membrane expansion, often in pacesetting fashion.

At the end of this long road lay the primitive phagocyte: a cell efficiently organized to feed on bacteria, a mighty hunter no longer condemned to reside inside its food supply but free to roam the world and pursue its prey actively, a cell ready, when the time came, to become the host of endosymbionts.

Such cells, which still lacked mitochondria and some other key organelles characteristic of modern eukaryotes, would be expected to have invaded many niches and filled them with variously adapted progeny. Yet few if any descendants of such evolutionary lines have survived to the present day. A few unicellular eukaryotes devoid of mitochondria exist, but the possibility that their forebears once possessed mitochondria and lost them cannot be excluded. Thus, all eukaryotes may well have evolved from primitive phagocytes that incorporated the precursors to

mitochondria. Whether more than one such adoption took place is still being debated, but the majority opinion is that mitochondria sprang from a single stock. It would appear that the acquisition of mitochondria either saved one eukaryotic lineage from elimination or conferred such a tremendous selective advantage on its beneficiaries as to drive almost all other eukaryotes to extinction. Why then were mitochondria so overwhelmingly important?

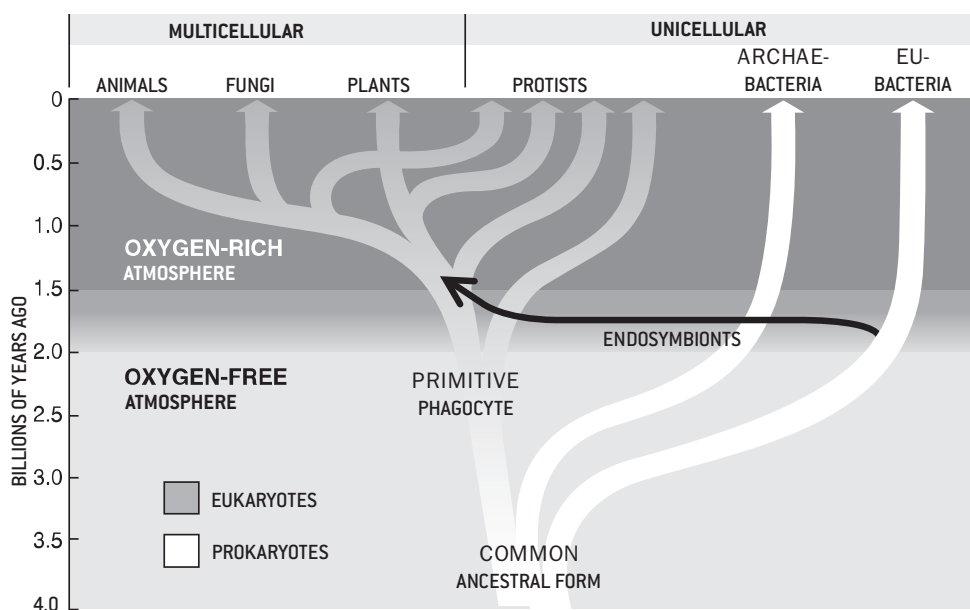
THE OXYGEN HOLOCAUST

The primary function of mitochondria in cells today is the combustion of foodstuffs with oxygen to assemble the energy-rich molecule adenosine triphosphate (ATP). Life is vitally dependent on this process, which is the main purveyor of energy in the vast majority of oxygen-dependent (aerobic) organisms. Yet when the first cells appeared on the earth, there was no oxygen in the atmosphere. Free molecular oxygen is a product of life; it began to be generated when certain photosynthetic microorganisms, called cyanobacteria, appeared. These cells exploit the energy of sunlight to extract the hydrogen they need for self-construction from water molecules, leaving molecular oxygen as a by-product. Oxygen first entered the atmosphere in appreciable quantity some two billion years ago, progressively rising to reach a stable level about 1.5 billion years ago.

Before the appearance of atmospheric oxygen, all forms of life must have been adapted to an oxygen-free (anaerobic) environment. Presumably, like the obligatory anaerobes of today, they were extremely sensitive to oxygen. Within cells, oxygen readily generates several toxic chemical groups. These cellular poisons include the superoxide ion, the hydroxyl radical and hydrogen peroxide. As oxygen concentration rose two billion years ago, many early organisms probably fell victim to the "oxygen holocaust." Survivors included those cells that found refuge in some oxygen-free location or had developed other protection against oxygen toxicity.

These facts point to an attractive hypothesis. Perhaps the phagocytic forerunner of eukaryotes was anaerobic and was rescued from the oxygen crisis by the aerobic ancestors of mitochondria: cells that not only destroyed the dangerous oxygen (by converting it to innocuous water) but even turned it into a tremendously useful ally. This theory would neatly account for the apparent lifesaving effect of mitochondrial adoption and has enjoyed considerable favor.

Yet there is a problem with this idea. Adaptation to oxygen very likely took place gradually, starting with primitive systems of oxygen



Evolutionary tree depicts major events in the history of life. This well-accepted chronology has newly been challenged by Russell F. Doolittle of the University of California at San Diego and his co-workers, who argue that the last common ancestor of all living beings existed a little more than two billion years ago.

detoxification. A considerable amount of time must have been needed to reach the ultimate sophistication of modern mitochondria. How did anaerobic phagocytes survive during all the time it took for the ancestors of mitochondria to evolve?

A solution to this puzzle is suggested by the fact that eukaryotic cells contain other oxygen-utilizing organelles, as widely distributed throughout the plant and animal world as mitochondria but much more primitive in structure and composition. These are the peroxisomes [see “Microbodies in the Living Cell,” by Christian de Duve; *Scientific American*, May 1983]. Peroxisomes, like mitochondria, carry out a number of oxidizing metabolic reactions. Unlike mitochondria, however, they do not use the energy released by these reactions to assemble ATP but squander it as heat. In the process, they convert oxygen to hydrogen peroxide, but then they destroy this dangerous compound with an enzyme called catalase. Peroxisomes also contain an enzyme that removes the superoxide ion. They therefore qualify eminently as primary rescuers from oxygen toxicity.

I first made this argument in 1969, when peroxisomes were believed to be specialized parts of the cytomembrane system. I thus included peroxisomes within the general membrane expansion model I had proposed for the development of the primitive phagocyte. Afterward, experiments by the late Brian H. Poole and by Paul B. Lazarow, my associates at the

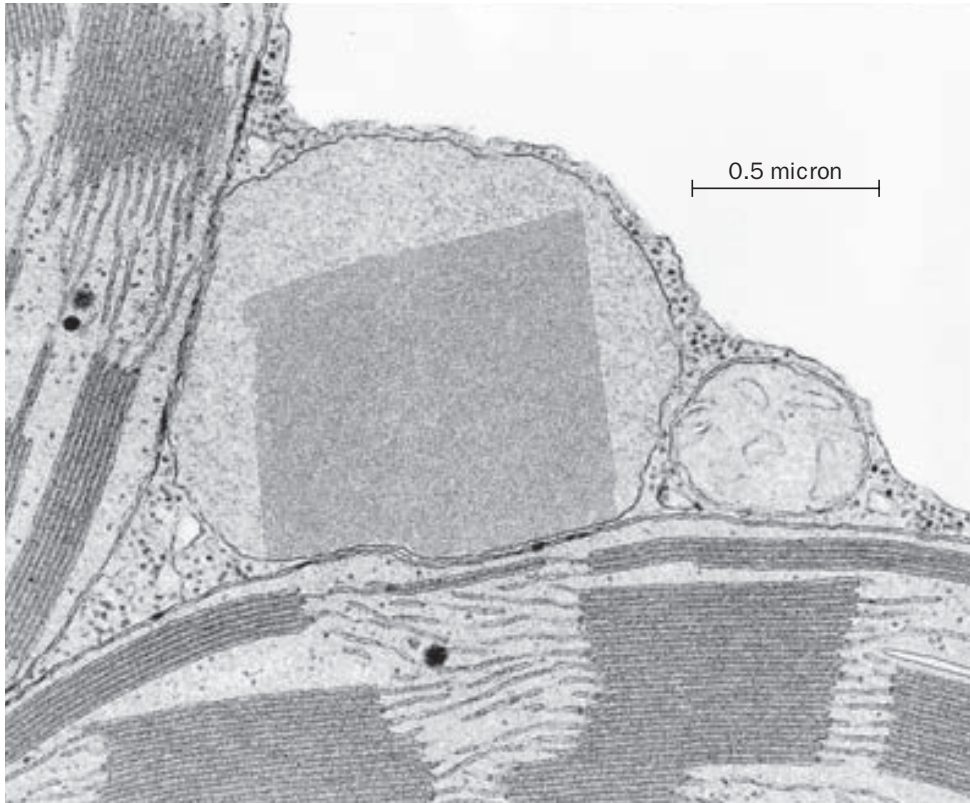
Rockefeller University, conclusively demonstrated that peroxisomes are entirely unrelated to the cytomembrane system. Instead they acquire their proteins much as mitochondria and plastids do (by a process I will explain shortly). Hence, it seemed reasonable that all three organelles began as endosymbionts. So, in 1982, I revised my original proposal and suggested that peroxisomes might stem from primitive aerobic bacteria that were adopted before mitochondria. These early oxygen detoxifiers could have protected their host cells during all the time it took for the ancestors of mitochondria to reach the high efficiency they possessed when they were adopted.

So far researchers have obtained no solid evidence to support this hypothesis or, for that matter, to disprove it. Unlike mitochondria and plastids, peroxisomes do not contain the remnants of an independent genetic system. This observation nonetheless remains compatible with the theory that peroxisomes developed from an endosymbiont. Mitochondria and plastids have lost most of their original genes to the nucleus, and the older peroxisomes could have lost all their DNA by now.

Whichever way they were acquired, peroxisomes may well have allowed early eukaryotes to weather the oxygen crisis. Their ubiquitous distribution would thereby be explained. The tremendous gain in energy retrieval provided with the coupling of the formation of ATP to oxygen utilization would account for the subsequent adoption of mitochondria, organelles that have the additional advantage of keeping the oxygen in their surroundings at a much lower level than peroxisomes can maintain.

Why then did peroxisomes not disappear after mitochondria were in place? By the time eukaryotic cells acquired mitochondria, some peroxisomal activities (for instance, the metabolism of certain fatty acids) must have become so vital that these primitive organelles could not be eliminated by natural selection. Hence, peroxisomes and mitochondria are found together in most modern eukaryotic cells.

The other major organelles of endosymbiont origin are the plastids, whose main representatives are the chloroplasts, the green photosynthetic organelles of unicellular algae and multicellular plants. Plastids are derived from cyanobacteria, the prokaryotes responsible for the oxygen crisis. Their adoption as endosymbionts quite likely followed that of mitochondria. The selective advantages that favored the adoption of photosynthetic endosymbionts are obvious. Cells that had once needed a constant food supply henceforth thrived on nothing more than air, water, a few dissolved minerals and light. In fact, there is evidence that eukaryotic cells acquired plastids at least three separate times, giving rise to green, red and



Four organelles appear in a tobacco leaf cell. The two chloroplasts (*left and bottom*) and the mitochondrion (*middle right*) evolved from prokaryotic endosymbionts. The peroxisome (*center*)—containing a prominent crystalline inclusion, most probably made up of the enzyme catalase—may have derived from an endosymbiont as well.

brown algae. Members of the first of these groups were later to form multicellular plants.

FROM PRISONER TO SLAVE

What started as an uneasy truce soon turned into the progressive enslavement of the captured endosymbiont prisoners by their phagocytic hosts. This subjugation was achieved by the piecemeal transfer of most of the endosymbionts' genes to the host cell's nucleus. In itself, the uptake of genes by the nucleus is not particularly extraordinary. When foreign genes are introduced into the cytoplasm of a cell (as in some bioengineering experiments), they can readily home to the nucleus and function there. That is, they replicate during cell division and can serve as the master templates for the production of proteins. But the migration of genes from endosymbionts to the nucleus is remarkable because it seems to have raised more difficulties than it solved. Once this transfer occurred, the proteins

encoded by these genes began to be manufactured in the cytoplasm of the host cell (where the products of all nuclear genes are constructed). These molecules had then to migrate into the endosymbiont to be of use. Somehow this seemingly unpromising scheme not only withstood the hazards of evolution but also proved so successful that all endosymbionts retaining copies of transferred genes eventually disappeared.

Today mitochondria, plastids and peroxisomes acquire proteins from the surrounding cytoplasm with the aid of complex transport structures in their bounding membranes. These structures recognize parts of newly made protein molecules as “address tags” specific to each organelle. The transport apparatus then allows the appropriate molecules to travel through the membrane with the help of energy and of specialized proteins (aptly called chaperones). These systems for bringing externally made proteins into the organelles could conceivably have evolved from similar systems for protein secretion that existed in the original membranes of the endosymbionts. In their new function, however, those systems would have to operate from outside to inside.

The adoption of endosymbionts undoubtedly played a critical role in the birth of eukaryotes. But this was not the key event. More significant (and requiring a much larger number of evolutionary innovations) was the long, mysterious process that made such acquisition possible: the slow conversion, over as long as one billion years or more, of a prokaryotic ancestor into a large phagocytic microbe possessing most attributes of modern eukaryotic cells. Science is beginning to lift the veil that shrouds this momentous transformation, without which much of the living world, including humans, would not exist.

FURTHER READING

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