

# The Birth of Complex Cells

*Humans, together with all other animals, plants and fungi, owe their existence to the momentous transformation of tiny, primitive bacteria into large, intricately organized cells*

by Christian de Duve

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 not

**PROKARYOTIC AND EUKARYOTIC CELLS** differ in size and complexity. Prokaryotic cells (*right*) 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 (*far right*), house a wide array of specialized structures—including an encapsulated nucleus containing the cell's main genetic stores.



**PROKARYOTIC CELLS**



for an illuminating clue that has come from the analysis of the numerous organelles that reside in the cytoplasm.

### A Fateful Meal

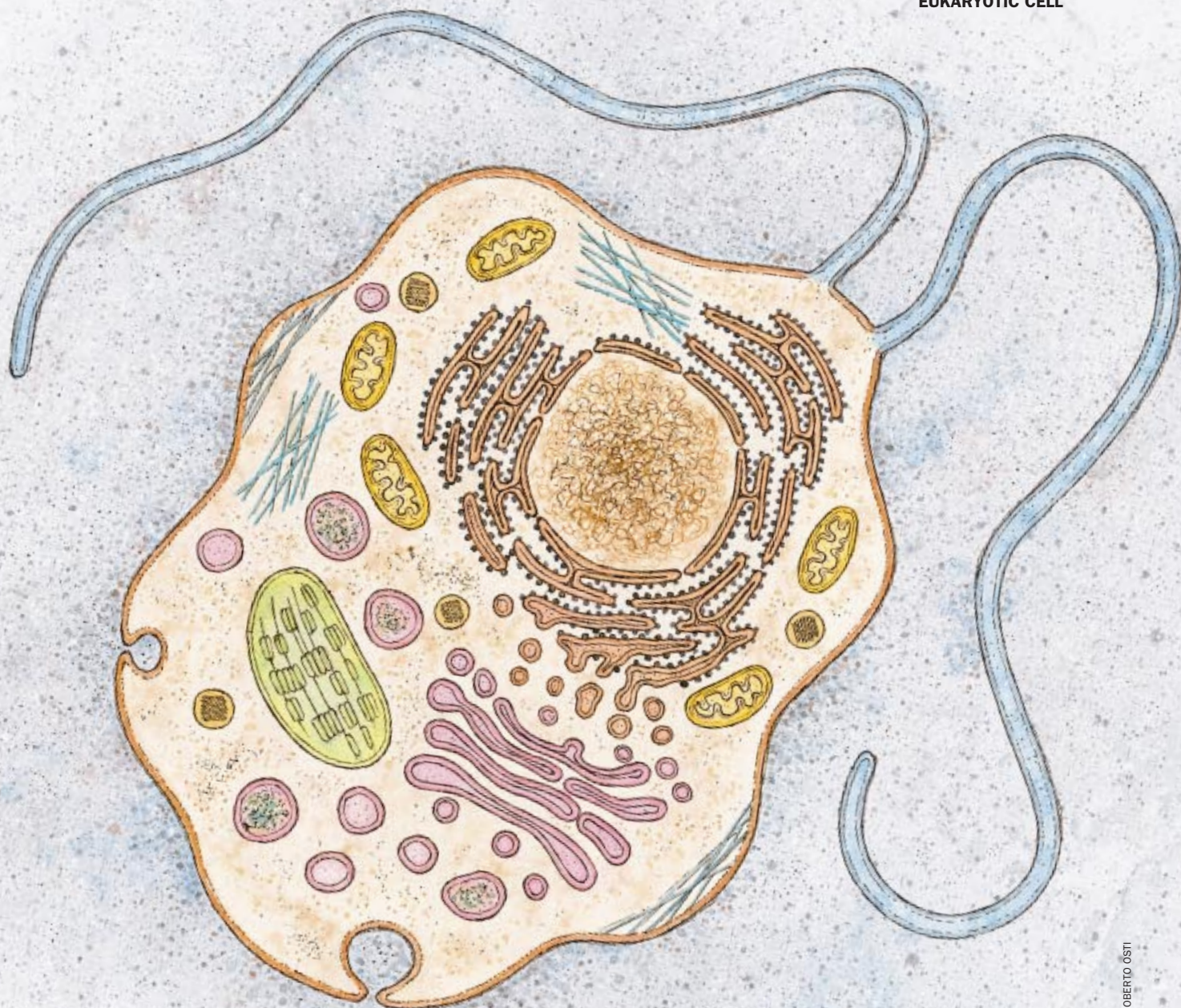
Biologists have long suspected that mitochondria and plastids descend from bacteria that were adopted by some ancestral host cell as endosymbionts (a word derived from Greek roots that means “living together inside”). This theory goes back more than a century. But the notion enjoyed little favor among mainstream biologists until it was revived in 1967 by Lynn Margulis, then at Boston University, who has since tire-

lessly 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 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

EUKARYOTIC CELL

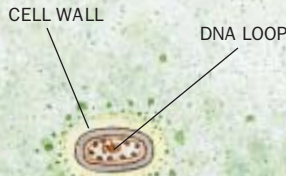


ROBERTO OSTI



## First Steps in the Evolution of a Eukaryotic Cell

The transformation of a prokaryote to a eukaryotic cell may have begun with the series of changes depicted on these two pages.



NAKED MEMBRANE



LOSS OF CELL WALL probably occurred first. The resultant cell was bounded only by a flexible membrane bearing many ribosomes (*black dots*)—sites of protein assembly that serve here to synthesize externally shed digestive enzymes.

CONVOLUTION



CONVOLUTION of the cell membrane enabled the cell to grow larger because the resulting folds increased surface area for the absorption of nutrients from the surrounding food supply (*green*). At this point, digestive enzymes broke down material only outside the cell.

INTRACELLULAR VESICLE



INWARD FOLDING of the membrane allowed pockets to pinch off, forming isolated interior compartments. Digestion then occurred both outside and inside the cell. Internalization of the patch of membrane to which DNA was anchored created a sac with DNA attached—a precursor of the cell nucleus.

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 pro-

vide 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



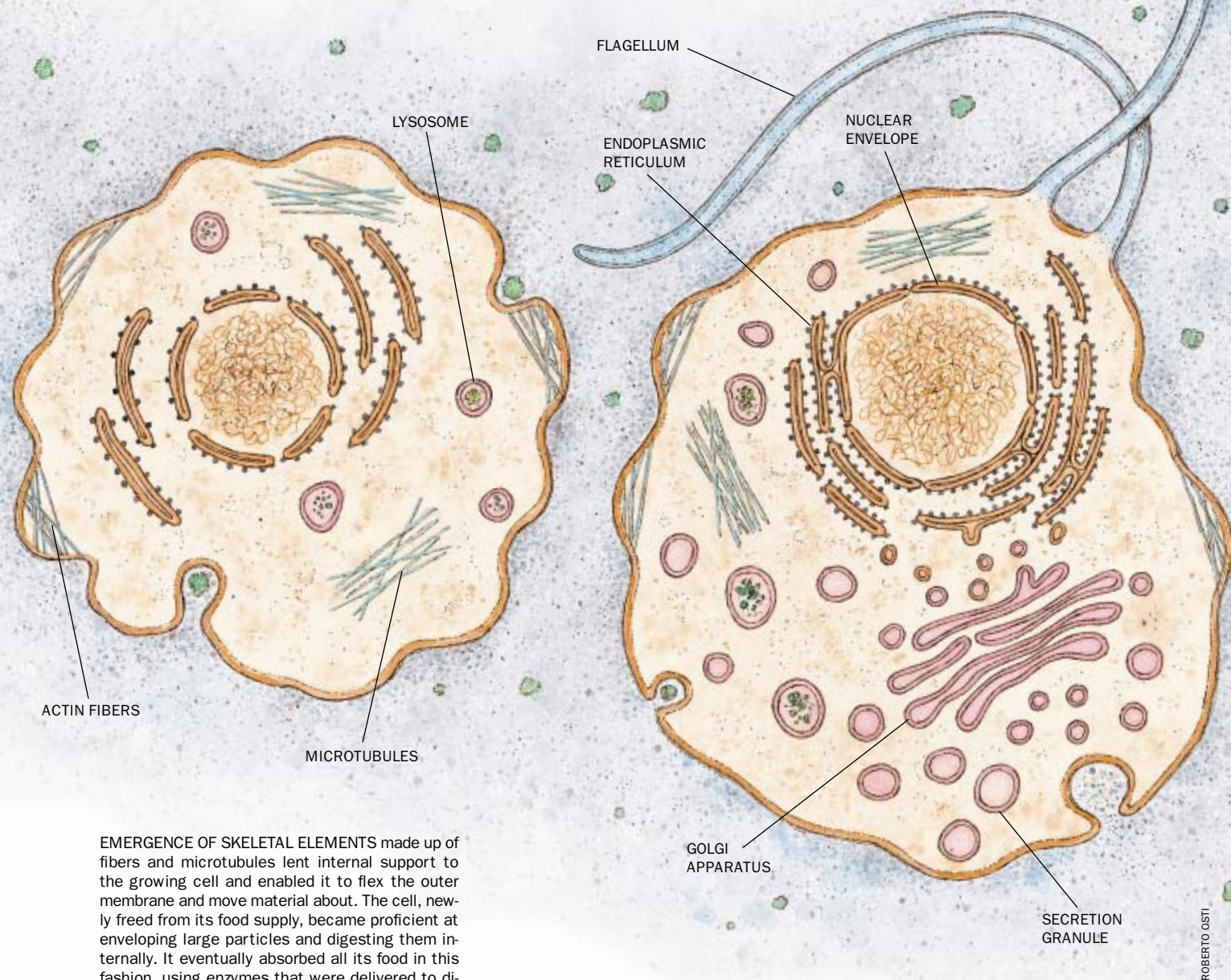
a cell wall, the rigid shell that surrounds most prokaryotes and provides them with structural support and protection against injury. Notwithstanding their fragility, free-living naked forms of this kind exist today, even in unfavorable surroundings. In the case under consideration, the stromatolite 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—al-

most 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



**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.



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 process-

ing 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 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.

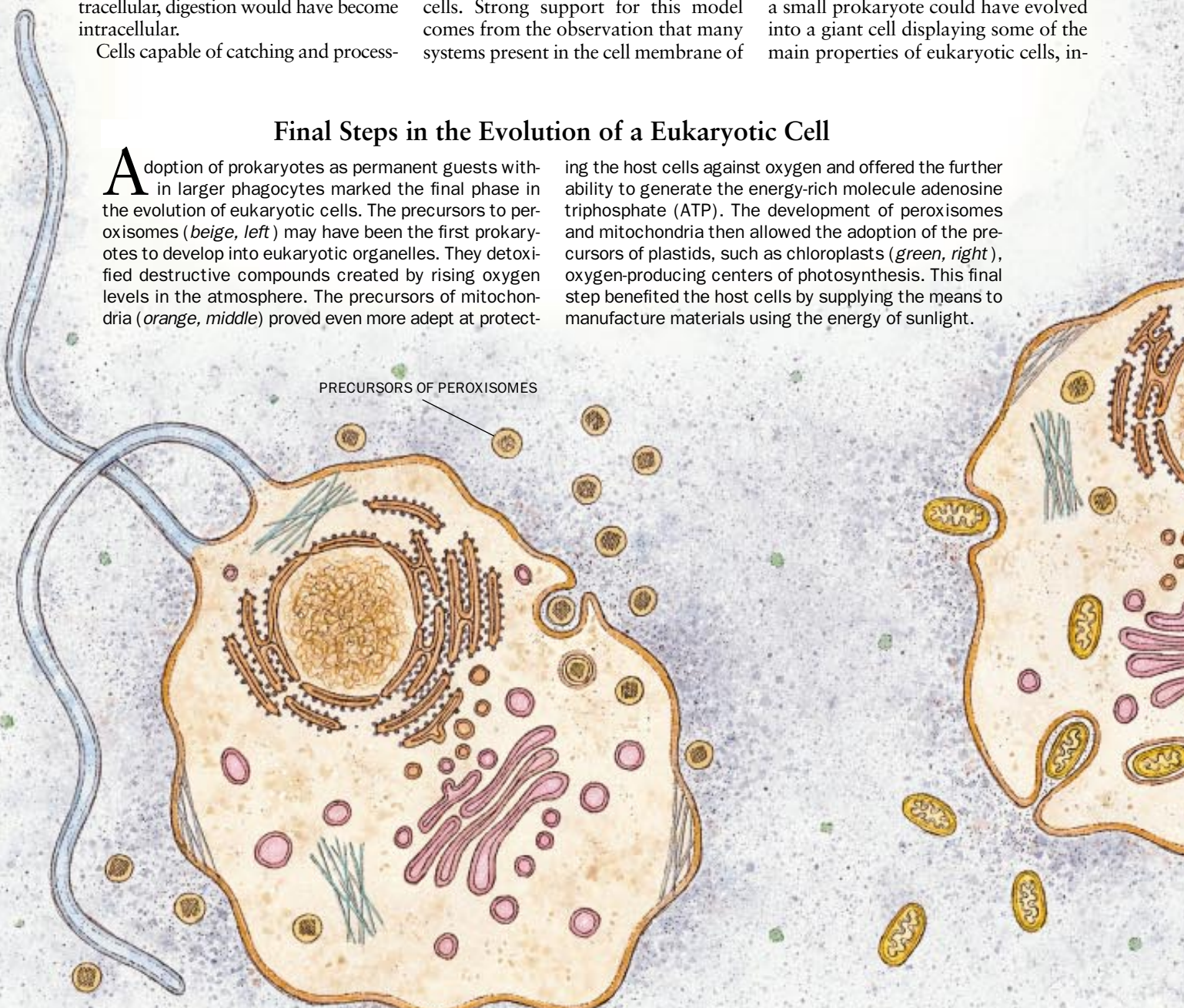
The proposed scenario explains how a small prokaryote could have evolved into a giant cell displaying some of the main properties of eukaryotic cells, in-

## Final Steps in the Evolution of a Eukaryotic Cell

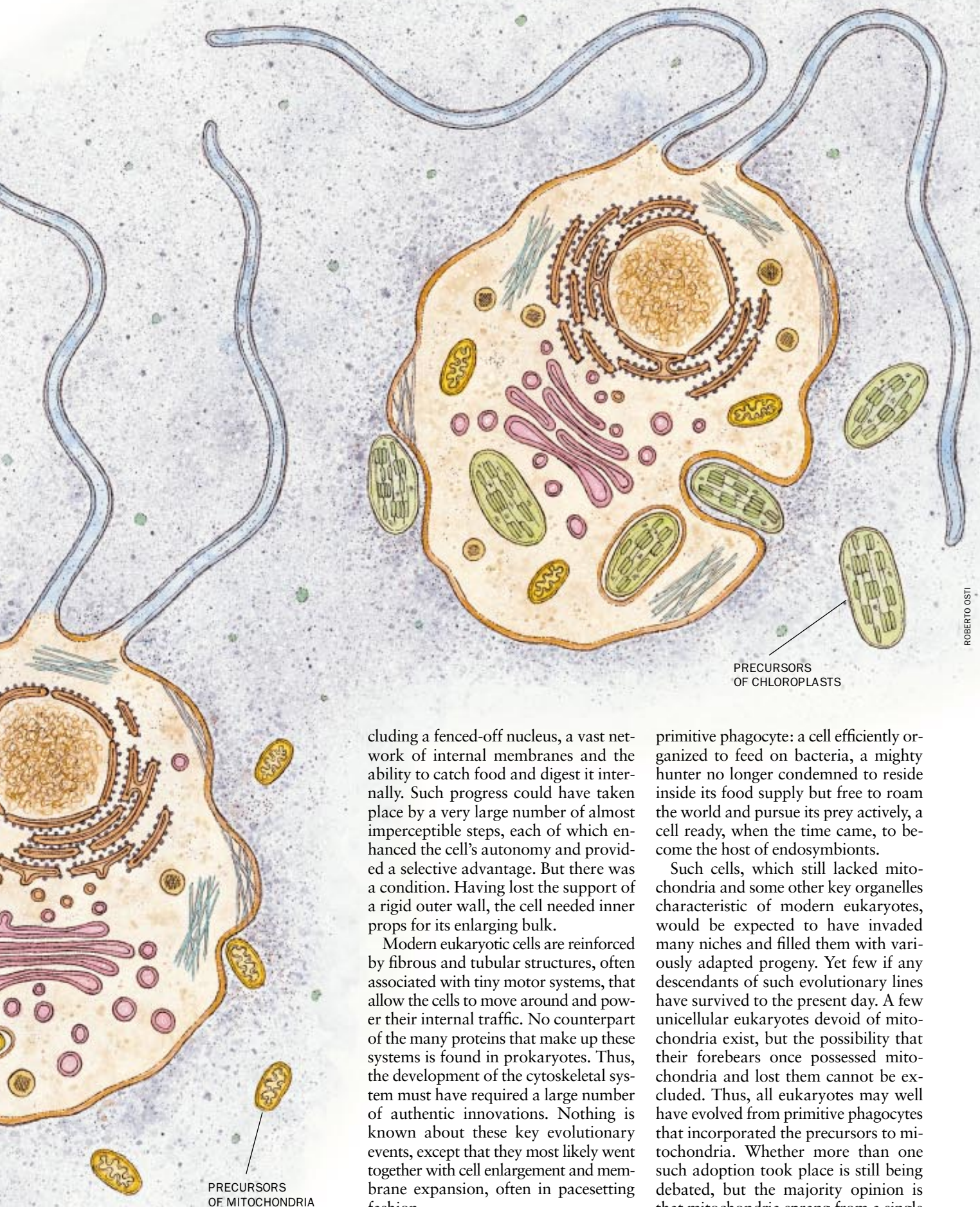
**A**doption of prokaryotes as permanent guests within larger phagocytes marked the final phase in the evolution of eukaryotic cells. The precursors to peroxisomes (*beige, left*) may have been the first prokaryotes to develop into eukaryotic organelles. They detoxified destructive compounds created by rising oxygen levels in the atmosphere. The precursors of mitochondria (*orange, middle*) proved even more adept at protect-

ing 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 (*green, right*), oxygen-producing centers of photosynthesis. This final step benefited the host cells by supplying the means to manufacture materials using the energy of sunlight.

PRECURSORS OF PEROXISOMES







cluding 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.

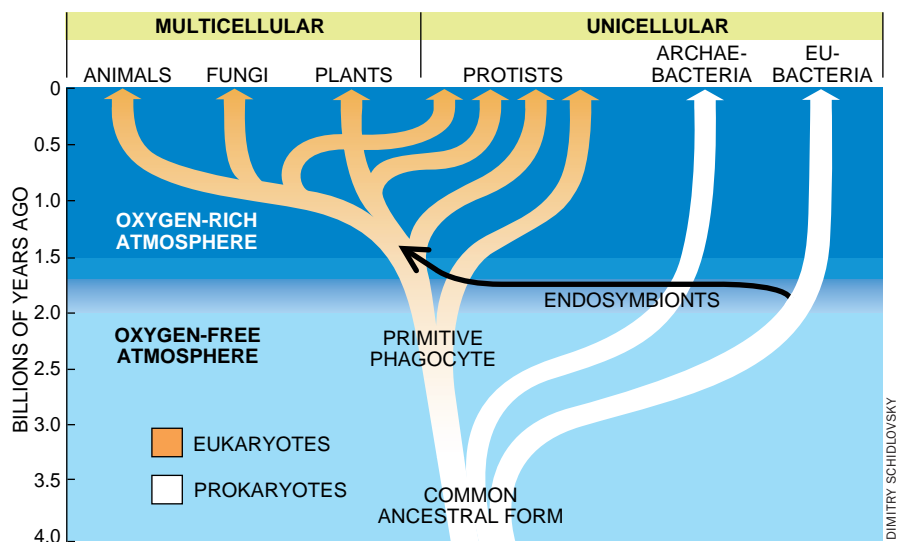
Modern eukaryotic cells are reinforced by fibrous and tubular structures, often associated with tiny motor systems, that allow the cells to move around and power their internal traffic. No counterpart of the many proteins that make up these systems is found in prokaryotes. Thus, the development of the 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 acquisi-





**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.

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

### From Prisoner to Slave

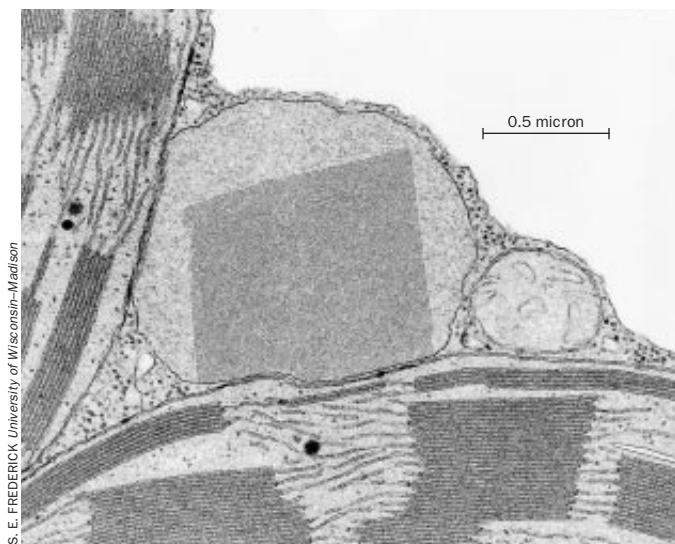
What started as an uneasy truce soon turned into the progressive enslavement of the captured endosymbiont prisoners by their phagocytic hosts. This subjugation was achieved by the

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



**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.

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. SA

### The Author

CHRISTIAN DE DUVE shared the 1974 Nobel Prize in Physiology or Medicine with Albert Claude and George E. Palade "for their discoveries concerning the structural and functional organization of the cell." He divides his time between the University of Louvain in Belgium, where he is professor emeritus of biochemistry, and the Rockefeller University in New York City, where he is Andrew W. Mellon Professor Emeritus. In Belgium, de Duve founded the International Institute of Cellular and Molecular Pathology. He is the author of *A Guided Tour of the Living Cell*; *Blueprint for a Cell: The Nature and Origin of Life*; and *Vital Dust: Life as a Cosmic Imperative*.

### Further Reading

THE ORIGIN OF EUKARYOTE AND ARCHAEABACTERIAL CELLS. T. Cavalier-Smith in *Annals of the New York Academy of Sciences*, Vol. 503, pages 17–54; July 1987.  
 BLUEPRINT FOR A CELL: THE NATURE AND ORIGIN OF LIFE. Christian de Duve. Neil Patterson Publishers/Carolina Biological Supply Company, 1991.  
 TRACING THE HISTORY OF EUKARYOTIC CELLS: THE ENIGMATIC SMILE. Betsy D. Dyer and Robert A. Obar. Columbia University Press, 1994.  
 VITAL DUST: LIFE AS A COSMIC IMPERATIVE. Christian de Duve. BasicBooks, 1995.