Archaebacteria

These unusual bacteria are genealogically neither prokaryotes nor eukaryotes. This discovery means there are not two lines of descent but three: the archaebacteria, the true bacteria and the eukaryotes

by Carl R. Woese

Early natural philosophers held that life on the earth is fundamentally dichotomous: all living things are either animals or plants. When microorganisms were discovered, they were divided in the same way. The large and motile ones were considered to be animals and the ones that appeared not to move, including the bacteria, were considered to be plants. As understanding of the microscopic world advanced it became apparent that a simple twofold classification would not suffice, and so additional categories were introduced: fungi, protozoa and bacteria. Ultimately, however, a new simplification took hold. It seemed that life might be dichotomous after all, but at a deeper level, namely in the structure of the living cell. All cells appeared to belong to one or the other of two groups: the eukaryotes, which are cells with a well-formed nucleus, and the prokaryotes, which do not have such a nucleus. Multicellular plants and animals are eukaryotic and so are many unicellular organisms. The only prokaryotes are the bacteria (including the cyanobacteria, which were formerly called blue-green algae).

In the past few years my colleagues and I have been led to propose a fundamental revision of this picture. Among the bacteria we have found a group of organisms that do not seem to belong to either of the basic categories. The organisms we have been studying are prokaryotic in the sense that they do not have a nucleus, and indeed outwardly they look much like ordinary bacteria. In their biochemistry, however, and in the structure of certain large molecules, they are as different from other prokaryotes as they are from eukaryotes. Phylogenetically they are neither prokaryotes nor eukaryotes. They make up a new "primary kingdom," with a completely different status in the history and the natural order of life.

We have named these organisms archaebacteria. The name reflects an untested conjecture about their evolutionary status. The phylogenetic evidence suggests that the archaebacteria are at least as old as the other major groups. Moreover, some of the archaebacteria have a form of metabolism that seems particularly well suited to the conditions believed to have prevailed in the early history of life on the earth. Hence it seems possible that the newest group of organisms is actually the oldest.

The Evolutionary Record

The earth is four and a half billion years old, and on the basis of the macroscopic fossil record it would appear to have been inhabited for less than a seventh of that time: the entire evolutionary progression from the most ancient marine forms to man spans only 600 million years. The fossil imprints of unicellular organisms too small to be seen with the unaided eye tell a different story. Microfossils of bacteria in particular are plentiful in sediments of all ages; they have been found in the oldest intact sedimentary rocks known, 3.5-billion-year-old deposits in Australia. Over an enormous expanse of time, during which no higher forms existed, the bacteria arose and radiated to form a wide variety of types inhabiting a great many ecological niches. This age of microorganisms is the most important period in evolutionary history not only because of its duration but also because of the nature of the evolutionary events that took place over those billions of years.

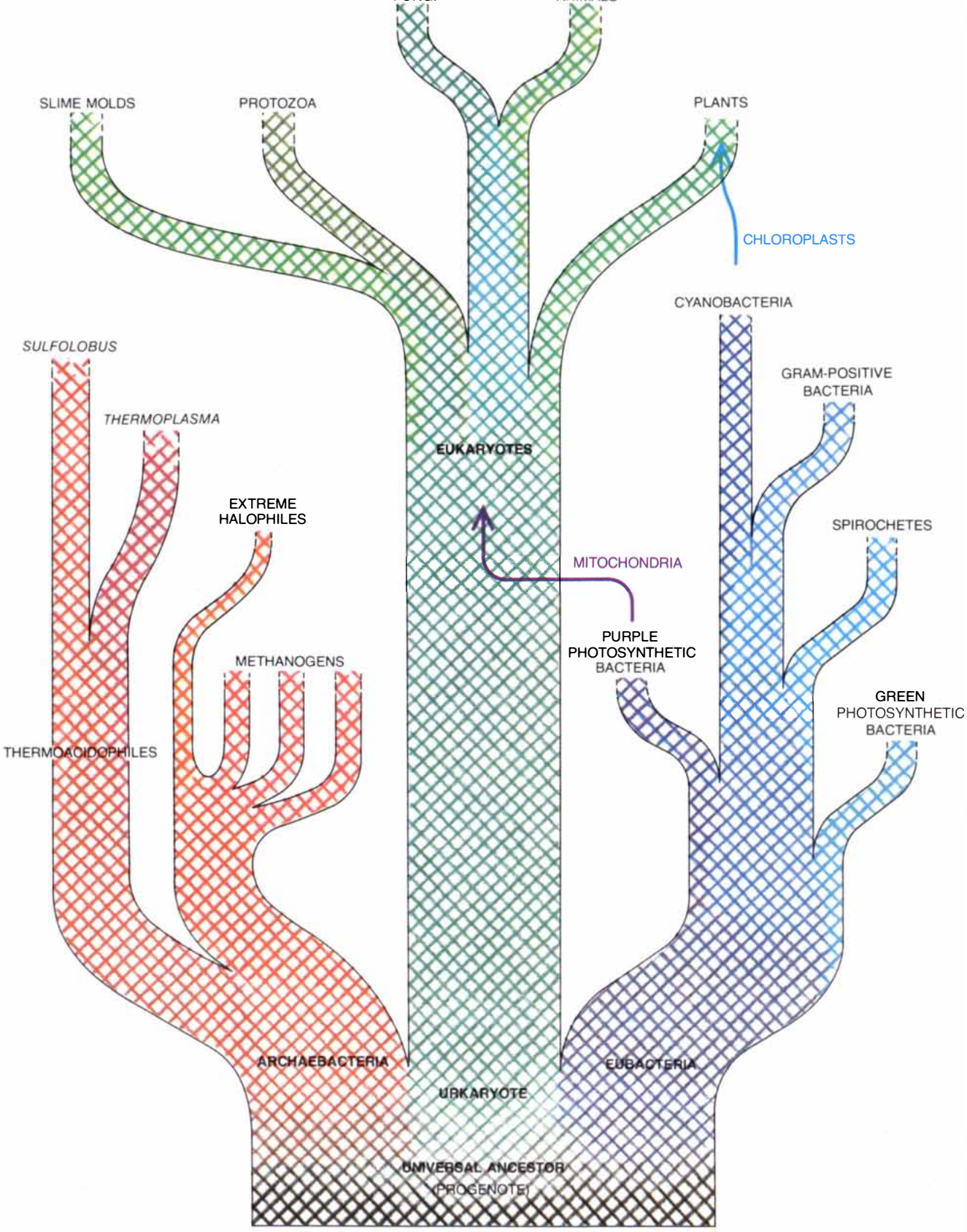
Until recently, however, almost nothing was known about the age of microorganisms. Bacterial microfossils are not very informative structures; little can be inferred from the imprint of a small sphere or rod. The main paleontological indications of the nature of the early bacteria have come not from the individual microfossils but from the macroscopic structures called stromatolites, which are thought to be fossilized bacterial mats: colonies of bacteria embedded with minerals. Today such structures are formed primarily by several kinds of photosynthetic bacteria, usually the cyanobacteria. Stromatolites fossilized recently resemble the ancient ones to such an extent that it seems entirely reasonable to think the ancient structures were also made by photosynthetic bacteria. Therefore at least some of the ancient bacteria must have been photosynthesizers. Apart from that one fact virtually nothing could be established with certainty about the earliest microorganisms. The entire evolutionary tree of the bacteria remained obscure, as did the base of the tree for the higher forms of life.

In reconstructing early evolutionary events, however, biologists are not limited to the geologic fossil record. The cell itself retains evidence of its past in the amino acid sequences of its proteins and in the nucleotide sequences of its nucleic acids: DNA and RNA. This living record is potentially far richer and more extensive than the fossil record, and it reaches back in time beyond the oldest fossils, to the period when the common ancestor of all life existed.

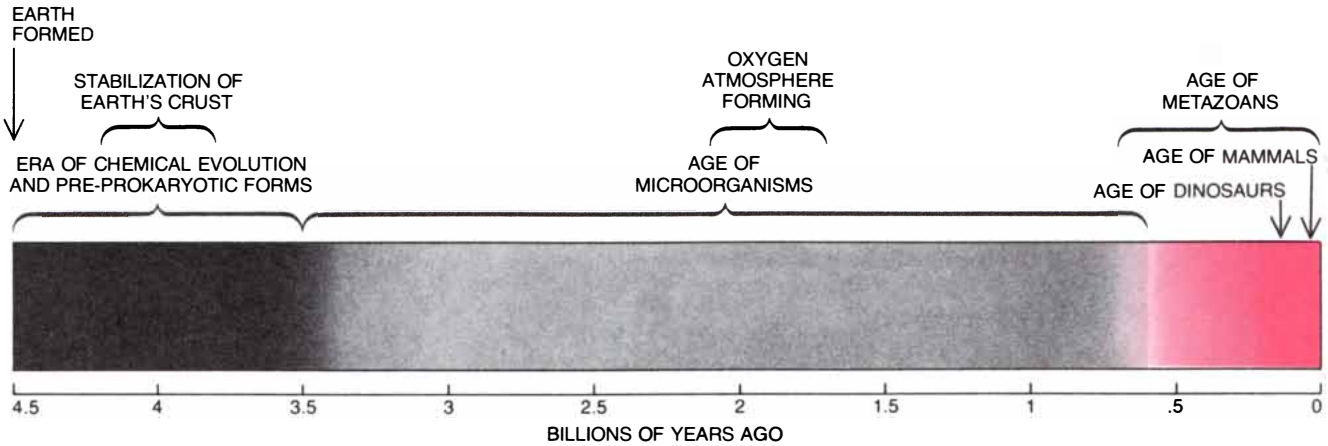
In order to read the biochemical record it was necessary to develop a technology for determining (at least in part) the sequence of a gene or of the RNA or protein product encoded by a gene. For proteins this has been possible for about 25 years, but the direct sequencing of DNA and RNA has been feasible for only the past five years or so. The new technology for sequencing nucleic acids should enable biologists to uncover in relatively short order far more about the history of life than had been thought possible. It was by applying techniques of sequencing to the century-old problem of the natural relations among bacteria that my colleagues and I recognized the archaebacteria as a third form of life.

Eukaryotes and Prokaryotes

In order to appreciate the special status of the archaebacteria it is helpful to consider some of the defining characteristics of eukaryotes and prokaryotes. The eukaryotic cell is comparatively large: roughly 10 micrometers on a side. It is surrounded by a double membrane,



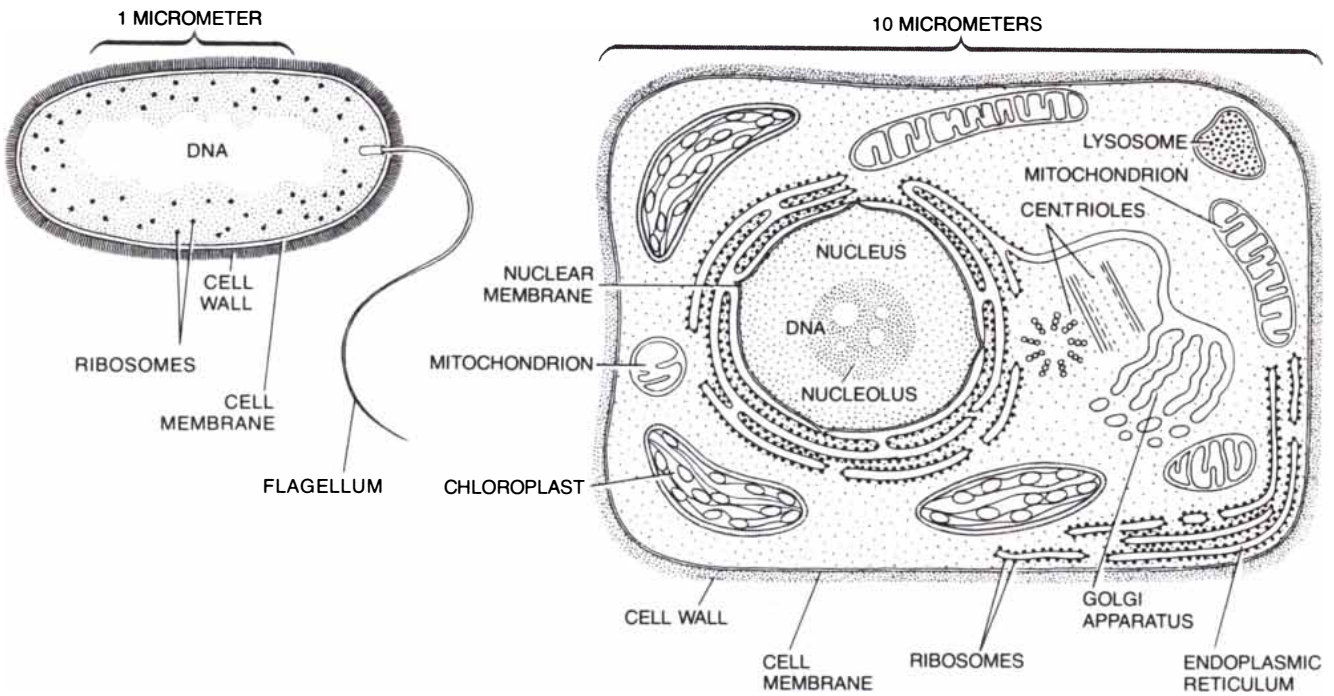
THREE PRIMARY KINGDOMS are proposed by the author to comport with the discovery that the archaebacteria are fundamentally different from all other bacteria, which are designated the eubacteria, or true bacteria. Both eubacteria and archaebacteria are alike in being prokaryotic cells: simple cells that lack a nucleus and are very different in their structural properties from eukaryotic cells, which have a nucleus and several other subcellular organelles. Genealogically, however, archaebacteria and eubacteria are no more closely related to each other than either group is to eukaryotes. It is proposed that the archaebacteria, the eubacteria and an urkaryote—the original eukaryotic cell—stemmed from a common ancestor (the progenote) much simpler than the simplest present-day cells (prokaryotes). Eukaryotes evolved after the urkaryote became a "host" for bacterial endosymbionts that developed into mitochondrion and chloroplast.



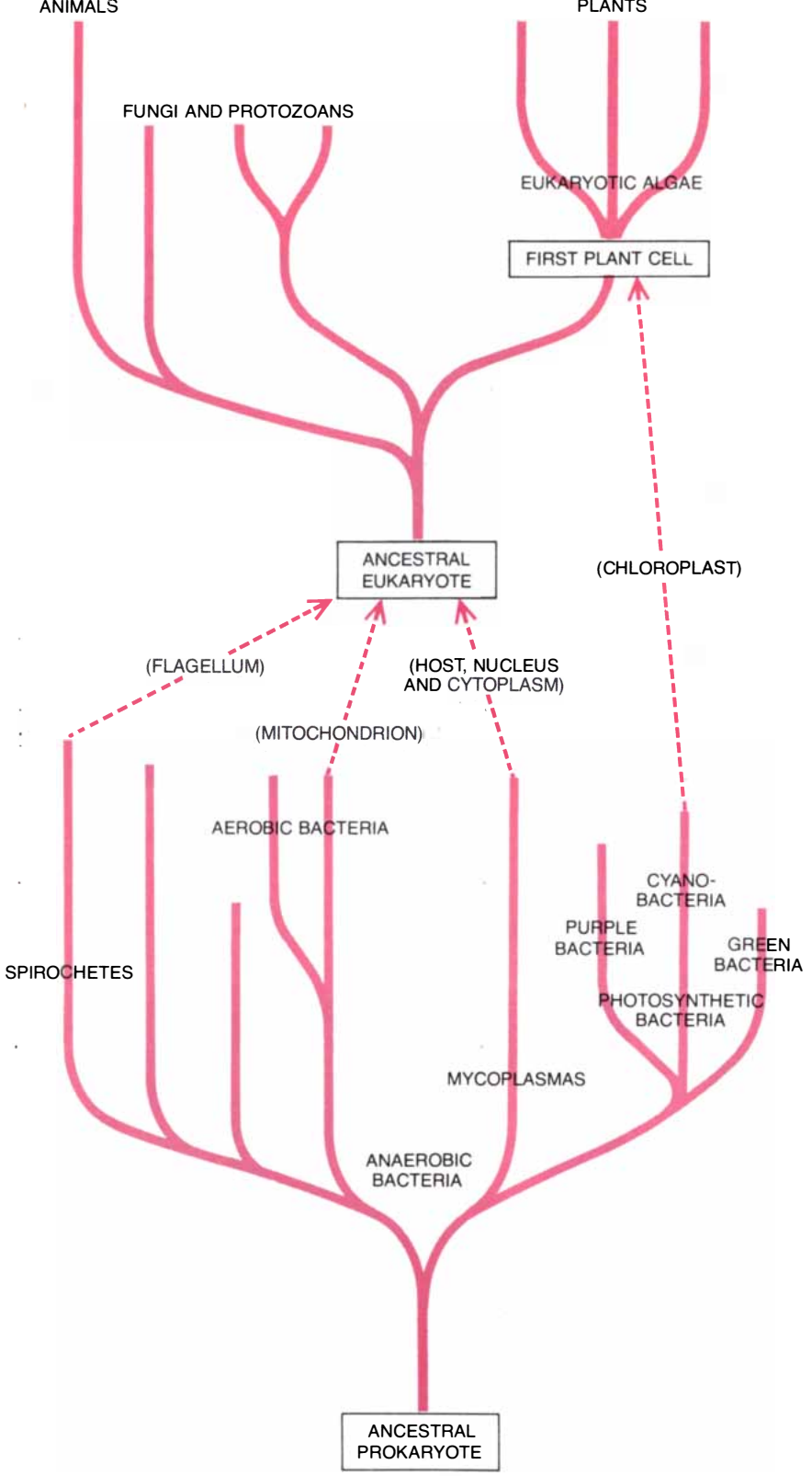
AGE OF MICROORGANISMS, which lasted some three billion years, dominates the time span of biological evolution. Microfossils of prokaryotic cells have been found in deposits 3.5 billion years old, and those cells must have been preceded by simpler ones. The earliest eukaryotic fossils are only about 1.3 billion years old. Almost nothing is known about evolution during the age of microorganisms. The macroscopic fossil record goes back only about 600 million years to the time of the earliest metazoans, or multicellular organisms.

within which a number of structures can be discerned that are themselves defined by membranes. The nucleus contains the bulk of the cell's genetic material. The rod-shaped mitochondria are the site of cellular respiration, which generates the cell's main energy currency, adenosine triphosphate (ATP). In plant cells the chloroplast, another rod-shaped body, converts the energy of light into the chemical energy of ATP. Other specialized structures such as the Golgi apparatus (for secretion) and cilia (for motility) are often present. Many eukaryotic cells are laced with a membrane system, the endoplasmic reticulum, that provides a surface on which important reactions such as the synthesis of proteins take place.

The prokaryotic cell is vastly different. It is typically far smaller than the eukaryotic cell: by a factor of 10 in linear measure and hence by a factor of 1,000 in volume. The prokaryotic cell too is circumscribed by a double membrane, and in addition it almost always has a rigid cell wall. On the other hand, none of the internal structures characteristic of the eukaryotic cell are present; there are no mitochondria or chloroplasts and of course there is no membrane-bounded nucleus. The genome—the total complement of genetic



PROKARYOTES AND EUKARYOTES are fundamentally different at the structural level, as is shown by these schematized drawings of a typical prokaryotic cell (*left*) and eukaryotic cell (*right*). The prokaryote is by far the smaller cell. Little subcellular structure is seen even at the scale revealed by the electron microscope; a single circular strand of the genetic material DNA lies loose in the cytoplasm. Both the archaebacteria and the eubacteria are prokaryotes and share prokaryotic structural properties. The eukaryotic cell is much larger and has a number of discrete subcellular structures. Its DNA, complexed with proteins, is organized into chromosomes within a membrane-bounded nucleus. Mitochondria carry out cellular respiration; in a plant cell there are chloroplasts, which conduct photosynthesis. The Golgi apparatus is a secretory organelle; the endoplasmic reticulum is a membrane system along parts of which some of the cell's ribosomes (on which genetic information is translated into protein) are arrayed. All cells more complex than the bacteria are eukaryotes.



CONVENTIONAL TREE OF LIFE prior to the discovery of archaebacteria had two primary lines of descent: prokaryotic and eukaryotic, the latter derived from the former. The first cells were assumed to have been anaerobic bacteria (prokaryotes) that derived energy from fermentation. They gave rise to a variety of sublines. After the atmosphere became enriched with oxygen, certain anaerobic cells that had lost their cell wall (mycoplasmas) established an endosymbiotic relation with smaller bacteria they had ingested. An endosymbiotic aerobic (oxygen-respiring) bacterium evolved into the mitochondrion, a photosynthesizing cyanobacterium into the chloroplast and (perhaps) a spirochete into the flagellum (an organ of motility). In this way an ancestral eukaryotic cell evolved, and it in turn gave rise to the protozoa, fungi, animals and plants. The drawing is based on a scheme devised by Lynn Margulis of Boston University.

material—is limited to between 2,000 and 3,000 genes in a prokaryotic cell; the typical eukaryotic genome is larger by several orders of magnitude.

The distinction between eukaryotes and prokaryotes was initially defined in terms of subcellular structures visible with a microscope. At that level all cells appeared to be either large and complex, and so eukaryotic, or small and simple, and so prokaryotic. The distinction between the two cell types was ultimately carried to the most basic biological level, the level of molecules. Here eukaryotic and prokaryotic cells have many features in common. For instance, they translate genetic information into proteins according to the same genetic code. Even where the molecular processes are the same, however, the details in the two forms are different; they are either characteristically eukaryotic or characteristically prokaryotic. For example, the amino acid sequences of various enzymes tend to be typically prokaryotic or eukaryotic. All these differences between groups and similarities within each group made it seem certain to most biologists that the tree of life had two main stems, one stem prokaryotic and the other eukaryotic.

That conclusion was drawn too hastily; the aesthetic appeal of a dichotomy was too great. Simply because there are two types of cell at the microscopic level it does not follow that there must be only two types at the molecular level.

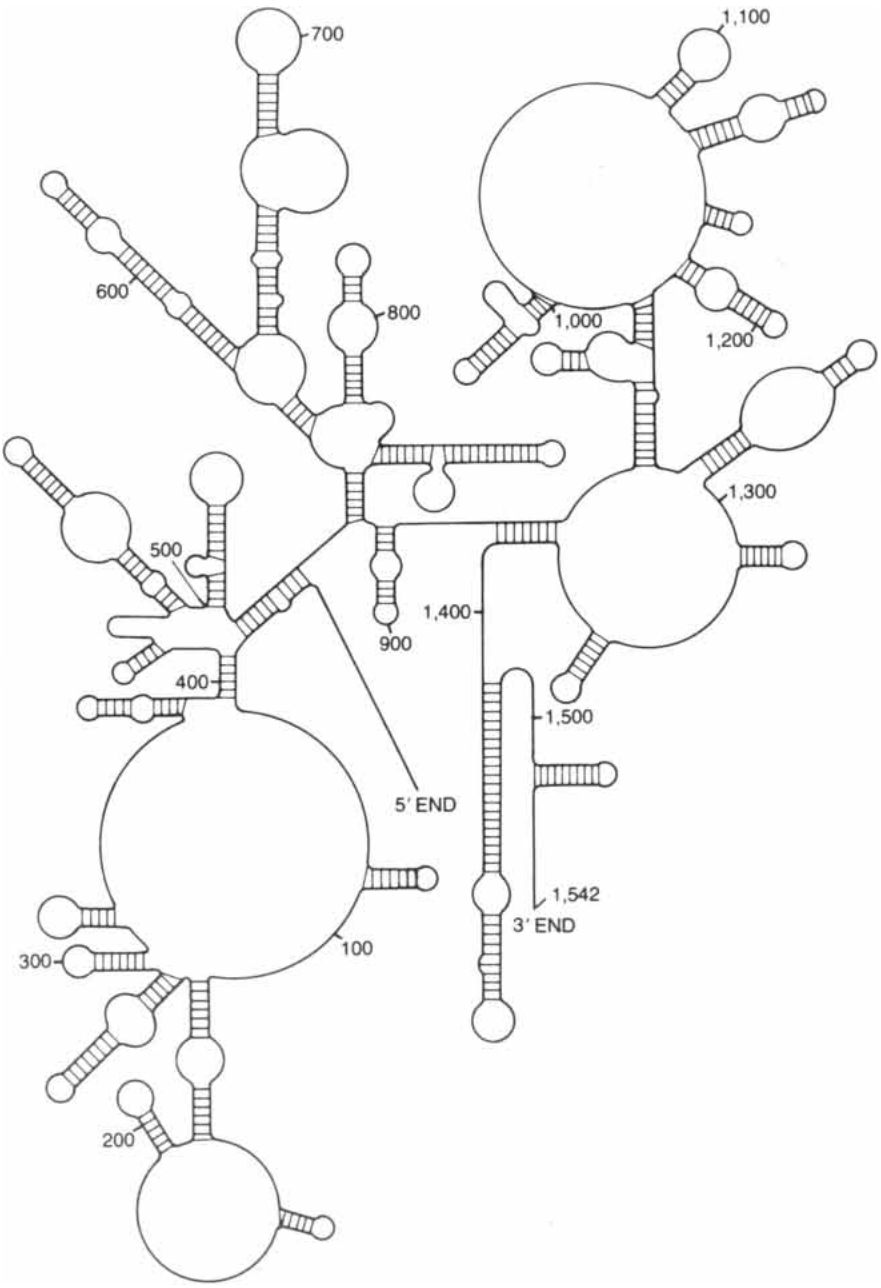
The evolutionary relation of prokaryotes and eukaryotes is actually more complicated than the evidence cited above would indicate. Two eukaryotic organelles, the mitochondrion and the chloroplast, each have their own DNA. Moreover, the pigments in the chloroplast (the chlorophylls and the carotenoids) are similar to those found in the cyanobacteria. Both mitochondria and chloroplasts are the size of bacteria; their apparatus for translating genetic information into proteins differs from the eukaryotic cell's own apparatus and has a number of properties in common with that of prokaryotes.

These facts and others have led to the idea that mitochondria and chloroplasts are descended from prokaryotes that became trapped in a larger cell and established an endosymbiotic relation with it. The mitochondrion is thought to have been a respiring bacterium and the chloroplast to have been a photosynthesizing relative of the cyanobacteria. This conjecture, which in its simplest form is more than a century old, was essentially proved in the case of the chloroplast by the demonstration that the nucleotide sequence of one of the kinds of RNA in the organelle, ribosomal RNA, is specifically related to ribosomal-RNA sequences in cyanobacteria. Similarly, the ribosomal RNA of the mitochondrion in plants appears to be of the bacterial

type. Thus it seems that at least two lines of prokaryotic descent are represented in the eukaryotic cell.

The Urkaryote

Logically the next question is: Where does the rest of the eukaryotic cell come from? What was the original host cell: the urkaryote? It is generally agreed that the bulk of the eukaryotic cell (the nucleus and the cytoplasmic structures) represents a separate line of descent. The exact nature of the ancestral cell is not clear. Most investigators have tended to believe the main eukaryotic line also arose from among the ordinary bacteria. The idea is that some anaerobic bacterium deriving its energy from the fermentation of nutrients (rather than from their oxidation) at some point happened to lose its tough cell wall. Organisms of this kind are known; they are the mycoplasmas. A strain of mycoplasma then evolved the capacity to engulf other organisms, an ability retained by many eukaryotes today. Among the many kinds of organisms such a mycoplasma might have ingested two appear to have established a stable endosymbiotic relation with their host and to have become the mitochondrion and the chloroplast. In this way the eukaryotic cell was born. (The origin of its defining characteristic, the membrane-bounded nucleus, is still far from clear.)



16*S* RIBOSOMAL RNA is the molecule whose nucleotide sequences in a number of organisms have been compared in order to establish phylogenetic relations. The molecule is a component of the ribosome, the molecular machine that synthesizes proteins; the designation 16*S* refers to the speed with which the molecule sediments in a centrifuge, measured in Svedberg units. The RNA molecule is a long chain of the subunits called nucleotides, each of which is characterized by one of four bases: adenine (*A*), uracil (*U*), guanine (*G*) or cytosine (*C*). The first two bases and the last two are complementary: they can be linked by hydrogen bonds to form pairs, *A* pairing with *U* and *G* pairing with *C*. Base pairing determines what is called the secondary structure of the molecule, or the way in which it initially folds, by forming some 50 short double-strand structures in which the bases are paired (*barred regions*). The drawing shows secondary structure of the 16*S* RNA of the eubacterium *Escherichia coli*, full sequence of which was determined by Harry F. Noller, Jr., of the University of California at Santa Cruz.

This view is satisfying in some respects, but it fails to explain the many differences between eukaryotes and prokaryotes. In particular it does not account for the different details of molecular processes or for the large differences in the amino acid sequences of functionally analogous proteins in the two kinds of cells. It is often taken for granted that the differences are merely a consequence of the many small changes in cellular design that would be necessary in passing from the simple prokaryotic condition to the more complex eukaryotic one. It is questionable that so many changes (changes in the composition of almost all enzymes, for example) can reasonably be accounted for in this way.

Essentially for this reason some biologists think the line of descent that gave rise to the putative urkaryotic species may have diverged from the prokaryotic line at some earlier point, before the ancestor of the bacteria had itself arisen. The urkaryote could then have evolved independently to a form comparable in complexity to that of the bacteria. Such an assumption would at least provide more time for differences to emerge between prokaryotes and the urkaryote. The urkaryote, then, would represent a line of descent distinct from that of the prokaryotes, in accordance with the basic phylogenetic dichotomy.

So it stood at the beginning of the 1970's. The phylogeny of the higher eukaryotes, spanning some 500 million years, was reasonably well understood except for the all-important joining of the main eukaryotic branches. There was a definite, widely accepted hypothesis concerning the way in which the eukaryotic cell had evolved. Tests of the hypothesis and answers to the remaining questions, however, lay in the unexplored recesses of bacterial phylogeny, in the age of microorganisms.

Genetic Sequencing

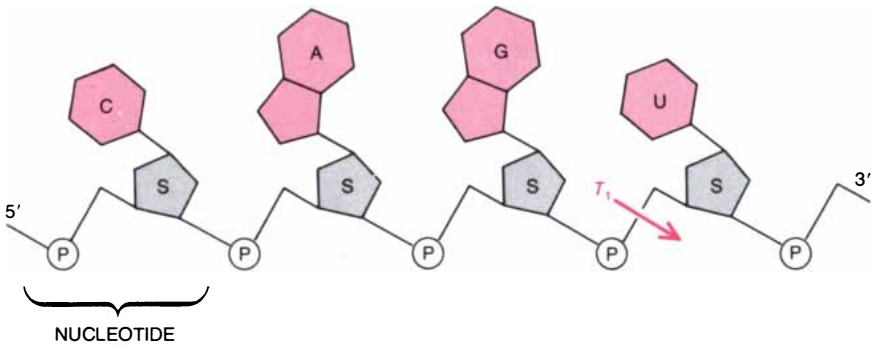
Bacteria constitute a world of extraordinary variety, far more than the microscope reveals. The ecological niches in

which they are found far exceed in variety those occupied by the higher forms of life. For a century microbiologists have tried in vain to understand the natural relations among bacteria and to impose some order on the bewildering array of forms, physiologies and ecologies. Variety among bacteria is mostly variety within simplicity, and so it provides little information about phylogenetic relations. In higher organisms the eye, for example, has evolved a number of times, but the eye is complicated enough for the independently evolved examples to be readily distinguishable from one another. Such is generally not the case for the form and structure of bacteria; rods, spheres and spirals, which are the typical bacterial shapes, are easily arrived at and have evolved many times. The same principle applies to bacterial biochemistry. Although some bacterial characteristics are valid phylogenetic indicators, it is impossible to tell in advance which ones are and which are not.

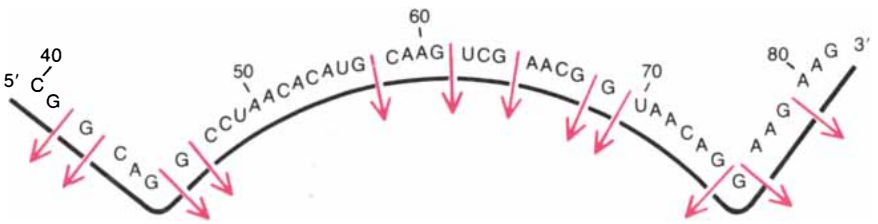
The simplest way in which the cell is a record of its past is in terms of genetic sequences. Every gene that exists in a cell today is a copy of a gene that existed eons ago. It is not an exact copy because mutations have altered the original genetic sequence, but vestiges of the original state often persist. What makes a gene a superb record of the past is its simplicity (it is a linear array) and the fact that genetic-sequence "space" is enormous, so that over the entire span of evolution only a small fraction of the possible genetic sequences can ever be realized. Hence if two genes are similar over a stretch comprising a significant number of nucleotides, this can only mean they have an ancestor in common; such genetically related molecules are said to be homologous.

A genetic sequence yields three kinds of evolutionary information. The sequence can reveal genealogical relations, it can measure evolutionary time and it is a record of ancestral characteristics. To the extent that two genes for the same function in different organisms are related, the organisms are related. The extent to which two such sequences differ measures the time since the organisms diverged from a common ancestor. From an extensive set of related sequences one can construct a phylogenetic tree in which the branch points measure (approximately) the relative times of the bifurcations. Finally, comparisons among an extensive set of homologous sequences make it possible to reconstruct with some accuracy various ancestral versions of a gene.

Since the relation between a gene and its product (either a protein or one of several kinds of RNA molecule) is generally a colinear one, the sequence of the product is ordinarily as useful for phylogenetic studies as the sequence of the gene itself. Because until recently only proteins could be sequenced it was through comparisons of proteins that the first phylogenies based on molecular evolution were constructed. Comparisons of the respiratory protein cytochrome *c* proved to be particularly valuable for confirming and extending the phylogenetic tree of the higher organisms. On the other hand, molecules such as cytochrome *c* are not as effective in establishing relations among bacteria. Such proteins are not universally distributed; they are not strictly constant in function and so are not entirely comparable, and because of the greater antiquity of bacterial lineages differences in sequence can be far greater among bacteria than they are among eukaryotes. These factors make bacterial phylogenies deduced from protein evolution incomplete and uncertain.



EACH NUCLEOTIDE in an RNA molecule is composed of a base, a ribose sugar (*S*) and a phosphate group (*P*). The enzymes called ribonucleases cleave the chain at specific sites. T_1 ribonuclease cuts it by hydrolysis (insertion of a water molecule) on the 3′ side of the phosphate that follows any guanine nucleotide. It therefore cuts a long RNA molecule into a number of short fragments, each consisting of one nucleotide or more and ending with guanine (*G*).



EFFECT OF T_1 RIBONUCLEASE on a short stretch of the *E. coli* 16*S* molecule is demonstrated. When a typical 16*S* RNA is cleaved in this way, its sequence is cut into fragments ("words") ranging in length from one nucleotide ("letter") to 20 nucleotides. The base sequence of each such word is determined. Words of six letters or more are compiled into a dictionary. Dictionaries of two organisms can be compared in terms of an association coefficient S_{AB}. The coefficient is a fraction equal to twice the number of letters in words (at least six letters long) common to organisms *A* and *B*, divided by the total number of letters in all such words in *A* and *B*.

Ribosomal RNA

There are other gene products that can serve as indicators of bacterial relations. All self-replicating entities necessarily have systems for maintaining and propagating genetic information and for translating it into the chains of amino acids that constitute proteins. Most of the large molecules engaged in these processes must trace their origin back to the very early stages in the evolution of the cell; they certainly emerged before cells became complex enough to be called prokaryotes. Therefore one would expect these molecules to have the requisite properties of a phylogenetic marker.

The most reasonable first choices among such molecules are the RNA molecules that are complexed with proteins to form the ribosomes. It is on the ribosomes that genetic information is translated into proteins. The ribosomal RNA is easy to isolate in workable quantities because a typical bacterial cell has from 10,000 to 20,000 ribosomes. Moreover, ribosomal-RNA molecules seem to have remained constant in function over great evolutionary distances. This is important because functional changes in a molecule bring with them additional changes in sequence that make it difficult or even impossible to compare one molecular sequence with another and thereby deduce phylogenetic relations. Still another advantage of the ribosomal RNA's is that at

least some portions of their sequences change slowly enough for the common ancestral sequence not to be totally obliterated. In other words, the sequences make it possible to detect the deepest phylogenetic relations.

There are three kinds of ribosomal-RNA molecules. In bacteria the "large" ribosomal RNA is the 23*S* RNA (*S* stands for Svedberg unit, a measure of the rate of sedimentation in an ultracentrifuge and hence an indirect measure of molecular size); it is approximately 2,900 nucleotides long. The "small" one, designated 16*S* ribosomal RNA, is about 1,540 nucleotides long. A very small one (5*S*) has only 120 nucleotides. The sizes are similar in eukaryotic cells: 18*S*, 25–28*S* and 5*S*. One might think that ease of characterization would make the small 5*S* RNA the most suitable one for phylogenetic studies. Actually it is not as accurate an indicator of phylogenetic relations as the larger ribosomal RNA's, chiefly for statistical reasons. (The 5*S* RNA sometimes exhibits anomalous large differences in sequence from one species to another.) The 16*S* ribosomal RNA is the molecule of choice, because the 23*S* molecule is almost twice as large and more than twice as difficult to characterize.

RNA Dictionaries

At the University of Illinois in 1969 I decided to explore bacterial relations by comparing the sequences of the 16*S* ribosomal RNA's in different species. It was not yet feasible (as it is now) to determine the nucleotide sequence of the entire molecule. The technology did exist, however, for sequencing short segments of the molecule. The enzymes called ribonucleases yield short fragments of RNA by cutting an RNA strand at specific sites. Each nucleotide of RNA is composed of a sugar called ribose, a phosphate group and one of four nitrogenous bases: adenine (*A*), uracil (*U*), guanine (*G*) or cytosine (*C*). The enzyme T_1 ribonuclease cuts an RNA strand at a particular bond on one side of each nucleotide that incorporates a guanine base. The T_1 enzyme therefore digests an RNA "text" into short "words," called oligonucleotides. Each oligonucleotide includes, and ends with, a single *G*, as in *AACUCG* or *UCCUAUCG*.

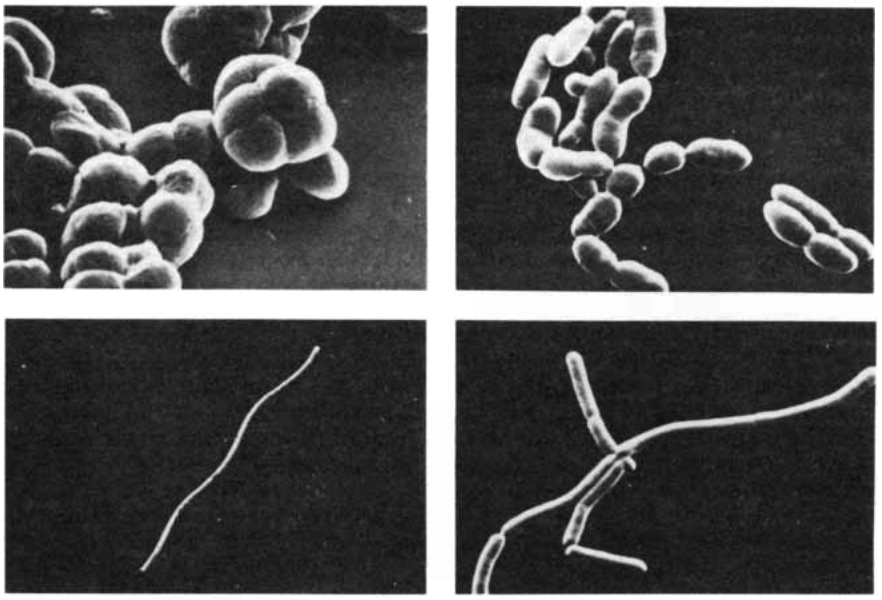
The oligonucleotides made in this way were short enough to be sequenced by the available techniques. The smallest words are of little value because they recur many times in each molecule. By the time the word length reaches six nucleotides, however, a particular sequence is unlikely to appear more than once in a 16*S* RNA molecule. (Given the constant terminal *G*, there are 3^5, or 243, possible six-letter sequences of this kind, and a typical 16*S* RNA molecule has roughly 25 such words.) When 16*S* RNA's from different organisms include the same six-letter sequence, it almost always reflects a true homology. By confining attention to words six letters long or longer one can generate a "dictionary" characteristic of a given organism, which can readily be compared with other such dictionaries to determine genealogical relations.

A simple way to analyze the data is in terms of an association coefficient S_{AB}, which is defined as twice the number of nucleotides in the words common to both of the dictionaries *A* and *B* divided by the number of nucleotides in all words in the two dictionaries. S_{AB} ranges from 1 when dictionaries *A* and *B* are identical to less than .1 when they are unrelated. (The coefficient is usually greater than zero even for unrelated sequences because of chance correspondences.) By compiling the S_{AB} values for a number of organisms in a matrix one can discern a pattern of relatedness or unrelatedness among organisms. Moreover, it is possible by straightforward statistical methods to construct from a set of S_{AB} values for a group of organisms a dendrogram, or tree, showing the relations among members of the group.

To date the ribosomal RNA's of almost 200 species of bacteria and eukaryotes have been characterized. Most of the bacteria form a coherent but very large (which is to say ancient) group. They are the eubacteria, or true bacteria, and as would be expected they are quite distinct from the eukaryotes. The relations among the various genera (represented by the branchings of the tree) determined through ribosomal-RNA analysis are at variance with many of the established prejudices about bacterial relations. What is important at this point is that the eubacteria are divided into a number of major branches and

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Saccharomyces cerevisiae*	—	.29	.33	.05	.06	.08	.09	.11	.08	.11	.11	.08	.08	.10	.07	.08
2	*Lemna minor*	.29	—	.36	.10	.05	.06	.10	.09	.11	.10	.10	.13	.07	.09	.07	.09
3	*L* cell	.33	.36	—	.06	.06	.07	.07	.09	.06	.10	.10	.09	.07	.11	.06	.07
4	*Escherichia coli*	.05	.10	.06	—	.24	.25	.28	.26	.21	.11	.12	.07	.12	.07	.07	.09
5	*Chlorobium vibrioforme*	.06	.05	.06	.24	—	.22	.22	.20	.19	.06	.07	.06	.09	.07	.05	.07
6	*Bacillus firmus*	.08	.06	.07	.25	.22	—	.34	.26	.20	.11	.13	.06	.12	.10	.07	.09
7	*Corynebacterium diphtheriae*	.09	.10	.07	.28	.22	.34	—	.23	.21	.12	.12	.09	.10	.10	.06	.09
8	*Aphanocapsa*	.11	.09	.09	.26	.20	.26	.23	—	.31	.11	.11	.10	.10	.13	.10	.10
9	Chloroplast (*Lemna*)	.08	.11	.06	.21	.19	.20	.21	.31	—	.14	.12	.10	.12	.12	.06	.07
10	*Methanobacterium thermoautotrophicum*	.11	.10	.10	.11	.06	.11	.12	.11	.14	—	.51	.25	.30	.34	.17	.19
11	*Methanobrevibacter ruminantium*	.11	.10	.10	.12	.07	.13	.12	.11	.12	.51	—	.25	.24	.31	.15	.20
12	*Methanogenium cariaci*	.08	.13	.09	.07	.06	.06	.09	.10	.10	.25	.25	—	.32	.29	.13	.21
13	*Methanosarcina barkeri*	.08	.07	.07	.12	.09	.12	.10	.10	.12	.30	.24	.32	—	.28	.16	.23
14	*Halobacterium halobium*	.10	.09	.11	.07	.07	.10	.10	.13	.12	.34	.31	.29	.28	—	.19	.23
15	*Sulfolobus acidocaldarius*	.07	.07	.06	.07	.05	.07	.06	.10	.06	.17	.15	.13	.16	.19	—	.13
16	*Thermoplasma acidophilum*	.08	.09	.07	.09	.07	.09	.09	.10	.07	.19	.20	.21	.23	.23	.13	—

MATRIX OF ASSOCIATION COEFFICIENTS reveals the degree of relatedness of any two organisms; the higher the S_{AB} fraction, the closer the relation. The pattern is significant. The eukaryotes (*1–3*), the eubacteria (*4–9*) and the archaebacteria (*10–16*) each form a distinct group (*color*). The archaebacteria are no more closely related to the eubacteria than to the eukaryotes. *Saccharomyces* is yeast; *Lemna* is duckweed; *L* cells are a line of mouse cells. Chloroplast is descended from endosymbiotic cyanobacterium and is therefore eubacterial.



METHANOGENS, anaerobic bacteria that generate methane (CH_4) from hydrogen and carbon dioxide, make up the largest group of archaebacteria identified so far. Four genera of methanogens that differ widely in size and morphology are seen here in scanning electron micrographs made by Alexander J. B. Zehnder of the Swiss Federal Institute of Technology. They are *Methanosarcina* (*top left*), *Methanobrevibacter* (*top right*), *Methanospirillum* (*bottom left*) and *Methanobacterium* (*bottom right*). The cells are shown enlarged respectively 2,500, 5,000, 1,000 and 5,000 diameters. The methanogens are found only in oxygen-free environments.

that several of the branches include photosynthetic bacteria. This finding suggests all eubacteria stem from a common photosynthetic ancestor.

The Discovery of Archaebacteria

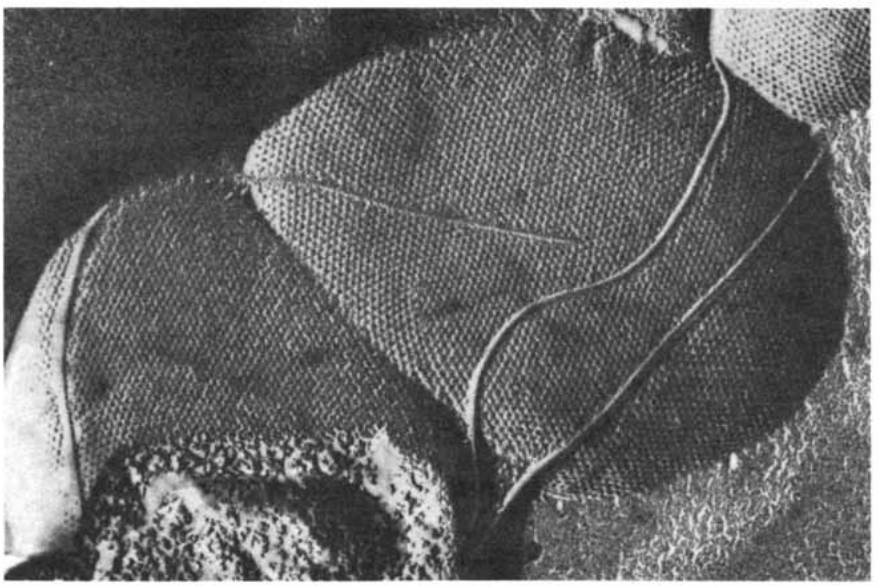
As the screening of bacteria continued a surprise emerged. In collaboration with Ralph S. Wolfe I looked at the ribosomal RNA of the methanogenic bacteria. These unusual organisms live only in oxygen-free environments and generate methane (CH_4) by the reduction of carbon dioxide (CO_2). We discovered that methanogens do not fall within the phylogenetic group defined by the other bacteria. Indeed, they appear to represent an evolutionary branching that far antedated the common ancestor of all true bacteria. Not only were the methanogens separate but also the group they formed seemed to be about as deep phylogenetically—as ancient—as the group defined by the eubacteria.

There can be no doubt that the methanogens and their relatives are bacteria. They are the size of bacteria, they have no nuclear membrane, they have a low DNA content and so on. Surely, then, one would have expected them to be related more closely to other bacteria than to eukaryotes. Our analysis showed they are not. Methanogens are related as closely to eukaryotes as to eubacteria.

How could this be? There were supposed to be only two primary lines of descent, the eukaryotic and the prokaryotic. Here was a new group of organisms: the methanogens and their relatives, which together have come to be called archaebacteria. They were obviously like other bacteria in their superficial characteristics, and so they had been assumed to be in the prokaryotic line of descent. It is not striking differences in morphological characteristics, however, that distinguish the prokaryote phylogenetically from the eukaryotic cell; it is the subtler and more ancient differences in molecular sequences and in details of function at the molecular level that distinguish them. Hence there is no reason two prokaryotic lines of descent cannot be just as distinct from each other as either one is from the eukaryotic line.

This idea was too novel to be easily accepted, and initially some biologists rejected out of hand the notion of a "third form of life." How could something that looked like a bacterium not be a bacterium and indeed not be related to bacteria? In time the simplicity of our argument and the accumulation of evidence prevailed. Although a few biologists still dispute our interpretation, the idea that archaebacteria represent a separate grouping at the highest level is becoming generally accepted.

The supposed great antiquity of the archaebacteria remains an unproved prejudice, but it is a plausible one. The methanogenic phenotype seems to cover a phylogenetic span as great as or greater than the span covered by any other comparable bacterial phenotype. This implies that the methanogens are as old as or older than any other bacterial group. Moreover, methanogenic metabolism (the reduction of carbon dioxide to methane) is ideally suited to the kind of atmosphere thought to have existed on the primitive earth: one that was rich in carbon dioxide and included some hydrogen but virtually no oxygen. The name archaebacteria implies that these organisms were the dominant ones in the primeval biosphere. When conditions changed, the methanogens' need



UNUSUAL CELL WALL of *Methanogenium marisnigri*, a methanogen found on the floor of the Black Sea, is enlarged 70,000 diameters in a scanning electron micrograph made by Frank Mayer of the University of Göttingen. The mosaic pattern of proteinaceous subunits is characteristic of several archaebacteria. It is different from the typical eubacterial cell wall made up of peptidoglycan subunits, which are not components of cell wall of archaebacteria.

for an anaerobic environment confined them to a limited range of relatively inaccessible niches.

The measurements that revealed the existence of the archaebacteria (differences in RNA sequences) were genetic ones and were purely quantitative. They revealed nothing about the quality of the differences—the phenotypic differences—between the archaebacteria and the true bacteria. If our interpretation of the archaebacteria as a primary kingdom separate from that of the true bacteria is correct, then on detailed inspection the archaebacteria should prove to be as different from true bacteria in their molecular phenotype as either group is from eukaryotic cells.

Archaebacterial Forms

The archaebacteria are indeed unusual organisms. The group is now known to include three very different kinds of bacteria: methanogens, extreme halophiles and thermoacidophiles.

The dominant form (in the sense that it constitutes a deep phylogenetic grouping) is the methanogen. Bacteria that give off methane have been known for some time. Alessandro Volta discovered in 1776 that "combustible air" is generated in bogs, streams and lakes whose sediments are rich in decaying vegetation, but the fact that a microorganism is responsible for generating "marsh gas" became known only much later. Methanogens are widely distributed in nature, but they are not commonly encountered because they are killed by oxygen and do not exist in the open.

In ancient times methanogens could have existed almost anywhere. Today they live only where oxygen has been excluded and where hydrogen and carbon dioxide are available. This generally means living in close association with other bacteria, such as the clostridia, that metabolize decaying organic matter and give off hydrogen as a waste product. Methanogens are found in stagnant water and in sewage-treatment plants (in amounts that have made it commercially feasible to manufacture methane). They are also found in the rumen of cattle and other ruminants and in the intestinal tract of animals in general. Methanogens can be isolated from the ocean bottom and from hot springs. In spite of their intolerance of oxygen they are obviously globally distributed.

The extreme halophiles are bacteria that require high concentrations of salt in order to survive; some of them grow readily in saturated brine. They can give a red color to salt evaporation ponds and can discolor and spoil salted fish. The extreme halophiles grow in salty habitats along the ocean borders and in inland waters such as the Great Salt Lake and the Dead Sea. Although the extreme halophiles have been studied by microbiologists for a long time, they have recently become particularly interesting for two reasons. They maintain large gradients in the concentration of certain ions across their cell membrane and exploit the gradients to move a variety of substances into and out of the cell. In addition the extreme halophiles have a comparatively simple photosynthetic mechanism based not on chlorophyll but on a membrane-bound pigment, bacterial rhodopsin, that is remarkably like one of the visual pigments.

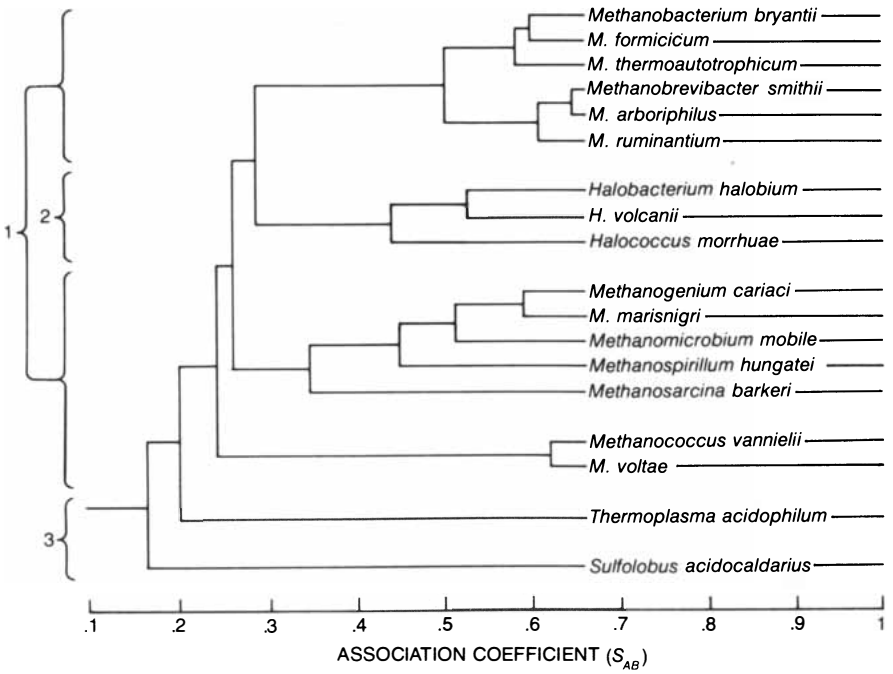
The Thermoacidophiles

The third known type of archaebacterium is the thermoacidophile, and the members of this group too are notable for their habitat. *Sulfolobus,* one of the two genera of thermoacidophiles, is found in hot sulfur springs. Its various species generally grow at temperatures near 80 degrees Celsius (176 degrees Fahrenheit); growth at temperatures above 90 degrees has been observed for some varieties. Moreover, the springs in which *Sulfolobus* flourishes are extremely acidic; *p*H values lower than 2 are common (a *p*H of 7 is neutral). *Thermoplasma,* the other genus of thermoacidophile, has so far been found only in smoldering piles of coal tailings. It is a mycoplasma: it has no cell wall but merely the limiting cell membrane.

Although archaebacterial thermoacidophiles can grow only in an acidic environment, the internal milieu of the cell has a quite moderate *p*H, near neutrality; this requires that a sizable *p*H gradient be maintained across the cell membrane. As in the extreme halophiles the gradient may play a role in pumping other molecules into and out of the cell. It is interesting that when the temperature is reduced and as a consequence *Sulfolobus* stops metabolizing, the cell's internal *p*H can no longer be maintained near neutrality and the cell dies.

For some time it had been recognized that various organisms now classified as archaebacteria are individually somewhat peculiar. In each instance the idiosyncrasy had been seen as just that: an adaptation to some peculiar niche or a biochemical quirk. The ribosomal-RNA phylogenetic measurement, however, showed at least some of the idiosyncrasies might instead be general characteristics of a new group of organisms. Thus informed, investigators in many countries have undertaken to find the general properties that link archaebacteria to one another and to see how those properties either distinguish the archaebacteria from the other two major forms or relate them specifically to one or the other of those forms.

One generalization about bacteria has



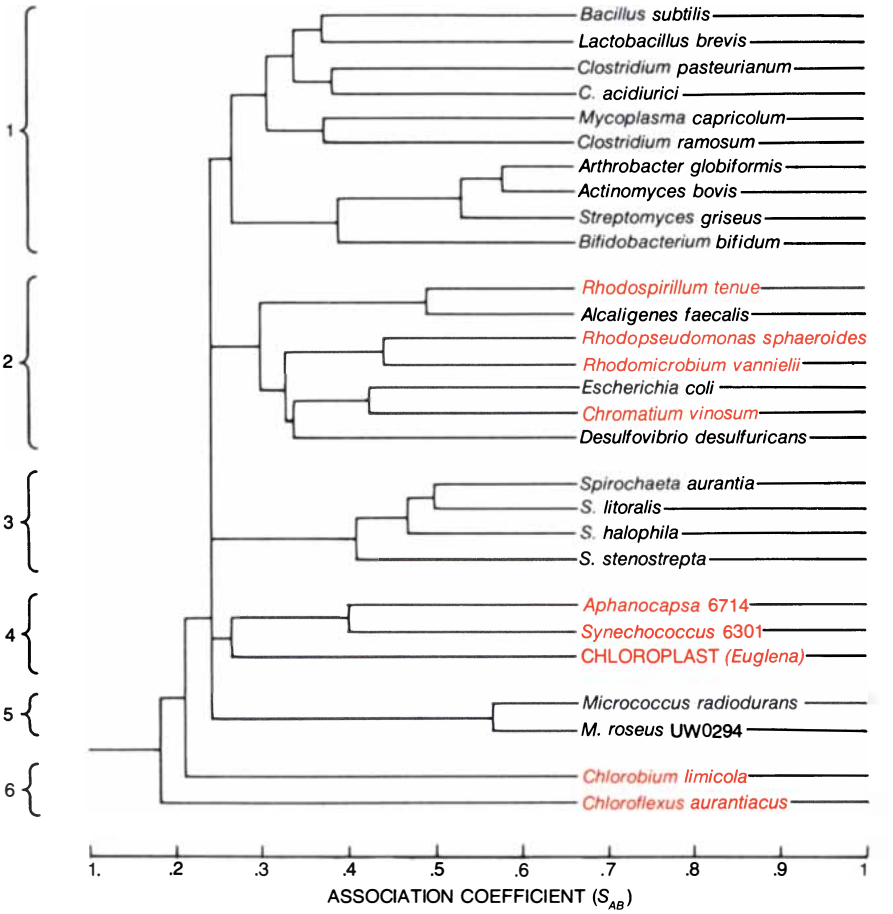
ARCHAEBACTERIAL DENDROGRAM, or tree, is derived from S_{AB} values and shows the phylogenetic relations among members of this primary kingdom. Most of them are methanogens (*1*), which are anaerobes (organisms that survive only in the absence of oxygen) and generate methane by the reduction of carbon dioxide or certain other very simple sources of carbon. The extreme halophiles (*2*) are aerobic (oxygen-respiring) bacteria that exist only in environments with a high salt concentration. The thermoacidophiles (*3*) are aerobic bacteria that live only in very hot, highly acidic environments. The methanogens appear to be an ancient group within which the halophiles arose; the thermoacidophiles may have arisen separately.

been that they have a cell wall incorporating the sugar derivative muramic acid, which is the basis of a complex polymer called a peptidoglycan. One extreme halophile and the thermoacidophile *Sulfolobus* were known to be exceptions to this generalization; they were considered to have an idiosyncratic wall structure. Otto Kandler of the University of Munich, collaborating with Wolfe, made a systematic study of cell-wall structure in other known archaebacteria. All of them turned out to be atypical. The archaebacteria have a variety of wall types, but none of them is of the muramic-acid-based peptidoglycan type.

Lipids and RNA's

It was also known that the cell membrane of the extreme halophiles and of the thermoacidophiles is composed of unusual lipids. The lipids of both eukaryotes and eubacteria consist in the main of two straight-chain fatty acids bound at one end to a glycerol molecule through an ester linkage (–CO–O–). The lipids of the extreme halophiles and the thermoacidophiles are also composed of a glycerol group linked to two long hydrocarbon chains, but the connection between the glycerol and the chains is an ether (–O–) link rather than an ester link. Moreover, the hydrocarbon chains are not straight but branched: every fourth carbon atom in the chain carries a methyl group (CH_3). The basic archaebacterial lipid, in other words, is a diether composed of glycerol and two molecules of an alcohol, phytanol. When a number of methanogens were examined for lipid composition, our expectation was confirmed: their lipids turned out to be typically archaebacterial branched-chain glycerol ethers.

In the course of the ribosomal-RNA studies another unexpected archaebacterial property emerged, one that was to provide the first clue to the significance of the differences between archaebacteria and true bacteria. Central to the process of translation is the transfer-RNA molecule. It recognizes a three-base "codon" in messenger RNA specifying a particular amino acid, and it delivers that amino acid to be incorporated into the protein chain. A number of the nucleotides in a transfer-RNA molecule are modified, that is, their structure is altered chemically after they have been incorporated into the molecule; most often a methyl group is added to the nucleotide at some position on either the base or the sugar. Biologists had come to believe one particular modification was characteristic of a certain position in almost all transfer-RNA molecules in almost all organisms: at that position the base uracil has been methylated to form thymine (which is normally present only in DNA, not in RNA). It turns out that all transfer RNA's of all archaebacteria lack this thymine unit; instead the uracil has been modified in one of two other ways to yield a pseudouridine or an as yet unidentified nucleotide.



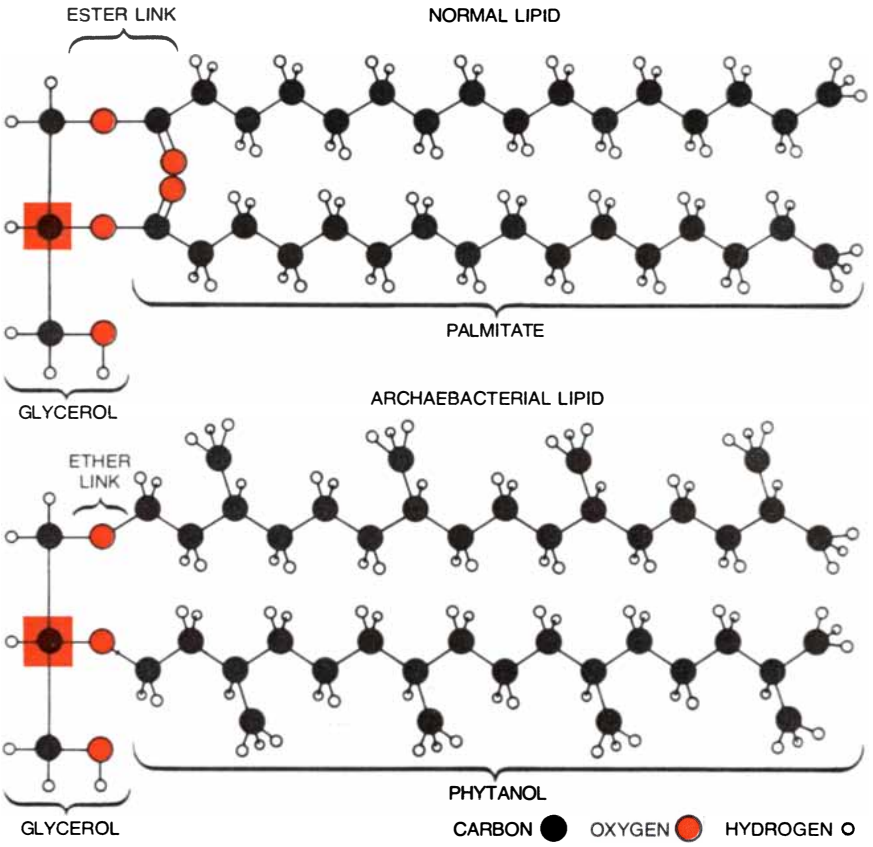
EUBACTERIAL DENDROGRAM shows six major subgroups, three of which include photosynthesizing bacteria (*color*); other groups remain to be defined. The Gram-positive bacteria (*1*) have a thick cell wall with a unique composition that absorbs and retains the Gram stain. The purple photosynthetic bacteria are grouped (*2*) with a number of close relatives that are not photosynthesizers, presumably having lost an ancestral ability. The spirochetes (*3*) are long, spiral bacteria. The cyanobacteria (*4*) are photosynthetic, oxygen-producing organisms; the chloroplast is descended from them. Some spherical bacteria with an atypical cell wall (*5*) are notable for their resistance to radiation. The green photosynthetic bacteria (*6*) are anaerobic.

If one compares both ribosomal RNA and transfer RNA in eukaryotes, eubacteria and archaebacteria, one finds a general pattern, of which the replacement of thymine in archaebacterial transfer RNA's is only one example. The same regions in the RNA's tend to be modified in all three primary lines of descent, but the nature of the modification tends to vary from one kingdom to another. The differences are of two kinds. Either the modification of a given base is different in each of the kingdoms, or a given base is modified in one kingdom and in another kingdom the modification is made to an adjacent base. These modes of variation suggest that the modifications have evolved separately in each major line of descent.

Several other molecular distinctions between the archaebacteria and the other groups are known (for example, in the subunit structure of the enzyme RNA polymerase), but the list is not long. The reason is not that additional differences do not exist; it is rather that the world of archaebacteria remains virtually unexplored. The study of archaebacterial genetics is in a primitive state; few mutants have even been isolated for genetic study. Nothing whatever is known about the control of gene expression in archaebacteria. The basic molecular biology of archaebacteria is not understood. And yet to the extent that the archaebacteria have been characterized they have been found to differ significantly from both of the other major groups.

A New Perspective

The discovery of a new primary kingdom of organisms is a major finding in its own right (comparable to going into



MEMBRANE LIPIDS of archaebacteria are different from the lipids found in other organisms. The lipids of both eubacteria and eukaryotes are glycerol esters of straight-chain fatty acids, that is, they are composed of a three-carbon alcohol, glycerol, attached to fatty-acid chains such as palmitate by an ester link. Archaebacterial lipids, on the other hand, are diethers in which a glycerol unit is connected by an ether link to phytanols: branched chains in which carbon atoms at regular intervals carry a methyl (CH_3) group. Moreover, glycerol has two optical isomers, distinguished by the configuration of the molecule about the central carbon atom (*colored box*); the optical isomers rotate polarized light in opposite directions. The configuration about the central carbon atom of glycerol that is found in archaebacterial lipids is the mirror image of the configuration that is found in both eubacterial and eukaryotic lipids.

the backyard and seeing an organism that is neither a plant nor an animal), but the real importance of the discovery lies in what it may reveal about the early history of life. When there were only two known primary lines of descent, one could not readily interpret the differences between the two. The recognition of three lines of descent equidistant from one another gives a much better perspective for judging which properties are ancestral and which have evolved recently. With the discovery of the archaebacteria two central evolutionary problems therefore become approachable: the nature of the common ancestor of all life and the evolution of the eukaryotic cell.

At what stage in the evolution of the cell did the fundamental division into the primary kingdoms take place? What was the nature of the universal ancestor? The assumption has been that the universal ancestor was a prokaryote, the simplest of today's living forms. Long ago, however, there must have been still simpler forms of the cell. Although nothing is known about such forms, one can make an educated guess as to certain of their general properties.

Consider the following argument. The translation mechanism is complex, comprising on the order of 100 large molecular components. It is also highly accurate in its functioning. In making proteins of the size common today (chains of from 100 to 500 amino acids), obtaining a flawless product 90 percent of the time or more requires an error rate of no more than a few parts in 10,000. Moreover, this accuracy must be attained by a mechanism of molecular dimensions. For such a mechanism to have evolved in a single step is clearly impossible. The primitive version of the mechanism must have been far simpler, smaller and less accurate.

Imprecision in translation would have required the synthesis of proteins that were smaller, and therefore less specific in their action, than proteins are today. (Otherwise the probability of error in making a protein strand would have been too great.) Among the smaller and less specific proteins would have been the enzymes required to process genetic information. If those enzymes were less precise than today's versions are, the cell's mutation rate must necessarily have been higher and the size of its genome correspondingly smaller. The translation process is the link between genotype and phenotype, between information and its expression; as the process evolved to become more precise, the cell itself necessarily passed through a corresponding series of evolutionary refinements. It evolved from an entity having simple properties, imprecise and general functions and a rather small complement of genes to an entity that functioned with many highly specific enzymes and a complex, precise genetic apparatus. To emphasize the primitive genetic and translational mechanisms of the earlier, simpler cells, I call them progenotes.

The Progenote as Ancestor

The discovery of the archaebacteria provides the perspective needed to approach the question of whether the universal ancestor was a prokaryote or a progenote. Although the question is far from settled, the initial indications are that the universal ancestor was indeed a progenote. First of all consider that true bacteria and archaebacteria have probably existed for at least 3.5 billion years. The time needed for the evolution of the first true bacteria or archaebacteria, then, had to be less than a billion years, and perhaps much less. Still, the kinds of evolutionary changes that have arisen within each of the bacterial kingdoms over the later interval of three billion years or more are minor compared with the differences that separate archaebacteria from true bacteria, such as the differences in lipids, in transfer-RNA and ribosomal-RNA sequences and modification patterns and in enzyme-subunit structure.

It would seem that the nature of evolution some four billion years ago was very different from what it was later. This implies that the organisms undergoing the evolution were also very different. The possibility that the universal ancestor was in the process of developing the cell wall is suggested by the fact that archaebacterial cell walls are as unlike true bacterial walls as eukaryotic walls are. Perhaps the universal ancestor was still developing or refining biochemical pathways as well; lipids are synthesized differently in the two bacterial kingdoms, and many coenzymes are different. If archaebacteria should be found to differ from true bacteria in their mechanisms for controlling gene expression (a possibility that has yet to be investigated), the implication would be that their common ancestor may

	ARCHAEBACTERIA	EUBACTERIA	EUKARYOTES
CELL SIZE (LINEAR DIMENSION)	ABOUT 1 MICROMETER	ABOUT 1 MICROMETER	ABOUT 10 MICROMETERS
CELLULAR ORGANELLES	ABSENT	ABSENT	PRESENT
NUCLEAR MEMBRANE	ABSENT	ABSENT	PRESENT
CELL WALL	VARIETY OF TYPES; NONE INCORPORATES MURAMIC ACID	VARIETY WITHIN ONE TYPE; ALL INCORPORATE MURAMIC ACID	NO CELL WALL IN ANIMAL CELLS; VARIETY OF TYPES IN OTHER PHYLA
MEMBRANE LIPIDS	ETHER-LINKED BRANCHED ALIPHATIC CHAINS	ESTER-LINKED STRAIGHT ALIPHATIC CHAINS	ESTER-LINKED STRAIGHT ALIPHATIC CHAINS
TRANSFER RNA'S:			
THYMINE IN "COMMON" ARM	ABSENT	PRESENT IN MOST TRANSFER RNA'S OF MOST SPECIES	PRESENT IN MOST TRANSFER RNA'S OF ALL SPECIES
DIHYDROURACIL	ABSENT IN ALL BUT ONE GENUS	PRESENT IN MOST TRANSFER RNA'S OF ALL SPECIES	PRESENT IN MOST TRANSFER RNA'S OF ALL SPECIES
AMINO ACID CARRIED BY INITIATOR TRANSFER RNA	METHIONINE	FORMYLMETHIONINE	METHIONINE
RIBOSOMES:			
SUBUNIT SIZES	30*S*, 50*S*	30*S*, 50*S*	40*S*, 60*S*
APPROXIMATE LENGTH OF 16*S* (18*S*) RNA	1,500 NUCLEOTIDES	1,500 NUCLEOTIDES	1,800 NUCLEOTIDES
APPROXIMATE LENGTH OF 23*S* (25-28*S*) RNA	2,900 NUCLEOTIDES	2,900 NUCLEOTIDES	3,500 NUCLEOTIDES OR MORE
TRANSLATION-ELONGATION FACTOR	REACTS WITH DIPHTHERIA TOXIN	DOES NOT REACT WITH DIPHTHERIA TOXIN	REACTS WITH DIPHTHERIA TOXIN
SENSITIVITY TO CHLORAMPHENICOL	INSENSITIVE	SENSITIVE	INSENSITIVE
SENSITIVITY TO ANISOMYCIN	SENSITIVE	INSENSITIVE	SENSITIVE
SENSITIVITY TO KANAMYCIN	INSENSITIVE	SENSITIVE	INSENSITIVE
MESSENGER-RNA BINDING SITE *AUCACCUCC* AT 3′ END OF 16*S* (18*S*) RNA	PRESENT	PRESENT	ABSENT

MOLECULAR TRAITS of archaebacteria, eubacteria and eukaryotes clearly distinguish the three primary kingdoms. In some instances the archaebacteria are unique; in others they are similar either to eubacteria or to eukaryotes, as is indicated by color pattern. Thymine and dihydrouracil are modified bases that replace uracil in transfer RNA's. Elongation factor is a component of translation machinery.

have had only rudimentary mechanisms of genetic control.

The key question is whether the universal ancestor was still developing the genotype-phenotype link when it gave rise to its descendant lines. Two observations suggest it may have been. RNA polymerase is the enzyme that transcribes the gene into its messenger-RNA complement (which is then translated into protein). The subunit structure of the RNA polymerase is quite constant among the true bacteria, whereas the archaebacterial polymerase structure is different. Could this mean the RNA-polymerase function was still being refined at the time the two bacterial lines separated?

The second observation concerns the modified nucleotides in transfer and ribosomal RNA's. As I mentioned above, the patterns of modification are almost invariant within any primary kingdom, but they tend to differ between kingdoms. Although the function of modified nucleotides in transfer and ribosomal RNA's is not understood, it is reasonable to assume most of them serve to "fine tune" translation: to make it more precise. If that is so, it would appear that many of the modifications have evolved independently in each of the primary kingdoms. The independence of these modifications implies in turn that the universal ancestor did not have today's highly specialized transfer-RNA and ribosomal-RNA molecules but made do with more rudimentary translation machinery. At this stage one can say only that the facts are consistent with, and indeed suggestive of, the universal ancestor's having had rudimentary transcription and translation mechanisms, and so having been a progenote.

The Origin of the Urkaryote

In terms of their ribosomal-RNA catalogues the archaebacteria, eubacteria and eukaryotes appear to be equidistant from one another genealogically; no specific relation between any two of the three has been detected. Nevertheless, in terms of the amino acid sequence of one protein, the ribosomal *A* protein, the archaebacteria clearly seem to be relatives of the eukaryotes. Therefore it may be that archaebacteria as well as true bacteria participated in forming the eukaryotic cell. Perhaps it is to the archaebacteria one should look for the origin of the unexplained stage of the eukaryotic cell: the urkaryote that played host to the endosymbiont ancestors of the mitochondrion and the chloroplast. (Rather than searching for the hypothetical host, however, one should instead question whether there was such an entity. This is not a time to shape new discoveries in accordance with old prejudices.)

As I have indicated, the differences between the eukaryotic cell and the other major cell types at the molecular level are more extensive and pervasive than any of the differences visible with a microscope. The eukaryotic nucleus appears to contain at least three kinds of genes: those of eubacterial origin (presumed to have been appropriated from the genomes of the eukaryote's organelles), those of archaebacterial origin (for example the gene for the ribosomal *A* protein) and those of an unidentified third origin (exemplified by the cytoplasmic ribosomal RNA). To what extent is the eukaryotic nucleus genetically a chimera: an entity composed of parts assembled from disparate sources? At what stage (or stages) of evolution did the presumed assembly take place? And what was the nature of the organisms that supplied the various genes and structures?

Biologists have tended to look at the eukaryotic cell as having been formed by the association of fully evolved prokaryotic cells; their association is assumed to have created a "higher" type of cell, the eukaryotic. (The term prokaryote—"before the nucleus"—carries just this implication.) The question that can profitably be asked now is whether the evolutionary events that gave the eukaryotic cell its basic molecular character really were of this nature. The eukaryotic cell appears to be a chimera at a very basic level. Even the eukaryotic ribosome seems to be chimeric, its component RNA's having come from a source other than that of at least one of its proteins. If this is a correct interpretation of the data (and future investigations must settle the point), the eukaryotic cell may be a different kind of entity than it is now taken to be. It may have been chimeric even before it reached a stage of complexity comparable to that of today's prokaryotes; it may have been chimeric as it emerged from the progenote condition. Rather than being an advanced, "higher" form, the eukaryotic cell may represent a throwback to the evolutionary dynamics of its long-gone ancestor, the progenote.

Perhaps the most exciting thing about the recent discoveries in molecular phylogeny is that they show how much information about the very early stages of evolution is locked into the cell itself. It is no longer necessary to rely solely on speculation to account for the origins of life. It has become customary to think of the last decades of this century as a time in biology when "genetic engineering" will make possible exciting developments in medicine and industry. It must also be recognized that biology is now on the threshold of a quieter revolution, one in which man will come to understand the roots of all life and thereby gain a deeper understanding of the evolutionary process.