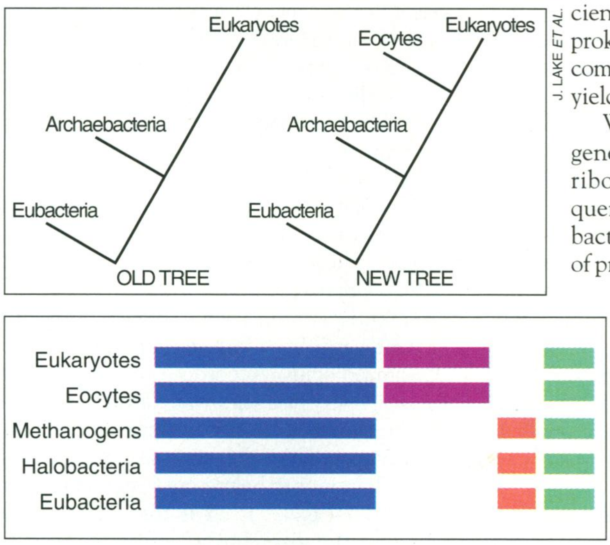
EVOLUTION

Researchers Find Organism They Can Really Relate To

When James Lake peers through his microscope at the bizarre heat-loving, sulfur-metabolizing organisms that thrive in temperatures at or above the boiling point of water, he sees his closest surviving bacterial relative. Lake, a molecular evolutionist at the University of California at Los Angeles (UCLA) and his colleague Maria Rivera present evidence on page 74 of this issue of *Science* that these strange unicellular creatures, known as eocytes, are more closely related to the cells of higher organisms than they are to other bacteria. This startling conclusion, says UCLA paleontologist Bruce Runnegar "makes a new split in the tree of life."

But it has also created a rift in the community of evolutionary biologists that has been trying to figure out the ancestry of eukaryotes—a class of cells that first evolved more than 2 billion years ago and whose members now include the cells of all known plants and animals, including humans. While some in the community enthusiastically welcome the new work, others simply don't agree with Lake's and Rivera's conclusions.



J. LAKE ET AL.

New branch in the tree of life. Lake and Rivera believe eocytes and eukaryotes share a common ancestor *(top)*. An 11 amino acid sequence *(pink)* in a protein called EF-Tu is present in eukaryotes and eocytes but not in other prokaryotes, which have a four amino acid sequence in its place *(orange)*.

The conventional wisdom has been that eukaryotes—cells that sequester their DNA in a nucleus, a structure lacking in bacteria and other prokaryotes—share a common ancestor with all the archaebacteria, a diverse bacterial "superclass" whose members lead pretty exotic lives: methane-producing methanogens; halophiles, which dwell in highly salty environments; and the eocytes. Now come Rivera and Lake with a different family tree: Eukaryotes, they believe, share a common ancestor only with the eocytes; the other archaebacteria branched off much earlier, according to their analysis.

The conventional wisdom relies largely on work by Carl Woese and his colleagues at the University of Illinois, Urbana, in the late 1970s. Woese looked for relatedness between various classes of organisms by comparing the nucleotide sequences of genes that code for ribosomes, structures required for synthesizing proteins that are common to every known cell type. He and his colleagues reasoned that these genes must also have been present in the ancient common ancestors of modern prokaryotes and eukaryotes, so that a comparison of the modern genes should yield clues to the family tree.

Woese focused specifically on genes that code for the RNA in the ribosomes. He analyzed their sequences in eukaryotes, in archaebacteria, and in the other superclass of prokaryotes: the eubacteria, which include pathogens such as *Escherichia coli*. His conclusion: The archaebacteria are all closely related to each other and, as a group, they are more closely related to eukaryotes than are the eubacteria. That implied, recalls Lake, "that [the archaebacteria] all descended from a common ancestor and all are more closely related to each other than to anything else." But Lake says he came to doubt that view in 1984, when he found that under the electron microscope, ribosomes from eocytes are structurally more similar to eukaryotic ribosomes than they are to other bacterial ribosomes. Now Lake and Rivera have found confirmation for that observation in a protein molecule.

Instead of using computer algorithms to compare the nucleotide subunits of DNA, Lake and Rivera sequenced and analyzed a protein called EF-Tu, one of the many proteins that participate in protein synthesis and is highly conserved among different species. They found a sequence of 11 amino acids in EF-Tu that was nearly identical in all the eocytes and eukaryotes they studied, whereas in its place in their sample of other prokaryotes there was a sequence of four amino acids (see diagram). The take-home message, says Lake, is that "eocytes are the closest relative to eukaryotes. Look at the data. They are black and white." Runnegar agrees: "Rivera and Lake have found something too complicated to have arisen by chance," he says.

Lake says he and Rivera chose to avoid gene sequence comparisons because they can give conflicting data for analyzing very early events in evolution, largely because individual nucleotides in DNA mutate easily compared to larger regions of genes. This feeling is shared by researcher Mark Chase at the University of North Carolina, Chapel Hill, who has tried to use DNA sequence data to study the more recent evolution of plant cells. "It is pretty clear to those of us who work with DNA sequences, where we already have a good idea of the relationships between organisms from the fossil record, as well as the anatomy and morphology of the organisms, that DNA sequences give silly results. At greater evolutionary depths, it is absolutely hopeless," says Chase. In contrast, he adds, Rivera and Lake have identified "a kind of character that ought to be more reliable in telling us relationships than can any coding sequence of DNA."

Many other evolutionary biologists remain skeptical, however. Take Norman Pace of the University of Indiana, Bloomington. He argues that you can't always infer family ties from the presence or absence of a protein sequence. "It is not true that insertions and deletions are conservative," he says. "There are many, many examples where inserts jump in and out of closely related genes." Indeed, evolutionary biologist Susan Golden of Texas A&M University says she found that deletions gave misleading information when she tried using them to trace the relatedness of a group of bacteria. Lake counters by pointing out that in cases of random insertion and deletion like those Pace refers to, one would not expect to see the insertion in all eukaryotes and eocytes. "In fact," says Lake, "they are in all eukaryotes for which sequences are known and in all phylogenetic subdivisions of eocytes."

Some researchers, like Gary Olsen of the University of Illinois, Urbana, are concerned that data from one protein are not sufficient to build a whole argument. And Pierrot Cammarano of the University of Rome argues that EF-Tu is the wrong molecule to analyze. "We show [in an upcoming paper] that EF-Tu is not a reliable marker. It does not have enough information," he says. By looking at EF-G, a related molecule that is twice as long, Cammarano has generated data that support Woese's tree.

Ultimately, says Chase, "researchers have to learn more about the organization of genomes and how things change over time. Then we could make it black and white." In the meantime, finding the roots for the tree of life will remain a thorny quest.

–**Michelle Hoffman**