MOLECULAR EVOLUTION

Proteins 'Clock' the Origins of All Creatures—Great and Small

At least 3.5 billion years ago, simple cells debuted on this planet, according to the fossil record. But among some of their more complex descendants—evolutionary biologists—the order of appearance of other forms has long been a matter of lively debate. Many scientists have argued that for hundreds of millions of years the only cells were prokaryotes, cells lacking a nucleus. Only much later did the tree of life branch, with the appearance of the eukaryotes, cells that do have nuclei, and they in turn led to everything from fungi to dinosaurs, butterflies, and humans. Yet another group of evolutionists argues that the tree branched near its base: Eukaryotes arose arm-in-arm with two types of prokaryotes, bacteria and the archaea.

To settle this argument about timing, researchers need a good clock. And on page 470, Russell F. Doolittle, a molecular evolutionist at the University of California, San Diego, and his colleagues describe an attempt to build a single timepiece that can encompass all groups of organisms. Their "molecular clock" uses the rate at which proteins shared by different groups of organisms change over time to indicate when those groups split off from a common ancestor. It suggests that the eukaryotes split off from the prokaryotes well into the history of life, some 2 billion years ago. Further, their study suggests that the archaea arose after this split, and that all extant eubacteria (common bacteria) last shared an ancestor even more recently.

These claims have prompted evolutionary clock-watchers to start punching in with opinions. "It's a very bold attempt—one that no one has ever done at this level before—and it's yielded many gratifying answers," says W. Ford Doolittle, a molecular evolutionist at the Canadian Institute for Advanced Research in Nova Scotia (and whose divergence from the other Doolittle lineage has been traced back 300 years). "If Doolittle's analysis is correct, then this is rather profound," agrees Bruce Walsh, an evolutionary biologist at the University of Arizona, Tucson. "It will shake things up."

It is certainly stirring the ire of researchers who disagree with it. "Forget it," snorts Norman R. Pace, a microbiologist at Indiana University in Bloomington, who argues based on other molecular evidence that eukaryotes and archaea extend back about 4 billion years. Paleobiologists, too, insist that at least one form of eubacteria, cyanobacteria, was present from 3.5 billion years onward. "Does the fossil record show the discontinuity in the cyanobacteria that Doolittle's data suggest?" asks paleobiologist J. William Schopf of the University of California, Los Angeles, who has found 3.5-billion-year-old microfossils that look like extant cyanobacteria. "No, not any way you cut the cake."

Researchers have built other clocks, based on RNA, that work within smaller groups of organisms. But Doolittle wanted a larger timepiece and so used proteins. "The data banks today are overflowing with sequence data from many of the extant lineages. That's what made this study possible," he says. So he and his colleagues selected 57 different sets of enzymes that are found in most of the 15 groups—from bacteria to baboons—that they were trying to compare.

To calibrate the clock, the researchers picked well-known split dates from the fossil record—among them the divergence of echinoderms and chordates, about 550 million years ago—and counted the number of amino acid changes that separate similar proteins in the two lineages. Then they could calculate a rate of amino acid change that could be used to date splits between other lineages. Because the same protein can evolve at different rates in different lineages, Doolittle's team took the rate derived from known splits and scaled it up or down in various other lineages, using established statistical tests to weed out biases produced by particularly slow- or fast-evolving groups. And the large number of sequences compared—531 in all—produced statistically robust results, Doolittle says.

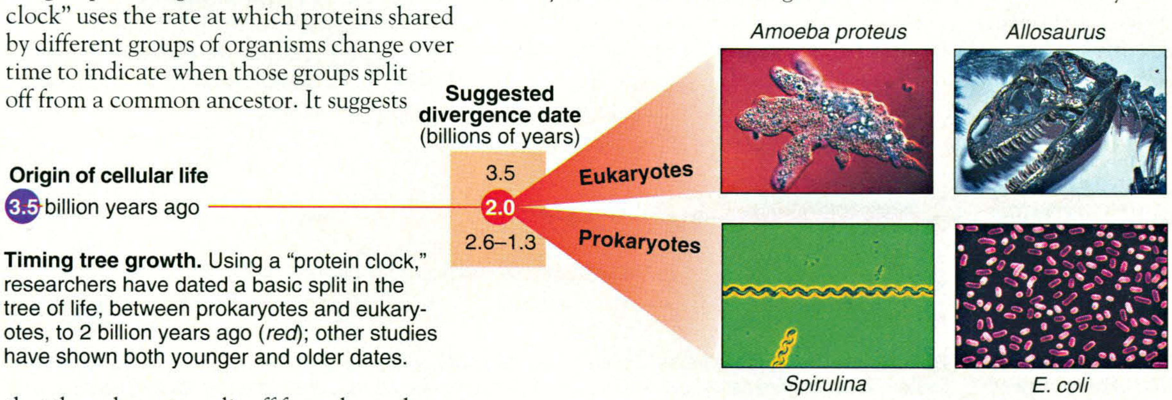
The results include not only the 2-billion-year-old prokaryote-eukaryote split but also a divergence between archaea and eukaryotes at 1.8 billion years ago. When archaea were first discovered, they were considered the very oldest organisms on Earth, because they are often found in hot, harsh environs. And Pace argues that the great diversity of RNA sequences found within the archaea confirms their antiquity. The protein clock implies, however, that their preference for harsh environments is a more recent adaptation.

The cyanobacteria might be younger still, according to the clock, which shows they last shared an ancestor with other eubacteria at 1.5 billion years ago, well after the big prokaryote-eukaryote split. Doolittle says that his finding doesn't preclude cyanobacteria existing prior to 1.5 billion years ago: The eubacteria common ancestor could have been a cyanobacterium, and it could have lived between 3.5 and 1.5 billion years ago. But the more recent molecular date does raise some doubts about whether the old fossils are what paleobiologists say they are. "I'm going to say the unthinkable," Doolittle says. "There is the possibility that the microfossil data at 3.5 billion years are not altogether correct, that they've been overinterpreted."

Critics say that it's Doolittle who is overinterpreting. Many of these objections center on his use of recent divergence dates to calibrate rates of change going back billions of years. "His dates are being extrapolated by anywhere from a factor of 4 to 20," says Gary J. Olsen, a microbiologist at the University of Illinois, Urbana, and that introduces a lot of uncertainty. And, notes Jeff Palmer, a molecular evolutionist at Indiana University in Bloomington, "it's all based on assumptions that the molecular clock is constant, when the more closely we look at molecular change, the more evidence we have that it is not."

Even Doolittle's supporters agree that his protein clock may not be the last word in the book of life; but it is, they insist, a valuable first chapter. "Russell has done the very best a person could do with the tools now available," explains W. Ford Doolittle. "If the answer is wrong, it probably means the tools are not adequate. So, if nothing else, his study has served as an excellent test of those tools and methods." Molecular watchmakers are already tinkering with them, trying for a still more accurate timepiece.

–Virginia Morell



Timing tree growth. Using a "protein clock," researchers have dated a basic split in the tree of life, between prokaryotes and eukaryotes, to 2 billion years ago (*red*); other studies have shown both younger and older dates.

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