meres hit a certain length, cells go into senescence and die. Cancer cells have a way of restoring their telomeres, however, and thus can continue dividing when normal cells would stop.

West became fascinated by the telomere research and its potential to short-circuit the aging process and possibly cancer as well. "Mike sought us out when he arrived here for medical school, and we were smart enough to let him work in our lab at night and on the weekends, when he wasn't in class," says Shay with a laugh. West was so taken with the potential of the work that he quit school in 1991 and started Geron to explore ways of exploiting it in the clinic. "He didn't want to waste time finishing the last year of medical school. He wanted to start work on this immediately and see what telomere biology could do for human medicine," Shay says.

West also got the firm to branch into the field of stem cell research, which led to last fall's developments. By then, however, he had left because of differences in corporate philosophy. Although neither Geron nor West would comment on his leaving, others involved with Geron who know West speculate that he pushed the firm too hard into areas of research that other executives felt were peripheral to its main mission. "Mike was the founder, and I'm sure he was frustrated with the fact that the people running the company weren't pushing certain research projects forward," says one academic researcher who receives funding from Geron. Says another, "I think it was a huge mistake for Geron to get rid of Mike, even though there were certainly clashes going on over research direction."

Since that departure, West has founded another business, Origen Therapeutics in South San Francisco, which is attempting to use avian stem cells to develop improved chicken varieties. That led him to his current job with ACT. During a meeting with officials from Avian Farms, the nation's largest poultry producer and ACT's financial backer, West learned that Avian Farms was looking for someone to run ACT. In short order, he had won the job, reportedly investing in the company, too.

As president, West is pushing ACT in two directions. The company's original goal was to use cloning and gene transfer technologies to engineer cows that produce pharmaceutical proteins in their milk. Two cloned cows born last year testify to the firm's success in this area; under West, ACT will continue to focus most of its efforts on this relatively noncontroversial research.

But West is also determined to move forward with the cow-human embryo work. Cibelli, who is now a senior scientist at the company, is currently repeating his earlier work on a large number of cells. West believes that if this approach succeeds, it will solve both ethical and practical problems. "First, we wouldn't be using [human] fetal tissue or frozen embryos," he says, "and second, should we eventually be able to turn stem cells into human organs for transplantation, we would be able to use a patient's own somatic cells to make the fusion and avoid any issues of rejection."

But a few critics say that in his enthusiasm, West slights the ethical dimensions of the work. "Michael West is a man who sincerely believes that what he is doing is right because it will ultimately benefit human health," says Richard Doerflinger, a theologian who works on pro-life issues for the U.S. Catholic Conference in Washington, D.C., and (like West) has testified before Congress on this issue. "But he really doesn't acknowledge the moral issues involved in what he's doing."

West disagrees. "First, I disagree that this is a moral issue, because these cells cannot become human beings. If they could, I would not be promoting this research. So then, where's the morality in blocking studies that can benefit millions and millions of people?"

—JOSEPH ALPER

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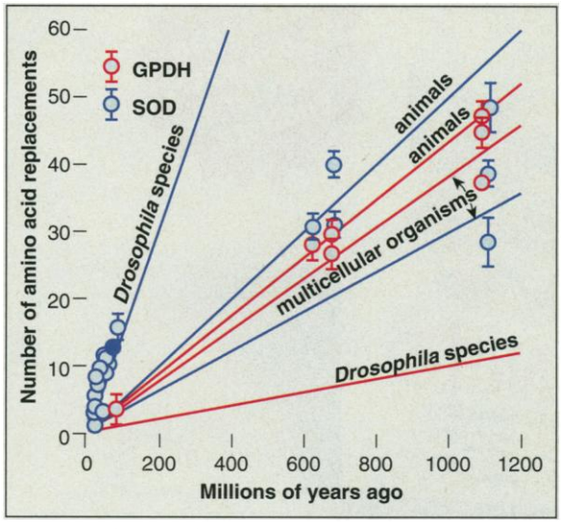
EVOLUTIONARY BIOLOGY

Can Mitochondrial Clocks Keep Time?

New data fuel fundamental challenges to the accuracy of molecular clocks, although researchers say they are tackling the problems

Put a scientist on the analyst's couch, say the word "mitochondria," and she's likely to blurt out "powerhouse of the cell" in response. But if she happens to be an evolutionary biologist, she might instead connect this organelle with a different type of power: the ability to illuminate evolutionary events deep in the past. For over two decades, biologists have been using mitochondrial DNA (mtDNA) to time the divergences of organisms from each other and to map human migrations. Such "molecular clock" studies have suggested that modern types of mammals and birds shared the Earth with the dinosaurs, that animals evolved hundreds of millions of years before their first fossils, and that "mitochondrial Eve," our common female ancestor, lived about 200,000 years ago in Africa.



Rate spread. Not only do the enzymes GPDH and SOD have different rates of evolution, the rates vary in different groups of organisms.

The DNA sequences pouring in from sequencing projects have fueled the effort and extended the clock approach to many genes in the cell nucleus. But the wash of data has uncovered some troubling facts. It's now clear that in many cases, the main assumption underlying molecular clocks doesn't hold up: Clocks tick at different rates in different lineages and at different times. And new work on the biology of mitochondria suggests that their evolution may be more complicated than researchers had suspected (see special issue beginning on page 1475).

"There's an emerging consensus that there are significant rate heterogeneities across different lineages," says John Avise, an evolutionary geneticist at the University of Georgia in Athens. "How big they are and how to deal with them is very much a matter of concern." Even those who once embraced the clocks are now somewhat skeptical. "Sure, there are mitochondrial clocks. A lot of them," says Wesley Brown of the University of Michigan, Ann Arbor, who no longer uses mtDNA sequences to time ancient divergences.

But even as scientists cast a newly critical eye on the clock results, researchers are proposing and applying sophisticated statistical methods to deal with the clock's idiosyncrasies. "People don't assume that a gene is evolving at a constant rate," says Blair Hedges, an evolutionary biologist at Pennsylvania State University in University Park. "We can test for different rates in different lineages and if a gene doesn't pass the test, you can throw it out." He and others say clocks can yield useful information even if they don't work perfectly. "A year ago people would have said we're sunk" if some of the model's assumptions didn't hold up, says David Penny, a computational biologist at Massey University in Palmerston, New Zealand. "Now it's just a nuisance. We have to add variability into our estimates."

Although researchers have varying degrees of confidence in the current statistical tests, biologists agree that clocks are worth fixing. "If you want to get a handle on the timing of events where there's no fossil record, this is your only option," says Gregory Wray, an evolutionary biologist at the State University of New York, Stonybrook. "Is this method so flawed that you want to abandon it completely? The debate boils down to whether you want to throw the baby away with the bathwater."

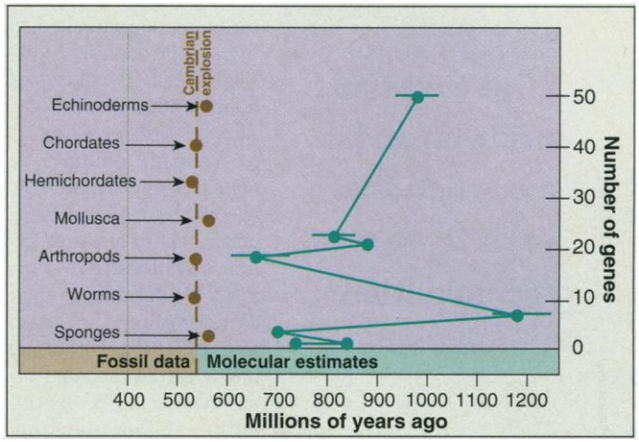
A steady rate?

Back in the late 1970s, mtDNA seemed the perfect choice for peering into the past. In multicellular animals it is almost always inherited from the mother, so researchers can track a maternal lineage, useful when trying to identify a single common ancestor. There are thousands of mitochondria in every cell, so mtDNA is abundant and relatively easy to obtain, even from partially decayed samples. Nuclear genes lack these advantages but tend to evolve more slowly than mtDNA, making it possible to extend the analysis further into the past.

For the clock to work with either sort of DNA, nucleotide changes must tick away steadily so scientists can convert the number of nucleotide differences seen between two organisms into the number of years since they diverged. Different genes evolve at different rates, depending on the selective forces upon them, but the model requires only that each gene's clock maintains its own rate.

Early work hinted that this might not always be true, and now a plethora of data shows that many genes don't conform to this model. For example, the nuclear gene that encodes an enzyme called Cu,Zn superoxide dismutase (SOD) has a variable rate of evolution depending on what groups of organisms are measured, according to a 1997 study by evolutionary geneticist Francisco Ayala of the University of California, Irvine. He found that the gene evolved very quickly among different species of *Drosophila*—5 times faster than it did among multicellular organisms in general. A different pattern of varying rates emerged for another gene, which encodes the metabolic enzyme glycerol-3-phosphate dehydrogenase (GPDH; see graph, p. 1435).

Even on a single branch of a phylogenetic tree, rates can fluctuate over time. For example, one stretch of DNA within the *Drosophila* male fertility gene Odysseus (*Ods*), has changed more in the past 500,000 years than in the preceding 700 million years, according to work by evolutionary geneticist Chung-I Wu at the University of Chicago and his colleagues (*Science,* 20 November 1998, p. 1501). If researchers assumed a standard rate, they would conclude that the last 500,000 years spanned a longer period of time than the previous 700 million.



Age confusion. Estimates of when animals originated have bounced up and down, but all are older than the fossil evidence.

Other problems arise in simply counting nucleotide substitutions, says Charles Marshall, a molecular paleobiologist at the University of California, Los Angeles. Certain substitutions are more likely to happen than others, depending on the organism, the gene, and a nucleotide's position in the gene. And a single change in nucleotide identity doesn't necessarily mean that only one substitution has taken place—there could have been multiple hits at the same site.

Paleontologists strike back

Such complexities may underlie some of the surprising results from clock methods, says Marshall. Many analyses of mitochondrial and nuclear DNA have arrived at dates that match those from fossils, but there are a few serious discrepancies.

For example, fossils record a burst of innovation that marked the emergence of many modern groups of animals about 530 million years ago, in the so-called Cambrian explosion. But molecular studies suggest that animals were diverging at least several hundred million years earlier (*Science*, 25 October 1996, p. 568).

One explanation is simply that creatures, especially soft, simple ones, can easily elude fossilization, so that multicelled animals could have lived before the Cambrian explosion without leaving a record. But paleontologists counter that the common ancestor of animals had a vascular system, a central nervous system, and body wall muscles, and that such a complex creature would eventually leave some trace. "Where are they going to hide this sophisticated animal for half a billion years?" wonders Doug Erwin, a paleobiologist at the Smithsonian's National Museum of Natural History in Washington, D.C.

Still, he and others caution that discrepancies between the molecular and fossil evidence don't necessarily mean that one type of data is wrong. Molecular data estimate the point at which genetic intermixing stops—usually some time before the appearance of diagnostic physical differences, which is what fossils record. "We're actually looking at two different things," says Erwin. "When the arthropods and the chordates split, the first species don't look like flies or J. Edgar Hoover. They look essentially identical. The morphological differences that we later recognize as being one or the other evolve later." But in this case, half a billion years of evolution is a bit much for even many molecular biologists to swallow, especially because the molecular estimates themselves have bounced around, from 1200 million years ago to 990 and even down to 670 (see graph above), suggesting that perhaps the fossils are right after all.

Fossils and molecules are also at odds over when most modern orders of birds and mammals appeared. Paleontologists haven't generally found them before about 65 million years ago, after the great Cretaceous-Tertiary extinction wiped out the dinosaurs. But molecular studies using both mitochondrial and nuclear DNA conclude that many living species diverged much earlier, up to 130 million years ago (*Science*, 1 May 1998, p. 675).

The molecular biologists argue that fossils documenting this early radiation have perhaps not been preserved or yet discovered. "Fossils are starting to get found on the opposite sides of barriers where people used to think nothing existed," says Alan

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Cooper, a molecular evolutionist at Oxford University. "Now [from fossils] we've got parrots and seabirds in the late Cretaceous."

But a recent study by paleontologist Mike Foote of the University of Chicago and his colleagues (*Science*, 26 February, p. 1310) suggests that the existing fossil record doesn't have huge gaps. The researchers assessed the quality of the record during the late Cretaceous, when molecular clock results suggest that modern placental mammals should exist. They concluded that the large number of other mammal fossils from that time make it implausible that modern placental mammals are there but undiscovered. "If the record really stinks, almost every species you find will be from single fossils," says Foote. "But the empirical record is something like 10 to 100 times greater than what would be required to allow for a 65-million-year gap in the fossil record." Hedges disagrees with that conclusion, noting that Foote's work assumes constant speciation across the Cretaceous-Tertiary boundary. "They ignored the biological effects of the asteroid impact that wiped out the dinosaurs," he says.

> "There was no such woman [as mitochondrial Eve] if there was recombination."
>
> —Svante Pääbo

Riddles of recombination

New information about the complexities of mitochondrial biology is also raising new questions about the mtDNA clock. Conventional wisdom has it that mitochondrial DNA comes only from the mother's egg. But electron microscopy and DNA detection studies have revealed that the sperm's mitochondria can enter the egg, says evolutionary geneticist Adam Eyre-Walker of the University of Sussex in Brighton. And several papers to be published this week make the surprising suggestion that, contrary to what scientists have thought, sperm-contributed mtDNA can recombine with that from the mother. If that's true, a single recombination event could instantly insert or erase multiple changes in a piece of DNA, throwing off the clock. Such a phenomenon may also explain how some people can have two different versions of mtDNA in their cells (*Science*, 2 January 1998, p. 28).

In work to be published in the *Proceedings of the Royal Society of London B*, Erika Hagelberg, a molecular geneticist at the University of Otago in Dunedin, New Zealand, and her colleagues report that when they sequenced a fast-evolving region of mtDNA from 452 inhabitants of some western Pacific islands, they found three distinct groups. In the inhabitants of one island, however, they detected something peculiar: a high frequency of a very rare nucleotide substitution, seen in individuals from all three groups. It seems unlikely that this mutation had occurred independently in the three lineages on this island but nowhere else, says Hagelberg. Instead, she suggests that the mutation arose once on the island, in a male, then recombined with egg mtDNA during fertilization. The male descendants of this lineage then spread the mutation into the other two lineages, again by recombination during fertilization. Such recombination is "probably ticking away in the background in other parts of the world," she says.

In an independent study, to be published in the same journal, Eyre-Walker and his colleagues draw similar conclusions. They looked for instances of multiple changes at the same site in mtDNA. "People have always interpreted that in terms of hypervariable sites," says Eyre-Walker. But in their 29 samples, the team found that these multiple hits occurred far more often than would be expected by random mutation. Instead, "it could all be due to recombination," he says. If so, "estimates of rates of nucleotide change are going to be incorrect."

Recombination could also be bad news for use of mtDNA in other questions of human ancestry. For example, in 1997 researchers led by Svante Pääbo of the Max Planck Institute for Evolutionary Anthropology in Leipzig retrieved ancient mtDNA from a Neandertal and concluded that the sequence was so distinct that Neandertals didn't contribute mtDNA to modern humans (*Science*, 11 July 1997, p. 176). But recombination tends to mix up lineages and create a more homogeneous population of DNA sequences over time. So if mitochondrial recombination occurred in our ancestors, one might expect greater diversity in mtDNA sequences in the past—and greater disparity between ancient sequences and the more homogeneous sequences of today, says Hagelberg. That would mean, she says, that "the Neandertals might be more closely related to present-day humans than the mtDNA data suggest."

"We should think about recombination as a possibility," agrees Pääbo, "but it's not proven by this work. It may still be possible to explain the mutations with sites that mutate frequently." He notes that Neandertals were confined to Europe and western Asia and so, if they gave rise to modern humans, they should be closest kin to people of these regions. But "Neandertal DNA is equally different from that of people everywhere in the world," making it unlikely that they were ancestral to Europeans, he says.

Recombination could also cause problems for mitochondrial Eve. Studies of mtDNA from living people on various continents show a surprising homogeneity, suggesting that we are all descended from a woman who lived a mere 200,000 years ago in Africa. But such homogeneity might be due to recombination rather than a common recent ancestor, says Hagelberg. Pääbo concurs. "Mitochondrial Eve is the one woman who carried the ancestral mitochondrial DNA," he says. "There was no such woman if there was recombination." Until scientists determine how frequently recombination in mtDNA occurs, however, "it's hard to know how it would affect these types of analyses," says Penny.

Despite these difficulties, researchers are already working to correct the clock's timekeeping. For starters, clock proponents argue that they can identify genes of variable evolutionary rate and avoid them. "Name a gene and there are going to be lineages that are fast or slow," says Hedges. "We do a statistical test to identify those problems." And some genes tend to evolve more steadily, such as those that encode serum albumin and enolase, he says.

Not everyone is convinced that the current statistics are powerful enough. But researchers such as Cooper are modeling the error bars in clock analyses, allowing them to answer well-defined questions with confidence. And researchers such as Jeffrey Thorne at North Carolina State University, Raleigh, are working to beef up the statistics. Thorne and others are developing new tools that allow for different probabilities for various nucleotide substitutions and for changes in the rate of substitutions at certain times during evolution. "One idea is that you expect closely related species to have more similar rates of molecular evolution than more distantly related species," says Thorne. "So we try that and then use statistical methods that are available to evaluate how well the model fits the data."

Even as the statistics improve, clock studies are benefiting from all of the sequencing under way, says Hedges. "We're gathering lots of genes from lots of organisms," he says. Because rates vary along branches in different directions for different genes, "the thing to do is to use a lot of genes." Far from giving up on clock analyses, researchers are still set on extracting whatever layers of meaning they can.

—EVELYN STRAUSS