AGING RESEARCH

Do Mitochondrial Mutations Dim the Fire of Life?

A sensitive technique shows that mutations accumulate over time in a key genetic region of the cellular power plants called mitochondria

Until about a decade ago, few researchers outside cell or evolutionary biology paid much attention to mitochondria, the tiny particles that generate most of the cell's energy. But now researchers have begun to see the more menacing side of these internal power plants. First, geneticists started tracing certain rare inherited disorders to mutations in the mitochondria's small circular genome. More recently, other researchers have speculated that mitochondria might contribute to aging, either by releasing tissue-damaging reactive oxygen molecules or by deteriorating and depriving the cell of the energy it needs to function.

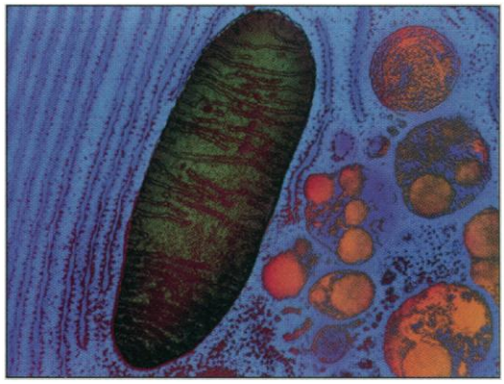
On page 774, a team led by Giuseppe Attardi, a human geneticist at the California Institute of Technology (Caltech) in Pasadena, reports some of the first hard evidence that mitochondria do deteriorate as people age. His team found that mutations in the 16,500-base mitochondrial genome accumulate with time and in a particularly important region: a 1000-base segment that controls the genome's replication. Other researchers had previously found low frequencies of mutations in mitochondrial DNA as people age, but given that cells have hundreds of mitochondria, each with multiple genome copies, skeptics argued that the changes were not extensive enough to alter cell function.

By carefully screening out contaminating DNA and using a technique that could detect single base changes, however, the Attardi team found that the damage can be extensive. The work "really shows that aging does something to the mitochondrial genome," says Manuel Graeber, a neuropathologist at the Max Planck Institute for Neurobiology in Martinsried, Germany.

Michael McKinney, a neuroscientist at the Mayo Clinic in Jacksonville, Florida, agrees. He says that while he's not sure that mitochondrial changes bring about aging by themselves, "even those of us who aren't mitochondrial people but who are worried about [aging] are going to be thinking about this [result]." Still, McKinney and others point out that a key link in the connection between mitochondrial mutations and aging is still missing: The Caltech team has not yet demonstrated that these changes alter mitochondrial—or cell—survival or activity.

For his current work, Attardi decided to study the main control region for replication of the mitochondrial genome, which "seemed the most [likely] to be involved in aging," he says. The control region must recruit cellular proteins to replicate itself and the rest of the mitochondrial genome and thus represents a pivotal connection between the mitochondria and the cell. If the connection goes bad and the mitochondria don't replicate properly, they might decline in number, causing the cell to have less energy than it needs.

The Caltech team began the project by sampling connective tissue cells, called fibroblasts, from 18 randomly picked healthy individuals ranging in age from less than a year to 101. The researchers also obtained two sets of stored cells, taken 9 to 19 years apart, from each of nine other individuals. To look for mutations in the DNA of the mitochondrial replication control region in the cells, molecular biologist Yuichi Michikawa first used DNA probes to pull out the region from each of the cell samples as seven separate segments.



Silent saboteur. Changes in the genomes of mitochondria like this one could contribute to aging.

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He then took comparable segments from the cells and sorted them on electrophoresis gels using a technique that can separate DNA pieces that have slight sequence differences, such as those that result from single base changes, based on the different rates at which they migrate through the gel. Finally, Michikawa cloned the DNAs in the bands on the gels in bacteria and then sequenced them. The analysis was "very, very laborious," notes Attardi. The labor paid off, however.

For one segment studied, for example, mutations were not present in any of the clones from younger individuals, but they were in 5% to 50% of the clones from the older individuals, eight of whom had exactly the same mutation. And by analyzing the nine pairs of samples separated in time, the Caltech researchers found three people who had at least one of these mutations in the older cells but not in the younger cells. Thus, their data indicate that specific mutations in the mitochondrial replication control region accumulate with age in some people, sometimes in high numbers. "The extent of the mutations is among the highest ever reported," says James Dykens, a biochemist with the biotech company Mitokor Inc. in San Diego, California.

Still, researchers say that much more work will be needed to show that the mutations have anything to do with aging. For starters, notes Eric Schon, a molecular biologist at Columbia University in New York City, the study needs to be replicated on a larger scale and in other types of cells, such as muscle or brain, that suffer the most harmful aging effects. Furthermore, says George Martin, a gerontologist at the University of Washington in Seattle, "There's no evidence [the mutations] functionally impair the cell or change [the rate] of mitochondrial replication."

Attardi concedes that he has not tied these particular mutations to a decline in cell or mitochondrial function. But he points out that the fact that his team found so many in one region suggests that other areas of the mitochondrial genome might be affected as well. And those hypothetical mutations may lead to mitochondrial changes such as increased production or release of the damaging oxidative free radicals that have already been linked to aging. Alternatively, or perhaps in addition, such mutations could make mitochondria less efficient at generating ATP, a molecule that fuels many of the cell's biochemical reactions, an idea suggested in 1989 by Anthony Linnane, now a molecular biologist at Epworth Medical Center in Melbourne, Australia. Linnane's own work shows that the amount of complete and active mitochondrial DNA declines as the years pass.

The new results now "give [that idea] ammunition," notes Vilhelm Bohr, a molecular biologist at the National Institute on Aging in Baltimore, Maryland. And Graeber, too, can see how it all might fit together. "The mitochondria have been compared to the fire of life," he explains. Perhaps "that fire is slowly extinguished as we grow old."

—ELIZABETH PENNISI