The Other Human Genome

Now that researchers have linked several diseases to mutations in the mitochondrial genome, mitochondrial DNA's role in other disease—even aging—is getting attention

WHEN DOUG WALLACE began studying mitochondrial DNA 20 years ago, the field was something of a backwater. His was one of only a handful of groups around the world trying to figure out whether changes in these genetic oddities—packages of DNA inside a cell that are entirely separate from the chromosomes that make up the nucleus—could be responsible for human diseases. But in the past 2 years, largely thanks to work by Wallace's group at Emory University and researchers at the University of London Institute of Neurology and Columbia University, the role of mitochondrial DNA (mtDNA) in disease has become a hot topic. So hot that as many as two dozen labs around the world are now studying mitochondrial genetics, with the number growing rapidly.

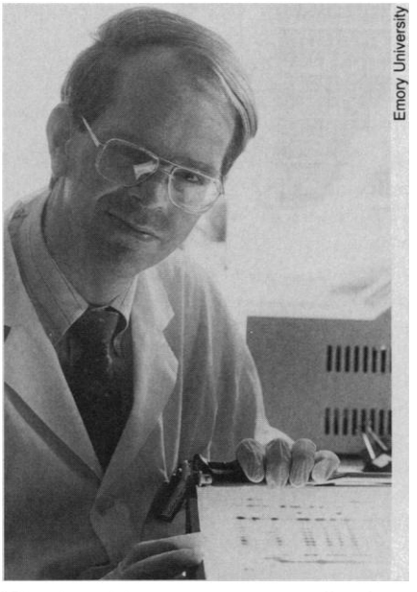
If there was one triggering event, it occurred in 1988 when Wallace and the London group, headed by Anita Harding and John Morgan-Hughes, independently linked specific mutations in mtDNA to specific diseases. The diseases now known to be associated with mtDNA mutations—Kearns-Sayre syndrome, chronic external ophthalmoplegia, and myoclonic epilepsy with ragged red fibers—are admittedly not everyday complaints. But as Eric Schon, a molecular geneticist at Columbia University College of Physicians and Surgeons who has been studying Kearns-Sayre syndrome, points out, "It's like many other diseases—a rare disease is rare until somebody finds something out, and all of a sudden things start popping up."

Things have been popping up all over for mitochondrial diseases. Not only are researchers ferreting out the specific mutations in mtDNA that cause these rare diseases, but they are also beginning to draw some intriguing links between defects in mtDNA and more common degenerative diseases like Huntington's and Parkinson's disease. Some have even proposed that the buildup of mutations in mtDNA over a person's lifetime may help explain the aging process.

Until these disease links were identified, mitochondria were generally viewed as simply providing a cell's power plant. Indeed, when Wallace started studying mtDNA in the early 1970s, no one knew just what it did. "It was known as a physical entity, because it could be isolated, but nobody had any idea of whether it had any information content," he recalls.

But gradually, researchers teased out its characteristics. For example, there can be as many as 100 mitochondria in cells that have high energy demands like nerve and muscle cells, or as few as one or two. Their job is aerobic respiration—taking energy from organic molecules ("food") and turning it into adenosine triphospate (ATP) that can be used to power the cell.

Mitochondria's evolutionary ancestors were once free-living bacteria that had perfected this process of respiration and were then appropriated by individual cells of higher organisms. Perhaps because their progenitors were separate organisms, mitochondria retain their own DNA, outside the cell nucleus. And compared to the nuclear DNA, mtDNA is like a flea next to an elephant: The entire mitochondrial genome has a mere 16,569 base pairs on a single, continuous double-stranded loop of DNA. Nuclear DNA, in contrast, consists of 3 billion base pairs separated into 23 pairs of chromosomes. Mitochondrial DNA encodes just 13 proteins—critical subunits in the respiratory process—and specifies the RNA for the ribosomes (the particles on which proteins are assembled) and 22 transfer RNAs for the mitochondria.



Mitochondria maven. *Doug Wallace has spent two decades seeking mtDNA disease.*

Wallace says he was convinced early on that mutations in mitochondrial genes could cause disease and that those diseases could be inherited. But proving it was another matter. "There were no criteria by which people could look for phenomena related to the mitochondria," he says.

That's because mtDNA doesn't follow the usual rules of genetic inheritance laid down by Gregor Mendel. Except with sex-linked traits, genetic inheritance is based on offspring receiving one copy of each gene from both parents. And either copy is potentially able to alter the physical characteristics of the offspring. But to understand mitochondrial genetics means "throwing away all your prejudices and starting over again," Wallace says. For example, mitochondrial genes are inherited only from the mother. And when a cell divides, mitochondria are randomly assigned to the daughter cells. Since there are many mitochondria per cell (and each mitochondrion contains between four and ten copies of the mtDNA genome), a mutation may be present in the mtDNA somewhere in the cell, but it can be far outnumbered by the normal, nonmutated copies that will also be present. Therefore, mtDNA-linked changes in an organism may not show up until the mutation spreads to a sufficient number of mitochondria.

Wallace and his colleagues began their hunt for a mtDNA disease with Leber's neuropathy, a disorder characterized by loss of central vision and degeneration of the optic nerve. The disease seemed like a good candidate because it was known to be exclusively maternally inherited, and the optic nerve is sensitive to fluctuations in the energy provided by the mitochondrion.

By sequencing the mtDNA from a Leber's neuropathy patient and comparing that sequence with a published sequence for a healthy subject, Wallace found a single base substitution in the middle of one of the mtDNA genes encoding an enzyme in the respiratory pathway: a guanine became an

adenine, changing the 340th amino acid in the protein encoded by the gene from an arginine to a histidine. Wallace and his colleagues looked at a total of 11 family pedigrees and discovered that individuals in 9 of the 11 families with Leber's blindness had the point mutation, whereas none of the control families did. The two discordant families suggest that there may also be another cause of Leber's blindness, or they may have another disease that mimics Leber's symptoms.

Armed with that success, Wallace's group quickly reported another disease caused by a single base pair mutation in the mtDNA: myoclonic epilepsy and ragged red fiber disease (MERRF). Patients with MERRF exhibit uncontrollable jerking in their skeletal muscles and ultimately a form of dementia. The ragged red fibers are a morphological description of the way their muscle fibers look when stained to show abnormal mitochondria. The Emory team reported earlier this summer that the substitution of an adenine for a guanine at nucleotide pair 8344 alters a portion of the transfer RNA molecule for the amino acid lysine, which ultimately interferes with the production of mitochondrial proteins.

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Power plant damage. *Magnified 35,000 times, this electron micrograph shows crystalline inclusions (solid rectangular structures), a hallmark of mitochondrial damage in neuromuscular disease.*

While diseases caused by these point mutations in mtDNA are clearly inheritable, another class of mtDNA diseases—those caused by deletions rather than point mutations—appear to arise spontaneously. Anita Harding's group was the first to link deletions in mtDNA with disease in a 1988 publication reporting that patients with a certain type of muscle weakness were missing a chunk of their mtDNA. There is no maternal inheritance pattern to these diseases, Harding notes, which suggests that the deletions are not inherited—most likely because a woman with a severe deletion in her mtDNA would be too sick to bear children and pass on her mutation.

Since Harding's study appeared 2½ years ago, groups around the world have been looking for diseases associated with mtDNA deletions. They soon found them. "All patients with [detectable] deletions of mtDNA have ophthalmoplegia—paralysis of the extraocular muscles—without exception," Salvatore DiMauro, a neurologist at Columbia University, told *Science*. DiMauro says it is likely that part of the explanation is that the eye movement muscles contain a lot of mitochondria, many more than limb muscles, but he does not believe that is enough to explain the uniformity of the finding.

Patients with progressive ophthalmoplegia fall into two groups. In one, there is the characteristic eye muscle paralysis, sometimes accompanied by minor muscle weakness. But in the other, the symptoms are severe and ultimately fatal. This is Kearns-Sayre syndrome, characterized not only by muscle weakness but also by elevated protein levels in the cerebrospinal fluid, damage to the cerebellum and ultimately complete heart block. The deletions in Kearns-Sayre syndrome range from 1.3 kilobases to 7.6 kilobases—an astonishing loss, amounting to nearly 46% of the mitochondrial genome.

Why do these deletions occur? Schon, DiMauro's colleague at Columbia, believes they may be happening all the time. When major deletions happen very early in development, they may be lethal to the embryo. If they occur somewhat later in development, the organism may survive, but with enough copies of the normal mtDNA to delay onset of disease. In practice, a shortened mtDNA has a reproductive advantage over a normal-sized molecule because the number of replications per unit time is directly proportional to the length of the molecule. This means that over time, the lethal mutation would take over. Wallace says a new study by Nils-Göran Larsson of Gothenburg University in Sweden shows that the proportion of mitochondria with deletion mutations increases over the course of Kearns-Sayre syndrome.

And mtDNA mutations may not just cause a few, rare diseases. "There's increasing evidence that other, more common diseases like Parkinson's disease, are due to mitochondrial abnormalities, which could be mitochondrial genetic abnormalities," says Harding. She points out that recent work has shown that some of the enzymes in the respiration pathway are depleted in the substantia nigra, the portion of the brain that is damaged in Parkinson's patients.

"When you start making a list of the things that you now expect of mitochondrial mutations," says Wallace, "you find that a large number of previously difficult to understand—and very common—problems could well be explained by this kind of phenomenon. One of the areas we're working on is the possibility that cardiomyopathies might have a common energy deficiency pathway" related to mtDNA damage. He adds that diseases resulting from mutations in nuclear DNA may also have a mitochondrial component. Wallace says an early onset form of Huntington's disease may be an example of this interaction. The idea, first proposed by neurologist Richard Myers of Boston University, is that there may be some incompatibility between the father's nuclear gene for Huntington's disease and the mother's mtDNA that causes the early disease onset in their children.

An even more intriguing possibility is that a buildup of mtDNA mutations during a person's lifetime could be the key to cellular aging, a notion that several labs around the world are presently considering. A group from St. Vincent's Hospital in Fitzroy, Australia, has shown that there is a decline in the functioning of mitochondria in skeletal muscles with age. Anthony Linnane of Monash University in Clayton, Australia, has proposed that this decline might be countered by restoring some of the enzymes that would otherwise be encoded by healthy mtDNA. Josef Müller-Höcker of the Pathologisches Institut der Universität München in Germany recently looked at 140 hearts obtained from autopsies and showed specific decreases in cytochrome c oxidase, an enzyme involved in respiration that is largely encoded by mtDNA. Schon sees this as the most direct evidence yet that mtDNA plays a role in the aging process and he is following it up in his lab.

For Wallace, this growing interest in mitochondrial genetics is a satisfying change from the years when few people accepted his ideas. "When we proposed that certain pedigrees were maternally inherited, people simply didn't believe us." Now, in ever increasing numbers, they're starting to.

■ JOSEPH PALCA

ADDITIONAL READING

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