

WHEN MITOCHONDRIA RUN AMOK:

These powerhouses of the cell belch harmful chemicals // Which tear up their surroundings // Which touch off a cycle of destruction // Which may be the culprits in a surprising range of diseases.

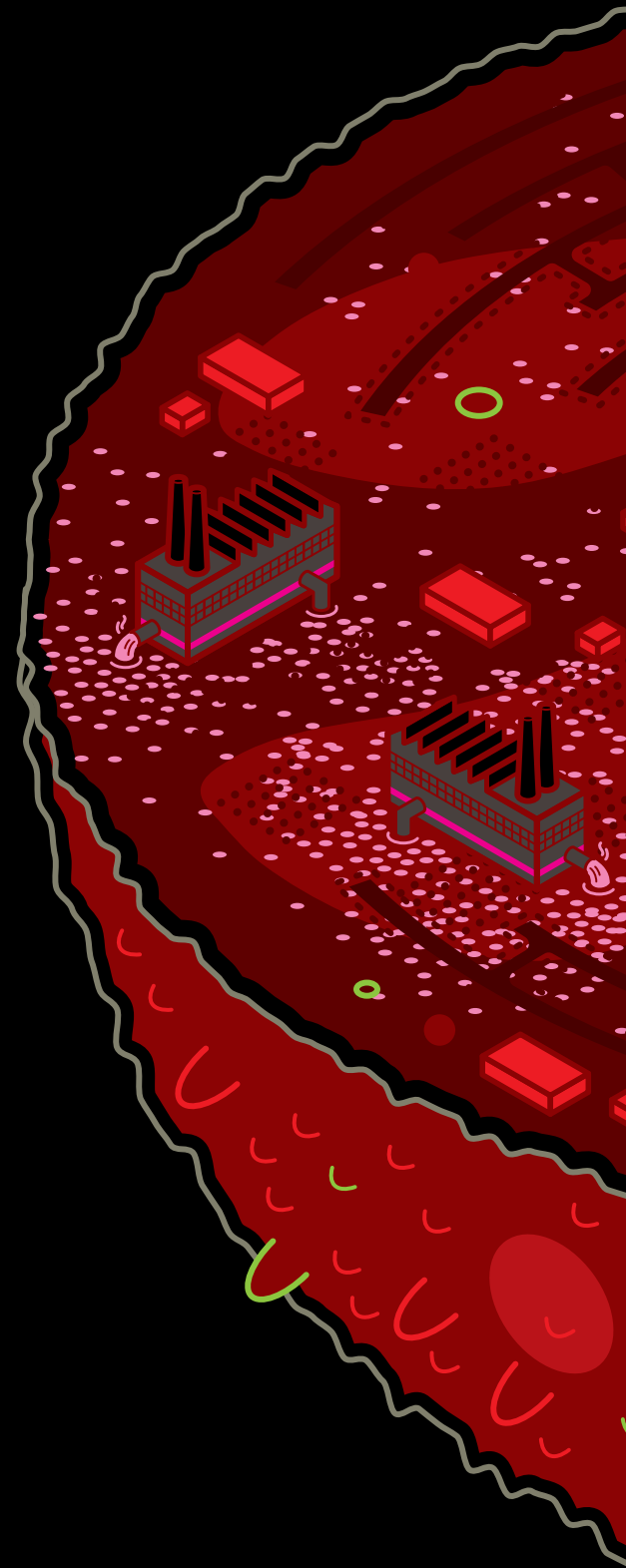
Energy Crisis

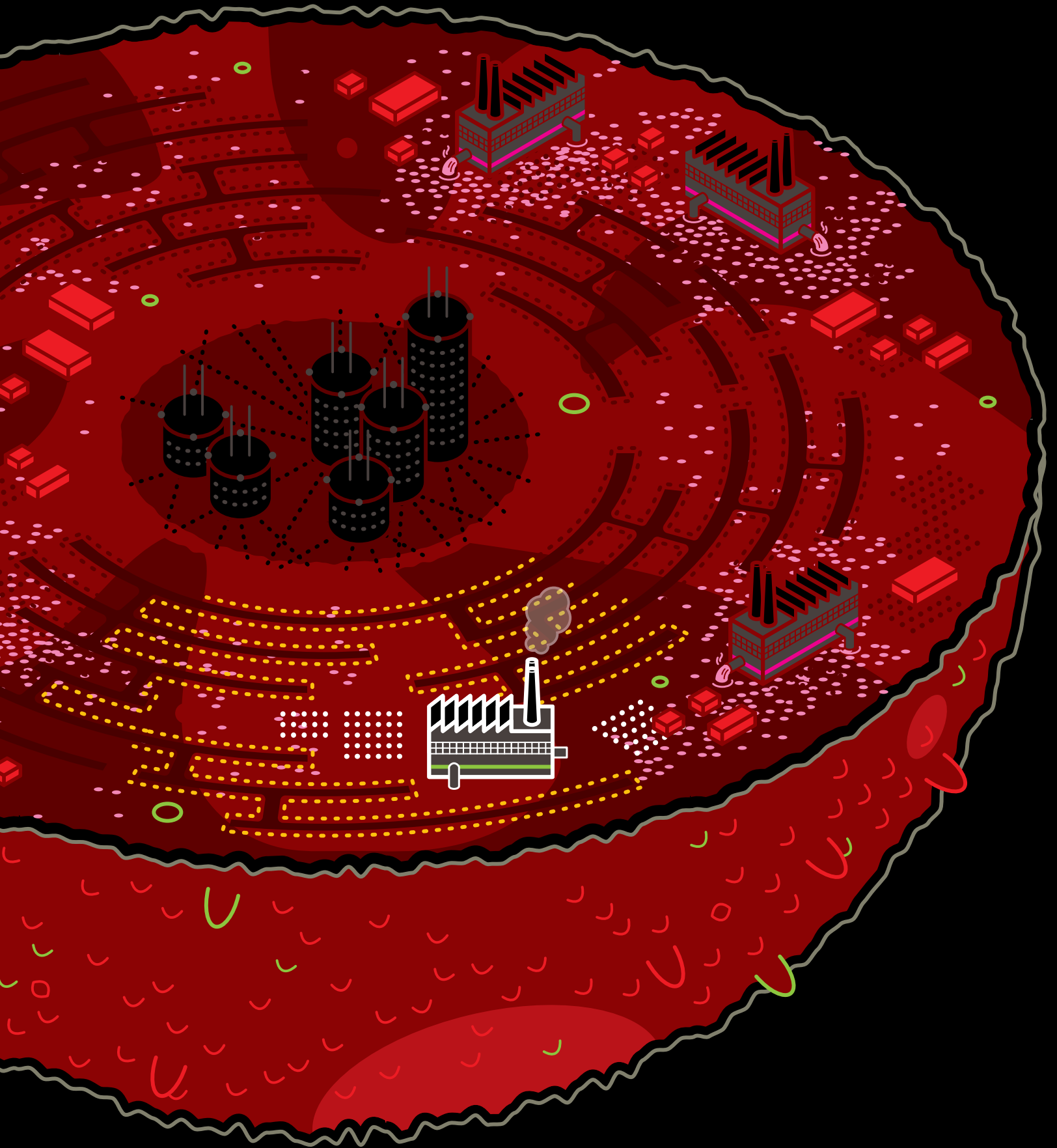
■ BY RACHAEL MOELLER GORMAN // ILLUSTRATIONS BY L-DOPA

The 120 people in Barry Snow's Phase II drug trial are clinging to the hope of a happy ending. They are still in the early stages of Parkinson's disease, and so far symptoms are mild—a tremor every now and then, occasional stiffness in the legs. Life feels almost normal, yet they all know that without some dramatic new treatment, their long-term prognosis is not good.

If they were regular Parkinson's patients, they could expect to return to their doctors' offices over the next several years having trouble walking and keeping their balance and with increasingly severe tremors. Their physicians would undoubtedly prescribe the only treatment (short of surgery or physical therapy) known to ease Parkinson's symptoms—increasing their brains' levels of dopamine, a chemical that helps control muscle movement, often in the form of the drug levodopa (L-dopa), which has helped Parkinson's patients since the 1960s. Yet any relief would be temporary. Their disease, with nothing to cure its root cause, would lead to a profound loss of muscle function and, eventually, dementia. Those with milder cases might lose only some muscle and brain function.

Snow, who heads the neurology department at Auckland Hospital in New Zealand, hopes the drug he's testing will do much more than treat Parkinson's symptoms. He believes that because his drug attacks what he and others think to be the crux of the disease, the drug will significantly slow its





advance. “Then we could delay the need for L-dopa,” he says.

The target of Snow’s drug is fairly novel in the realm of disease treatment, although it should be familiar to anyone who has taken high school biology. He’s aiming for mitochondria—tiny powerhouses in every cell—which recent research shows may play an important role in the progression of Parkinson’s. The problem could be an overabundance of destructive reactive oxygen species (called ROS, and which include a type called

Some disabled mitochondria in a cell may completely shut down, while others function so poorly that they trigger a chain reaction of destructive events.

free radicals) in the mitochondria; the drug, MitoQ, gets inside these organelles and mops up free radicals. MitoQ has been in development since the late 1990s, when biochemist Mike Murphy and organic chemist Robin Smith at the University of Otago in New Zealand developed it; since then, its effects have been studied in human cells and animals.

If MitoQ is able to slow the progression of Parkinson’s, it might also be effective in treating other diseases believed to result, at least in part, from a falloff in mitochondrial function. Studies during the past 10 years have identified a host of common disorders with apparent ties to mitochondria, including type 2 diabetes, cancer, even aging. Each disease is different, with unique triggers and symptoms, and much of the science seeking to establish mitochondria’s role is still in the early stages and could fail to confirm causal links. Yet the potential payoff is considerable. If mitochondria prove to be involved with varied disease processes, they could become a major target of treatment.

Two billion years ago (give or take a few hundred million), a bacterium invaded a larger single-celled organism. Because the bacterium was very good at something the single cell needed—making energy—the cell kept it around. During millions of years of dividing and evolving, the bacterium

became the mitochondrion, which powers almost every cell in every multicelled organism, helping the organism digest food, beat its heart and move its muscles. Cells now have hundreds or even thousands of mitochondria.

To generate energy, mitochondria manipulate sugars. First, a cell breaks apart glucose into a smaller molecule called pyruvate, which the cell then imports into a mitochondrion. Via a multistep process, the mitochondrion strips each pyruvate molecule of its carbon atoms to create two other molecules, reduced nicotinamide adenine dinucleotide (NADH) and 1,5-dihydroflavin adenine dinucleotide (FADH₂). Machinery within the mitochondria, the electron transport chain, extracts electrons from NADH and FADH₂ and pumps positively charged protons into the intermembrane space (mitochondria have two cell membranes). The protons are then drawn back inward to the negatively charged mitochondrial matrix at the mitochondrion’s center, and as they surge across the

membrane, they turn a waterwheel-like molecule, adenosine triphosphate (ATP) synthase. That generates energy, which is stored in ATP and used by every cell and organ in the body.

Mitochondria’s 37 genes code for the manufacture of key proteins and helper molecules in the energy production pathway (including the electron transport chain) and are all inherited from the mother’s mitochondria-packed egg. In 1981 Nobel laureate Frederick Sanger and his colleagues in Cambridge, England, determined the sequence of these genes. That enabled researchers to compare mitochondrial DNA in healthy people with that in patients suffering from a number of rare, maternally inherited diseases. Scientists eventually discovered 50 mutations and linked them to a series of devastating disorders known by such acronyms as MELAS, LHON and MERRF. In all these diseases, the genetic mutations have crippled the mitochondria’s energy-producing machinery.

Intriguing findings in small studies of humans and in animal research have since directed scientists toward a closer examination of mitochondria’s involvement in other, more complex diseases—those with multiple genetic and environmental causes, including Parkinson’s, type 2 diabetes and cancer. The researchers suspected that smaller mutations in a series of nuclear genes that encode mitochondrial proteins, or slight mitochondrial defects stemming from environmental factors or

even simple aging, could help trigger these diseases. Disabled mitochondria do a poor job producing energy, and that allows electrons to leak out of the electron transport chain and interact with oxygen inside the mitochondria, creating free radicals that further damage the mitochondria. Some mitochondria in a cell may completely shut down, while others function so poorly that they trigger a chain reaction of destructive events. The diseases that may result from this dimming and flickering are less immediately severe than those that are caused by mutations in mitochondrial DNA, but they may end up being just as deadly.

The link between mitochondria and Parkinson's disease was discovered serendipitously in the early 1980s, when addicts began injecting a contaminated version of a euphoria-inducing drug called meperidine. In fact, the drug had been inadvertently poisoned by a meperidine by-product

called MPTP. After the drug users developed symptoms strikingly similar to those of late-stage Parkinson's, researchers found that MPTP inhibits mitochondria's energy-producing machinery in neurons that make dopamine—the same neurons that are killed in Parkinson's disease.

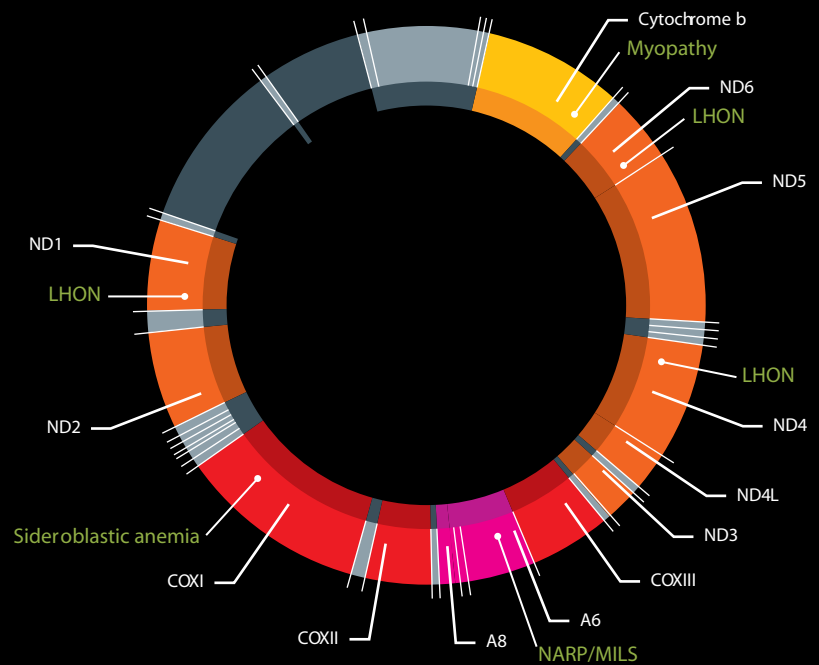
Since then, researchers have identified at least three nuclear genes whose proteins are associated with mitochondria. Preliminary evidence suggests that these genes are all involved in managing oxidative stress—the damage caused by free radicals. This points to a possible vicious cycle of deterioration in which mutations hinder the mitochondrion's ability to generate energy while churning out higher levels of polluting ROS, which, in turn, tear up a cell's protective membranes and further mutate mitochondrial DNA. The damaged mitochondria may create still more ROS, and the cycle continues.

If, indeed, oxidative stress is implicated in Parkinson's disease, finding a way to relieve it might slow mitochondrial

When Mitochondria Mutate //

Sections of nuclear DNA dictate certain mitochondrial functions. When these sections mutate (in other words, when the DNA sequence within a gene is altered), mitochondria can malfunction, though the role of these malfunctions in various diseases—such as Parkinson's and diabetes—remains murky. But mutations in the mitochondrion's own 37 genes point more clearly to certain rare disorders. Here, dotted along the mitochondrion's circular genome (its set of hereditary information), is a sampling of conditions relating to the 13 major genes that code for proteins that make up complexes. Each complex performs a specific step in the energy-producing chain.

- | | |
|---|---|
| ■ Complex I genes | ■ Complex V genes |
| ■ Complex III genes | ■ Ribosomal RNA genes |
| ■ Complex IV genes | ■ Transfer RNA genes |



LHON: When inherited mutations cause complex I genes to malfunction, the result can be the death of retinal ganglion cells, whose axons compose the optic nerve. That can trigger the disease LHON (Leber's hereditary optic neuropathy), which primarily strikes young men and results in the sudden loss of vision within just days or weeks.

MYOPATHY: Sporadic mutations (alterations in genes that accumulate during development or life and are not inherited) in the cytochrome b gene can cause a certain type of myopathy, or muscle disorder, which manifests as severe muscle weakness and the inability to exercise.

SIDEROBLASTIC ANEMIA: Sporadic mutations in the COXI gene disrupt complex IV. This can lead to sideroblastic anemia, which results in fatigue, weakness, breathing difficulties and even liver damage and kidney failure. The mutation may impair the production of heme, the iron-rich part of blood cells that carries oxygen to the body's tissues.

NARP/MILS: An inherited mutation in the A6 gene can lead to neuron death and brain atrophy. Diseases like NARP (neuropathy, ataxia and retinitis pigmentosa) and MILS (maternally inherited Leigh's syndrome) can result, causing impairment in coordination, vision loss, dementia and seizures.

deterioration. Typically, antioxidant dietary supplements don't help, probably because they can't get through the mitochondrial membrane into the matrix, where free radicals are produced. To address that problem, Murphy designed MitoQ with a positive charge so that it would be drawn in by the mitochondria's negative charge.

Now the larger Phase II trials should show how well MitoQ, a powerful antioxidant, works in humans and help determine whether oxidative stress does play a major causative role in Parkinson's—and, potentially, in a number of other diseases, including Alzheimer's, amyotrophic lateral sclerosis (ALS) and even migraines. If MitoQ works for Parkinson's, it might also prove effective against those other diseases.

In the case of type 2 diabetes, one of today's most prevalent diseases, evidence for a possible mitochondrial link stems from the observation that mitochondria of diabetics have a reduced ability to produce energy. In 2003, Vamsi Mootha, a systems biologist at the Massachusetts General Hospital and the Broad Institute in Cambridge, decided to test whether a genetic approach, using a device known as a gene chip, could help isolate the problem. (Gene chips are quartz plates the size of a postage stamp spotted with every human nuclear gene, but not mitochondrial DNA. Manufacturers chemically attach more than 20,000 bits of DNA at specified coordinates on the plate.)

In his study, Mootha and his team poured a batch of RNA—the intermediary molecule DNA uses to make proteins—from the muscles of diabetics over 17 chips. To 18 others they applied RNA from the muscles of nondiabetics. He knew that if the RNA stuck to the DNA on the chip and fluoresced under a special light, the genes were actively producing proteins.

The results were striking. The dots for genes involved in energy production pathways—and linked to mitochondria—were much dimmer in diabetics than in those without the disease. It appeared that whole sets of energy production genes were associated with diabetes. “No single gene showed a striking difference in expression between the diabetics and the controls, probably because diabetes is a complex disease,” Mootha says. “Rather, we're seeing a general decline in mitochondrial numbers and activity,” suggesting diabetic muscles can't produce energy as well as nondiabetic muscles.

Mootha and others don't yet know whether dysfunctional mitochondria cause diabetes, are a by-product of the disease or are simply an unrelated correlative condition. Still, these results strengthen the hope for one simple remedy. “It has long been known that exercise increases mitochondrial content and efficiency,” Mootha says.

Is This Why We Get Old?

Mitochondrial deterioration, fueled by free radicals, may speed the aging process. But the link is far from certain.

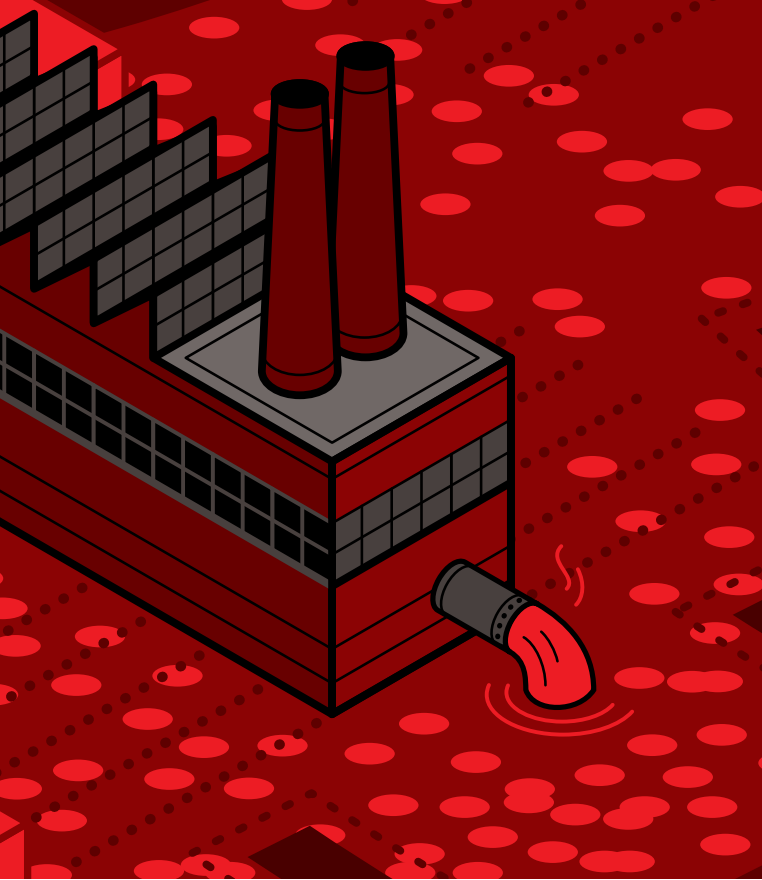
The vicious-cycle theory of aging is also known as the mitochondrial theory, and it holds that as time passes, molecules called free radicals accumulate in the mitochondria. Free radicals are a normal result of energy production, yet they are also destructive to mitochondrial DNA. Damaged mitochondrial DNA hinders the ability of mitochondria to generate energy efficiently, causing them to churn out more polluting free radicals, which in turn cause further mitochondrial DNA damage. The theory suggests that this vicious cycle goes on in all human cells, eventually leading to sagging skin, loss of cognitive abilities and muscle decline, among other degradation.

“My mitochondrial DNA are slightly more mutated today than they were yesterday,” says neurogeneticist Nick Wood of the Institute of Neurology, University College, in London. Wood studies mutations in genes, targeting the mitochondria that result in Parkinson's disease. “But are those mutations sufficient to cause aging, or are the mutations just a result of being on the planet for 70 years?”

Whether mitochondrial mutations actually promote aging or are coincidental—and whether free radicals even play a role—is controversial. The best study addressing the question was performed on mice in 2004 at the Karolinska Institute in Sweden. The mice had mitochondrial DNA that accumulated mutations at a faster-than-normal rate. They developed hunched backs and enlarged hearts at an early age and typically survived about half the normal life span. The researchers were able to establish a direct link between the increased mutations in mitochondrial DNA and this rapid aging, and hypothesized that free radicals were to blame. But when the same Karolinska group, using the same type of mice, repeated the experiment, this time measuring the mice's levels of free radicals, they found the levels remained constant, making it unlikely that free radicals were the cause of premature aging.

Other studies have suggested free radicals are involved in the aging process, so it's important to settle the debate. But if free radicals are not to blame, there's no vicious cycle to target as a way to slow aging.

Mitochondria's role in cancer has been hypothesized since the 1930s, when Otto Warburg, a Nobel Prize-winning German physician, discovered that mitochondria in cancer cells do a poor job of producing energy. And although the past 70 years have seen often halting progress in research on the subject, it's now clear there is at least a coincidental connection between mitochondria and cancer. “All cancer cells examined so far have mutations in mitochondrial DNA,” says Keshav Singh, a mitochondrial geneticist at the Roswell Park Cancer Institute in Buffalo and founder of the Mitochondria Research Society. “And most cancer cells have defective mitochondria.”



to screen for early signs of disease. (Because there is a lot of mitochondrial DNA in cancer cells, mutations are easy to spot, and they appear early in cancer development.)

One diagnostic approach involves the MitoChip, a gene chip created by Johns Hopkins researchers in 2004 to detect mutations in mitochondrial genes. Singh is working on another method, the NucleoMito Chip, which looks for changes in expression of mitochondrial proteins encoded by nuclear genes. Both methods can potentially detect a variety of cancers rapidly, although clinical use is a few years away.

Singh is also working with scientists at the Sandia National Laboratories in Albuquerque on a device known as the Biocavity laser, which detects fluorescence in individual mitochondria. Some proteins in the mitochondria's energy production machinery glow; when mitochondria malfunction, the glow is affected. "We can detect changes in a single cell," Singh says. "Most of our success against cancer has been due to early detection, and if our tests become sensitive enough to find one bad mitochondrion, that may help us find cancer very early on."

If this and other clinical outgrowths of research into the role of mitochondria succeed, they could open a fruitful focus for detecting and treating disease. "Mitochondria are like a bridge connecting two island suburbs—one island represents the many causes of a disease, the other the resulting symptoms," says Auckland Hospital's Barry Snow. "Knocking out all the houses on either side"—in other words, relieving all symptoms or eliminating all environmental and genetic causes—"is difficult. Targeting the narrow bridge"—mitochondrial dysfunction, which appears to link causes and symptoms—"is much easier." ■

But as with diabetes, the question is whether mitochondrial mutations or defects cause cancer or are merely correlated with it. Singh believes that defective mitochondria harm other parts of the cell, particularly the nucleus. "When mitochondria don't work properly, they cause genetic instability in the nuclear genome," Singh says, "and instability is a major cause of cancer." The result is that mutations occur more frequently than normal, increasing the likelihood that genes involved in cell division or cell death will fail to work. (Uncontrolled cell division leads to cancer; programmed cell death prevents it.)

That instability may stem from mitochondria that produce an excess of free radicals, which can damage nuclear DNA. Or, Singh says, deficient mitochondria may trigger a process called error-prone repair—instead of fixing damaged DNA, it inserts incorrect nucleotides into the genetic code, increasing the likelihood that a cell will mutate and become cancerous.

No one yet knows which comes first—a malignant tumor or mitochondrial mutations—but the association itself may aid cancer detection. The existence of mutations could signal a cancer's presence, and with recent breakthroughs in scanning bodily fluids for mitochondrial mutations, it may be possible

→ DOSSIER

1. *Power, Sex, Suicide: Mitochondria and the Meaning of Life*, by Nick Lane (Oxford University Press, 2005). A tale of mitochondria's evolution from free-floating, single-celled bacteria to the most important organelles in our bodies.
2. "Powerhouse of Disease," by Nick Lane, *Nature*, March 2006. A fascinating summary of the hunt for nuclear genes that encode mitochondrial proteins, detailing the role such genes play in initiating a variety of common diseases.
3. "Mitochondria, Oxidants, and Aging," by Robert S. Balaban et al., *Cell*, Feb. 25, 2005. Balaban presents a clear picture of the mitochondrial theory of aging, supported by studies of aging worms, flies and mice.