

THE MITOCHRONICLES: PART 2

BY JOE MIKSCH

# MITOCHONDRIA'S MANY MISSIONS

*In the first half of this series, scientists were going gaga over the likely role of mitochondria, a long-neglected organelle, in cancer. Pitt mitochondriacs are also tackling Parkinson's disease, heart health, and a devastating childhood disease. Here, you'll read stories of resurrection, vital dances, and very sick flies.*

**T**he addicts of America needed a fix. War in poppy-producing Afghanistan and Turkey made it tough to get the real stuff. In 1976, a graduate student in chemistry, a Maryland man named Barry Kidston, tinkered around in his home lab and mixed up a super-potent synthetic called MPPP.

The drug had been around since the 1940s and, back then, had 70 percent of the potency of morphine. Through Kidston's labors, he made it even stronger and, therefore, all the more desirable to addicts. Then, in 1977, the 23-year-old injected some of his "home brew" as he often had in the past. But something went wrong. Within three days he developed symptoms of Parkinson's disease.

In 1982, 42-year-old George Carillo arrived at a hospital in San Jose, Calif. He was bent, twisted, drooling, and mute. His muscles were frozen. Six similar cases followed. Doctors became detectives. All seven Parkinsonian "frozen addicts" were found to have used a synthetic opiate called China White.

Members of Charleen Chu's lab have found that mutations that cause Parkinson's disease decrease the number of mitochondria (green) in dendrites (red), which are vital for receiving signals in the brain. This image shows mitochondria in healthy neurons.

RUBEN DAGDA AND CHARLEEN CHU

Then, a toxicologist involved in the California case recalled Kidston's misadventure. Though it wasn't big news at the time, authorities who examined Kidston's lab found a very nasty impurity—MPTP—in the batch that had rendered him ill. Six years later, that same impurity was found in the garage-lab-made California China White.

And thus began an explosion in Parkinson's research. Investigators found that when the brain metabolizes MPTP, the byproducts include a toxin called MPP+. This MPP+, they eventually learned, attacks mitochondria—especially the beginning of the electron transport chain, what's known as complex I. This group of enzymes is associated with the mitochondria's best-known role: producing energy for the cell. Later, researchers would discover that complex I is inhibited in all cases of Parkinson's, even in the rare inherited form.

there was to know—that mitochondria make ATP [fuel for cells]. It's turned out to be much more complicated than that.”

In the late 1990s, while he was on the faculty at Emory University in Atlanta, Greenamyre began thinking that it might be useful to create a better animal model of Parkinson's. He had a particular interest in replicating the manner in which damaged mitochondria create conditions that make neurons interacting with dopamine susceptible to the disease. He started with a naturally derived pesticide called rotenone, which was known to cause mitochondrial damage and remains one of several environmental factors thought to cause Parkinson's.

Sure, other researchers had met with some success; but their techniques were limited. First off, the dominant approach at the time—injecting rotenone directly into a mouse's

rotenone, administered intravenously, to replicate in mice what they had found in people. Success here produced systemic mitochondrial dysfunction, hit only dopaminergic neurons, and produced Parkinsonian symptoms in the mice. Even more useful: The mice developed tangles of alpha-synuclein proteins (the same proteins that aggregate in the brains of human Parkinson's patients) called Lewy bodies. The resulting paper, published in 2000, remains the most cited in *Nature Neuroscience*.

“This model was the first proof of concept that generalized mitochondrial dysfunction could lead to very specific neural degeneration,” Greenamyre says. “And since then the genetics of Parkinson's disease have really advanced. Now we've found several genes that, when mutated, affect the mitochondria and produce Parkinson's.”

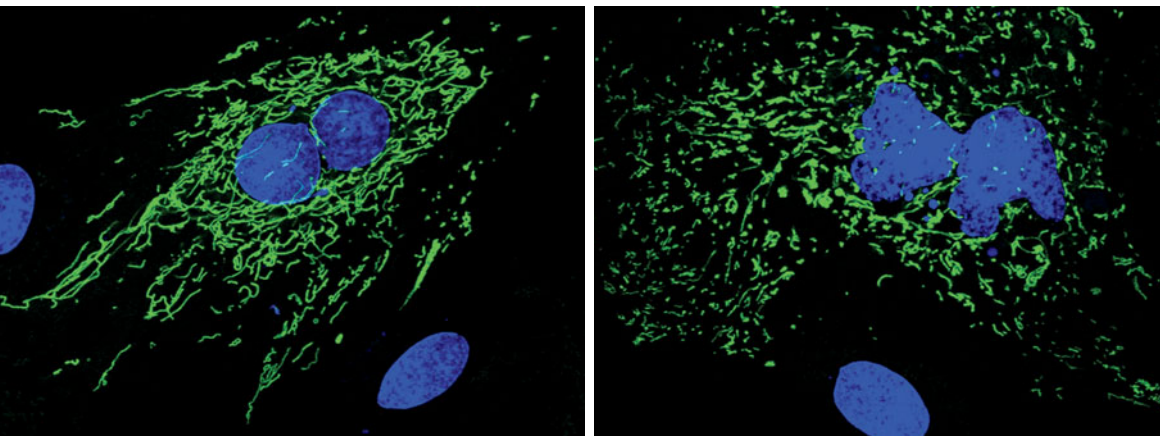
The common thread, then, is that regardless of whether a particular case of Parkinson's is spurred by environmental toxins such as rotenone or genetic mutations accumulated over time, mitochondria remain central.

“That's true in many, probably the majority, of Parkinson's cases,” Greenamyre says. “If you're thinking about the pathogenesis of Parkinson's, you can start the degenerative process in numerous places. In some cases, it starts with the mitochondria, in some cases not. But mitochondria will play a vital role in the disease at some point.”

Charleen Chu is confident that Greenamyre is right. So much so that the MD/PhD professor of pathology and PIND member is looking at five models of Parkinson's, all of which affect the mitochondria's favored organelle. To Chu, regardless of whether the models involve neurotoxins or mutated genes, the upshot is that something is quite wrong with mitophagy, the process by which damaged mitochondria are safely destroyed. Or, if it's not mitophagy per se that's gone awry, it's the related and equally important cellular step of generating new mitochondria to replace the bad ones that have been eaten up.

“We've studied the recycling of mitochondria for some time,” Chu says. “And the question we're faced with is, ‘Well, if mitophagy is good for you, why, in some cases, does it cause neuronal cell death?’”

In the spring, Chu and colleagues published a paper in *Cell Death & Disease* that gives some inkling of an answer. She found that there are



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**Mitochondria, the green stuff, fusing (left) and undergoing fission (right). Fusing mitochondria are longer; fissioning ones are shorter; and the entire process is vital for cellular health.**

As the disease progresses, mito dysfunction kills off the dopamine-producing neurons in the substantia nigra, a part of the brain that controls voluntary movement.

Enter Pittsburgh's mitochondriacs.

The University of Pittsburgh's J. Timothy Greenamyre—MD/PhD UPMC Endowed Professor of Neurology, chief of Movement Disorders, and director of both the Pittsburgh Institute for Neurodegenerative Diseases (PIND) and the American Parkinson Disease Association Advanced Center for Parkinson's Disease Research at Pitt—was among those intrigued by the relationship between toxins, mitochondria, and Parkinson's.

“In the '80s, mitochondria research was a backwater,” says Greenamyre, whose investigations of the organelle stretch back to that decade. “People thought they knew everything

brain—indeed killed dopaminergic neurons, but it also obliterated plenty of other brain cells not implicated in Parkinson's. You might expect that a random and nonspecific brain cell death would result no matter what toxin was injected directly into the brain, especially in such high doses. Further, because of the injection method, such models damaged mitochondria only in the brain. Greenamyre and his colleagues believed, and emerging literature suggested, that Parkinson's patients had systemic mitochondrial damage, not just damage to the mitochondria in their dopaminergic neurons.

Greenamyre and his Emory group examined cells from humans—Parkinson's patients. They determined the degree to which mitochondrial function was inhibited and then slowly but surely found the right level of

times when mitophagy outpaces a neuronal cell's ability to regenerate mitochondria. "In any recycling process, it's not good enough to degrade the damaged stuff," she says. "You have to rebuild." Neural cells require and burn more energy than other types of cells, making them particularly dependent on mitos. When there aren't enough mitos, there's not enough energy; calcium is mishandled; death comes calling and causes, in this case, neurodegenerative diseases like Parkinson's.

The discovery was based on a slightly counterintuitive observation: Inhibiting ERK, a protein vital to cellular proliferation, resulted in recovery of healthy, functional mitochondria. You see, neurons don't proliferate, and slowing down mitophagy gave them time for mitochondrial repair.

"When we inhibit ERK, not only do we slow down degradation, we boost biosynthesis, and the cells are completely protected," she says. "In our other studies—we've used three genetic models and two toxin models—although mitochondria are all impacted differently, all these processes feed into this cycle of damage-mitoph-

agy-resynthesis." which mitos meet each other, swap genetic material, and separate could have a significant role—when it goes wrong, that is—in the progression of Parkinson's.

Sarah Berman (PhD '98/MD '00), a PIND member and assistant professor of neurology, became interested in mito dynamics and Parkinson's as a grad student here at Pitt. During her training, she studied reactive oxygen species (ROS), the nasty stuff that mitochondria make when the energy production process has gone bad. The idea was that ROS was one of the factors that accounted for the slaughter of dopamine neurons in Parkinson's.

By Berman's grad student days, this was important and interesting, but no longer a groundbreaking concept. The body of evidence was strong suggesting that trouble with complex I and, subsequently, mitochondrial respiration, was a precursor to the development of Parkinson's. Berman, in her own lab, decided to delve a bit more deeply into a different aspect of mitos and Parkinson's.

"What I study is not so much respiration, but mitochondrial dynamics," she says. "It

find a way to alter the fission and fusion processes, to keep them healthy, "maybe then we can develop a corrective therapy we can use before cells die and Parkinson's develops."

Sitting in her office, Berman brings up a movie showing fission and fusion in real time. Mitos blink red and are tagged with a photo-active green fluorescent protein. When a pair fuses, yellow flashes on the screen.

"See!" she says excitedly.

"When the membranes merge and the contents get mixed up, the whole thing turns yellow. Then you see it divide into two, and that's fission. You can monitor and quantify these events, and it's neat. I mean, the geeky scientist that I am, I look at this and think, *It's so cool!*"

In the lab, Berman manipulates the proteins responsible for fission and fusion. Something a bit unexpected happened after Berman started playing with those proteins—something that could, down the line, prove to be an important step in preventing cell death in Parkinson's before the process really gets going.

"When we add a fission inhibitor to the rotenone model—and I wasn't sure which

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agy-resynthesis."

One path to a possible treatment for Parkinson's and like diseases is obvious: maintain the balance between the destruction of ailing mitos and the generation of healthy replacements. But doing so isn't easy. Bennett Van Houten, a Pitt cancer researcher featured in the first installment of the Mitochronicles, calls mitochondrial degradation over time the "rust of life." As time passes, he suggests, mitos might just accumulate too much rust; and the balance between death and resurrection, the intracellular circle of life, then becomes intractably screwed up.

Chu puts it this way: "Even with increased damage, as long as the cycle is able to roll, you might be able to compensate for it, and maybe that's why diseases like these don't present until decades have passed."

Rolling. An interesting word choice in the realm of mitochondria. The conventional wisdom, for quite a while, was that these organelles were static. They just hung about in the cell's interior and produced ATP. That view turned out to be wrong. Mitos are not only very active; but movement, it is now known, is vital for mitochondrial health. Further, the process by

sounds like an obscure sort of thing, but the fact that mitochondria divide and fuse together and get transported up and down neurons, this is really critical—in neurons, in particular."

Neurons, Berman says, are different from other cells in that they require more energy to work, thereby increasing the importance of mitochondrial health. (Not that it is unimportant in other cells.) Further, she says, the process of fission and fusion, the movement of mitochondria from the cell body all the way down the axons to the synapses, seems to be absolutely crucial to the life of the neuron.

"If you take away the ability for them to divide, they don't get down to the synapses, and the synapses don't work right," she says. "That's really intriguing, because one of the things that happens in Parkinson's is that it's not the cell bodies that die first, but the long axon terminals. Something is happening very far away from the cell body, and then the cell dies."

Berman uses an in vitro version of Greenamyre's rotenone model to reveal the music that makes mitochondria dance up and down axons.

Her hunch and hope are that, if she can

way this was going to go—it turned out to be protective. And if we prevented fission, we didn't lose the [axons]. Maybe we can alter the neuropathology, but we don't know why this is protective.

"Yes, this is exciting," Berman continues. "But at this point we have many more questions than answers."

**I**n the world of mitochondria research—still a pretty young field—questions abound. Sruti Shiva has taken questions about the organelle's role—with the aid of nitrite—to heart. As in heart attacks. Shiva is investigating how mitos, exposed to extra nitrite, protect cells from damage when blood, and the oxygen it carries, floods back into organs after a period of deprivation.

For the past decade or so, it's been known that nitrite, which is native to our bodies and also found in green, leafy vegetables, plays a role in cell signaling. (It had previously been considered nothing more than a physiologically inert product of nitric oxide.) What's new, out of Shiva's lab, is confirmation that increasing the amount of nitrite in the bloodstream can increase the number of mitochondria in a

cell. (Shiva's "cell of choice" comes from the heart.) And a boost in the number of mitoses has been shown to facilitate adaptation to high altitude, increase energy efficiency, lengthen lifespan, and ease weight loss.

An emerging bit of Shiva's research (which the PhD assistant professor of pharmacology and chemical biology does as a member of the Vascular Medicine Institute), though, skips over the number of mitochondria generated by the introduction of extra nitrite. Rather, she has examined how nitrite spurs mitochondrial activity, leading to a series of cellular events that offer protection to cells stressed by a lack of oxygen and its subsequent reintroduction in the wake of a heart attack.

"What we've found is that, if you give a small dose of nitrite (pre-heart attack), you get a small burst of ROS [those unpleasant reactive oxygen species] from the mitochondria," she says. "This actually turns on a signaling cascade that signals for more protection. It somehow causes a cell to increase its production of antioxidants (counteracting the damaging reactive oxygen species released by a stressor) and bump up protective signaling messages."

Animal studies, Shiva says, have shown a massive reduction in organ damage.

Pursuing and understanding the mecha-

and then at some point later—in severe cases we're talking days or weeks after birth—the disease develops," says Michael Palladino. "And being diagnosed with primary mitochondrial encephalomyopathy is pretty much a death sentence, particularly in its [very early] childhood-onset form." The Pitt associate professor of pharmacology and chemical biology notes that when faced with ME, clinicians prescribe cocktails of vitamins and other supplements, but, "No one has any real confidence that those are anything near a cure or even of any therapeutic value." Yet the disease is horribly painful, he says, and "a child could linger for a couple of months, a couple of years, maybe more."

Palladino has developed the only animal model of ME with a mitochondrial DNA (mtDNA) mutation; he uses *Drosophila*, the fruit fly. There are about 200 different such mutations that cause ME, which affects one in 11,000 children. Until Palladino's flies came along, it was difficult to study the disease.

The mutation in Palladino's flies causes them to have poor motor control and exceedingly short lives. It was serendipity that led Palladino to this particular strain. He and members of his lab were studying a fly model of neurodegeneration. (He is also a member of PIND.) The process of sorting out its genetic

makeup led to the discovery that a mutation of the ATP6 gene in the fly's mitochondria was also causing symptoms akin to the rare but devastating MEs that affect humans.

"And this put us in a beautiful place," Palladino says. "There's all this interest in human primary mitochondrial encephalomyopathies, but no one had a model system of them. So we went from having very little interest in mitochondria to *BOOM*. We had to become experts in this pretty quickly, because we had a novel resource that no one else in the world had."

The most important feature of the model, says Palladino, is the fact that endogenous mitochondrial diseases are progressive. Yes, the basics can be studied in vitro, but to get the full picture of how such diseases take root and devastate over time, you need to look at a living creature.

So with a compelling and unique model, Palladino has set off to find some sort of viable treatment for the disease. And he's made some progress.

The first front is pharmacological therapy. Drug screening has yielded a handful of compounds that somewhat improve the condition of Palladino's sick flies.

"The drugs we've identified do make the animals better—they live 5 to 8 percent longer, and we've seen a 10 to 15 percent improvement in locomotor function," he says. "But the thing about pharmacological therapies is that these are such complex diseases that few people think there's going to be a magic pill that fixes them. The rationale is that mitochondria do so many important things that there can't be just one target. A large cocktail of drugs probably wouldn't even work."

Knowing this led Palladino to aim closer to the source: mtDNA. Scientists have become adept at inserting and removing components of nuclear DNA, but they've had less success directly affecting the mitochondrial genome. So although he knew his target, he was unsure about the best weapon to deploy. Palladino chose allotopic expression. The idea here—and this is a technique developed about 15 years ago at Columbia University—is that while no one knows how to directly manipulate mtDNA, it is possible to tweak the nucleus. Before inserting a gene into the nucleus, a researcher can attach chemical directions that will drag the protein, generated by the gene in the nucleus, into the mitochondria.

"We've made some huge improvements to the efficacy of this process," Palladino says. "We're getting 10 to 15 percent rescue in longevity and 35 to 45 percent improvements with locomotor function."

"Within a year or two, we're going to have some real successes here. Our mutant's disease is so incredibly severe—worse than it ever is in humans—and we're very excited about the levels of improvement we've seen so far. Nevertheless, we're pushing for a 40 to 50 percent improvement in longevity."

"We'd like to achieve that before we publish, and I think we can."

Palladino is so excited about the therapy's potential—an excitement that seems both natural and a possible consequence of the espresso machine in his office—that he's already in the early stages of planning for clinical trials.

Why so optimistic? "Why not?" Palladino asks. "It keeps you motivated!" ■

**For further mitochondrial motivation, visit [www.upci.upmc.edu/trmad/](http://www.upci.upmc.edu/trmad/)**

## "So we went from having very little interest in mitochondria to *BOOM*."

nisms by which nitrite and mitoses convey cytoprotection are, naturally, preconditions to bringing any related therapy to the clinic. Thus far, it appears that when nitrite is administered before or during ischemia and reperfusion, it keeps the now obviously vital mitochondrial complex I from going off the rails. Keeping that first step in ATP production on point, it seems, reduces the production of ROS and bolsters production of the antioxidants that tame those ROS that are released.

The takeaway, Shiva says, is that a little more of something that's already inside us can help our mitochondria protect our hearts from a second, and profoundly damaging, insult in the wake of a heart attack.

**C**hildren with mitochondrial encephalomyopathy (ME)—which causes seizures, headaches, strokes, lactic acidosis, dementia, and, typically, death before age 20—are not born with evidence of the disease.

"You're born and deemed healthy at birth