One fish, two fish, red fish, see-through fish. This zebra fish has been bred to be transparent. With this model, Edward Burton, Simon Watkins, and others at Pitt are seeing mitochondria misbehaving in real time. Many labs are now turning their focus to mitochondria; when the organelle is not functioning correctly, it can lead to Parkinson’s, Alzheimer’s, and Huntington’s diseases. Mitochondria also seem to have difficulty doing their job in the presence of other neurological disorders and brain injury.
About 2 billion years ago, a couple of bacteria swimming in the primordial soup collided and tried to eat each other. Instead, they merged and formed an unexpected truce that changed life on Earth. Over eons, their descendants slowly evolved—one acquired novel genes and became the home structure, while the other moved in, gave up most of its genes, and slowly morphed into mitochondria, those tiny organelles dedicated to producing energy. With all that extra energy available, life was free to invent a dazzling array of creatures far more complex than bacteria—from hyacinths to hyenas, mushrooms to mice, fruit flies to falcons. Today, mitochondria are scattered through our cells like stars in a night sky. They mostly allow themselves to be regulated by the nuclear genome, and the energy they produce is so critical to cellular function that we now suspect mitochondrial impairment to be centrally involved in many diseases of aging and neurodegeneration.
“Mitochondria is where everybody’s research is leading now,” says the University of Pittsburgh’s J. Timothy Greenamyre—MD/PhD vice chair and Love Family Professor of Neurology, chief of the Division of Movement Disorders, and director of both the Pittsburgh Institute for Neurodegenerative Diseases and the American Parkinson Disease Association Advanced Center for Parkinson’s Disease Research at Pitt. “We’re all studying mitochondria, especially for neurodegenerative diseases.” And what Greenamyre and colleagues are uncovering is changing our understanding of diseases like Alzheimer’s, Parkinson’s, and Huntington’s, and paving the way for potential new therapies and drugs based on novel insights into mitochondrial function.

Take Greenamyre’s collaborative work with Edward Burton, an MD/DPhil associate professor of neurology at Pitt. The two recently demonstrated that gene therapy can prevent Parkinson’s symptoms in rats. Their study used a small, harmless virus called AAV2, engineered to safely transport a piece of genetic code into the brains of rats. That genetic code blocks production of a protein, α-synuclein, which builds into damaging clumps in the substantia nigra—a streak of tissue in the midbrain—of people with Parkinson’s disease. The substantia nigra regulates motor function and is studded with long and delicate dopamine-producing neurons; it slowly loses function in Parkinson’s, producing the disease’s abnormal movements, stiffness, and immobility.

In the experiment, gene therapy was delivered to the right side of the rat brain, which controls the left side of the body, and then the rats were given a precise dose of the pesticide rotenone. Exposure to rotenone can lead to Parkinsonian symptoms. After the rats were injected with the pesticide, motor function on their “treated” left side remained normal, while the untreated side developed symptoms. In contrast, untreated rats, as well as rats given a control virus that contained no gene therapy, developed full-blown Parkinson’s symptoms after rotenone exposure. The findings were published in July 2015 in the Journal of Clinical Investigation. Eventually, this approach could be translated into clinical trials at UPMC and Pitt. (Other Pitt investigators are already set to begin another gene therapy in Parkinson’s patients, one that enhances a brain enzyme that converts the most common Parkinson’s drug, levodopa, into dopamine.)

This remarkable study builds on the rotenone model of Parkinson’s that Greenamyre developed 15 years ago. “Rotenone is unique,” says Greenamyre, “in that it is a pesticide, and pesticide exposure is a known risk factor for Parkinson’s. But it also inhibits a very complex [mitochondrial] enzyme we simply call complex 1 that is impaired in Parkinson’s disease.” In Parkinson’s, complex 1 deficits appear in multiple places—blood, muscle, platelets—suggesting that mitochondrial function is defective throughout the body. But the very selective neurodegeneration happens only in the substantia nigra of the brain, leading to the actual disease. Rotenone exposure reproduces both those conditions, so it’s an exquisitely accurate model. “There was a very big reaction, a lot of press, a lot of hubbub about that first paper on the rotenone mouse model in 2000,” says Greenamyre. “It has been cited 2,000 times.”

Building on the gene therapy study, Greenamyre and colleagues have now shown how the dance between the nuclear genome and the mitochondria—the dance first started in that symbiotic union 2 billion years ago—is impaired in Parkinson’s and why α-synuclein may be so crucial. It turns out that mitochondria can’t function without importing proteins made and regulated by the cell’s nucleus, and...
STUTTERS AND STOPS

More than two decades after the cause of Huntington’s disease was discovered, there is still no treatment—largely because it had been unclear how the mutant HTT gene led to the death of neurons. A recent Nature Neuroscience paper uncovers this mechanism.

“It’s becoming more and more obvious that mitochondria are a central player in neuronal health,” says principal investigator Pitt’s Robert Friedlander, MD, the Walter E. Dandy Professor and chair of neurological surgery, and head of cerebrovascular neurosurgery at UPMC.

Friedlander’s group found that mutant huntingtin, the protein transcribed from HTT, blocks other proteins from entering mitochondria. Lacking these important proteins, mitochondria gradually begin to function inappropriately. Ultimately, this leads to the activation of the cell death pathway. This process occurs very early in the progression of Huntington’s disease, and it is specific to neurons, despite the fact that the Huntington’s gene is present in every cell in the body.

“It’s a very direct link between the cause of the disease and a relevant disease pathology,” says Friedlander.

Understanding the significance of this finding requires a little background about why it isn’t feasible to treat Huntington’s, a wholly heritable disease, with gene therapy. It isn’t a good idea to remove the HTT gene entirely, because this manipulation is lethal in mice at the embryonic stage. Targeting the mutation alone is difficult, because its presentation is subtle—just a stutter in the DNA sequence. Friedlander’s newly discovered pathway from mutant huntingtin to the demise of neurons opens up new avenues for drug development.

Even more exciting, Huntington’s disease shares many of the same mitochondria-mediated cell death pathways with Parkinson’s, ALS, and Alzheimer’s, so a drug that works to treat one disease may very well work on others, Friedlander notes.  —Erin Crowder Hare
that excess α-synuclein impairs that ability—interrupting their pas de deux.

“Although mitochondria contain their own genome,” says Greenamyre, “they must import 99 percent of the proteins they own genome,” says Greenamyre, “they must import 99 percent of the proteins they need. The mitochondrial import machinery is highly regulated and dependent on the nuclear genome.” In new work, forthcoming in Science Translational Medicine, Greenamyre shows that α-synuclein binds to an outer membrane receptor on mitochondria that imports proteins. It is at that receptor’s door that excess α-synuclein begins the vicious cycle. “We’ve seen that gene therapy may help downregulate production of α-synuclein,” says Greenamyre, “but we are also going to try to upregulate the [outer membrane] protein, so that the receptor is more active and can import more proteins. That might be another effective approach.”

Meanwhile, Burton is building on his collaborative work with Greenamyre by turning

“α-synuclein begins the pas de deux.

“Perhaps α-synuclein enhances production of reactive oxygen species by mitochondria, or inhibits the repair of damaged mitochondria,” he says.

What’s most remarkable is the way Burton has applied genetically encoded fluorescent probes to mitochondrial function. With color-coded fluorescent tagging—cerulean blue, green, cherry, and other colors—he can light up different structures in the see-through fish. The outline of neurons might be green, the mitochondria themselves red. Then, using microscopes so powerful they can capture three-dimensional views of organelles and record the actual movement of mitochondria inside cells, they reveal a once invisible wonderland.

“It takes us about a year to make a [line of] transgenic zebra fish with a green outline to the neuron and a red outline to the mitochondria,” Burton explains. “But the investment of time is worth it.”

Burton has already used fluorescent proteins sensitive to chemical changes in dopamine neurons, and plans to use the fish to recreate the Parkinsonian susceptibility of dopamine neurons to mitochondrial inhibitors in the presence of α-synuclein.

“We will be able to see dynamic biochemical changes in the neurons of a living brain. Nobody has done this before. Combined with the rapid screening capacity of zebra fish, there is tremendous potential to understand the basis for cellular susceptibility in diseases like Parkinson’s, and to develop novel therapies.”

To watch mitochondrial dynamics in these brightly lit transgenic fish, Burton has been working with Sarah Berman, an MD/PhD associate professor of neurology. Berman mastered fluorescent tagging work during a postdoc at Johns Hopkins, where

those associated with traumatic brain injury (including in football players). Burton hopes to discover just how and why α-synuclein makes nerve cells vulnerable to mitochondrial inhibitors like rotenone.

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haps to exchange genes, discard damaged DNA, and replace it with healthy DNA. Berman’s work focuses on how dysfunction in these mitochondrial events contributes to neurodegeneration.

“It’s really cool to see red fluorescent mitochondria moving up and down the neuron. We don’t understand why they’re leaving and then stopping and coming back.” But she hopes to find out. Already, by using fluorescent tagging on neurons in cell culture, Berman has learned that inhibiting mitochondrial fission is beneficial for neurons exposed to low, chronic doses of rotenone.

“One of the beauties of working with zebra fish,” says Berman, “is we will be able to look in detail at the pathology and the triggers of degenerative diseases like Parkinson’s.” The researchers plan to knock out the Parkin gene in zebra fish and see how it affects the mitochondria.

As a practicing neurologist who treats Parkinson’s,” says Berman, “I have good symptomatic medicines to help patients manage their motor symptoms for many decades. But why are the cells dying in the first place? We want, as researchers, to find the earliest changes and target our therapy to those systems before they start dying.”

Burton’s transgenic fish are generating a bit of a neuro-buzz on campus. Funded by a grant from the National Institute of Environmental Health Sciences, Pitt’s Bennett Van Houten is now working with Burton and coprincipal investigator Patty Opresko, a PhD associate professor of environmental and occupational health, to discover how mitochondria, by generating free radicals, may potentially damage telomeres. Telomeres are the caps at the end of each strand of DNA that protect our chromosomes, and they shorten with age.

“The ability to see into the developing zebra fish brain and actually track living mitochondrial behavior is unprecedented,” says Van Houten, a PhD, the Richard M. Cyert Professor of Molecular Oncology, and associate director for basic research at Pitt’s Aging Institute. And, he adds, “None of this zebra fish work would be possible without our imaging center.”

At Pitt’s Center for Biologic Imaging, Simon Watkins collaborates with all of these researchers, offering the skills of his team and the use of a 6,500-square-foot suite that houses about 30 microscopes. (Watkins, a PhD, is a Distinguished Professor and vice chair of cell biology.)

“I pinch myself every day when I interact with Watkins,” says Van Houten. “The ability to go over to that facility to use the tools he’s assembled is truly extraordinary.”

Each image from a microscope may have 80 or 90 moving mitochondria. To quantify the movements, the images are fed into computers and analyzed.

Van Houten thinks that this kind of work will ultimately, well, illuminate mitochondrial dysfunction as part of the pathophysiology of many common neurodegenerative diseases. Burton concurs:

“We’ve made zebra fish models of Parkinson’s disease, progressive supranuclear palsy, dystonia—we can subject them all to this level of analysis. Mitochondria are dynamic, and neurons are constantly shuffling their mitochondria around. We’ve been looking closely at the axon—the projection from the nerve cell that is communicating with other nerve cells. It’s a long, narrow projection, and we see the mitochondria moving down it a bit like cars on the freeway. Some are moving out from the cell; some are moving in. Is the neuron trying to pull some of them back into the cell body to repair them? Nobody’s ever seen this in dopamine neurons in the brain before. It’s really quite striking. Our zebra fish models will provide the tools we need to understand why this is happening and how it contributes to disease.”

A BIOMARKER FOR PARKINSON’S DISEASE?

Working in collaboration with J. Timothy Greenamyre’s laboratory, Laurie Sanders, a PhD, assistant professor of neurology, and member of the Pittsburgh Institute for Neurodegenerative Diseases, has discovered a potential blood biomarker for Parkinson’s disease—both the hereditary and sporadic forms. That biomarker manifests as increased mitochondrial DNA damage. “We have a common phenotype between the two kinds of Parkinson’s now. The assay may ultimately help catch Parkinson’s in its earliest stages.” Sanders hopes to explore the dysfunctional repair pathways that lead to the damage, then find drugs to target them. In the genetic, familial forms of Parkinson’s disease, mutations in a gene called LRRK2 are common. These lead to an increase in kinase activity, and as kinase activity increases, mitochondrial damage increases, as well. “When we use gene editing to return the LRRK2 gene to its non-mutated wild type state, there is no damage in the neurons,” says Sanders. “We are looking at kinase inhibitors, but what is unique about our approach is we are using our biomarker to monitor their effectiveness.” —JN

NEUROPROTECTOR

What causes neurons to shrink and dwindle in people with Parkinson’s disease? Charleen Chu’s laboratory at Pitt has discovered a mechanism that regulates both quality control and growth for neurons. Chu, an MD/PhD, holds the A. Julio Martinez Chair and is a professor of pathology in the School of Medicine.

When mitochondria are damaged, molecules such as PINK1 accumulate on the surface, alerting the cell to consume the misbehaving organelle. Clearing non-functional mitochondria staves off cell death. Healthy mitochondria import PINK1, cleave it, and release the shortened protein. Shortened PINK1 signals the growth of long and elaborate dendrites. These bushy branches allow neurons to stay in touch with their neighbors—and that’s critical for health and functionality. So one molecule, whose function is lost in familial Parkinson’s disease, facilitates neuroprotection through two distinct avenues. Looking forward, we can imagine the use of this potent messenger in treatments that slow or halt the progression of neurodegenerative diseases brought on by age, toxins, and genetic predisposition. —ECH