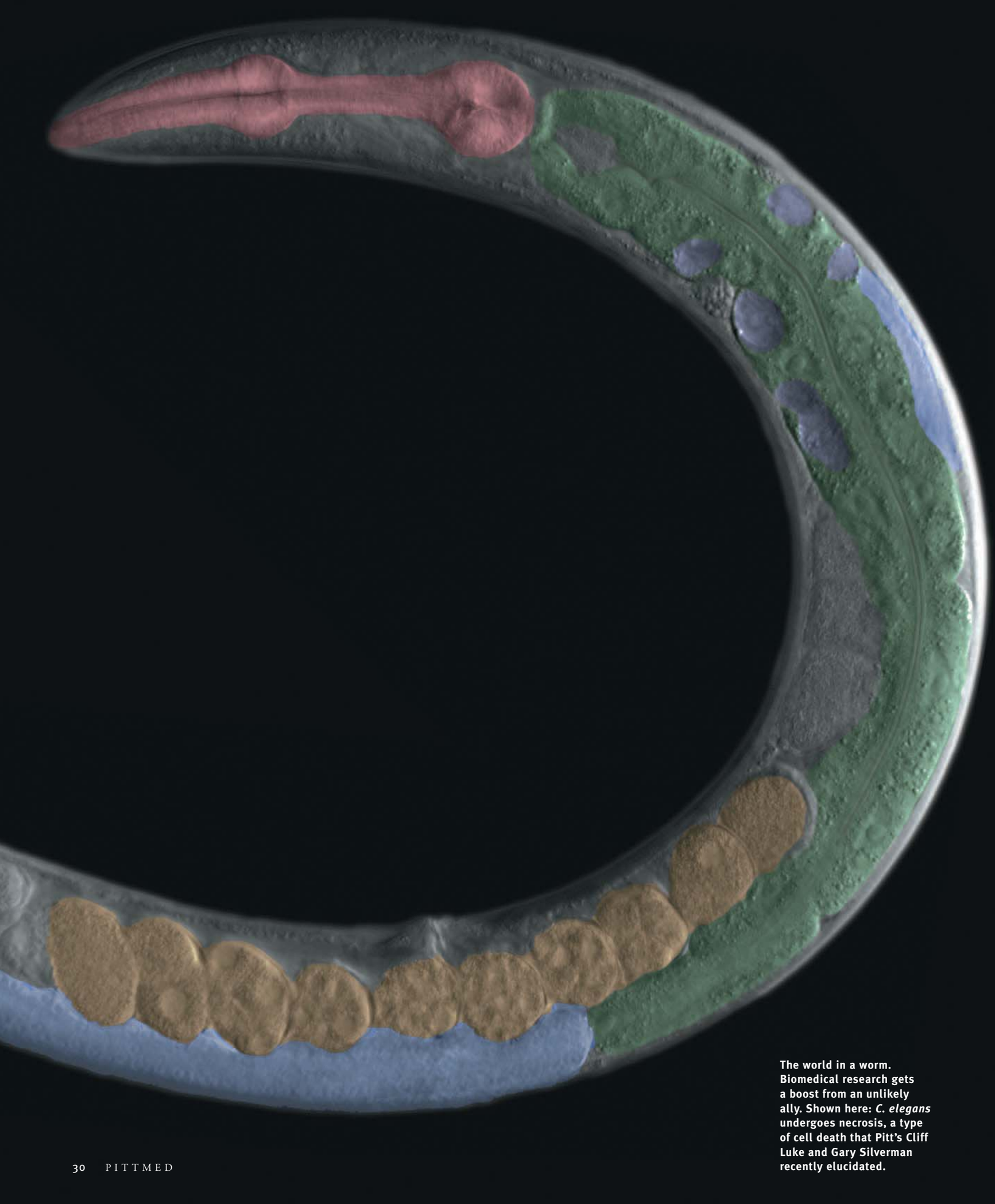


The world in a worm. Biomedical research gets a boost from an unlikely ally. Shown here: *C. elegans* undergoes necrosis, a type of cell death that Pitt's Cliff Luke and Gary Silverman recently elucidated.

OUR PRIMAL ROOTS

BY MELINDA WENNER MOYER

MISSION CREEP

Physicists may need 17-mile-long particle colliders, and chemists expensive chromatographs, but biologists answer some of their most difficult questions using tiny tools. A number of this century's celebrated biomedical discoveries—three of which were honored with Nobel prizes this decade—have relied on the help of one particularly basic and infinitesimal laboratory tool: a one-millimeter-long worm called *Caenorhabditis elegans.*

Thankfully abbreviated as *C. elegans*, the worm—or nematode, as it is called by those in the know—has a number of things going for it. It matures from embryo to adult in three days (just feed it bacteria), reproduces by itself, and is completely transparent, so researchers can observe its biological processes in real time, much like the internal workings of a Swiss watch. It's also the first multicellular animal to ever be used to test drugs in robotic drug discovery systems.

COURTESY C. LUKE

Best of all: "If you look at a human and you look at a worm, they look very dissimilar," says Lewis Jacobson, a PhD professor of biological sciences at the University of Pittsburgh. "But the more you go down toward the molecular level, the more similar they look."

It's astonishing. These worms have 19,000 genes to our 25,000, but up to three-quarters of the ones we have in common are identical. "In fact, they are often interchangeable," Jacobson posits. It would theoretically be possible, then, to engineer a human so that three-quarters of his genes came from this worm—and perhaps no one would be able to tell the difference.

In the 1960s, biologist and 2002 Nobel Laureate Sydney Brenner chose the worm as his second-in-command to chip away at one of the biggest biological mysteries of all: how, exactly, our genes all come together to make us functional organisms. By eliminating genes one by one, or tinkering with them so they didn't work properly, Brenner defined the roles that many individual genes play in making us, well, *us*. Today hundreds of labs and thousands of investigators around the world use *C. elegans* to answer burning biological questions.

The University of Pittsburgh is certainly no exception. In recent years, Pitt scientists have employed the worm to probe the causes of human disease, aging, and infertility. One lab has even sent the worms into space to observe how their muscle cells respond.

The more time Pitt researchers spend peering inside these tiny transparent tools, the more of life's mysteries they crack.

When asked which diseases his lab's research could eventually help treat, Cliff Luke, a PhD assistant professor of pediatrics in Pitt's School of Medicine, laughs. "Pretty much everything," he says. He's hardly exaggerating. In 2007, Luke and his colleagues, who include his lab's lead investigator, Gary Silverman, MD/PhD chief of newborn medicine in Pitt's Department of Pediatrics, made the cover of *Cell* after discovering that a common form of cell death called necrosis—a direct cause of much of the damage triggered by heart attacks, strokes, and lung disease—is not, as had been previously assumed, disorganized and uncontrolled. Using *C. elegans*, they found that necrosis actually proceeds in an organized, stepwise fashion. This begs the question: Might it be possible to undo it in a stepwise fashion, too?

Their 2007 discovery centers around a protein called SRP-6, whose job is to protect cellular organelles called lysosomes. Lysosomes are like the stomach of the cell—they digest proteins that the cell uses for food. Sometimes, they suffer from their own form of acid reflux disease, leaking dangerous enzymes called peptidases into the cell body. SRP-6, one of a class of proteins called serpins, is like the lysosome's antacid. It "sequesters the peptidases from damaging the normal parts of the cell," Luke explains.

Luke and his colleagues bred *C. elegans* so that they lacked the gene for SRP-6 (see "An Elegans Solution" in our Spring 2008 issue). The worms quickly died from the damage caused by necrosis, a finding that suggested that this particular form of necrosis is "actually a very structured routine that is controlled by peptidases" rather than some uncontrolled violent reaction, Luke says. Recently, the lab has shown that a mammalian version of the SRP-6 protein works in a similar manner. In an experiment they conducted in 2009, they inserted the mouse version of the gene into the genome of a SRP-6-deficient worm. The worm survived.

It would be tough to insert these genes into people, but it's not ridiculous to wonder whether simple compounds might be able to halt necrosis, too. To find out, Silverman's lab has teamed up with Stephen Pak, a research assistant professor of pediatrics at Pitt, to develop one of the world's first, and the highest-throughput, drug discovery platforms using *C. elegans*. Typically, when labs and companies screen compounds as possible drugs, they use molecules or single cells as targets. Problem is, these screens say nothing about whether the compound actually has the intended effect on a whole animal. But by using *C. elegans*, their platforms is much more powerful, because if a compound "has an effect on one animal, there's a good chance it'll have an effect on another," Silverman says.

Here's how it works: The lab has engineered thousands upon thousands of *C. elegans* to contain markers that light up when a compound interferes with the biochemical pathway involved in necrosis. Robots plop one worm into each well of a 384-well microtiter plate and add possible antinecrosis compounds to the mix. Then, a fluorescent-monitoring microscope scans the wells to see which cells light up.

These "hits" are then cherry-picked as possible drugs that may one day find their way to the clinic to safely and effectively prevent the cellular damage that is the hallmark of so many conditions.

Luke points out that it might sometimes be useful to induce necrosis, too: Some cancers may be resistant to chemotherapy because serpin levels in the tumor are too high, preventing the necrotic damage necessary to eliminate the tumor. By manipulating serpin activity in various ways, it would be possible to prevent damage when you don't want it, and boost it when you do.

In collaboration with David Perlmutter, Vira I. Heinz Professor and chair of pediatrics at Pitt, Silverman and Pak are also using *C. elegans* to model a disorder called alpha-1 antitrypsin deficiency. Caused by a mutation in the gene for alpha-1 antitrypsin—another serpin—the disorder results in an accumulation of the mutant protein in the human liver and is the primary cause of liver transplantation in children. *C. elegans* doesn't have a liver, but it's been enormously useful in investigating the disease. Using their *C. elegans* screening platform, the team has already identified one molecule that breaks down and removes the toxic protein from the worm.

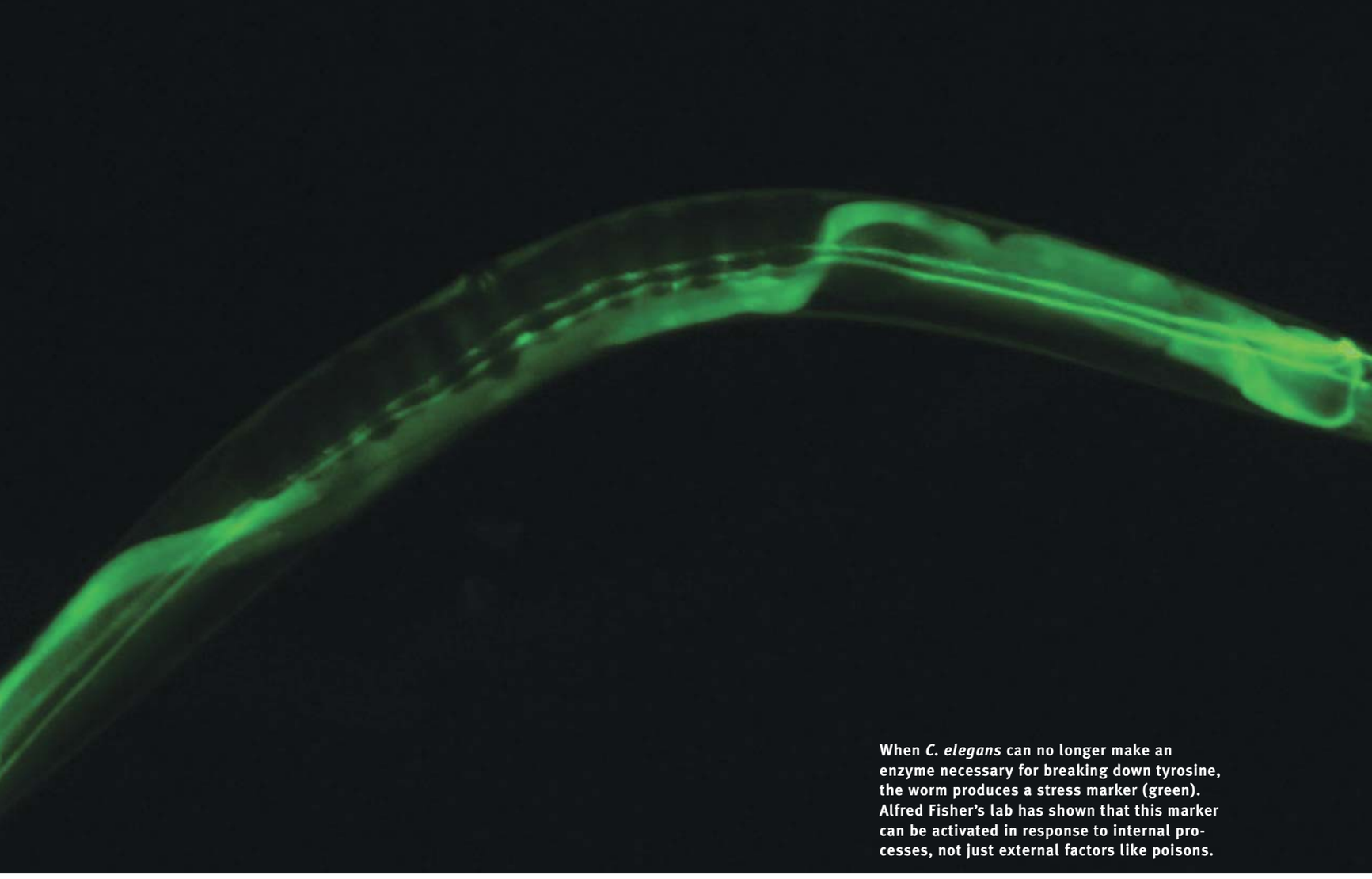
Their findings could have far-reaching implications, because drugs that clear this particular mutant serpin may also help clear the protein aggregates that characterize other neurodegenerative diseases.

"What's so exciting is that this class of drugs, called autophagy enhancers, has the potential to work on multiple substrates," Perlmutter says.

Serpins are, of course, only one of many classes of proteins that are protective. Others include the Aip-1/AIRAP proteins, short for arsenic-inducible RNA-associated proteins, which are within the purview of Alfred Fisher, an MD/PhD assistant professor in the Division of Geriatric Medicine in the School of Medicine. These proteins were first discovered for the role they play in the body's response to arsenic exposure: Their job is to clear other proteins from the cell that have been damaged by the poison. Since then, researchers have learned that the Aip-1/AIRAP proteins are also produced after other external stresses like heat shock, and that they do their dirty work by binding to a protein-degrading cellular body called a proteasome, ramping up its activity.

In research published in the June *Molecular and Cellular Biology*, Fisher showed, using *C. elegans*, that these proteins are also produced when the body has trouble breaking down the amino acid tyrosine, which it frequently does to create energy. Fisher bred *C. elegans* so that they

MOLECULAR AND CELLULAR BIOLOGY, VOL. 30, ISSUE 11, PP. 2651-2667 WITH PERMISSION FROM AMERICAN SOCIETY OF MICROBIOLOGY, © JUNE 2010



When *C. elegans* can no longer make an enzyme necessary for breaking down tyrosine, the worm produces a stress marker (green). Alfred Fisher's lab has shown that this marker can be activated in response to internal processes, not just external factors like poisons.

could not produce an enzyme necessary for tyrosine degradation; they suffered from intestinal problems and early death. But before they died, the worms also produced Aip-1/AIRAP. This was the first example of a completely internal process—as opposed to exposure to heat or poison—triggering the production of the proteins.

Fisher is now working to uncover what other genes and internal processes might have the capacity to turn the proteins on. His hunch is that ramping up the activity of these proteins in a safe manner might alleviate damage caused by a number of environmental insults and stresses. It might even open up doors for treating the many conditions linked to proteasome dysfunction. These include neurodegenerative diseases like Alzheimer's and Parkinson's, as well as viral and bacterial infections, he says.

There's another question percolating in Fisher's mind. Previous research has shown that animals that cannot make Aip-1/AIRAP die early. So will animals live longer if they make lots of it? "My guess is, they might," he says. To find out, his lab is developing worm constructs that produce different amounts of the proteins. Then he will wait and see which, if any, end up with a particularly long life span.

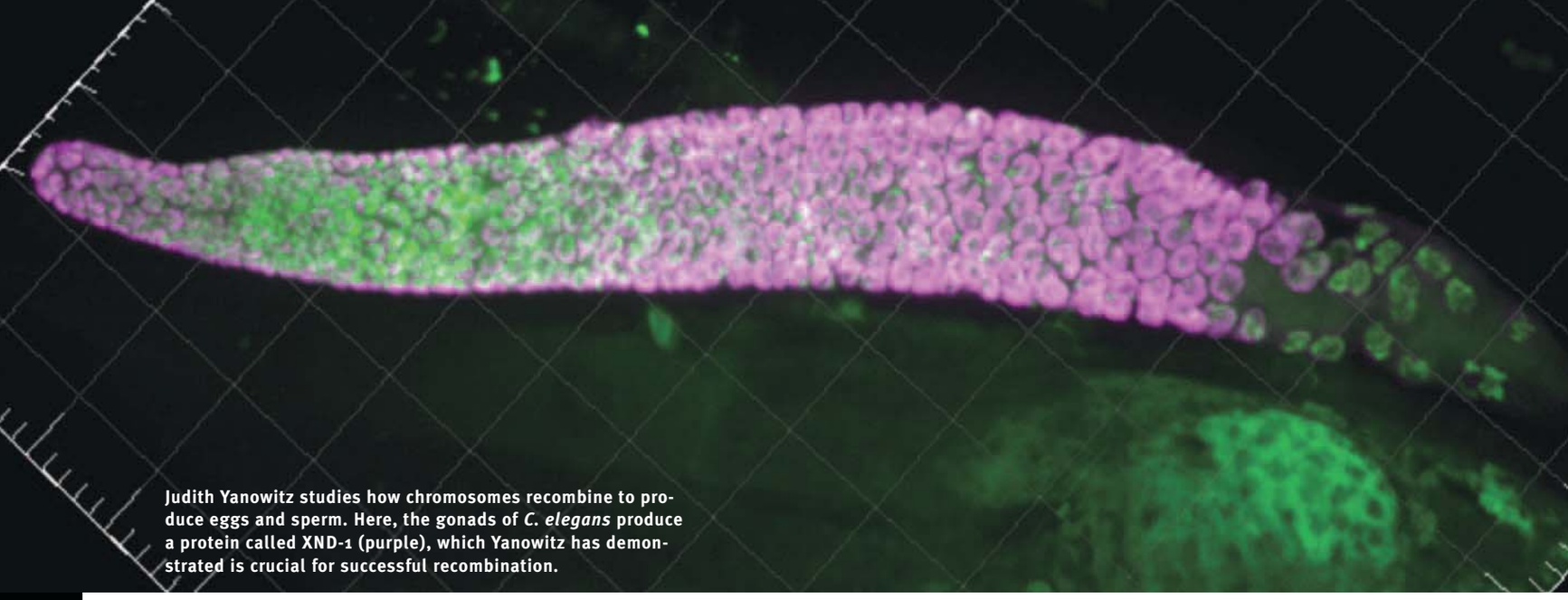
"For a lot of the things we do in the lab, we say, 'Let's see what happens if you do that,'" Fisher says. "You often find interesting and surprising things that way."

While Fisher tinkers away at extending life, Judith Yanowitz, a PhD assistant professor in the Department of Obstetrics, Gynecology, and Reproductive Sciences at Pitt and member of the Magee-Womens Research Institute, is conducting experiments with *C. elegans* in the hopes of ensuring that life can happen in the first place. A newcomer to Pitt—she joined the faculty last year—Yanowitz is striving to understand the many things that can go awry when gametes (eggs and sperm) form.

To do so, she focuses on meiosis, the form of cell division that produces gametes. One key aspect of meiosis is the crossing over, or recombination, that occurs between homologous parental chromosomes, as this ensures DNA exchange—and such exchange is necessary for the production of offspring that are genetically different from each parent. As it turns out, "that process of crossing-over is actually essential for the chromosomes to segregate properly," Yanowitz explains. "If you don't do it, you end up with a disease like Down syndrome—with too many or too few chromosomes per gamete," or what scientists call nondisjunction. Other forms of nondisjunction lead to miscarriage.

C. elegans is the perfect organism to use to study chromosomal recombination. Because the worm is transparent, "We can see the chromosomes going through what we call the meiotic dance," Yanowitz says. What's more, the events that take place during worm recombination are identical to those in humans. "Some of the proteins that are specifically involved are different, but the process itself is highly conserved," she says.

In 2005, Yanowitz performed a screen from which she identified 20 proteins that play a role in regulating recombination on *C. elegans* chromosomes. These proteins, she discovered, affect the higher-order structure of DNA known as chromatin. This makes sense. For crossing over to occur, each homologous parental chromosome first has to be "snipped"



Judith Yanowitz studies how chromosomes recombine to produce eggs and sperm. Here, the gonads of *C. elegans* produce a protein called XND-1 (purple), which Yanowitz has demonstrated is crucial for successful recombination.

COURTESY J. YANOWITZ

so that DNA can be exchanged; the proteins her screen uncovered seem to facilitate this strand-cutting by affecting DNA structure. "They're like the homing proteins," she explains. They say, "Come over here, there's nothing around, so you can make your break." Recently she has zeroed in on understanding the mechanism employed by one of these proteins, XND-1, which influences crossing-over on all *C. elegans* chromosomes.

Another mystery that Yanowitz's lab is trying to solve: What, exactly, happens to cause older women to be more likely than younger women to have miscarriages and babies with genetic defects (what's known as "reproductive aging")? Worms also experience these problems: During the late stages of their reproductive capacity, their gametes are 100 times more likely to form improperly. "Now we're testing to see if we can see changes in higher-order DNA structure during reproductive aging," she says. By uncovering the molecular problems at the root of this phenomenon, it might be possible, she says, to prevent them.

As any of us age, many things can go wrong, of course. And lots of them involve our skeletal muscles, which make up a whopping half of all of our tissues. "It is immensely expensive in a biochemical sense, and in terms of nutrition, to build and keep all of that protein," explains Jacobson. Muscle "is something that you can afford if and only if there's a good justification for it."

Our bodies are constantly evaluating whether to keep muscle or get rid of it—the latter being much more common during aging—and Jacobson is striving to understand, with the help of his favorite worm, exactly how they do this.

C. elegans' muscle cells do something surprising: They constantly produce a muscle-degrading protein called fibroblast growth factor (FGF). Although that might sound suicidal, Jacobson discovered why it isn't. In a 2007 paper he published in *EMBO Journal*, Jacobson reported that worms have a signal moderator molecule that notes the presence of both FGF and a muscle-saving hormone called insulin-like growth factor (IGF). When both signal to the muscle, he found, the cell is spared. But when Jacobson prevented IGF signaling in the worms, their muscles broke down. The finding could explain why people with diabetes, who have impaired insulin signaling, frequently suffer from muscle atrophy.

The question that Jacobson's lab is now trying to answer is what, exactly, naturally shifts this balance in favor of degradation. One cause is well known: lack of use. When muscles are not utilized, as anyone who has ever taken a six-month exercise hiatus and then attempted to run three miles knows, they break down.

This is partially because the muscle cells stop releasing calcium, a natural byproduct of muscle use. Jacobson has recently found that the same signal moderator that monitors FGF and IGF also pays attention to calcium levels. When they are low for long periods of time, the molecular moderator tips the balance in favor of breakdown.

Muscles break down naturally in the elderly in part because "it is a pro-survival trait in any organism, from worms to humans, to give up a muscle if giving up the muscle is important," Jacobson says. Better to lose your biceps and save your brain and heart. Historically, scientists have had difficulty teasing out the molecular mechanisms involved in the body's decision to sacrifice muscles during aging, but Jacobson is breeding *C. elegans* with different muscle signaling profiles in the hopes of finding out. To date he has produced more than 280 mutant strains, because many signals are likely involved. "It's clear that muscle is listening to a lot of things," he says.

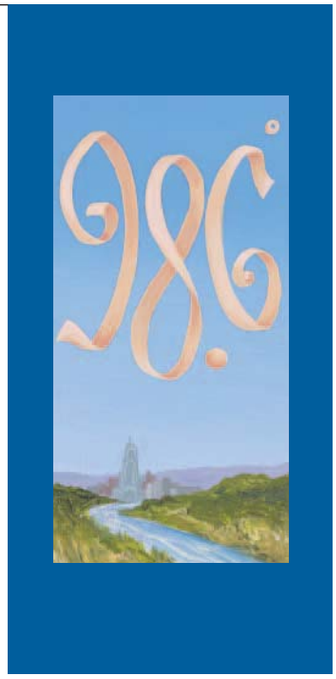
Jacobson's lab has a side project: sending worms into space. Astronauts have long lamented the fact that even if they exercise, their muscles atrophy more quickly in space than on Earth. In a recent experiment, Jacobson and his colleagues measured the levels of expression of two-thousand worm genes while the worms were aboard the International Space Station. Several genes involved in the attachment of muscle cells to each other and to skin changed their expression patterns. "It looked like the attachment complexes that were keeping the muscles tightly glued together were being down regulated in space," Jacobson explains.

So what does that mean? Space flight "causes protein breakdown in the muscles, and the issue is why and how," Jacobson says. "I don't think we have a lot of answers yet."

It may seem crazy to think that a one-millimeter-long worm could be at the root of so many discoveries. But nature knows when it has found something good. "Even though worms and humans separated probably a billion years ago or more in the course of evolution, nature is very parsimonious," Jacobson says.

So as researchers study the delicate *C. elegans*—as they watch the inner workings of its evolutionarily ancient organs—they are, in a sense, peering inside every animal that has evolved in the epochs since. Looking inside these worms, then, isn't all that different from looking inside ourselves. "The important stuff doesn't change," Jacobson says. ■

People and programs that keep the school healthy and vibrant



A PATHOLOGIST CHAMPIONS FAMILY MEDICINE

BY JOE MIKSCH

As Larry Nichols puts it, he's at the age where having to go to the doctor is, let's say, not particularly uncommon. Along the way he has noticed, firsthand, the need for more family physicians. "We have a situation in this country where there are not enough primary care physicians," he says.

This fact got the MD associate professor of pathology at the University of Pittsburgh wondering whether he could do anything about it. Nichols, who has not only an MD but also an MA in philosophy from the University of Wisconsin, is the chief of the autopsy service for UPMC and teaches basic pathology and cardiac pathology in the School of Medicine.

"As a pathologist—and an old pathologist—I really can't be going and doing family medicine," he says. "So how can I help?"

Nichols did a little math. Med students graduate with crushing debt. This debt can compel someone whose heart may be in family medicine to instead become a specialist who earns much more than a family practitioner. *What could help stem this tide to other fields?*, he wondered. *Money!*

Nichols began to seriously consider establishing a scholarship for family medicine practitioners.

"I figured out how much I can afford," he says, "and contacted the family physicians I know to ask them if this sort of donation might help students decide to go into family medicine. They said, 'Yes.' They loved it!"

And so was born the Larry Nichols Family Medicine Scholarship Award. Any Pitt med student who is matched to a family medicine residency is eligible for the annual $20,000 prize. The winner is selected by the attending physicians who supervise the candidates on their family medicine rotations. "They just try to determine who is most likely to make the greatest contribution to the field," Nichols says. The first recipient is Nadine Champsi (MD '10), who is doing her residency at UPMC St. Margaret.

Champsi first learned of the scholarship at this spring's senior class award ceremony. Nichols says she seemed happy enough when he summoned her to the podium to receive an award she'd never heard of. Her happiness turned to sheer joy when she was told the amount of the award. "You should have seen the expression on her face. Absolute glee," Nichols recalls with a laugh.

"The award from Dr. Nichols was a total shock to me," Champsi says. "I was in awe of his generosity and of his commitment to supporting a cause we both believe in—primary health care in this country."

Champsi, who had her first child in April, says the debt relief will give her greater flexibility in her career choices. Her husband, Kevin Carl (MD '09), is a psychiatry resident at Western Psychiatric Institute and Clinic.

Still, Nichols thinks, $20,000 isn't enough.

"The sad part is, that's only about 10 percent of the debt," he says. "My goal is to get more people to join in this with me. It would be so good if we could pay off the whole debt." Nichols adds that though the scholarship bears his name, it doesn't have to. "If others join the cause, we can pool the money and call it the Family Medicine Scholarship. I just want to help as much as possible." ■

J. BURKE/ CIDDE



Nadine Champsi was thrilled when she learned she would receive $20,000 as the winner of the Larry Nichols Family Medicine Scholarship Award. She's shown here with her benefactor, Professor Nichols.

BOOSTER SHOTS

During their typically long stays, young patients in the transplant unit at Children's Hospital of Pittsburgh of UPMC have a new remedy for boredom—24 donated Xboxes. In April, **Make Room for Kids**, a fundraising project undertaken by **Virginia Montanez**, of the Pittsburgh blog *That's Church*, teamed up with the **Mario Lemieux Foundation** and local **Microsoft** workers to fund the venture. Together, they raised more than $15,000.

Microsoft donated many gaming units and videogames, yet patients benefited from more than Xboxes. Montanez's readers made donations used to bring in handheld gaming devices, iPads, and Toughbooks. Nancy Angus, executive director of the Mario Lemieux Foundation, says there are still more accessories coming in. "It was very gratifying to be able to assist in this project," she says.

When **Anette Duensing**, an assistant professor of pathology, presented her research on gastrointestinal stromal tumors at a conference in Boston in 2005, the **GIST Cancer Research Foundation** (GCRF) took notice and began donating to her lab at the University of Pittsburgh Cancer Institute. Recently, the Duensing Lab received another gift, this time $165,000 from GCRF.

Duensing and her lab colleagues are attempting to develop new therapies and screening approaches to GIST, a fairly rare form of cancer. But, as medical researchers know, it's costly to buy new equipment and conduct experiments. The pathologist says she feels lucky to receive GCRF's donation. "The extra boost of money lets us do things we otherwise wouldn't be able to do," she says. —*Keith Gillogly*

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