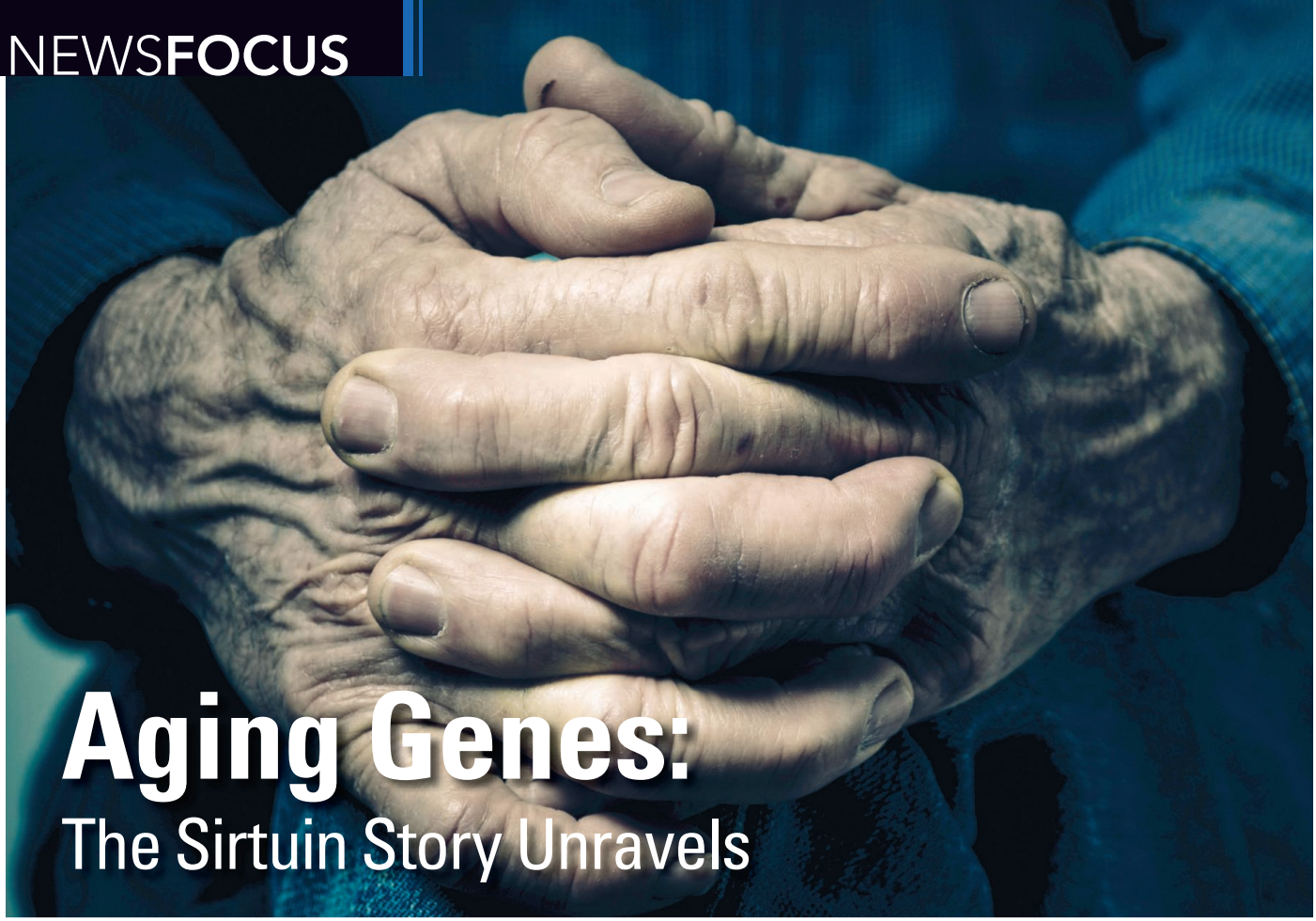

Aging Genes:
The Sirtuin Story Unravels

Work that pinpointed the control of aging in a handful of genes is being taken apart by some of the scientists who made early discoveries. Efforts to replicate studies are producing conflicting results

SEATTLE, WASHINGTON—The lush, drizzly campus here at the University of Washington (UW) is 4000 kilometers from the concrete jungle of the Massachusetts Institute of Technology (MIT) in Cambridge, but Matt Kaeberlein keeps finding himself pulled back to the East Coast institution where his unusual scientific career began. Thirteen years ago, as a graduate student from a little-known western school, he stepped into a highly competitive lab and helped launch a new field in the biology of aging. For the past 7 years, he's been systematically dismantling the building blocks he laid, arguing that some of those early discoveries—and many since then—are wrong.

"It's been an adventure," says Kaeberlein, who turned 40 this year. Wearing jeans and glasses with square metal frames, he comes across as a mix of science nerd and Seattle cool. His fifth-floor office is as neat as they come. "I don't think that I could have ever predicted that things would happen the way they happened," he says.

Kaeberlein's journey began in the lab of MIT professor Leonard Guarente back in 1998, chasing a then-heretical idea in science: that certain genes can prolong life. The work started slowly but captured the attention of nearly everyone in the lab, particularly Guarente, an intense, brilliant biologist. The group churned out a series of influential papers that transformed how scientists and the public think about aging. The idea that life span was a malleable part of biology was no longer science fiction. Discoveries from Guarente's lab linked a set of genes to calorie restriction, which had been known for years to stretch life span in animals. This suggested that drugs to mimic the effects of calorie restriction might not be far behind.

About 10 years ago, Kaeberlein and his cohort of Guarente lab members wrapped up their Ph.D.s and postdocs and scattered. Most went on to start labs of their own at top institutions. From there, the story gets peculiar.

Guarente and some former lab members pushed forward with the new aging genes and vigorously promoted their findings. Others, such as Kaeberlein, experienced nagging doubts that grew with time. Kaeberlein wasn't finding what others were reporting in recent experiments. Outside the Guarente circle, some scientists had similar problems while others reported success.

The result is mass confusion over who's right and who's wrong, and a high-stakes effort to protect reputations, research money, and one of the premier theories in the biology of aging. It's also a story of science gone sour: Several principals have dug in their heels, declined to communicate, and bitterly derided one another. Tensions reached a crescendo in September, when Kaeberlein and colleagues in the United Kingdom published one of their most damning papers yet, finding no effects from a key aging gene in worms and flies.

Almost everyone "is from the same place," the MIT lab run by Guarente, says Stephen Helfand, a fly biologist at Brown University who studies aging and who did not start his career there. What's happening now, he says, is "either Shakespearean or Freudian." Or maybe both.

Online
sciencemag.org
Podcast interview with author Jennifer Couzin-Frankel.

Hooked

Kaeberlein was not programmed for a career in science. His father was a postal worker and his mother a homemaker, then an office

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clerk after his parents divorced. Neither graduated from college.

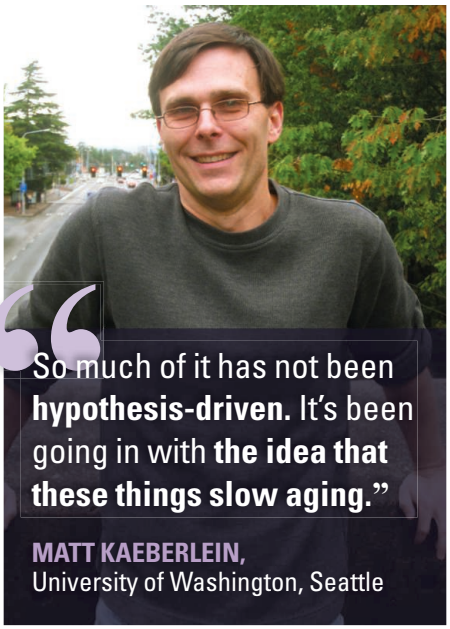
Kaeberlein spent a few years working for United Parcel Service after finishing high school in Seattle, loading trucks at dawn. He enrolled in community college and moved on to Western Washington University, a state school that overlooks Puget Sound about a half-hour south of the Canadian border. When his wife uprooted to Boston to study marine plant biology at Northeastern University, Kaeberlein tagged along, winding up across the Charles River at MIT. In January of his first year he attended a lecture by Guarente—part of a series by faculty members to attract graduate students to their labs—and was hooked.

"Lenny got up and he talked about how his lab was working on aging using yeast and applying genetics and molecular biology," Kaeberlein recalls. "It wasn't so much the specific story as the idea that you could take what I believe is one of the most complex problems in biology and apply biochemistry and genetics and molecular biology to try and study it. … That had just never occurred to me before. It was like, wow, that's really cool." In the spring, he signed on with Guarente.

The lab back then was crackling with electricity. Several years earlier, two graduate students named Brian Kennedy and Nicanor Austriaco Jr. had announced they wanted to study aging. Guarente's focus then was on gene regulation; diving into aging research was high-risk. But life experience had left Kennedy fearless. Less than 2 years before joining the lab, when he was 22 years old, he was hit head-on by a drunk driver traveling the wrong way on a highway without any headlights. That driver was killed. Kennedy spent 6 months in a wheelchair recovering from badly broken legs, a torn diaphragm, and a collapsed lung. He postponed the start of graduate school for a year.

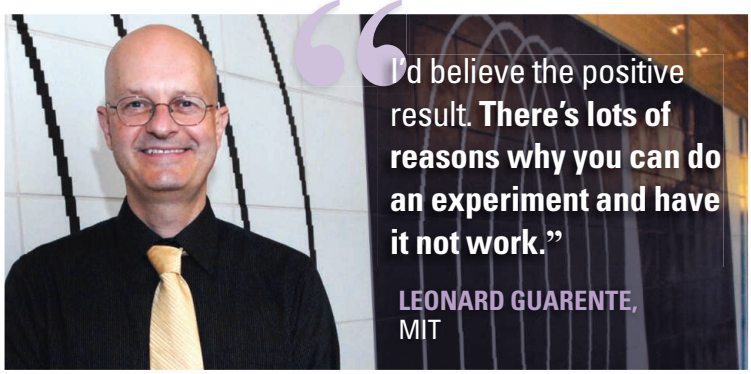
The accident changed him. "You never know what's going to happen in life," Kennedy says now from his perch as president and CEO of the Buck Institute for Research on Aging in Novato, California. "You might as well see what you can achieve and not be afraid of failing." (Austriaco wasn't your average graduate student either; after finishing in the Guarente lab, he went on to become a priest.)

Kennedy and Austriaco focused on yeast, a single-celled fungus. Although Kennedy admits that "I felt like we would have to be lucky to find something about human aging from studying something like yeast," he figured it was worth a shot. In 1995, along with Guarente, Kennedy and Austriaco published the prologue to much of what followed: They found that, when mutated to make it more active, a gene called *SIR4* could extend life span in yeast by as much as 30%. "We were all very excited but also very naïve," Guarente says now. "You find things very new and refreshing, [but] you're not quite certain" what they mean.



"So much of it has not been **hypothesis-driven.** It's been going in with **the idea that these things slow aging."**

MATT KAEBERLEIN,
University of Washington, Seattle



"I'd believe the positive result. **There's lots of reasons why you can do an experiment and have it not work."**

LEONARD GUARENTE,
MIT

Kaeberlein joined the lab 2 years after Kennedy's departure and, building on work by his labmates, turned to a different gene: *SIR2*. It belongs to a family of five genes in yeast that produce proteins called sirtuins. (There are seven sirtuins in mammals.) In yeast, it turned out that Sir4 was actually acting through Sir2; Sir2 was the master regulator.

Kaeberlein's yeast paper, published in *Genes & Development*, got relatively little attention. But 2 years later, in 2001, MIT postdoc Heidi Tissenbaum and Guarente repeated the work in worms, showing that overexpressing *SIR2* extends life span. Three years after that, Helfand, now at Brown, did the same with *SIR2* in flies. In the insular world of aging biology, sirtuins were suddenly of modest interest.

Two discoveries catapulted them to fame. First, a postdoc in Guarente's lab, Shin-Ichiro Imai, found that *SIR2* could sense the metabolic state of a cell, a topic of long-standing interest in longevity research. Scientists knew that cutting calories significantly altered metabolism, but they didn't understand how that led to longer life. *SIR2* might be a missing domino in the lineup, and that's what Guarente's lab described. Calorie restriction impacts a particular chemical, called NAD, which guides how the cell uses energy. NAD also affects *SIR2*. "So anything that changes the levels of NAD in cells will change the activity of sirtuins," Guarente says. "And one thing that does that is diet." Guarente was proposing the solution to a decades-long puzzle of how cells lived longer on fewer calories. Sirtuins were the answer.

Another leap came in 2003. An ambitious Australian postdoc of Guarente's, David Sinclair, who had recently taken a post at Harvard, reported that resveratrol, a molecule found in red wine, boosted sirtuin activity in a test tube and extended life in yeast. Sinclair presented the resveratrol work at a meeting in the Swiss Alps at the same time it was published in *Nature*. "I've been waiting for this all my life," he told a *New York Times* reporter.

The red wine connection "hit the public consciousness in a way that nothing else in the field has," Kaeberlein says. After all, scientists were not only saying they could find a way to mimic calorie restriction without the untenable diet. They were saying that the treatment of choice might be red wine. How much more appealing could an antiaging prescription get?

Nagging questions

By 2002, Kaeberlein was out of the loop. He had finished his Ph.D. with Guarente and wanted a break from academia. He worked a stint at a start-up biotechnology company, which rented a shuttered video rental store in Waltham, Massachusetts, and filled it with lab benches and equipment purchased

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1999

At MIT, Matt Kaeberlein and Leonard Guarente show that in **yeast**, the *SIR2* gene extends life span.

2000

2001

Other members of the lab show the same in **worms** and suggest that the protein responds to calorie restriction.

2002

Kaeberlein completes his Ph.D.; he spends some time at a biotech company before moving to Seattle.

2003

2004

Stephen Helfand at Brown University repeats the *SIR2* finding in **flies**.

At the University of Washington, Matt Kaeberlein and another Guarente alum, Brian Kennedy, question whether *SIR2* and calorie restriction are related in yeast.

at an auction. Soon the venture flopped, Kaeberlein's wife finished her Ph.D., and the two decided to move back west. Kaeberlein landed at UW Seattle, where, it happened, Brian Kennedy had already settled.

One afternoon they met for coffee in the rotunda, an open cafeteria with stained green carpet and white pillars. Kennedy and Kaeberlein reminisced about their years in the Guarente lab. Then they started talking about yeast and *SIR2*. Was *SIR2* really the whole story when it came to yeast aging, they wondered? "We both felt that the field had become very narrowly focused," Kaeberlein says. Publications in top journals nearly all spotlighted *SIR2*. "We know there's got to be other stuff out there."

Finding it seemed an overwhelming task. Yeast have about 6000 genes. Kennedy and Kaeberlein knew they would have to study tens of thousands of yeast cells, each with a different gene deleted, to determine which played important roles in aging. To do that, they'd need to sit over their microscopes, hour after hour, watching daughter cells bud off the mother cell and counting them one by one, until the mother stopped producing them and yeast life, as it's measured, came to a halt.

They forged ahead and used a yeast strain whose genetic background was different from that of the yeast that Kaeberlein had studied in the Guarente lab. Yeast are fickle; not all strains, it turns out, respond the same way to *SIR2* and calorie restriction. One strain in Guarente's lab lived longer when it was calorie restricted but not when it was flooded with *SIR2*; another did exactly the opposite, living long with extra *SIR2* but not when calories were restricted. Kennedy and Kaeberlein happened to find a strain that responded to both environments, and the young scientists experimented with combining them. What they found suggested that the early work was off the mark: The yeast cells lived far longer when glucose was reduced and *SIR2* was overexpressed than when just one factor was modified. The clincher was that even when *SIR2* was deleted, calorie restriction stretched life span—running counter to the idea that calorie restriction worked by increasing *SIR2* activity.

Piecing this together, Kennedy and Kaeberlein reasoned that *SIR2* overexpression and calorie restriction were acting through different pathways and didn't have much to do with each other, at least in yeast. That would explain the extra-long life when they were combined. "Occam's razor says go with the simplest model that explains the data," Kaeberlein says. They published their findings in 2004 in *PLoS Biology*.

The gauntlet thrown by Guarente's former disciples foreshadowed contretemps to come. For one, there was the problem of comparability. Using genetically different organisms made it difficult to replicate experiments between labs. Even in worms, where there's been a concerted effort to study the same strain originally collected from mushroom compost in Bristol, U.K., in the 1950s, specimen collections evolve independently. Emotions also ran high. Guarente reacted with displeasure to the 2004 paper, Kaeberlein says: "That was when Lenny first got really upset."

Guarente describes being taken aback by the challenge. "Initially, I didn't know what to think," he says, "until I looked really closely and saw the conditions were different." Kaeberlein and Kennedy, he argues, starved their yeast much more aggressively than he had.

Kaeberlein, then a postdoc with Stan Fields, a UW professor who uses yeast as a way to develop new technologies for biological discovery, wouldn't back down. Kennedy saw no need to retreat either. Guarente's argument about different dietary conditions, Kaeberlein says, is a "red herring"; the yeast in the 2004 paper were tested under varying glucose concentrations, including one commonly used by Guarente.

Meanwhile, the sirtuin field was charging ahead. The same year Kennedy and Kaeberlein first raised public doubts about how sirtuins functioned, Sinclair launched a company to capitalize on the molecules and the red wine connection he'd uncovered. He had published a second paper in *Nature* showing that resveratrol also extended life in worms and flies and that it acted through *SIR2*, just like calorie restriction. Sirtris, Sinclair's company, planned to test resveratrol and other sirtuin activators in animals and eventually in people in hopes of preventing disease and ultimately extending life.

But Sinclair would soon have to contend with Kaeberlein and Kennedy. The pair turned back to their pet yeast strain to test resveratrol. They drew a blank. "We went through a year of trying every different concentration, every protocol you can think of, back and forth with David, trying to figure out … why we were getting different results," Kaeberlein says. Resveratrol wasn't doing anything—not extending life, not activating *SIR2*. They tested it in the same yeast strains Sinclair was using, to no avail. Sinclair proffered various explanations: The glucose concentrations used to restrict calories or the plastic on the petri dishes might be throwing results off.

Kaeberlein and Kennedy went ahead and published in 2005 in *The Journal of Biological Chemistry*. Their bottom line: Contrary to Sinclair's *Nature* papers, resveratrol did nothing to help yeast cells live longer.

Life versus health

Plenty of scientists are fascinated by yeast, but for the rest of the world the big question has always been what sirtuins do in mam-

2004–2005

Guarente lab alum David Sinclair forms the company Sirtris to develop drugs for age-related diseases. The compounds include resveratrol, found in **red wine**.

Kaeberlein and Kennedy argue that in yeast, resveratrol has no effect on *SIR2* or life span.

2006

2007

Guarente publishes the first paper showing that ovexpressing *SIRT1* in **mice** doesn't help them live longer, though the mice are healthier in old age.

2008

Pharma giant GlaxoSmithKline buys Sirtris for $720 million.

2009

2010

2011

Kaeberlein and colleagues in Britain publish in *Nature* saying that ***SIR2*** doesn't extend life in worms or flies.

mals. The answer appears to be complicated.

Researchers set out to mimic earlier yeast, worm, and fly work in mice, overexpressing the *SIR2* gene—which in mammals is called *SIRT1*—and testing whether it had the same effect. Three groups—one led by Guarente, one by Manuel Serrano of the Spanish National Cancer Research Center, and one by Domenico Accili of Columbia University—all tried their hand at this. In no study did the mice live longer than usual.

But they weren't your average mice, either; the rodents looked unusually healthy. Guarente's mice, which he described in 2007 in *Aging Cell*, had lower cholesterol and better glucose tolerance and looked a lot like animals deprived of calories. The other groups reported similar results: less type 2 diabetes, healthier metabolic profiles, and healthier livers.

Sirtuin supporters quickly focused on the positives. These mice didn't live longer, but they stayed healthier longer, and wasn't that what mattered to most people? "It's really unclear whether mammalian sirtuins have a role in" extending life, says David Lombard, another Guarente alum now at the University of Michigan, Ann Arbor. "What is undeniable is that sirtuins promote health span" or extended good health. To Lombard, "even if no one showed longevity extension by sirtuins, it doesn't mean they're unimportant."

Skeptics, including Kaeberlein, ask whether life span and health span are really separable. "It's hard for me to imagine a way in which you would slow the progression of multiple age-related diseases without doing something about the molecular damage that is causing the aging process," Kaeberlein says. As it happens, this is a rare point on which Guarente and Kaeberlein agree. His former mentor doesn't believe they can be disentangled, either.

Another doubter is David Harrison, who studies aging in mice at the Jackson Laboratory in Bar Harbor, Maine. Harrison, who is part of a federally funded consortium testing various antiaging compounds in mice, is critical of many mouse studies because they focus on only one strain. "Each mouse strain is a different individual," he says, and individuals often respond differently to the same treatment. The Jackson Lab and its collaborators have so far offered more than 20 compounds to genetically diverse mice to test whether they slow aging.

Resveratrol didn't work, Harrison says. But even skeptics agree that resveratrol and related molecules might help reduce the risk of type 2 diabetes and other metabolic diseases, as well as fatty liver disease—a benefit that's been recorded in mice given the drug. Last month, researchers reported in *Cell Metabolism* that 11 obese men given resveratrol had lower glucose levels, triglycerides, and markers for inflammation in their blood. Sinclair says that resveratrol extends life in mice fed a high-fat diet—but Harrison notes that might be simply because the compound prevented type 2 diabetes.

"There's a view that seems to be current, that **somehow one doesn't engage in quarrels. Sometimes, you have to.**"

DAVID GEMS, University College London

As for whether sirtuins stretch life in mice and mimic calorie restriction, that puzzle remains unsolved. Unlike people, most mice die of one disease: the cancer lymphoma. And overexpressing *SIRT1* in mice, although it does appear to protect against cardiovascular disease and loss of muscle mass and cognitive decline, doesn't do much to target lymphoma. So the animals still live an average life span, as several experiments, including Guarente's, have shown.

Those studies might not be the last word, however. Shin-Ichiro Imai of Washington University in St. Louis in Missouri is trying to take mouse studies of sirtuins to a new level. Imai, the former Guarente postdoc who helped link *SIR2* to calorie restriction early on, thinks that many past studies are incomplete because *SIRT1* was overexpressed throughout the animals' body. Levels may vary tremendously from one site to another, or from one mouse to another. Imai is trying a different approach: overexpressing *SIRT1* selectively in different tissues. "We do have some very critical results," but they're not yet published, Imai says. "We are 100% convinced that mammalian *SIRT1* plays a role in caloric restriction," and by extension, in aging.

Loose cannons

Kaeberlein's latest, and arguably most acrimonious, venture into sirtuin land began a few years back, when he ran into a British scientist named David Gems at a conference. Gems studies the biology of aging at University College London and by his own description excels at finding errors. "I've tended to be kind of a loose cannon," Gems confesses, sorting out "pitfalls" that others overlook. In 2005, he heard gossip about worm work Guarente and his postdoc Tissenbaum had published back in 2001, linking *SIR2* to longer worm life. The rumor was that *SIR2*'s potent effect on life span disappeared when genetic differences between control animals and those overexpressing *SIR2* were minimized. Gems hesitated to get involved but in the end chose to investigate.

Based on experiments in his lab, Gems concluded that the rumors were true. So-called outcrossing of the worms—mating them repeatedly with worms from the same strain—smoothes out differences between the genetically modified strain and the con-

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trol group. Scientists then verify which worms are still overexpressing *SIR2*. The hope is that other genetic variables have been largely erased. Outcrossing the worms up to six times revealed that *SIR2* had no effect on life span.

Gems asked Kaeberlein to try to replicate these results, which a graduate student in Kaeberlein's lab did. Meanwhile, a collaborator of Gems's, Linda Partridge, found the same problem in flies: *SIR2* overexpression, she concluded, didn't have an effect on their life span, either. This contradicted work that Helfand had published in 2009 showing that in genetically identical flies, *SIR2* overexpression extended life. "It's basically a boring little story that says if you do the experiment properly," you arrive at the correct results, Partridge says.

Gems, Partridge, Kaeberlein, and their colleagues published their report in September in *Nature*, the journal in which so much earlier work heralding sirtuins first appeared.

Partridge and Gems took the unusual step of not alerting Guarente, who led the worm work, or Helfand, who led the fly work, before publication of their conflicting findings. Partridge says she challenged some aging work of Helfand's in the past "and got a very dusty response, so I didn't think contact would be helpful." Gems made a similar point. "Normally we would" reach out, he says. But in this case, "based on some of the previous interactions, we thought it would be futile."

Guarente believes otherwise. If he'd heard from his British counterparts, he says, "I think this would have gotten sorted out without dueling papers. That's certainly how we would operate if we found we couldn't reproduce something in someone else's lab." As it happens, a colleague of Guarente's had alerted him to a problem in the worm strain, and he was already looking into it.

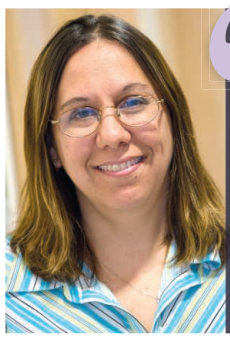
Kaeberlein was more conflicted. He encountered Guarente at a meeting in 2010 and updated him, offering to test any worm strains Guarente wanted to supply. Guarente provided specimens, but in Kaeberlein's hands the experiment didn't work. Oddly, some worms with more *SIR2* lived longer than expected, but so did the control animals. "We can't interpret that," Kaeberlein says.

Guarente tested the worms himself. He discovered that unbeknownst to him and Tissenbaum 10 years earlier, the animals carried a second genetic mutation unrelated to sirtuins, and that eliminating it left only about a 10% to 15% extension of life span from *SIR2*, not the 30% reported.

"Nobody's falsifying data; people are just getting different results, and I think there's room for discussion in all of this," says Tissenbaum, now at the University of Massachusetts Medical School in Worcester. She left the sirtuin field several years ago, weary of all the controversy.

"I believe that all the results are likely to be correct," says Felipe Sierra, director of the Division of Aging Biology at the U.S. National Institute on Aging, which has funded much of the academic sirtuin research. "What matters is that there seems to be an effect in some circumstances."

This line of thinking nags at Kaeberlein. Eventually, he says, if you try hard enough, you might be able to extend life span with almost any gene. This doesn't mean that the gene is actually behind aging in a real, living, breathing organism.



> **"Nobody's falsifying data; people are just getting different results,** and I think there's room for discussion in all of this."
>
> **HEIDI TISSENBAUM,** UMass Medical School

And although Kaeberlein agrees that ultimately mammals matter most when it comes to human health, he also believes that the unfolding story line in lower organisms should worry those working with mice and people. "All of this mammalian sirtuin hype is based on the worm and fly work," he says. "Now that that's looking a little questionable, you have to wonder about the rationale for even doing these experiments in the first place."

Billion-dollar question

In 2008, GlaxoSmithKline bought Sinclair's company, Sirtris, for $720 million. Sinclair remains a professor at Harvard Medical School and is an adviser to the company. Speaking by phone during a recent trip to Sydney, Australia, Sinclair said he stood by his data. "Rumors of the death of sirtuins and aging are greatly exaggerated," he said. "There are now over 1000 papers on the subject every year." (A PubMed search of sirtuins found just over 2000 dating back to 1994.) Sinclair is examining how sirtuin activators impact physiology.

Gems is through with sirtuins. "We never really worked on them anyway; it was just a tidying-up operation," he says. Gems worries about the effect on aging research, even science generally, if sirtuins don't pan out. "If it turns out that this was a giant bubble—how is it possible for so many publications, so much money, it shouldn't have got that far, it shouldn't have happened," he says. He knows of some groups that chose not to publish negative results in the field. "There's a view that seems to be current, that somehow one doesn't engage in quarrels. Sometimes, you have to."

Looking back on those heady days in his lab, Guarente feels "like we were flailing around trying to find some interesting genes." He's still convinced, without a doubt, that he and his mentees did. "There's an overwhelming case in mammals" that sirtuins are linked to calorie restriction, "and you cannot negate that," he says. He believes, too, that the worm and fly data are correct but that *SIR2* needs to be overexpressed at particular levels to extend life, something that's not easy to do. "I'd believe the positive result," he says. "There's lots of reasons why you can do an experiment and have it not work." About his former student Kaeberlein, he wouldn't say much.

Kennedy, now juggling administrative and research duties in northern California, where he moved from UW last year, agrees that the mammalian work looks promising. But "I have a hard time believing that one protein has been placed on Earth to do positive things in every tissue—we're crossing over into the divine." Still, part of him hopes it will all work out. He finds himself in the odd position, he says, of being the only scientist he knows on the fence about sirtuins.

As for Kaeberlein, his unusual trajectory has taught him as much about how high-stakes science is done as it has about the nitty-gritty of these intriguing molecules. "So much of it has not been hypothesis-driven," he says in frustration. "It's been going in with the idea that these things slow aging." The massive media attention paid to sirtuins has lent them more fame than he believes they deserve. He has no regrets about challenging the dogma, even if he was one of those who pioneered it. "I get that it's embarrassing and people feel bad," he says. But "getting the right answer is more important than people's egos."

–JENNIFER COUZIN-FRANKEL

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