

Lenny Guarente and his former postdoc David Sinclair can dramatically extend the life span of yeast. They're battling over how this works, and competing head-to-head to grant extra years to humans

Aging Research's Family Feud

BOSTON AND CAMBRIDGE, MASSACHUSETTS—At 34, David Sinclair is a rising star. His spacious ninth-floor office at Harvard Medical School boasts a panoramic Boston view. His rapidly growing lab pulled off the feat of publishing in both *Nature* and *Science* last year, and it made headlines around the world with a study of the possible antiaging properties of a molecule found in red wine. In a typical day, he fields calls from a couple practicing a radical diet to extend life span, and from an actor hunting for antiaging pills and the chance to invest in Sinclair's new company.

There is, however, another side to this glossy picture of success. Sinclair is engaged

tenure-track spot on Harvard's faculty in late 1999. He then made clear that his old professor's pet theories weren't off limits. At a meeting at Cold Spring Harbor Laboratory in late 2002, Sinclair surprised Guarente by challenging him on how a key gene Guarente discovered extends life in yeast. That sparked a bitter dispute that crescendoed this winter, when the pair published dueling papers.

Researchers who study aging are finding the quarrel both intellectually provocative and a lively source of gossip. And the reverberations extend well beyond that community. At its core, the argument involves one of the hottest topics in longevity research: how cutting calories may increase life span and how its effects can be translated into antiaging therapies. But the dispute, which remains unresolved, involves molecular biology so intricate that many scientists are uncertain how to assess it. It's also not clear how it applies to other organisms, such as mammals.

Guarente and Sinclair are also rivals in business. Both have bold ideas for translating yeast studies into ways to stall mammalian aging and

share many traits common among successful scientists. Both are deeply ambitious, relentlessly competitive, and supremely self-confident. Both savor the limelight. Both love science and show little interest in other pursuits.

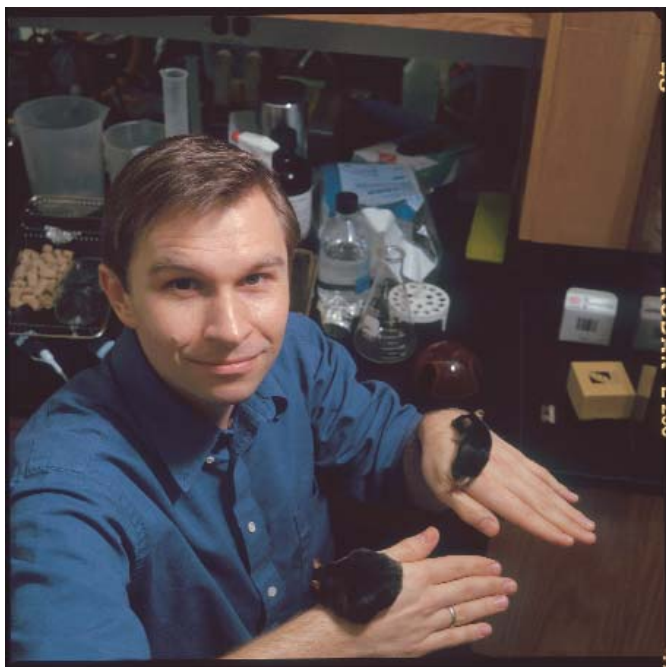
Still, they've retained something of the parent-child relationship that can shape interactions between senior researchers and their students. Like a father dismayed when his son joins a punk rock band, and then dismayed further when it attracts devoted fans and favorable reviews, Guarente exhibits a mix of pride, anger, and disappointment when the conversation turns to his former postdoc. At the same time, Guarente confesses that Sinclair's choices aren't altogether startling. "There's a side of me that identifies with him," he says. "The young Lenny Guarente was not all that different."

Endurance

A native of Massachusetts, Guarente's academic life has revolved around two of the most high-powered institutions in the country. He attended MIT as an undergraduate—where biology at first "felt squishy to me"—and completed graduate and postgraduate work at Harvard. Then he moved two subway stops back up Massachusetts Avenue and settled again into MIT, where he has remained ever since.

With tenure under his belt at 34, Guarente began thinking beyond the mainstream assignments to which he had gravitated early

"They're doing exactly what we're doing, and it's a race." —David Sinclair



in a tense and very public battle with his mentor, a renowned scientist based across the Charles River at the Massachusetts Institute of Technology (MIT). Leonard Guarente, 51, is an undisputed leader in the field of aging, an author of major discoveries about genes that prolong life. For 4 years, Sinclair all but lived in Guarente's lab as a postdoctoral fellow, and the two grew extremely close.

Thanks in part to a glowing recommendation from Guarente, Sinclair nabbed a

treat age-related diseases. Sinclair recently announced plans to launch a biotechnology company that he says will compete directly with one co-founded by his mentor. "They're doing exactly what we're doing, and it's a race," says Sinclair, clearly relishing the prospect.

The two have spoken little since their Cold Spring Harbor falling-out. Some who know the pair say the clash is unsurprising; despite the generation gap, Guarente and Sinclair

on, like studies of gene regulation. In the early 1990s, two of his graduate students, Brian Kennedy and Nick Austriaco, sat down with him to discuss a project they wanted to pursue: dissecting the causes of aging.

At the time, aging was considered fringe science, a topic few reputable researchers would touch. But "Lenny likes a challenge," says Kennedy, now at the University of Washington, Seattle. "He said, 'You've got a year to learn something.'"

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The clock ticking, Kennedy and Austriaco focused on yeast, a single-celled organism that lends itself to laboratory manipulation. They began hunting for mutant yeast cells that lived abnormally long life spans, measured by the number of daughter cells they produce. (A mother cell will typically produce a daughter every 1 to 4 hours; an average cell generates about 20 daughters.) In those early days, when Kennedy and Austriaco were testing hundreds of strains, they organized round-the-clock vigils to track their yeast cells; one of them was always there,

Sinclair had studied genetic regulation in yeast—he and Guarente first met after a genetics conference in Australia—and Sinclair's dissertation was the thickest in the lab. And he had already earned a reputation for pushing limits. He racked up traffic violations in his red sports car, regularly skating close to losing his license and once having it confiscated altogether. "You're allowed to get 12 points, and at one stage he had 14," recalls Geoff Kornfeld, the lab manager in Ian Dawes's lab at New South Wales, where Sinclair studied.

SIR2 wasn't the only project stirring excitement in Guarente's lab. A graduate student, David Lombard, had just cloned the mouse gene for Werner syndrome, a rare disease that mimics accelerated aging. Along with postdoc Robert Marciniak, Lombard was trying to understand how the Werner's protein behaved in mouse cells. "There were ideas and debates flying through the air constantly," says Brad Johnson of the University of Pennsylvania in Philadelphia, who was then a postdoc in the lab. Guarente, never one to coddle his stu-

"This has run me through so many emotions, some of which I didn't know I had."

—Lenny Guarente

gazing at the cells under a microscope and delicately counting off the daughters.

It quickly became obvious that some strains lived unusually long, which piqued Guarente's interest. He hovered nearby, checking in with the students two or three times a day and grilling them on what they'd found. The news was good: About 1 in 1000 strains both produced an abundance of daughter cells and survived well under the stress of a chilly refrigerator, underscoring known links between longevity and stress tolerance. Of these, one mutant caught their attention. It was sterile—unable to mate with other yeast cells—and lived 50% longer than normal.

Guarente canceled golf games with Kennedy to spend more time in the lab. Gradually, the group homed in on a trio of genes called *SIR* genes; deleting them seemed to shorten a yeast cell's life.

It was around this time, in early 1996, that 26-year-old David Sinclair boarded a plane in his hometown of Sydney, Australia, and flew halfway around the world to Cambridge. "Before I even knew I got the [post-doctoral] fellowship, I said to Lenny, 'I'll take out a loan, I'll sell my car'" to come to the lab, says Sinclair. His wife-to-be, a German biologist, was moving to Australia on a fellowship of her own, but Sinclair couldn't pass up the opportunity to study with Guarente, whom he idolized. Still, he knew that the science Guarente favored was then heretical: "The idea that you could use yeast to study human aging was a joke."

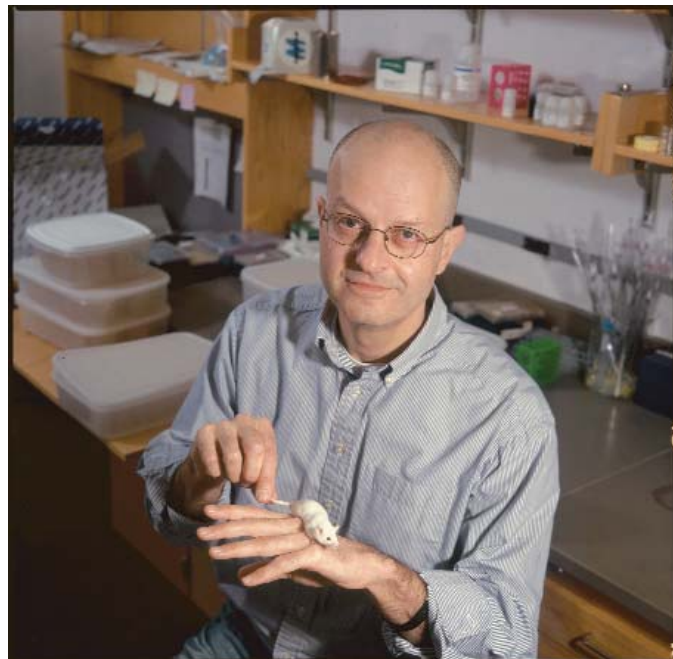
Just as heresy had not deterred Guarente, it didn't stop Sinclair. He already knew yeast: As a graduate student at the University of New South Wales in Sydney,

The atmosphere in Guarente's lab when Sinclair joined was electric. Other students were building on the yeast work by Kennedy and Austriaco, and they pinpointed a specific *SIR* gene, called *SIR2*, that could dramatically extend the cells' life span. Extra copies of *SIR2* also enabled worms to live longer.

Furthermore, Guarente's lab had found that activity of the protein generated by *SIR2*, called SIR2p, was dependent on an enzyme called NAD. All cells carry NAD, which helps govern metabolism. SIR2p, it appeared, could sense the metabolic state of a yeast cell.

This was significant because it dovetailed with what scientists had observed for decades in animals: Curtailing calories and altering metabolism dramatically extends life span. Biologists theorize that, in the face of scarce resources, organisms trade off reproduction, which is hindered by calorie restriction, for survival. No one knows, though, precisely how a meager environment slows aging.

Guarente turned the connections over in his mind. Like calorie restriction, *SIR2* lengthened life span. Its connection with NAD and metabolism suggested something more: Maybe it was a gene that could explain why cutting calories slowed aging. Furthermore, when manipulated, perhaps *SIR2* could mimic the antiaging effects of calorie restriction without a near-starvation diet.



dents, pushed for results.

Sinclair proved to be a brilliant and prolific researcher, often the first to arrive, at 8:00 a.m., and the last to leave, at 12:30 a.m., running to catch the final subway train of the night. He quickly became a favorite of Guarente. But many lab members began regarding him warily, especially after the 1997 publication of a *Cell* paper by Sinclair and Guarente. The paper reported that buildup of ribosomal DNA, a kind of repetitive DNA sequence, in yeast cells caused them to age (*Science*, 2 January 1998, p. 34). Although Sinclair had conducted the experiments himself, Johnson had also proposed similar studies. When lab members learned that Johnson was not a co-author on the paper, they began guarding their work more closely.

Sinclair has a ready reply. He says that when he first thought of the *Cell* experiments, he was concerned that others might accuse him of poaching the ideas. So, to prove that the concept was his, he wrote and mailed himself a letter describing the experiments before they'd been done. Sinclair still

has that letter, its seal intact.

Sinclair agrees, though, that he was unpopular in Guarente's lab, but he explains it differently. "Lenny didn't hide his favoritism" for his Australian postdoc, says Sinclair.

Molecular elixirs

Guarente's arms are sore from shoveling after a December blizzard dumped nearly 2 feet of snow on Boston, but that doesn't stop him from gesticulating to underline his side of the story. "This has run me through so many emotions, some of which I didn't know I had," he says of the falling-out with Sinclair.

Some facts are not in dispute: Calorie restriction extends life in nearly every species tested so far. In yeast, restricting calories boosts activity of SIR2 proteins, and extra SIR2p slows aging. The critical question on which Guarente and Sinclair disagree is how calorie restriction makes the SIR2 protein more active.

Guarente believes the answer lies in the ratio of NAD to a related molecule, NADH. Like NAD, NADH is found in species from yeast to humans, and it helps cells translate food into energy. Metabolic reactions in cells convert NAD to NADH, and vice versa. In 2000—soon after Sinclair joined Harvard's faculty—Guarente's lab reported in *Science* that with less NAD than normal, calorie-restricted yeast don't outlast regular yeast (22 September 2000, p. 2126).

Then, in 2002, a *Nature* paper by Guarente's lab pulled NADH into the picture. It described how knocking out electron transport prevented calorie-restricted yeast cells from living longer. Electron transport is governed in part by NADH. "That really made it look like the NAD:NADH ratio would be a good candidate" for stimulating SIR2p activity, says Guarente, "but there was still no evidence one way or the other."

Sinclair's announcement at Cold Spring Harbor Laboratory that his data diverged from this theory took Guarente by surprise. In his talk, Sinclair presented an alternative model. His candidates were not NAD and NADH. Instead, he focused on a vitamin B precursor called nicotinamide, which is also a breakdown product of NAD, as well as a gene, *PNC1*, that converts nicotinamide into another molecule, nicotinic acid. Nicotinamide was already known to inhibit SIR proteins.

Sinclair found that without *PNC1*, calorie-restricted yeast didn't live longer than normal. Furthermore, adding copies of *PNC1* to yeast receiving normal amounts of

glucose extended their life span. They behaved, in other words, as if they were on a low-calorie, low-glucose diet, even though they weren't.

Sinclair explains his model this way: *PNC1* senses when yeast cells are exposed to low glucose. That boosts the gene's expression, which depletes nicotinamide, which boosts activity of SIR2p, which extends life span. Mammals don't have a *PNC1* gene. But Sinclair believes that nicotinamide and genes that deplete it may guide SIR2p and related proteins in those organisms. *PNC1*, he notes, could also explain why other stressors such as

absence of *PNC1* didn't stop the cells from sensing calorie restriction and living longer, as Sinclair's theory supposed.

Guarente did admit defeat in one arena: He agreed with Sinclair that NAD fluctuations weren't mediating SIR2. But he and Lin reported in their *Genes and Development* paper that the NAD:NADH ratio is crucial nonetheless. To their surprise, they say, calorie restriction appears to lower NADH levels rather than increase NAD. The drop in NADH, in turn, boosts the NAD:NADH ratio and extends life.

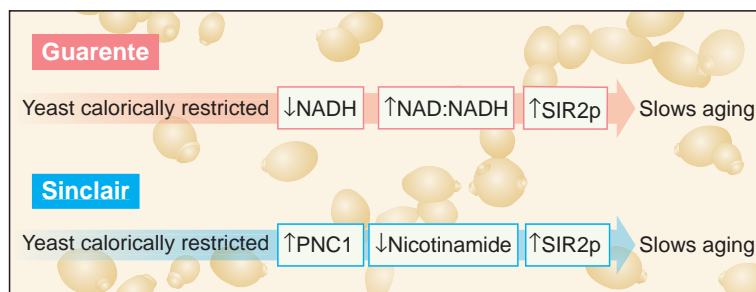
The response from outsiders to this burst of studies has mostly been bafflement. To begin with, no one can agree on whether Sinclair's and Guarente's theories are mutually exclusive, or whether they can coexist. (Even Guarente, Sinclair, and Lin don't agree on this score.)

"My gut view is that one can't be right," says Steven McKnight, a biochemist at the University of Texas Southwestern Medical Center in Dallas. A longtime fan of Guarente, his tendency is

nonetheless to side with Sinclair—partly, he confesses, because the high ratio of NAD to NADH and the high NAD levels that Sinclair reports jibe with McKnight's own work.

The molecular biology that Guarente and Sinclair are tackling is so complex that biochemists have spent decades squabbling over some important details. Among them is a normal cell's ratio of NAD to NADH. McKnight falls into the school that endorses a high ratio of at least 20, similar to what Sinclair reports; some others favor a much lower ratio of 1 to 3, which Guarente stands behind. For NADH fluctuations to significantly impact the ratio, as Guarente postulates, the ratio must be low.

Richard Veech, a metabolism researcher at the National Institutes of Health in Bethesda, Maryland, takes issue with parts of both studies. "It's been well known since 1958 to any biochemist that [a ratio] of two or three is nonsense," he says of the Guarente paper. And as for Sinclair: "Our primary observations differ from his primary observations" when it comes to Sinclair's report that the ratio of free, unbound NAD to unbound NADH molecules has no impact on *SIR2* activity. (Guarente's paper measured the total levels of NAD and NADH, which includes molecules bound to structures in the cell.) Ultimately, though, Veech and some others conclude that more than one mechanism must be regulating *SIR2*. "You're going to control life span with one enzyme for one effect?" he says. "Please!"



Devil in the details. Guarente and Sinclair have different explanations for how slashing calories makes yeast live longer. Their colleagues say the jury's still out on whether one, both, or neither of them is right.

heat shock extend life in yeast: The gene is upregulated by many environmental stresses, not just low glucose.

Last May, Sinclair's lab published this work in *Nature* (*Science*, 9 May 2003, p. 881). And to hammer home his point, he tested Guarente's theory and published a separate paper on the subject in *Science* last December. Its title didn't bode well for Guarente: "Yeast Life-Span Extension by Calorie Restriction Is Independent of NAD Fluctuation" (19 December 2003, p. 2124). The battle lines were drawn.

Guarente shot back. With his postdoc Su-Ju Lin, who recently moved to the University of California, Davis, he published a counterpoint in January's issue of *Genes and Development*. It addressed this question: Did the yeast strains Sinclair used, which lacked *PNC1* altogether and hence accumulated substantial levels of nicotinamide, mask fluctuations in the NAD:NADH ratio that occur in normal, although calorically restricted, yeast cells? In other words, Guarente wondered whether what Sinclair saw in his cells, while accurate, wasn't what happened in nongenetically altered yeast exposed to low glucose.

To test Sinclair's theory against their own, Guarente and Lin created yeast that lacked the *PNC1* gene. Everyone agreed that the cells' extra nicotinamide would inhibit SIR2p regardless of other factors, so they depleted the excess nicotinamide. When calorically restricted, the yeast still lived extra-long. The

In business

Beyond advancing his own case in the *SIR2* clash, neither Guarente nor Sinclair is keen to discuss it. Both are now eyeing a world beyond yeast, pursuing mechanisms of aging in mammals. And both are chasing pet theories they hope will combat diseases of aging and potentially extend life.

Four years ago, Guarente and his colleague Cynthia Kenyon, a worm researcher at the University of California, San Francisco, helped found Elixir Pharmaceuticals, which is a short walk from Guarente's lab in Cambridge. Roughly half the company's research is focused on *SIR2* and molecules that modulate its effects. (The other half revolves around a separate pathway identified by Kenyon in worms.) One of the toughest challenges in targeting the protein made by *SIR2*, however—or *SIRT1*, as it's known in mammals—is that “this protein is all over the body,” says Peter DiStefano, the chief scientific officer of Elixir. The company is currently experimenting with various animal models and has raised more than \$40 million from investors.

Elixir has also operated with almost no direct competition; pharmaceutical companies have hesitated to enter this market, and very few other biotechnology firms are devoted to aging research. If Sinclair has his way, that won't last long. Last fall, he asked Andrew Perlman, a 28-year-old millionaire who made his money selling two technology companies he founded, to help Sinclair build a new company called Sirtris Pharmaceuticals. Sirtris, which hasn't yet raised funds, will focus largely on Sinclair's most recent obsession—a compound called resveratrol, an antioxidant in red wine and other foods.

In a paper published last August in *Nature*, Sinclair and his colleagues reported that in yeast, resveratrol appeared to stimulate *SIR2*, hence mimicking calorie restriction and slowing aging (*Science*, 29 August 2003, p. 1165). Various studies in animals also suggest that resveratrol protects against cancer. It's “as close to a miraculous molecule as you can find,” says Sinclair. “One hundred years from now, people will maybe be taking these molecules on a daily basis to prevent heart disease, stroke, and cancer.” A Montreal company, Royalmount, is beginning human trials of resveratrol in herpes and colon cancer prevention; Sinclair hopes Sirtris will partner with it. He's also experimenting with modified versions of the compound.

Because resveratrol occurs naturally, it's already widely advertised in health food

stores and over the Internet. Sinclair purchased a dozen samples peddled as resveratrol and tested them in his lab. Only one passed the test—the compound is quite unstable at room temperature—and Sinclair briefly became a paid consultant to the company that makes it, Longevinex. In late December, he announced that he had severed ties with Longevinex after the company broadcast comments from him on its Web site that Sinclair claimed were inaccurate.

Guarente, who tried to recruit Sinclair to Elixir before their falling-out, even bringing him to some of the company's board meetings, wasn't expecting to hear that his former postdoc was starting a company of his own. Sinclair's choices, however, mirror Guarente's years ago: As a young scientist, Guarente rejected an offer from his Harvard adviser, Mark Ptashne, to join Ptashne's new company. Instead, says Guarente, “I and a bunch of young turks at Harvard started a competitor company. ... That's what young people do.” The company eventually folded. Ptashne, he

says Elixir's DiStefano. Sinclair hopes to avoid this by tinkering with the compound's chemical makeup.

The big question facing *SIRT1*, meanwhile, is what it does in mammals. The skepticism Guarente confronted in the early 1990s, when he backed yeast as a model for human aging, has abated, but fundamental mysteries remain. Perhaps most importantly, is calorie restriction in mammals mediated by the *SIRT1* gene? “It would surprise me only somewhat” if it's not, says Marciniak, a former Guarente lab member now at the University of Texas, San Antonio.

Both Guarente and Sinclair are wrestling with this question, and, as in other areas, they're racing along parallel paths. Earlier this month, a paper in *Cell* by Guarente's team and a paper in *Science* on which Sinclair was an author (www.sciencemag.org/cgi/content/abstract/1094637) both explored how *SIRT1* helps mammalian cells withstand environmental stress.

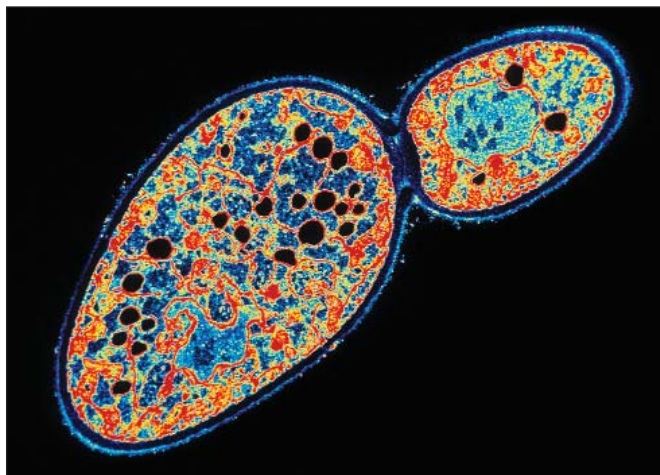
Guarente also talks animatedly about a project that's captured his attention: fat and its links to some of the seven *SIRT* genes in mice. He and his lab members are feverishly working to link these genes with fat accumulation and sensitivity to insulin—which could lead to new therapies for obesity and diabetes. “That's what I think the 5-year plan is,” says Guarente. He never imagined, he adds, that his work on life span might translate into diabetes drugs.

Sinclair is more coy, but he admits to exploring connections between fat and *SIR2* in worms and mice. The workaholic in him hasn't abated, even with a 1-year-old daughter at home. He keeps a microscope, an incubator, and a refrigerator for yeast plates in his house. Reluctant to take time off, he's returned to Australia just once in the last 3 years. And Sinclair guards his work with extraordinary care: After a notebook filled with data went mysteriously missing, he installed a safe in his Harvard office. There the lab's notebooks sit, locked inside.

Guarente's lab is calmer these days than it was when Sinclair and others toiled there. “Now we have birthday cakes,” says Marcia Haigis, a postdoc. “Lenny says the lab is too touchy-feely.”

A more relaxed atmosphere hasn't stopped Guarente, like Sinclair, from sticking resolutely to his theory of aging. The truth, if and when it surfaces, may well embrace a synthesis of what the two—and others—propose. Or, of course, it may show that only one of them is right.

—JENNIFER COUZIN



Mother-daughter division. Days-long vigils in Guarente's lab identified yeast that bud more than usual, a measure of longevity.

says, was “like my father, the establishment.”

Certainly, Sinclair still views his mentor as something of a father figure. Asked if he's read Guarente's 2003 memoir, *Ageless Quest: One Scientist's Search for Genes That Prolong Youth*, he looks less than enthusiastic. “I don't want to see into his mind,” says Sinclair. “It's a bit like learning about your parents' sex life.”

Although many scientists agree that both the resveratrol molecule and the *SIRT1* drug target seem promising, they don't foresee smooth sailing. “I can't believe that it's going to be the magic bullet that cures aging,” says James Joseph, a neuroscientist at Tufts University, of resveratrol; he has extensively studied the family of compounds to which it belongs. Resveratrol is also known to target a broad swath of molecules in the body, something that could be problematic in a drug,