Much ado about ageing

Questions about a laboratory assay are making Sirtris, a high-profile biotechnology company, the talking point of the ageing field. **Heidi Ledford** investigates.

Konrad Howitz wasn't looking for a fountain of youth. As director of biochemistry at BIOMOL International in Plymouth Meeting, Pennsylvania, Howitz wanted to add new molecular assays to the company's catalogue. A protein called a sirtuin had recently been shown to lengthen lifespan in yeast[1], and Howitz was developing methods to measure the activity of one of its mammalian forms, called SIRT1.

But when Howitz and his team stumbled on the discovery that a compound called resveratrol seemed to activate SIRT1, they quickly realized the implications. Resveratrol was rumoured to be the ingredient in red wine that kept the French healthy. In 2003, Howitz shared his findings with David Sinclair, a molecular biologist at Harvard Medical School in Boston, Massachusetts, who had studied sirtuins extensively. Sinclair immediately saw commercial potential for resveratrol as a human anti-ageing drug. Fearing corporate espionage, Sinclair code-named the compound 'R'.

A year later, 'R' became the centrepiece of Sirtris, a company Sinclair co-founded in Cambridge, Massachusetts. Four years after that, Sirtris became a biotechnology success story when London-based pharmaceutical giant GlaxoSmithKline (GSK) purchased it for US$720 million. Yet behind the scenes, researchers have been voicing concerns about some of the research that forms the basis for the company. Most recently, questions have resurfaced[2] over whether the interpretation of Howitz's original SIRT1 assay[3] was flawed.

It is a technical debate with big implications, not just for Sirtris but also for the rapidly expanding research community that is now studying sirtuins. Some researchers are questioning whether resveratrol, and other compounds like it that Sirtris is now testing in clinical trials, really does activate sirtuins. Until the mechanism is clear, they are cautious about pursuing drugs that might have unanticipated biological targets and effects. "It is an exciting time in the ageing field," says Brian Kennedy, a molecular biologist at the University of Washington in Seattle. "But this issue has had a polarizing effect. It needs to be resolved."

Helped in no small part by Sirtris's public-relations efforts, sirtuins have been trumpeted in the press as the key to boosting human lifespan. The compounds have a compelling biological narrative: Sinclair and others have proposed that resveratrol and other SIRT1 activators imitate the effects of 'caloric restriction', a drastic reduction in calories that lengthens lifespan in some animals. Compounds that activate sirtuins might therefore have all the benefits of caloric restriction without the starvation.

Biologist David Sinclair co-founded Sirtris to explore sirtuins, enzymes linked to lifespan and ageing.

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Seeing the light

Those were the exciting implications when, in 2003, Howitz, Sinclair and their colleagues showed that sirtuin activators could extend yeast lifespan by 70%. Their paper, published in *Nature*[3], also showed results from Howitz's assay. The sirtuins are members of a class of enzyme called deacetylases, which strip acetyl groups from proteins. To measure this activity, Howitz designed an acetylated protein fragment bearing a chemical tag. When activated, SIRT1 deacetylated this peptide substrate and the tag started to fluoresce. The greater the activity of SIRT1, the greater the fluorescence — and in the paper, resveratrol and other chemicals thought to activate SIRT1 generated a mighty glow.

Doubts about the assay first surfaced publicly two years later. Two papers[4,5] showed that resveratrol boosted the activity of SIRT1 only when its peptide substrate contained the fluorescent tag: no tag, no activity (see graph). The papers caused a stir in the field, but failed to register among investors or the public. "The sirtuin stuff has just sort of been a runaway train," says Kennedy, who was lead author on one of the papers[4]. "We might have caused the train to wobble a little, but it kept barrelling down the tracks."

The assay controversy wasn't the only stone on the tracks. Some labs have been unable to consistently reproduce Sinclair's life-extending results in model organisms such as fruitflies and nematodes[6], and debate has simmered over whether SIRT1 activation truly mimics caloric restriction. For Sirtris, however, the arguments have little practical bearing — the company hopes to market its drugs as a way to stave off diabetes and other diseases associated with ageing, rather than a way to extend lives. And by 2006, Sinclair and his colleagues had shown that resveratrol improved the health of mice fed extremely high-fat diets[7]. Then, in a high-profile *Nature* paper published at the end of 2007, Sirtris unveiled a set of compounds that its researchers said were 1,000 times more potent than resveratrol[8]. In April the following year, GSK announced that it planned to

purchase the tiny biotech for $22.50 per share, nearly double its $12 trading price.

In the paper, the team used mass spectrometry instead of the fluorescence assay to show that resveratrol and the more potent newcomers activated SIRT1, allaying some researchers' concerns. "A lot of people read 'mass spec', considered that a different assay, and moved on," says Joseph Baur, a molecular biologist at the University of Pennsylvania in Philadelphia and a former member of Sinclair's lab.

Yet closer inspection showed that the peptides used in the mass spec experiments still carried a fluorescent tag, hinting that this assay, too, worked only when the tag was present. "That should have thrown up a red flag to one of the reviewers, but apparently it did not," says Ronen Marmorstein, a structural biologist at the Wistar Institute in Philadelphia, Pennsylvania. Sinclair says that they used peptides originally prepared with a fluorescence screen in mind. When they switched to mass spectrometry, they simply used the substrates they had on hand.

The debate blew up again this year when a team led by Pfizer researchers in Groton, Connecticut, published a paper in the *Journal of Biological Chemistry*[2]. The group claimed that several of the new Sirtris compounds did not activate SIRT1 without the fluorescent tag. Worse, they presented evidence that the compounds were inhibiting a slew of other proteins — and some of the mice taking high doses of the drugs died. The paper concluded that the Sirtris compounds and resveratrol were pharmacological dead-ends owing to "their highly promiscuous profiles".

Biotech blogs have been abuzz about Sirtris ever since. What did GSK know about these problems before it bought the company? Do the drugs work as claimed? Do they confer metabolic benefits at all? Most researchers seem to agree with Sirtris that the SIRT1-activating compounds do have beneficial effects in mice. Rafael de Cabo at the National Institute on Aging in Baltimore, Maryland, and a co-author on the 2006 *Nature* paper, says that he has safely tested one of the compounds, SRT1720, in more than 1,000 mice, some of which received the compound for two years. At least three other groups have published data showing that SRT1720 is beneficial and non-toxic in mice[9–11].

But the debate about how the compounds act rages on. Some interpret the Pfizer results to mean that the assay had yielded an artefact, and the compounds, resveratrol included, may not act on SIRT1 at all. "If this drug is just binding to this fluorigenic group, then why would it really talk to SIRT1 *in vivo*?" asks Kennedy. Instead, the compounds might be acting directly on the tag, and they could be bestowing beneficial health effects by acting on other, unknown, targets.

Sinclair, Howitz and their colleagues at Sirtris acknowledge that the assay does not work with some substrates in the absence of a fluorescent tag. But they stand by their argument that the drugs are acting on SIRT1, saying that when the peptide is attached to the fluorophore, it mimics a natural SIRT1 substrate found in the cell. "To my mind, these results are structural clues," says Howitz, who says that the biochemical data in the Pfizer group's paper are solid. Howitz, whose company is now called ENZO Life Sciences, says that the assay was still worthwhile because it yielded drug candidates that are looking promising in follow-up work.

If the assay was useless, Sinclair points out, then it would have been highly unlikely to have repeatedly identified compounds that all identically improve the health of mice. "What's going on? These compounds just happen to hit random targets in the cell and most targets just happen to lower blood sugar, increase endurance and boost mitochondrial function?" asks Sinclair. "I wish finding drugs was that easy."

"The sirtuin stuff has just sort of been a runaway train."

In fact, several companies, including GSK, dropped their pursuit of SIRT1 activators after the results of their high-throughput fluorescence screens failed to stand up to closer scrutiny. A former GSK employee says that the firm's internal hunt for SIRT1 activators was killed well before the Sirtris deal. The same fate met putative SIRT1 activators fished out of a screen at Elixir Pharmaceuticals, a company founded two years before Sirtris, says chief scientific officer Peter DiStefano. Whereas Sirtris's fortunes soared, Elixir is now down to five employees at its office in Cambridge, Massachusetts.

The same fate might have met Sirtris's programme, says Baur, but for the compelling animal data from Sinclair's lab and from the company itself. Ad Rawcliffe, head of worldwide business development at GSK, said in a statement that the company was aware of the controversies surrounding the fluorescence-based assays before it purchased Sirtris. GSK fully evaluated those risks, he says, and remained "confident that the consistency of the activity in cell-based and animal studies is driven through a SIRT1-dependent mechanism".

Not everyone shares that confidence, but few are willing to dismiss sirtuin activators until they see the outcomes of Sirtris's clinical trials. These include phase IIa trials against type 2 diabetes, inflammation and cardiovascular disease.

Meanwhile, the field is intent on working out exactly how resveratrol and these compounds act, something that is likely to be important for drug development, says Marmorstein. "If something goes wrong with the compound, you can't modify it based on an understanding of sirtuin function if you don't know that is the target."

Several academic labs hope to tackle the assay question head on. Anthony Sauve, an enzymologist at Weill Cornell Medical School in New York and a member of Sirtris's scientific advisory board, is considering treating cells with SIRT1 activators to look for 'native' substrates that are deacetylated by SIRT1. And biologist Leonard Guarente from the Massachusetts Institute of Technology in Cambridge, whom Sinclair calls the "grandfather" of the sirtuin field and who is co-chair of Sirtris's scientific advisory board, says he is so anxious to have the matter resolved that his lab may begin a hunt for other proteins that may be required to activate SIRT1 alongside resveratrol.

At Sirtris, the labs are just as intent on clarifying how the drugs work, says company president George Vlasuk. "I care what the mechanism is," he says. "I think everybody cares what the mechanism is." ■

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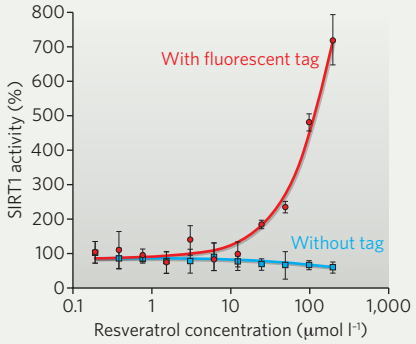
SIRTUINS UNDER SCRUTINY

Some researchers claim that an assay designed to measure activation of SIRT1 by resveratrol works only in the presence of a fluorescent tag — as suggested by these data from M. Kaeberlein *et al. J. Biol. Chem.* **280,** 17038–17045 (2005).



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