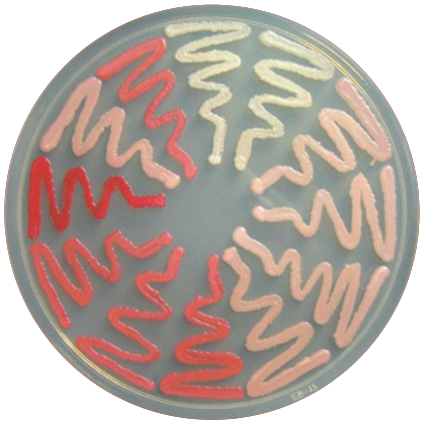

Outside the fold

Susan Lindquist has challenged conventional thinking on how misfolded proteins drive disease and may power evolution. But she still finds that criticism stings.

BY BIJAL P. TRIVEDI

On a frigid winter's morning in 1992, Susan Lindquist, then a biologist at the University of Chicago in Illinois, trudged through the snow to the campus's intellectual-property office to share an unconventional idea for a cancer drug. A protein that she had been working on, Hsp90, guides misfolded proteins into their proper conformation. But it also applies its talents to misfolded mutant proteins in tumour cells, activating them and helping cancer to advance. Lindquist suspected that blocking Hsp90 would thwart the disease. The intellectual-property project manager she met with disagreed, calling Lindquist's idea "ridiculous" because it stemmed from experiments in yeast. His "sneering tone", she says, left an indelible mark. "It was actually one of the most insulting conversations I've had in my professional life." It led her to abandon her cancer research on Hsp90 for a decade. Today, more than a dozen drug companies are developing inhibitors of the protein as cancer treatments.

Lindquist seems able to shrug off such injustices, now. Her work over the past 20 years has consistently challenged standard thinking on evolution, inheritance and the humble yeast. She has helped to show how misfolded infectious proteins called prions can override the rules of inheritance in yeast, and how this can be used to model human disease. She has also proposed a mechanism by which organisms can unleash hidden variation and evolve by leaps and bounds. She was the first female director of the prestigious Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, and has received more than a dozen awards and honours in the past five years. In a paper being published this week in *Nature*, she and her colleagues show that in wild yeast, prions provide tangible advantages, such as survival in harsh conditions and drug resistance[1].

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To hear an interview with Susan Lindquist:
go.nature.com/alzlss

What is most striking about Lindquist, however, is that despite having the self-confidence to take on controversial projects, she remains remarkably sensitive to criticism. The sting of rejection from the Chicago intellectual-property office may have dulled, but she recognizes and is dismayed by what she sees as a growing incivility among colleagues, a meanness that she thinks threatens the progress of science. "I feel like the profession is getting less and less genteel and more and more cut-throat," she says.

Prion proteins are responsible for the colour differences in some strains of yeast.

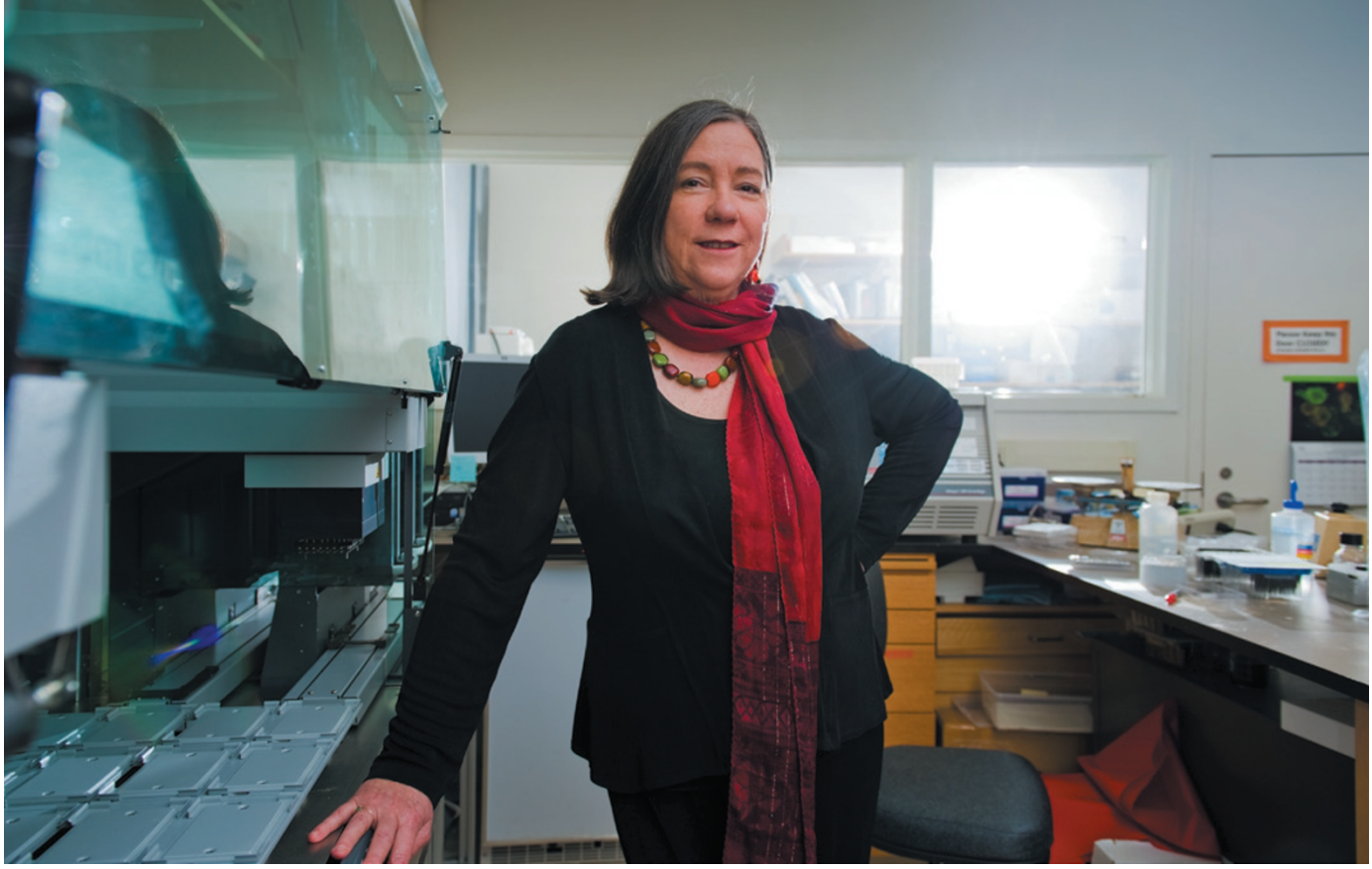
R. KRISHNAN/S. LINDQUIST

HEATING UP RESEARCH

Lindquist began her career at Harvard University in Cambridge, Massachusetts, in 1971, in the laboratory of Matthew Meselson, a biochemist famous for helping to show how genetic information is copied and inherited. "He was a brilliant scientist," Lindquist says, but when she started he was spending much of his time lobbying for a federal ban on biological weapons in the United States. "So he was never here."

She found the lack of a mentor very stressful in those early days. "It was terrifying and I almost left a couple of times," she says. Working more or less on her own, Lindquist decided to probe a mysterious phenomenon that researchers were exploring, called the heat-shock response. When fruitfly larvae are exposed to high temperatures, certain regions of their chromosomes 'puff up' as genes at these sites frenetically produce RNA. In work that would culminate in her PhD and eventually shape her career, Lindquist showed that applying heat to cultured fruitfly cells triggers an emergency response in which the cells manufacture heat-shock proteins, such as Hsp90, to protect themselves[2].

When Lindquist published her data, she says, "an awful lot of people thought it was nonsense". Colleagues dismissed the findings as an artefact — the result of heat denaturing proteins. Although the work was published in a prestigious journal, Lindquist took the criticisms hard. Her lab



Susan Lindquist's career has been shaped by her investigations of proteins produced in response to 'heat shock'.

SAM OGDEN FOR *NATURE*

mate, collaborator and close friend at the time Steven Henikoff — now at the Fred Hutchinson Cancer Research Center in Seattle, Washington — wondered, "How can such a nice person survive" in this field?

With her newly minted PhD, Lindquist started a postdoctoral fellowship in 1976 at the University of Chicago. Two years later, the university offered her a tenure-track position. Lindquist became interested in studying heat-shock proteins in yeast, partly because it would allow her to manipulate genes more easily than in flies. A faculty member warned her against changing organisms until she had tenure, but Lindquist ignored the advice, assuming that she had little chance of getting tenure anyway. "It was really very, very difficult being a woman in science then," she says. So she pursued what she found most mysterious and fascinating.

That courage often seems to be lacking in younger scientists these days, Lindquist laments. She recalls struggling to convince students or postdocs to take on risky projects, only to learn later that when they did, their lab mates mocked them. "That shocks me," says Lindquist. She has often been afraid of being wrong — a fear that led to lots of repeated experiments — but "I didn't have a fear of a new idea".

Most of the new ideas Lindquist has developed met with resistance. In late 1993, when she proposed that a heat-shock protein called Hsp104 could untangle and dismantle clumps of protein, *Nature* initially rejected her paper. The ideas struck many as absurd, Lindquist says. "When I gave a talk about it, reactions ranged from scepticism to outright disbelief." The work was eventually published the following year[3].

"I FEEL LIKE THE PROFESSION IS GETTING LESS AND LESS GENTEEL."

Still, she was literally staring at her rejected manuscript when she received a call from Yury Chernoff, then a postdoc in Susan Liebman's lab at the University of Illinois at Chicago, who had found that Hsp104 influenced a bizarre colour trait in some yeast strains. Geneticist Brian Cox, then at the University of Liverpool, UK, first described this trait[4], called [*PSI*$^+$], in yeast in 1965. Cox noted that when white yeast strains mate with red ones their progeny produce only white offspring, rather than the mixture of red and white predicted by conventional genetic theory. According to one hypothesis, the trait was actually passed on not by genes but by a misfolded protein that worked like the self-replicating, disease-causing prions known to trigger fatal neurological disorders such as Creutzfeldt–Jakob disease.

Prions join together to form long, 'amyloid' fibres. Working with Chernoff, Lindquist showed how Hsp104 controls the [*PSI*+] trait by chopping up fibres of a protein called Sup35 (ref. 5). Short segments of these Sup35 fibres are passed to daughter cells and act as a template for more to form. Watching the yeast prions pass from mother cell to daughter cell was "pretty magical", Lindquist says. Moreover, the results suggested that simple yeast cells could be used to study the proteins that cause neurodegenerative disorders in humans — another idea that colleagues found hard to swallow.

For the next 15 years, Lindquist expanded her study of yeast prions. Chernoff, now editor-in-chief of the journal *Prion* and based at the Georgia Institute of Technology in Atlanta, says that Lindquist pioneered many of the biochemical and molecular techniques now used for studying yeast prions. But her controversial hypotheses, he says, have really driven the field forward and provoked discussion and new experiments. Lindquist suggested that yeast prions are widespread and may be beneficial in some cases because they are able to switch between soluble, active states and fibrous, inactive states[6].

Many have suggested that the prions she has been observing are artefacts of laboratory culture techniques that force proteins to behave in unnatural ways. But in her most recent paper[1], Lindquist has shown that about one-third of the 700 or so wild yeast strains she examined harboured prions. In almost half of those strains, the prion seems to confer a beneficial trait. For example, a strain isolated from white wine is resistant to acidic environments and to the anti-fungal drug fluconazole; and a strain harvested from Lambrusco grapes is resistant to a DNA-damaging agent. When the prions in these strains are eradicated or 'cured', these useful traits disappear.

Lindquist has also continued her studies of Hsp90. When, in the 1990s, she disabled, or knocked out, both copies of the gene that makes Hsp90 in fruitflies, the creatures died; but when she knocked out just one copy, something mysterious happened. Flies were born with a hodgepodge of physical deformities, such as shrunken or square eyes, shrivelled wings and crooked legs[7].

Lindquist realized that Hsp90 was chaperoning proteins that contain detrimental mutations into a working form, thereby hiding their effects. Removing half the Hsp90 meant there wasn't enough of it to go around, proteins could no longer fold correctly, and the effects of all the hidden mutations became apparent. Lindquist hypothesized that the same thing happens during a natural crisis such as starvation or a change in temperature or pH. The environmental shock makes more proteins misfold and these suck up the available Hsp90, leaving a surplus of incorrectly folded proteins that could spawn the evolution of new traits. Most of this misfolding will be bad, says Lindquist. But if any of it yields a cell that is well adapted to the new conditions, some organisms could survive and thrive.

Lindquist calls Hsp90 a "capacitor" for evolutionary change. Just as an electrical capacitor stores electrical energy, Hsp90 lets hidden variation build up in the genome. When an environmental stressor trips the switch, dramatic variations can be unleashed. She found the same kinds of effects in the plant *Arabidopsis thaliana* — upturned and extra roots, exotic leaf whorls and darker hues appeared when the heat-shock protein system was put under stress[8]. Lindquist suggests that studying this phenomenon would be a powerful approach for discovering hidden variation in plants — unlocking the basis of traits such as drought resistance or salt tolerance.

The mutations responsible for these flies' deformities are present in normal-looking flies, but their effects are usually hidden by 'chaperone' proteins.

Lindquist says she was unaware that these ideas would upset people. Many in the evolutionary-biology community adhere to the idea that evolution proceeds in slow, tiny steps, not the big bursts she was proposing. Nick Barton, an evolutionary geneticist at the University of Edinburgh, UK, says that the suggestion that the chaperone system releases "useful" variation when needed is controversial. "I really don't think there is much evidence for an adaptive role," he says.

Others are more open to the hypothesis. This mechanism should be incorporated into evolutionary theory, says Massimo Pigliucci, an evolutionary biologist and philosopher at the City University of New York Graduate Center. Pigliucci says that Lindquist "put empirical meat on ideas that have been around for a while". Still, he asks, "How important are these in the evolution of lineages?" It may take another 20 years to work that out, he says.

In August 2001, Lindquist moved from the University of Chicago to take the helm of the Whitehead Institute. It was an honour, but also a draining position that she held for only three years. She oversaw the separation of Whitehead from its genome centre, a sequencing powerhouse that had contributed much of the data for the Human Genome Project. It was a financially messy ordeal that left her desperate to focus on science, and particularly on disease-related research.

Even though she hasn't been the one to develop them, Hsp90 inhibitors have already begun to show some promise. More than 20 clinical trials are exploring their effect in cancer. "It's a hot topic," says Len Neckers, a cancer biologist at the National Cancer Institute in Rockville, Maryland, who identified the first Hsp90 inhibitor 20 years ago. The inhibitors might also target drug-resistant fungi that cause deadly infections in people with suppressed immune systems[9].

Lindquist's expertise in protein folding fuelled an interest in neurodegenerative diseases. Amyloid fibres are also present in Alzheimer's, Parkinson's and Huntington's diseases, and Lindquist has championed yeast as a model for studying their effects in these conditions. In a study published last year[10], she showed that a pile-up of the amyloid-β protein, a hallmark of Alzheimer's, is toxic to yeast, slowing its growth. She then used the model to screen 5,000 yeast genes for ones that might affect this toxicity. The approach was successful: it turned up 40 genes, 12 of which had human homologues, and one of which is a known risk factor for Alzheimer's. Two others interact with known risk factors.

Her hope is to pin down in yeast the initial steps that lead to amyloid formation in Alzheimer's, then to identify drugs that prevent it. The approach continues to raise eyebrows, however. "Many wondered how she could possibly model things such as Alzheimer's and Parkinson's in yeast — which are a single cell, have a short life span and, of course, don't have a brain," says Nancy Bonini, a neurogeneticist at the University of Pennsylvania in Philadelphia.

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Her grant applications have received "very mixed reviews", says Lindquist — a charitable description, she adds. Many hardworking scientists with great ideas get their proposals turned down, she says, but she worries that the tough funding climate is dragging down the tone of grant and paper reviews. "They get exhausted, tired, and they get cranky. And then they get a paper to review." She pauses, leans forward and says emphatically, "I think we have to stop and say, 'No, let's not do this. Let's not be mean to somebody else because someone was mean to you.'"

Meselson, she says, instilled in her the importance of ethical and compassionate scientific conduct. It is something she has worked hard to emulate and pass on to her own trainees. In late-2010, she wrote a short commentary[11] entitled 'Three quite different things that matter to me'. Think and train broadly, she wrote; be kind, be generous, don't try to destroy someone; and, have faith.

Her work and the testaments of colleagues speak to her success with the first two, and her own words attest to the last: "When I think about my kids' future I feel very concerned," she says, tearing up as she lists the world's environmental, social, economic and political woes. "And then I go to a lecture. I'll hear someone get up and talk about their work, and they've done something amazing. The profession that I live and breathe gives me hope." ■

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