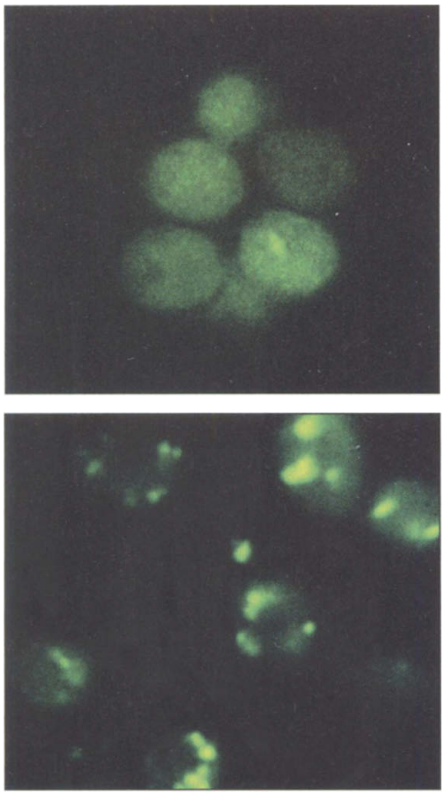
Researchers studying misfolded proteins have found prions in yeast and fungus; unlike those in mammals, they don't seem to harm the host

In Yeast, Prions' Killer Image Doesn't Apply

Twenty years ago, a curious new agent emerged from obscurity to join the cast of biology's villains. Dubbed prions, these infectious proteins have been fingered for causing an array of rare but horrific brain illnesses. They are suspected of triggering "mad cow disease," for example, which is thought to have crossed the species barrier and killed more than 100 people in Europe.

Prions' fearsome reputation is enhanced by mystery: Researchers have not been able to determine exactly how they do their dirty work. And although they are the prime suspect in several diseases, they haven't been experimentally proven to be the sole cause of any. But researchers have identified one feature that all prions appear to share: a pernicious shape. Whereas many proteins bend into alternate forms and still function properly, the hallmark of human prion proteins is that they have morphed from a normal, harmless protein into a contortion that seems to turn them deadly. And having made that change, according to prion researchers, they become unrelenting bullies, forcing other proteins to adopt their misfolded shape.



Innocent? Yeast cells dotted with clumps of [PSI$^+$] prions (*bottom*) seem no worse off than normal cells (*top*).

This standard picture is shifting, however. A radical new line of inquiry is casting human prions not as prototypical evildoers but more like the rare bad guys in an extended family of eccentrics. Among the prions' mild-mannered siblings and cousins, according to studies published since 1994, are certain prionlike agents in yeast and fungus. Like their malevolent kin, they appear to cause other proteins to adopt their shape, yet their hosts usually seem to suffer little or no harm. Some research even indicates that these prions might perform useful functions such as helping cells survive in tough environments or transmitting beneficial qualities from one generation of cells to the next. Although controversial, these ideas are gaining support. "We have to get out of the mindset of considering a prion as a disease [agent]," says Susan Liebman, a molecular geneticist at the University of Illinois, Chicago.

Even if the new prions do turn out to be benign, they might still be useful models for researchers studying human diseases. Increasingly, biomedical scientists are turning to colleagues in the yeast and fungus fields for help in understanding how proteins fold, misfold, and possibly trigger brain lesions. For example, Alzheimer's disease and prions are both associated with distinctively arranged clusters of misfolded protein, called amyloids. In addition, a number of other neurodegenerative diseases, including Parkinson's and Huntington's diseases, are marked by comparable clumps of misfolded protein in the brain. The amyloids of Alzheimer's and the protein clusters of Parkinson's are not considered transmissible from one cell to another, but similar mechanisms might be behind all the protein misfolding in these structures.

Surprising finds

As early as the 1950s, before prions were identified, researchers had noted bizarre behavior displayed by some strains of yeast and fungus. French scientists observed that heritable, non-DNA elements of certain fungi could be passed on to their offspring. In the 1970s, another French group found a similar anomaly in yeast: An inherited element that was not produced by a gene seemed to appear spontaneously. "That at first seemed very strange to me," says Reed Wickner, a geneticist at the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Maryland. In 1989, he began considering parallels between the odd yeast traits and those of a human prion, then a relatively new term referring to the misfolded version of the mammalian protein PrP, the normal form of which is especially prevalent in nerve cells.

Wickner focused on two heritable, non-DNA elements: [URE3] and another that had been described in the literature, [PSI$^+$]. (Prion names are normally bracketed.) Both had already been found to be transmissible from one yeast cell to another in cytoplasm—similar to viruses. Wickner proposed in *Science* in 1994 that the pair represented "self-perpetuating" versions of two normal yeast proteins, Ure2p and Sup35p, respectively (*Science*, 22 April 1994, p. 566). Somehow, these proteins could spontaneously convert to the [URE3] and [PSI$^+$] forms, which in turn converted other normal proteins around them—just as the prion form of PrP is thought to do in human brains as it destroys them. This would explain why genes didn't produce these proteins: As prions, they appeared only when the normal protein form misfolded. Neither [URE3] nor [PSI$^+$] had an obviously remarkable effect on yeast, though. [URE3] alters the cell's nitrogen metabolism, and [PSI$^+$] allows the synthesis of certain proteins to continue when it would normally be stopped. Despite the apparent absence of disease, Wickner labeled the pair prions; at the time, they were the only proteins other than [PrP] to garner that title.

Yeast researchers went on an extended

CREDITS: S. LINDQUIST

Susan Lindquist: Prion Expert Leads the Whitehead Institute

Susan Lindquist likes to say she "stumbled into" a career in science. She doesn't mean she hesitated. It's just that she never planned to be a member of the National Academy of Sciences, a leader of a fast-developing area of protein studies (see accompanying story), or the director of a major academic center: the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, which she was chosen to head last fall. As Lindquist tells it, her strategy was pretty simple: to follow her curiosity.

"As a kid," Lindquist said in an interview earlier this year, "one of my favorite activities was to make 'mixtures,' " concoctions of berries and "weird things" gathered in the neighborhood and fermented under plastic wrap "to see what would happen." Looking back, she sees this "enticing" aspect of discovery as drawing her into science, the "inventiveness ... of poking at something and having an idea."

Her Swedish- and Italian-American parents did "not have a chance to go to college," Lindquist says, and she was not programmed to be an academic superstar. During her adolescence in Chicago in the 1960s, "becoming a housewife was pretty much the program. ... That's what a woman was supposed to do." But she was attracted to medicine as an undergraduate at the University of Illinois and got hooked on biology one summer working in the lab of biologist Jan Drake. She was accepted into Harvard's graduate school in 1971 and wound up in the high-powered lab of biochemist Matthew Meselson, her thesis adviser, later famous as a MacArthur award "genius" and expert on biological warfare. Even then, she didn't entertain the "expectation that I would ever have a lab of my own." After all, she says, there were hardly any women on the faculty.

New energy. One of Lindquist's main tasks, observers say, will be recruiting younger faculty members to the Whitehead.

Today, Lindquist runs not just a lab but an entire institute, and she will need more than curiosity as she takes on some difficult management challenges. She took over from Gerald Fink, a yeast genetics expert like herself, who remains on the faculty. The change came at a critical time.

The Whitehead, which is marking its 20th anniversary this year, has been a pioneer in basic research but is perhaps best known now for its contribution to sequencing the human genome. Led by molecular biologist Eric Lander, the mass-production effort boosted the Whitehead's profile—and its income. In fact, the genome center's income—mostly federal—accounts for 71% of the Whitehead's $140 million budget. But as sequencing winds down, Lander reportedly has been exploring ways to spin off his evolving interests in a new center (*Science*, 21 December 2001, p. 2451). Such a spinoff, which would leave a hole in the institute's finances and faculty, has yet to materialize. But if it never does, this could be difficult, too, for the genome group and the rest of Whitehead might need to sort out their overlapping, postsequencing agendas.

Steering the Whitehead through these changes is Lindquist's job. Neither she nor Lander wants to discuss it. The topic of the genome center's future, a Whitehead spokesperson explained, is "off the table" for public discussion. Nor is Lindquist prepared to comment on other strategic issues that came up at an institute retreat earlier this year—such as how to bring more youthful leaders into the faculty, which is top-heavy with Whitehead founders.

Lindquist is forthcoming, however, when she talks about her work, which includes prions: misfolded proteins suspected of triggering a variety of brain disorders including "mad cow disease." She got into this field, she says, through her graduate research on heat shock proteins (HSPs), a category of agents also called "chaperones" because they guide other proteins within cells and help fold them into the proper shapes.

Lindquist chose HSPs as a thesis topic on her own, because Meselson was "distracted" with weapons-control activities at the time, she recalls. After spending "2 weeks" in the library, she decided to investigate proteins produced by fruit fly larvae in heat stress, the biochemical products associated with heat shock "puffs" visible on fly chromosomes then being studied by a Harvard faculty member, Sarah Elgin.

Now a professor at Washington University in St. Louis, Missouri, Elgin says her own work on chromatin benefited when Lindquist and another member of the Meselson lab, Steven Henikoff, characterized the heat shock proteins and made it possible to identify some of the genes involved. Elgin thinks Lindquist has an "intuition for what's interesting" and is "fearless about going in new directions." Meselson doesn't recall Lindquist's struggle to find a thesis topic, but he does recall her work: "Susan was very careful to make herself familiar with all aspects of what she was doing; she focused on important problems—and she has not changed."

Lindquist moved to the University of Chicago in 1976 and stayed for more than 2 decades, working with yeast to explore heat shock proteins, protein folding, and, later, prions and diseases that might involve protein folding. Among the lab's achievements, she says, was showing that the alternative shapes a prion takes correlate with its biological properties. It was "quite hard" to leave Chicago, she says, because she had lived there "all my life, and most of my family is there." But the lure of the Whitehead—and the rich scientific infrastructure of Cambridge—were too much to resist.

Although Lindquist is reluctant to talk about management decisions, she says she launched a salary review that significantly raised the Whitehead's pay for postdocs. This had "complex" repercussions, she notes, but she adds that she would like to go further in "reducing hierarchy." She has set up a lab of her own at the Whitehead and will investigate links between protein folding and human disease. For the institute, her main goal is to support individual excellence: "I want to ... allow people a broad scope and palette and let them go off in different directions." More specific plans will become clear, according to a spokesperson, after an outside consulting group delivers a "top-to-bottom strategic review," due in September.

—ELIOT MARSHALL

CREDIT: PAUL FETTERS

dig to confirm Wickner's theory and uncover additional prions. Biologists examined [URE3] and [PSI$^+$] for similarities, which they hoped would guide them. One critical feature quickly emerged: Both yeast prions contained unusually long stretches of two amino acids, glutamine and asparagine. Some believe that these boost the chance that proteins will form certain flat structures, called β sheets, which likely enable protein-protein interactions.

Another link turned up in studies by a team including Liebman, Yury Chernoff, now a yeast prion expert at the Georgia Institute of Technology in Atlanta, and Susan Lindquist, a yeast geneticist then at the University of Chicago and now director of the Whitehead Institute at the Massachusetts Institute of Technology. They found that at least one yeast prion, [PSI$^+$], seemed to require the presence of a so-called heat shock protein, HSP104, to maintain its prion structure. Heat shock proteins are molecules that protect a cell from stress and guide protein folding. Since these researchers reported on HSP104 and [PSI$^+$]'s relationship 7 years ago, Lindquist and others have found that heat shock proteins play a role in both formation and inhibition of additional yeast prions (see profile).

Metaphysical questions

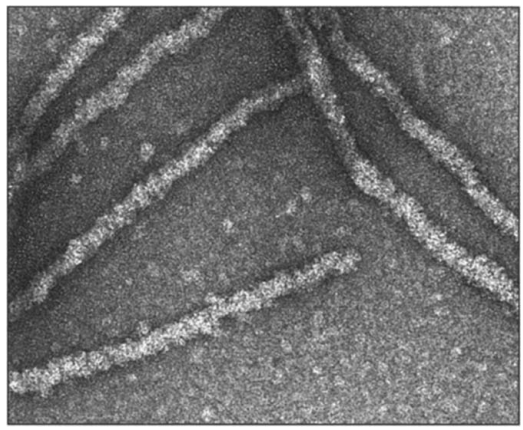
The hunt is on for new prions, and labs are stalking them in different ways. One popular approach involves removing the rich glutamine-asparagine portion of a known prion protein—considered the "active" bit that permits conversion—and replacing it with a chunk of candidate protein that is suspiciously prionlike. Researchers can then test whether the revamped protein still contorts into a prion. Another test is to flood a cell with a suspected prion protein and then determine whether the normal form of the protein loses its standard function.

With these and other tactics, researchers have pinpointed at least four prions in yeast and are debating the properties of several more. They have also found a candidate prion in a species of fungus. Lindquist predicts that an additional 20 yeast prions await discovery, a conviction supported by a paper Liebman published in *Cell* last summer. While screening to find the gene behind a possible prion, Liebman hit on 11 that passed the test. Two were known or suspected of generating prion proteins; the rest all produced proteins rich in glutamine and asparagine and, she concluded, might potentially be responsible for still-undiscovered prions in yeast.

"We know that these sequences [rich in glutamine and asparagine] are preserved and are very prone to forming prions," says Jonathan Weissman, a cell biologist and biochemist at the University of California, San Francisco. "Exactly why they're there is much harder to answer." Such teleological quandaries are the subject of polite disagreement. Lindquist reported in *Nature* in 2000 that almost half the time, yeast cells containing the [PSI$^+$] prion react differently to their environment. In more than a quarter of these cases the effect is positive, such as tolerance of harsh conditions. She hypothesizes that in certain situations the ability to "switch on" prions is beneficial and that these self-perpetuating proteins play a major role in guiding yeast evolution.

But others aren't so sure. Skeptics point out, for example, that although yeast prions are abundant in laboratory strains, they're almost unheard of in the wild. (The strain commonly used in the lab and the source of much prion data, *Saccharomyces cerevisiae*, is itself difficult to unearth in a natural environment.) Mick Tuite, a molecular biologist at the University of Kent at Canterbury, U.K., has tested 15 yeast strains taken from AIDS patients and found the [PSI$^+$] prion in none of them; a couple of strains, though, have yielded another yeast prion, [RNQ$^+$].

A fungal prion, [Het-s], isolated from *Podospora anserina*, has been found to exist naturally, says Sven Saupe, a geneticist at the University of Bordeaux in France. This oddly folded protein appears to inhibit *Podospora* organisms from fusing with one another—a widespread phenomenon among certain fungi. This might help prevent the spread of infection, Saupe says, but it's not clear why a prion would be selected for this role.



Connections. Yeast cells with prions develop amyloid fibrils, as in the brains of Alzheimer's patients.

CREDIT: (TOP) R. WICKNER *ET AL.*

What makes a murderer?

As researchers such as Lindquist and Saupe struggle to outline the role prions might perform in yeast and fungi, they keep running into perhaps the most vexing question of all: Why don't prions such as [URE3] and [Het-s] kill the way [PrP] does? That mystery is bringing yeast and fungi experts together with those who study [PrP]—still the only mammalian prion known—and certain diseases marked by amyloids and misfolded proteins.

Huntington's disease is the target of one such collaboration. Michael Sherman, a biochemist at Boston University School of Medicine, saw yeast as a tool for investigating what makes the culprit protein, called huntingtin, toxic. Huntingtin damages yeast cells, and varying levels of toxicity can easily be measured in this system. To his surprise, the yeast prion [RNQ$^+$] seemed to increase huntingtin's ability to do damage. Sherman teamed up with Chernoff, the Georgia Tech yeast prion expert. The pair determined that converting [RNQ$^+$] to its normal protein shape prevents huntingtin from aggregating and killing the yeast cells. This suggested that huntingtin alone isn't sufficient to launch disease in yeast. And in the 10 June issue of *The Journal of Cell Biology*, the group hints that still-undiscovered prions or prionlike proteins in humans might also be critical to forcing huntingtin to aggregate. "The question is whether there's something similar in mammalian cells," says Sherman. "There are probably many, many

PRIONS: A CAST OF CHARACTERS

Prion	Where it's found	Function of prion	Kills organism?
[PrP]	Mammals	Unknown	Yes
[Het-s]	Fungi	Prevents fusion with another fungus	No
[URE3]	Yeast	Regulates nitrogen metabolism	No
[PSI$^+$]	Yeast	Alters protein synthesis	No
[NU$^+$]	Yeast	Unknown	No
[RNQ$^+$]*	Yeast	Unknown	No

* Also known as [PIN$^+$].

other prion-type proteins ... but we don't know of them."

Lindquist champions the view that prionlike proteins are common in mammals, including humans, but that they might not normally cause disease. "It depends entirely on the kinds of proteins they interact with," she says. It's also possible that some prions are intrinsically more prone to toxicity than others. Despite their potential for harm, Lindquist adds, prions likely extend benefits, too. "It's a wonderful means for very stably transmitting information," she says, referring to the prion's ability to convert proteins in cells around it to the same form. "Once you set up certain states, having structures that tend to be self-perpetuating makes a lot of sense." The logic is finding support in at least one provocative new line of inquiry.

Tantalizing evidence for benign prion-like proteins—they don't match up to true prions—is coming from Nobelist Eric Kandel's lab at Columbia University in New York City. Kandel and lab member Kausik Si are studying a common protein in neurons called CPEB, a section of which resembles parts of prions in yeast. Preliminary evidence suggests that the protein can self-perpetuate in mammalian brains, the pair reported at a National Academy of Sciences meeting in March. Although cautioning that the evidence is extremely preliminary, they speculate that it might play a role in storing information—in other words, in memory.

Lindquist argues that further study of prions—or self-perpetuating proteins, as she likes to call them—will help explain what makes at least one of them harmful. Hazardous and not, she and others believe, many more prions are out there, waiting to be revealed.

–JENNIFER COUZIN

SENSING

Brainstorming Their Way to An Imaging Revolution

In June, a handpicked team of researchers locked themselves away in an R&D hothouse to produce a new detector of elusive terahertz waves. Their prototype is already being tested

OXFORD, U.K.—Terahertz waves penetrate fog, peer through paper and clothes, and look into human tissue, but their useful properties are terra incognita to most because of the huge cost of existing sensors. Last week, however, a team of scientists from across Europe began testing an imaging chip that could open up this long-neglected part of the electromagnetic spectrum to new applications, from medical imaging to satellite observations of Earth. The device itself is intriguing enough, but equally novel is how it's being developed.

The process began in November 1999, when a pair of physical scientists embarked on a breakneck effort to fabricate a new material that can completely block out terahertz waves. This radiation, in the nether region between infrared and radio waves, is hard to detect, but a so-called photonic bandgap material impervious to terahertz waves could revolutionize imaging devices, greatly improving their ability to peer through materials opaque to light of many other wavelengths. Chris Mann of Rutherford Appleton Laboratory (RAL) near Oxford, U.K., and Ramón Gonzalo of the Public University of Navarra in Spain cloistered themselves away in RAL for a month to come up with the goods.

The duo succeeded, producing a prototype terahertz-blocking silicon material. Musing over their accomplishment in a Pamplona bar in May 2000, Mann, Gonzalo, and Peter de Maagt of the European Space Agency (ESA) agreed that this kind of forced, intense teamwork—a mini–Manhattan Project approach—might be just the ticket to take the next, more difficult, step in terahertz imaging. They hatched a plan that night to assemble a crack R&D team to design a terahertz imager that could be deployed in space and elsewhere.

Hear them roar. The Star Tiger team in a rare moment of relaxation with U.K. science minister David Sainsbury, seated third from left.

CREDIT: CCLRC

Two years later, the Star Tiger project, funded by ESA, is yielding its first fruits. A team of 11 researchers from across Europe, under the leadership of de Maagt, Mann, and RAL colleague Geoff McBride, last week began putting a prototype terahertz imager through its paces at an RAL lab. The scientists still face big technical hurdles if they are to reach their goal: production of a much more sophisticated chip by the end of the project in October. But they are zealous converts to the agency's novel multidisciplinary team approach. "If you want to find something innovative, it's the best way," says Luisa Deias, an electronics engineer from Italy and the team's sole female member. "This isn't work," adds British materials scientist James O'Neill. "We're just having fun."

The seed that germinated in the Spanish bar 2 years ago fell on fertile ground at ESA. Back at the agency's technology research center in Noordwijk, the Netherlands, de Maagt mentioned the idea to his superiors. It quickly moved up the hierarchy, and in April 2001, ESA launched a feasibility study into building a terahertz imaging chip. Six months later, Mann and de Maagt got the go-ahead for a $650,000 project.

All objects emit terahertz waves, just as they emit infrared radiation, but terahertz waves are much harder to detect. Existing imaging devices were originally designed by the military to help land aircraft in fog, but they are complex, bulky, and expensive. Chip-sized detectors could be mass-produced and thus could open up new markets. A terahertz imager at an airport, for example, would be able to see through passengers' clothes and reveal hidden weapons, which emit more terahertz waves than the human body does. Every airliner could have a detector in its nose cone, allowing the pilot, on a foggy night, to see the runway. And—the reason ESA got involved in the project—satellites could use them to look down at Earth through cloud cover or up at the stars at this little-studied wavelength.

To make a tiny chip-sized detector practical, you have to ensure that as many of the weak incoming waves as possible make it into the detector rather than leaking into the surroundings or into the chip material itself. The semiconductors that chip substrates are normally made of are a big impediment: