BIOCHEMISTRY

Yeast Protein Acting Alone Triggers Prion-Like Process

The notion that a misshapen protein can transmit disease—a feat long thought to be the exclusive preserve of living pathogens—has moved from the heretical to the mainstream in the past decade. A growing body of circumstantial evidence has convinced many researchers that rogue proteins, called prions, are the villains behind diseases like Creutzfeldt-Jakob disease (CJD) in humans and "mad cow disease" in cattle. Yet nobody has produced a smoking gun: clear proof that a prion protein can wreak havoc without an accomplice.

Researchers studying a similar phenomenon in yeast are getting close to a conviction, however. On page 381, molecular geneticist Michael Ter-Avanesyan and his colleagues at the Institute of Experimental Cardiology in Moscow report that in the test tube, a purified protein can cause changes in other protein molecules, converting them into an insoluble lump of proteins like those seen in affected yeast. The scientists think the twisted molecule acts as a template, inducing others to change shape—the same process by which prions are theorized to cause disease in mammals.

The evidence is still not watertight, but the work is a huge leap forward, says Reed Wickner, a geneticist at the National Institute of Diabetes and Digestive and Kidney Diseases, who first proposed that a prion-like process might explain why two types of yeast, called [*PSI*$^+$] and [URE3], are able to pass on a new trait to their offspring without changing their DNA. Biochemist Byron Caughey, who studies mammalian prions, agrees. "They have done a lot of things that we would hope to do [in mammals]," he says. And, although it's a big leap from the test tube to living cells—and an even bigger one from yeast to mammals—supporters of the prion hypothesis say the growing consensus among yeast researchers strengthens their case.

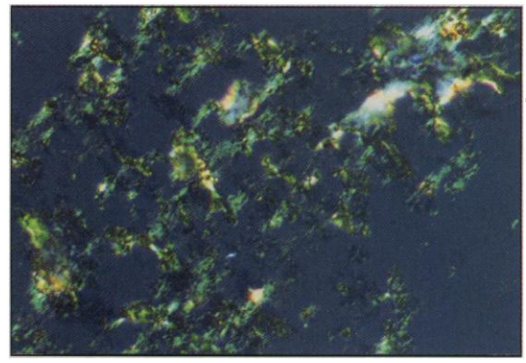
Ter-Avanesyan's work focuses on a protein called Sup35 that normally helps the yeast proofread during the DNA translation process, which converts the DNA code into proteins. In [*PSI*$^+$] strains it is inactive, allowing the yeast to make proteins from certain stretches of DNA that would otherwise be ignored as unintelligible. A year ago, yeast biologist Susan Lindquist and her colleagues at the University of Chicago showed that in living cells, the Sup35 protein is evenly distributed in normal [*psi*$^-$] cells but is clumped together in [*PSI*$^+$] cells. At about the same time, Ter-Avanesyan and his colleagues showed that the clumping inactivates the protein, and that it takes place when Sup35 proteins stick together at one end. That still left a question, however: What caused the clumping?

Ter-Avanesyan and his colleagues set out to show that the [*PSI*$^+$]'s Sup35 protein alone—not a combination of proteins or a more complicated series of events—was the culprit. They combined cell extracts from normal [*psi*$^-$] yeast, in which Sup35 is soluble, with cell extracts from [*PSI*$^+$], in which Sup35 is insoluble, and then spun the mixture in a centrifuge to separate the soluble and insoluble proteins. In as little as 20 minutes, much of the soluble protein had become insoluble, indicating that the protein had clumped together. After 2 hours, no Sup35 was left in the cell solution; it was all in the insoluble pellet.

To eliminate the possibility that, in [*PSI*$^+$] cell extracts, something other than Sup35 triggered the clumping, Ter-Avanesyan and his group purified just the sticky end fragment of the protein and added it to a solution of normal Sup35. The protein duly formed a clump. The researchers then used a piece of the newly formed pellet to convert a fresh solution of normal Sup35. By the third pass, the signature of the original protein fragment had disappeared from protein-detecting blots, suggesting that the newly converted proteins can indeed pass along their new conformation.

These results fit in well with two other recent studies of Sup35. In the 30 May issue of *Cell*, Lindquist and her colleagues showed that Sup35 produced in genetically engineered *Escherichia coli* can spontaneously form long fibrils in the test tube—similar, at first glance, to the fibrils present in the brains of Creutzfeldt-Jakob disease patients and bovine spongiform encephalopathy (BSE)–infected cows. They also showed that adding the fibrils to freshly prepared solutions of the protein caused new fibrils to form much more quickly than in "unseeded" solutions. And in the 24 June *Proceedings of the National Academy of Sciences*, biophysicist Kurt Wüthrich and molecular biologist Chih-Yen King of the Institute for Molecular Biology and Biophysics in Zurich and their colleagues showed that Sup35's sticky end alone also will form long fibrils in the test tube. In addition, they report two lines of evidence that a shape change is responsible for the fibril formation. While the freshly prepared protein solution shows little evidence of specific shape under polarized light, the fibrils look like they might contain β sheets, the same conformation that is suspected in mammalian proteins. In addition, when the researchers stain their fibrils with Congo Red—a stain used to identify characteristic plaques in the brains of CJD patients—they found that, like CJD plaques, the stained aggregates appear yellow-green under polarized light, a characteristic of an ordered pattern in the protein aggregate.

So far, researchers studying mammalian prion proteins haven't been able to conduct the kinds of serial experiments Ter-Avanesyan carried out. One reason is that so much of the abnormal mammalian protein appears to be required to trigger changes in the normal version that it is impossible to separate out just the newly converted protein to see if it can, in turn, convert a fresh batch of normal proteins.



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Signs of order. Yellow-green color under polarized light is characteristic of ordered pattern in yeast fibrils.

But at least one longtime critic of the idea that infectious proteins can cause disease praises the new work in yeast. Yale neuropathologist Laura Manuelidis, who thinks CJD and BSE are caused by an as yet undetected virus, reported in the 4 July issue of *Science* (p. 94) that she and her colleagues were able to induce a BSE-like disease without any evidence of abnormal PrP protein. She says the new work is interesting for its insights into how proteins may be part of the disease process, but cautions that showing that proteins can induce others to change shape in the test tube is very different from showing that they can cause infectious disease.

Even in yeast, researchers acknowledge that the protein-only case is not yet closed. Doubts will remain until someone is able to insert purified protein into a living cell and show that it causes the [*PSI*$^+$] trait. "That's the final nail, but that little hole is still there, and it needs to be filled in," says Lindquist, adding: "There's the same Holy Grail as for the mammalian prion story."

–Gretchen Vogel