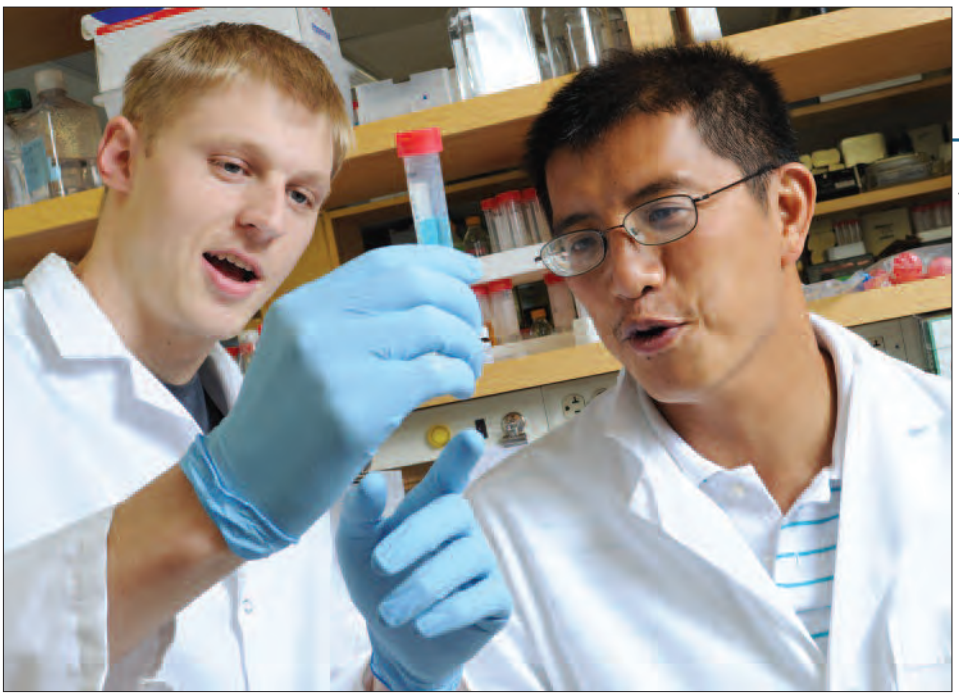

JON GILBERT FOX

Supattapone, right, and Miller, left, were surprised their test tube findings didn't hold up in experiments in mice.

Insight into prions, *in vitro* as well as *in vivo*

When proteins' elaborate shapes are altered, the result can be as dangerous as an infectious brain disorder or as benign as a cooked egg. Proteins are made of long, intricate chains of amino acids folded into many different shapes. Changing their shape, either through denaturation or misfolding, can have dramatic results. Cooking an egg, for example, turns a soupy mess into breakfast.

Scientists have long suggested that several infectious neurodegenerative diseases—such as scrapie in sheep and Creutzfeldt-Jakob in humans—are caused by abnormally folded prions, proteins found in the brain. How a normal prion protein becomes infectious has remained a mystery, however. But the mystery is slowly being unraveled in the lab of Surachai Supattapone, M.D., Ph.D., a professor of biochemistry at DMS.

Shape: To explore how a diseased prion can "infect" a normal prion by passing on its misfolded shape, scientists have been studying prion behavior in test tubes, or *in vitro*, and using those results to predict what happens in a living body. "Most people predicted that if you have an infectious prion in a test tube that it will be able to convert a normal prion protein into its own abnormal shape, and will make all the converted prion protein infectious," says Supattapone. "People in the medical field have been pretty quick to adopt the idea that when this occurs in test tubes it causes disease invariably."

Bind: But that's not the case, as he and Michael Miller, an M.D.-Ph.D. student, discovered. For several years, they have been studying prion binding sites called polybasic domains. Polybasic domains are positively charged parts of normal proteins that scientists previously presumed were necessary for a normal protein to be able to bind to an infectious prion. Miller and Supattapone were curious what would happen if they removed them.

The mutant prions showed diminished infectivity in the mice.

So they engineered three types of prion proteins by removing the polybasic domains from different locations on the normal protein molecules. "Our hypothesis was that deleting these domains would prevent interaction" between the normal prions and the infectious prions, says Miller. They then tested the binding, conversion, and spreading capability of the engineered prion proteins *in vitro*. Lacking polybasic domains, these mutant prions were expected not to be able to bind to the infectious scrapie prions.

Deleting the domains did reduce the interaction between the engineered prions and the infectious prions but didn't prevent it entirely, Miller says. After five days of incubation, one of the engineered prions unexpectedly transformed into a mutant scrapie form. Next, he ran a test that monitors the ability of the newly formed mutant prion to propagate its shape. Indeed, the scrapie prion converted the rest of the neighboring proteins into the abnormal shape, too.

Seed: "Once it was formed it became a very efficient seed," explains Supattapone. "They must have been interacting through some other domain that has not been removed, an alternate form."

Miller repeated the experiment four times. Each time, on the fifth day, he saw the conversion of engineered proteins into the mutant scrapie shape. "In science there are a lot of things you could see once and you may not see it later, again," he says. "When you see it over and over, you start believing that you are observing a real event in nature."

Wondering what would happen *in vivo*, or in a live organism, Supattapone and Miller injected the mutant prions into mice, expecting that all or most of the mice would acquire the disease. But the mutant prions, which had been highly effective in propagating the scrapie shape in test tubes, showed diminished infectivity in the mice.

"It was surprising to us," says Miller. "Not all of the animals got sick." This was the first time that prion findings *in vitro* did not predict infectivity *in vivo*. Both results, which were published in the journal *PLoS Pathogens*, have major implications. They may have opened the door to learning more about the detailed mechanisms of prion misfolding.

Clear: And it's clear that prion scientists will now have to keep in mind the limitations of *in vitro* findings. "You've got to be careful about what you study in the test tube and saying that's the cause of disease in the brain," says Supattapone. NILU NURINOVA