Like a transformer? Protein unfolds and refolds for new function

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New research has shown that a protein does something that scientists once thought impossible: It unfolds itself and refolds into a completely new shape.

This protein, called RfaH, activates genes that allow bacterial cells to launch a successful attack on their host, causing disease. The researchers determined that RfaH starts out in its alpha form, composed of two spiral shapes. Later, in its beta form, it resembles spokes on a wheel and is called a barrel.

When RfaH refolds, it acquires a new function – yet another finding that researchers would not have predicted.

"We showed that RfaH refolds, which is a big enough deal already. You would think this is impossible. That's what you're told in school," said Irina Artsimovitch, professor of microbiology at Ohio State University and a lead author of the study. "But in this case, it's even better than that because we show that when RfaH refolds, it acquires a new function. It can do something that it couldn't do before."

Though the process happens in seconds, Artsimovitch likened the refolding to "having a knitted sweater that you rip out and then knit into a sweater with a different pattern."

The research is published in the July 20, 2012, issue of the journal *Cell*.

The findings have significant implications for studies of gene expression control and protein structure. This remarkable ability to refold suggests that RfaH and similar proteins might be able to bind in ways and to other molecules that had never been considered. Scientists who engineer proteins might have an entirely new step to add to their models. And now that the first case of a complete alpha to beta structural change has been demonstrated, chances are good that researchers will find other proteins that can do the same thing.

In particular, these findings are reminiscent of prion proteins, infectious agents that cause fatal brain diseases in humans and mammals. Prions refold from a harmless alpha into contagious beta forms, which initiate a chain reaction leading to the formation of large prion aggregates, the causative agents of diseases that include Creutzfeldt-Jakob Disease and bovine spongiform encephalopathy in cattle, also known as "mad cow disease."

All proteins fold into their designated shape, determined by DNA sequence, and have one job in a cell. For instance, some proteins, including RfaH, turn on gene expression – and typically that's the end of the story.

At the time of this study, the scientific community considered RfaH a transcription factor – a molecule that binds to specific DNA sequences to control the movement of genetic information from DNA to messenger RNA. Messenger RNA, also known as mRNA, carries those protein-building instructions as the gene expression process continues.

Artsimovitch and colleagues recently determined that RfaH closes a critical gap in the enzyme RNA polymerase, allowing it to maintain its grip on DNA and start the activation of genes. RNA polymerase is responsible for setting gene expression in motion in all cells by wrapping itself around the double helix of DNA, using one strand to match

nucleotides and make a copy of genetic material.

But the researchers observed that when RfaH binds to DNA and RNA polymerase, its two halves separate, with one remaining bound to the enzyme and another holding on with just a string of a few amino acids. And then it refolds into a completely different shape.

This new work shows that refolding allows RfaH to participate in translation, a completely different step in gene expression. During translation, a molecule called a ribosome binds to mRNA and uses its instructions to select amino acids and join them into chains, which then fold to compose proteins, the final products of gene expression.

"You have factors that work on transcription and factors that work on translation. They are typically not the same," Artsimovitch said.

Research had long ago established that RfaH was a transcription factor, and that this function is universally conserved – meaning it is present for this role in all living organisms and has been for generations.

But this new research shows that RfaH is even more effective as a translation factor – about 100 times more powerful than it is as a transcription factor, Artsimovitch said. RfaH has more power during translation because it recruits the ribosome molecule under circumstances when there isn't enough information in the mRNA for the ribosome to do its protein construction.

"Normally the ribosome binds to a specific sequence. If there is no sequence, it doesn't know where to bind. We think that the ribosome recognizes RfaH instead of this sequence and starts translation," Artsimovitch said. "This is what we're going to be studying now, because it is completely unprecedented.

"RfaH can interact with the ribosome once it refolds. It's a really smart way to do it. It's a tiny protein, yet it can bind to both the RNA polymerase and the ribosome at the same time and link them together."

Artsimovitch and colleagues actually proposed several years ago that RfaH refolds because its observed structure was so different from what they expected. However, RfaH sequence could be folded in silico – via computer simulation – into the "correct" shape.

Paul Rösch, a scientist at the Universität Bayreuth in Germany and a lead author on this paper, tested this idea using high-tech nuclear magnetic resonance imaging. His lab found that RfaH can indeed fold into two very different states. The two labs then collaborated as Artsimovitch set out to determine the functional significance of RfaH refolding.

Artsimovitch has been using Escherichia coli as a model system for these studies. In these cells, the RfaH protein is a virulence factor that gives bacteria their ability to infect and cause disease. Though that role is not emphasized in this research, it makes sense that this particular protein would have some special qualities, Artsimovitch explained.

Genes that control bacterial virulence are famous for being designed in a way that makes their translation very inefficient – hence, they need specialized proteins to help their expression. If RfaH didn't refold and enable translation, certain genes would not be expressed, and in the wild, these bacteria could not survive.

The researchers determined that the separation of the protein's domains, or sections, triggers the refolding because they could reproduce that event experimentally. But they haven't yet determined exactly what allows that dissociation to happen, and are designing models in which to further investigate the refolding process.

"There is actually a big question here: How does RfaH know where to begin binding to the message to recruit the ribosome? We can tell that the ribosome knows where to start, but how it knows, we have no idea," Artsimovitch said.

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