Proteins enable essential enzyme to maintain its grip on DNA

July 21 2011, by Emily Caldwell

Scientists have identified a family of proteins that close a critical gap in an enzyme that is essential to all life, allowing the enzyme to maintain its grip on DNA and start the activation of genes.

The enzyme, called RNA polymerase, is responsible for setting gene expression in motion in all cells. RNA polymerase wraps itself around the double helix of DNA, using one strand to match nucleotides and make a copy of genetic material.

RNA polymerase cannot fall off of the DNA or stop this process once it starts. If it does, no proteins will be made, and the cell will die.

A team led by Ohio State University researchers demonstrated in a bacterial model that a specific protein binds to two sides of a space in the RNA polymerase molecule at a critical point in its connection to DNA, effectively closing the gap and creating a clamp around the two strands.

In bacteria, two related proteins perform this function. One is NusG, which is required for bacterial growth. Another is RfaH, a virulence factor that gives bacteria their ability to infect and cause disease. Depending on the gene, either NusG or RfaH bridges the critical gap in RNA polymerase in bacteria to maintain the enzyme's attachment to DNA, the researchers found.

"DNA could be imagined as a cylinder, and RNA polymerase encircles

it," said Irina Artsimovitch, associate professor of microbiology at Ohio State and senior author of the research. "Before, we had a structural model where these proteins sit at a site where RNA polymerase contacts the DNA. But even if you see something binding, you still have to prove this binding has a functional consequence. We show here that RNA polymerase forms two halves of a clamp, and these proteins bind in the middle and make the clamp complete."

Though understanding this mechanism was the main goal of the study, the findings could contribute to research in antibiotic development. With these proteins known to have a critical role in supporting cell life, they could function as targets for drugs designed to either kill bacteria or take away their ability to cause disease.

The research is published in the July 22, 2011, issue of the journal *Molecular Cell*.

RNA polymerase is an unusual enzyme because of its processivity, a quality that both requires and enables it to do its extremely long and complicated job perfectly every time, without pausing or making a mistake. Scientists have known that RNA polymerase is processive, but until now didn't know how it remained so. Because RNA polymerase is universally conserved – meaning it is present and has the same function in all living organisms and has for generations – these findings in bacteria apply to all other forms of life, including humans.

"RNA polymerase has to make very long messages. In humans, RNA chains can be up to 1 million nucleotides long. If RNA polymerase stops prematurely, it loses the RNA chain and has to start over again. To prevent this futile cycle, some factor has to help RNA polymerase to stay bound to the DNA and RNA," Artsimovitch said. "Our major argument is that RNA polymerase can run longer if it makes a ring around the DNA."

Artsimovitch pursued the roles of RfaH and NusG because these proteins, too, are universally conserved, just as the RNA polymerase enzyme is. In other single-celled and also more complex organisms, they have different names than those found in bacteria, but their roles as transcription factors – proteins that control gene expression – are the same. And they are the only family of transcription factors known to be universally conserved.

"It makes sense – if something is universally conserved, it is likely doing something very important," said Artsimovitch, also an investigator in Ohio State's Center for RNA Biology.

She and colleagues conducted a series of genetic and biochemistry experiments in cells and test tubes, respectively, to define the roles of the RfaH and NusG proteins in Escherichia coli, their model system. Their findings helped confirm recent reports from other researchers studying single-celled Archaea organisms suggesting that the structures of these proteins allow them to close the clamp on RNA polymerase and contribute to its processivity.

There is additional context from Artsimovitch's work, however, that determines which protein fills the gap.

"So we know the mechanism by which these proteins work is similar in all organisms, but you can have different scenarios," said Anastasia Sevostyanova, a postdoctoral researcher in microbiology at Ohio State and first author of the study.

In most cases, a bacterial cell needs to turn on genes just so it can continue to grow. In those cases, NusG would close the gap. However, under circumstances when specialized control of genes is in order – such as when bacteria infect their human host – then RfaH, the virulence factor, will fill that gap in the RNA polymerase clamp instead.

The researchers hope to further elucidate how other factors from the same universally conserved family of proteins orchestrate the gene expression programs that control cell life.

Provided by The Ohio State University