Protein plays DNA matchmaker role

UC Davis postdoctoral fellow makes important discovery

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Researchers have directly observed an essential three dimensional DNA damage repair process using a special microscopy technique.

Anthony L. Forget, who now works at MIT in Cambridge, Massachusetts, worked as a postdoctoral fellow with Stephen Kowalczykowski in the department of microbiology and the department of molecular and cellular biology at UC Davis to make the discovery.

Single-molecule microscopy is an exciting technique created by "the merging of two scientific disciplines, physics and biology," Forget said. "A laser beam is manipulated to form molecular tweezers that hold a single piece of DNA in place under a microscope."

Forget and Kowalczykowski used microscopic fluorescent tags to view the cellular machinery one molecule at a time. Forget used the technique to study DNA repair using a key protein from the E. coli bacteria called RecA.

"The E. coli RecA protein is fairly easily purified," said Kendall Knight, Forget's former graduate advisor who is a professor at the University of Massachusetts Medical School. Knight was not involved in the current study.

"It's a well characterized protein that is ideal for these types of single-molecule studies," Knight said.

Certain types of repair processes involve searching segments of DNA in order to find matches between two strands, Knight said . For E. coli, the RecA protein is involved in this type of repair.

Forget, during an important turning point in the study, noticed something unexpected while reviewing the data.

"Some of the single molecules that I usually disregarded looked interesting," Forget said . "I noticed that these DNA molecules actually had nucleoprotein filaments (RecA/damaged DNA complexes) stuck to them. I discovered that the intact template DNA had to be 'relaxed,' not stretched out, for the nucleoprotein filaments to interact with it."

Forget explained that when the target double strand DNA is relaxed, or "curled up," in a three-dimensional way, then the nucleoprotein filaments, made up of single strand DNA and RecA, can contact it in two or more places at a time. This interaction leads to the single strand DNA finding the stretch of matching DNA on the target (template) strand onto which it can align completely to finish the repair process.

The RecA protein uses this three-dimensional search process to ensure that the correct sequences of DNA can come together — a process that is called recombination.

The RecA-protein search process is only one of a number of potential mechanisms used by DNA binding proteins.

"Other DNA binding proteins carry out searches for specific DNA sequences using a hopping mechanism, while others can 'sit down' and slide on the DNA," Knight said .

"Recombination is a very important process for all living creatures," said Ryan Jensen, an assistant professor at Yale University who was not involved in the current study. "It's absolutely essential for life as we know it by repairing lethal DNA damage and generating genetic diversity."

Jensen previously worked as a postdoctoral student in Kowalczykowski's lab at UC Davis with Forget.

Forget expects that the current work with the RecA protein will lead to future studies involving the more complex process in human cells, which involves the Rad51 protein.

“This experimental design could be utilized to probe the activity of Rad51 and its various poorly understood ‘helper’ proteins to see how they contribute to making the process more efficient,” said Forget.

The current study by Forget and Kowalczykowski was published in the scientific journal Nature on Feb. 16, 2012.

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