Scientists Are First To Observe The Global Motions Of An Enzyme Copyinng DNA

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Zucai Suo

(PhysOrg.com) -- Scientists here have identified how the motions of an enzyme are related to correctly copying genetic instructions, setting the stage for studies that can uncover what happens when DNA copying mistakes are made.

Perpetuation of DNA mistakes can cause mutations that lead to cancer and other diseases.

But before scientists can determine how and when errors are made during DNA replication, they must first fully understand what's going on when the copying process works properly - a monumental research

pursuit that remains incomplete.

The Ohio State University researchers are the first to observe real-time behavior of all four sections, or domains, of an enzyme called Dpo4, a model Y-family enzyme.

The research defines critical steps in the process that starts when the enzyme binds to the correct nucleotide and ends when that nucleotide is put in place to become part of the string of molecules that compose a copied strand of DNA.

"Gaining insight into complex DNA transactions is fundamental to understanding the molecular basis of disease," said senior study author Zucai Suo, associate professor of biochemistry and an investigator in the Ohio State University Comprehensive Cancer Center.

In the study, fluorescent markers on the enzyme's sections showed how three of four components of Dpo4 tighten their grip on the DNA to achieve precise alignment while a nucleotide is added. The sections then relax their grip.

By looking at all four pieces of this enzyme in real time, the scientists may already have uncovered one clue about how DNA mistakes are made. The unexpected movement of one of those four pieces - called the little finger domain - in an opposite direction suggests this domain could make room for DNA mistakes to slip through the process.

"By moving away from the DNA, the additional space on the enzyme's active site may accommodate a distorted DNA structure," Suo said. "We are investigating that possibility now."

The research appears online today (10/27) in the journal *PLoS Biology*, published by the Public Library of Science.

Human cells contain enzymes - called polymerases - that perform the process of copying DNA. The double helix of the DNA contains the genetic instructions for building an organism, and when cells in any living being divide, they copy DNA to pass on those instructions to new cells.

During this replication, the two halves of the DNA strand separate, and each half becomes a template for a new partner. The polymerases are responsible for "reading" the templates to determine where to place nucleotides to create the matching partners - resulting in the creation of two identical double helices.

Polymerases are divided among six families of enzymes based on their amino acid sequence and function: A, B, C, D, X and Y (the most recently discovered family).

The A family has been well-studied and takes care of most of the DNA copying. But enzymes in this family stall if they come across DNA damage, and that stalling endangers the replication process and could lead to cell death.

The Y family of polymerases, on the other hand, can step in to bypass damage and allow copying to continue. This keeps the cell alive but also has led scientists to wonder if these enzymes could also be a major source of mistakes.

Polymerases are attractive research subjects because they have already been used as targets in anti-cancer and anti-viral drugs, so fully understanding their behavior could open up avenues for further drug development, noted Jessica Brown, a doctoral student in the Ohio State Biochemistry Program and a co-author of the study.

"In the case of polymerases, you want to know how these dynamics

relate to the correct nucleotide incorporation so you can then determine the dynamics when the wrong molecule is incorporated," Brown said.

The research group used an enzyme called Dpo4, or DNA polymerase IV, for the experiments. Before now, scientists had determined crystal structures of relationships between this enzyme and DNA that represented their relationship frozen at a single point in time.

To see the interactions in real time, Suo and colleagues were the first to insert fluorescent markers into each of the four components of the enzyme - called the palm, thumb, finger and little finger. They also placed markers in a strand of DNA. Previous studies have emphasized just one enzyme domain at a time.

The polymerase interacts with DNA to start the copying process in the presence of a nucleotide - in this case, a molecule called dNTP. The scientists used fluorescence resonance energy transfer technology to see the action take place.

They described the process as a series of eight critical steps that proceed with three distinct movements in the presence of a dNTP. The enzyme stands alone until it binds to DNA. The enzyme and DNA undergo repositioning when the nucleotide binds to the polymerase, and the enzyme's domains begin to move, gripping the template DNA strand. The DNA strand must move from what is known as the pocket to allow binding and incorporation of the nucleotide in the correct place.

By step five in the process, the DNA copy has been extended, and in the following steps the enzyme begins its relaxation motions - allowing the DNA to shift to allow for placement of the next nucleotide.

Those who study DNA polymerases seek to define what is called a rate-limiting event, which could be thought of as a moment when all parts of

the process pause and check themselves over. Because errors occur in the DNA copying process, the rate-limiting step must be known to completely understand the copying mechanism, Suo noted.

To date, scientists have believed that the rate-limiting step in this process occurred long before the placement of the nucleotide. To check this, Suo and colleagues applied mathematical equations to the fluorescently lit observations to assign time frames to each part of the process.

They determined that the rate-limiting step of dNTP placement occurs during a brief and subtle shift of amino acids that ensures correct alignment of all structures just before the nucleotide is placed.

The rate-limiting step also requires the most energy of any step in the dNTP placement process. Suo and colleagues measured energy requirements for the key steps preceding placement of the nucleotide and found that the step requiring the most energy is that same subtle alignment shift.

The Suo lab is now using these findings and the fluorescent marking system to explore the dynamics of replication when DNA is damaged.

Source: The Ohio State University (news : web)