Crucial step in human DNA replication observed for the first time

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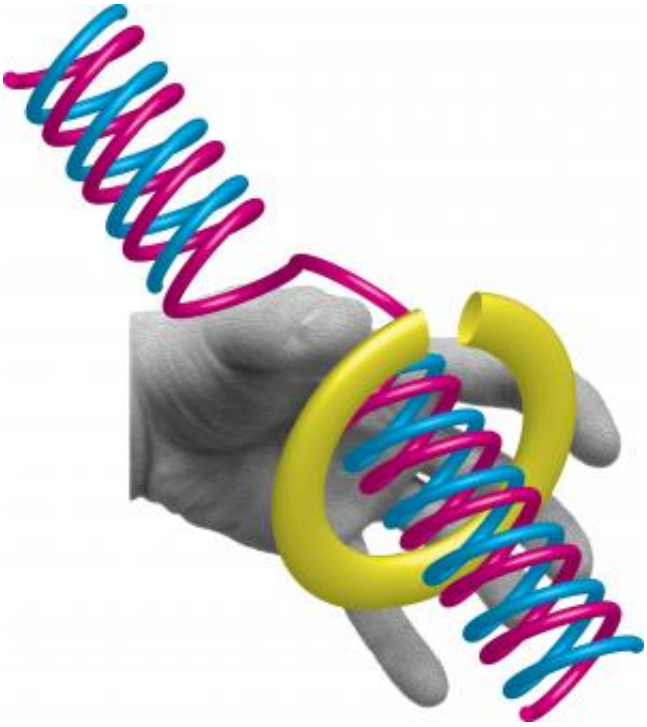

An elusive step in the process of human DNA replication has been observed, for the first time, by scientists at Penn State University in the lab of Stephen J. Benkovic. The step, which is crucial for DNA replication in humans and other forms of life, previously had remained mysterious and had not been well studied in human DNA. For illustrative purposes, this image represents a crucial molecular player in the process, by a hand, which is loading the sliding clamp ring onto DNA. Credit: Benkovic lab, Penn State University

(Phys.org) —For the first time, an elusive step in the process of human

DNA replication has been demystified by scientists at Penn State University. According to senior author Stephen J. Benkovic, an Evan Pugh Professor of Chemistry and Holder of the Eberly Family Chair in Chemistry at Penn State, the scientists "discovered how a key step in human DNA replication is performed." The results of the research will be published in the journal *eLife* on 2 April 2013.

Part of the DNA replication process—in humans and in other life forms—involves loading of molecular structures called sliding clamps onto DNA. This crucial step in DNA replication had remained somewhat mysterious and had not been well studied in human DNA replication. Mark Hedglin, a post-doctoral researcher in Penn State's Department of Chemistry and a member of Benkovic's team, explained that the sliding clamp is a ring-shaped protein that acts to encircle the DNA strand, latching around it like a watch band. The sliding clamp then serves to anchor special enzymes called polymerases to the DNA, ensuring efficient copying of the genetic material. "Without a sliding clamp, polymerases can copy very few bases—the molecular 'letters' that make up the code of DNA—at a time. But the clamp helps the polymerase to stay in place, allowing it to copy thousands of bases before being removed from the strand of DNA," Hedglin said.

Hedglin explained that, due to the closed circular structure of sliding clamps, another necessary step in DNA replication is the presence of a "clamp loader," which acts to latch and unlatch the sliding clamps at key stages during the process. "The big unknown has always been how the sliding clamp and the clamp loader interact and the timing of latching and unlatching of the clamp from the DNA," said Hedglin. "We know that polymerases and clamp loaders can't bind the sliding clamp at the same time, so the hypothesis was that clamp loaders latched sliding clamps onto DNA, then left for some time during DNA replication, returning only to unlatch the clamps after the polymerase left so they could be recycled for further use."

To test this hypothesis, the team of researchers used a method called Förster resonance energy transfer (FRET), a technique of attaching fluorescent "tags" to human proteins and sections of DNA in order to monitor the interactions between them. "With these tags in place, we then observed the formation of holoenzymes—the active form of the polymerase involved in DNA replication, which consists of the polymerase itself along with any accessory factors that optimize its activity," Hedglin said. "We found that whenever a sliding clamp is loaded onto a DNA template in the absence of polymerase, the clamp loader quickly removed the clamp so that free clamps did not build up on the DNA. However, whenever a polymerase was present, it captured the sliding clamp and the clamp loader then dissociated from the DNA strand."

The team members also found that, during the moments when both the clamp loader and the clamp were bound to the DNA, they were not intimately engaged with each other. Rather, the clamp loader released the closed clamp onto the DNA, allowing an opportunity for the polymerase to capture the clamp, completing the assembly of the holoenzyme. Subsequently, the clamp loader dissociated from DNA. "Our research demonstrates that the DNA polymerase holoenzyme in humans consists of only a clamp and a DNA polymerase. The clamp loader is not part of it. It disengages from the DNA after the polymerase binds the clamp," Hedglin added.

Benkovic noted that this mechanism provides a means for the cell to recycle scarce clamps when they are not in use for productive replication.

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