Study suggests enzyme crucial to DNA replication may provide potent anti-cancer drug target

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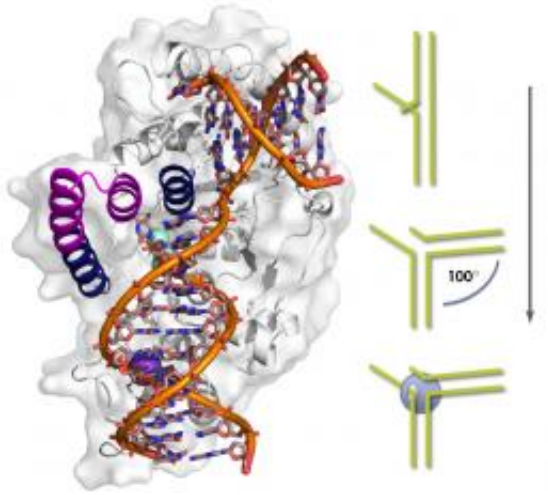

During DNA replication of the lagging strand, numerous Okazaki fragments must be joined. The newer fragment ends in a short flap call the 3' overhang, while the previous fragment leaves a long 5' flap after its primer is removed. The junction opens when the template strand is bent 100 degrees. FEN1 grasps the DNA at the bend, threads the flap through an archway, and trims the flap to match the overhang.

(PhysOrg.com) -- An enzyme essential for DNA replication and repair in humans works in a way that might be exploited as anti-cancer therapy, say researchers at The Scripps Research Institute and Lawrence Berkeley National Laboratory.

The research, published in the April 15, 2011 issue of the journal *Cell*, focused on a member of a group of enzymes called flap endonucleases, which are essential to the life of a cell. The findings show new, clearly defined crystal structures of the enzyme FEN1 in action—demonstrating it functions in a way opposite to accepted dogma.

"This work represents a seminal advance in the understanding of FEN1," said team leader John Tainer, professor and member of the Skaggs Institute for Chemical Biology at Scripps Research and senior scientist at Lawrence Berkeley National Lab. "The research produced very accurate structures showing DNA before and after being cut by FEN1 activity, providing a basis for understanding a whole superfamily of enzymes that must cut specific DNA structures in order for DNA to be replicated and repaired."

This superfamily includes important targets for the development of new cancer interventions, Tainer added. Many cancers show high levels of FEN1 expression, which in some cases is correlated to tumor aggression. For these cases, FEN1-specific inhibitors may have chemotherapeutic potential.

"A better understanding of FEN1 structure and function may have long-term positive benefits to human health," noted co-author Andy Arvai, a scientific associate at Scripps Research.

Working rapidly with exquisite precision

In order for DNA to replicate, it has to unwind its double helix, which is formed out of two strands of amino acids coiled together. This unwinding is done by a replication fork whereby the two strands are separated. These strands, which form two branching prongs of the replication fork, serves as a template for production of a new complementary strand.

That task is fairly straightforward on what is known as the "leading" of the two strands. The replication fork moves along from the so-called 3' (three prime) end to the 5' (five prime) end, and DNA polymerase synthesizes a 5' to 3' complementary strand.

But because the two strands are anti-parallel, meaning they are oriented in opposite directions, the work of DNA polymerase, which can only work in the 5' to 3' direction, is more difficult on the so-called lagging strand. This strand needs to be replicated in pieces, which are known as Okazaki fragments, located near the replication fork. These fragments include a "primer," a strand of RNA that serves as a starting point for DNA synthesis.

This is where FEN1 comes in—it removes that RNA primer on the 5' flap, which occurs every 100 base pairs or so on the lagging strand, said Tainer. It's an enormous job that has to be done rapidly and accurately in order to glue the ends of replicated DNA on the lagging strand together to eventually provide an intact chromosome. "To replicate one DNA double helix in one cell you have to cut off a 5' flap so that you don't have one base pair too many or one base pair too few, and you have to do this accurately with 50 million Okazaki primers in each cell cycle," Tainer said. "It has always been a mystery as to how FEN1 can precisely cut this flap so efficiently and so rapidly. It's an amazing, efficient molecular machine for precisely cutting DNA."

To determine what FEN1 looked like in action, Arvai led the difficult but ultimately successful effort to grow crystals of the human FEN1 protein bound to DNA. The team then used X-ray crystallography to determine the atomic structure of the complex. Using Lawrence Berkeley National Laboratory's Advanced Light Source beamline, called SIBYLS, the scientists solved three different crystal structures.

The end result was a highly detailed and accurate model showing the

structures of DNA before and after being cut by FEN1.

Earlier crystal structures suggested that FEN1 first grabs onto the flap of the 5' single stranded DNA, slides down to the joint where DNA is duplicated, and cuts and patches the primer there. But the new study found that, in fact, FEN1 binds, bends, frays, and then cuts the DNA.

"It binds duplex DNA, bends it into a single-stranded DNA right at the flap, flips out two base pairs, and cuts between them," said Tainer. "This gives FEN1 very precise control—a sophistication we had not expected."

Clues to cancer control

Researchers know that mutations in FEN1 can predispose humans to cancer growth because errors in flap removal can create unstable DNA that promotes cell growth and division. And studies in mice have shown that when one of two inherited FEN1 genes are knocked out, the mice are predisposed to cancer development if their DNA is damaged.

While other DNA repair systems can help compensate for FEN1 mistakes, or for missing FEN1 activity, "you need a lot of FEN1 for DNA repair and replication to work properly," Tainer said.

This suggests that, in tumors already missing one set of repair proteins, selectively inhibiting the function of FEN1 in rapidly replicating cells may prove to be an effective anti-cancer therapy. "The Achilles heel of cancer cells is defective DNA repair pathways," said Tainer, "because that makes them more sensitive to traditional therapies, such as chemotherapy and radiation. If cancer can't repair the damage these therapies do to tumors, they will die."

This is the paradox of DNA repair: while a defect in DNA repair can cause cancer, knocking out a number of backup repair systems may

make tumors vulnerable to anti-cancer therapies.

"My hope is that our finding of how FEN1 works mechanistically might provide a foundation for a next-generation cancer drug," said Tainer. "We need to cut as many lifelines as possible in cancer cells in order to provide an effective treatment."

More information: "Human Flap Endonuclease Structures, DNA Double-Base Flipping, and a Unified Understanding of the FEN1 Superfamily," by Susan E. Tsutakawa et al. *Cell*.

Provided by The Scripps Research Institute