

OCTOBER 27, 2005

DNA Enzyme Shows Unexpected Acrobatic Flair

New experiments by Howard Hughes Medical Institute researchers show that the helicase enzyme, which normally crawls along the length of a DNA strand during its function in replication and other processes, exhibits stunning acrobatic flair when it encounters an obstacle. The studies show that the enzyme snaps back to its original position on the DNA strand so that it can begin the process again.

This unexpected finding invites speculation that the enzyme's shuttling motion—which is repeated multiple times—may serve a useful biological purpose. For example, it might preserve the integrity of DNA by stripping the DNA strand of potentially toxic proteins that accumulate during replication.

Understanding how the helicase enzyme functions could aid in deducing the mechanism underlying genetic diseases caused by abnormal helicase activity, said the study's senior author Taekjip Ha, a Howard Hughes Medical Institute investigator at the University of Illinois Urbana-Champaign.

"It reminded us of the Greek character Sisyphus, who had to get up every morning and push the same boulder up the same hill."

- Taekjip Ha

Ha and Illinois colleagues, Sua Myong, Ivan Rasnik and Chirlmin Joo, collaborated on the studies with Timothy Lohman at Washington University School of Medicine in St. Louis. The studies were published in the October 27, 2005, issue of the journal *Nature*.

Helicases are a critical part of the DNA replication process because they unwind double-stranded DNA to create single strands suitable for copying by the replication machinery. This and other helicase activity in the cell depends on the ability of the helicase's protein "engine" to crawl along the DNA strand. This locomotion is powered by ATP, the cell's ubiquitous energy

source.

In their test tube experiments, Ha and his colleagues used only the engine component of a helicase called Rep from the bacterium *E. coli*. They followed the motion of the Rep engine along the DNA strand by tagging it with a green fluorescent dye. They also attached a molecule of a red fluorescent dye to the destination end of the DNA strand. This technique, called fluorescence resonance energy transfer (FRET), enables researchers to determine how the tagged molecules move relative to one another by observing how one dye molecule transfers energy to the other.

The researchers observed the molecules through a fluorescence microscope, and as Rep approached a blocked end of the DNA strand, they expected to see a gradual reduction in the red signal and an increase in the green signal.

“We saw the gradual FRET increase as we expected, and we knew that the protein was moving in a particular direction that would bring the two dyes close to each other,” said Ha. “We expected that the protein would simply stop at the blockade and fall off the DNA. But we were surprised to see that it somehow showed up back where it began on the DNA. Then it repeated that gradual movement toward the barrier again and repeated the snap-back movement. It was like a shuttle bus going back and forth between two stations, except that the backward movement was instantaneous,” said Ha.

To their surprise, the experiments showed that the Rep protein wasn't letting go of the DNA strand at the end of its journey. Rather, once it reached the blockade, it stretched out to grab the DNA strand near its starting point, formed a loop in the DNA and then released its stopping point to bring it back to its beginning.

“It reminded us of the Greek character Sisyphus, who had to get up every morning and push the same boulder up the same hill,” said Ha. “We wondered what the reason was for all this suffering that Rep was going through. So we did additional experiments, and the results suggested that the repetitive shuttling could prevent the accumulation of unwanted proteins on the single-stranded DNA that could prove toxic to the cell. It's sort of like the reverse of dental floss, in that the Rep protein acts like the teeth that clean the DNA `floss,’” said Ha.

Ha and his colleagues would next like to explore whether the shuttling phenomenon they observed in their test tube studies actually functions in other helicases and in the living cell. Also, he said, there are rare disorders in humans that could possibly involve malfunction of the helicase engine. People with one such disorder, called Werner syndrome, grow normally until they reach adolescence, when they begin to age very rapidly. The rapid aging suggests that helicase may play a role maintaining genomic integrity, said Ha.

Ha noted that Bloom syndrome, another intriguing helicase-related disease, is caused by a defect in a different helicase. “This disorder is especially interesting because people with this defect show an increased propensity for many types of cancer,” said Ha. “Again, the helicase involved in this disorder appears to play a role in the general maintenance of genomic integrity. And if we can understand its regular function and how it is altered when mutated, it may be possible to understand a more general mechanism underlying cancer,” he said.