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New Imaging Tools Show Protein Slip-Sliding Along DNA



Image Title: Single stranded DNA (gray tube) around a single stranded DNA binding protein (SSB, colored ribbons) mimicking a seam on the baseball surface. Scientists found that SSB protein does not sit on the DNA. Instead, SSB rapidly migrates on the DNA, likely utilizing the thermal fraying of ends of the DNA. - Taekjip Ha

The job of single-strand DNA binding protein has always been to protect and preserve. The sticky surface of the protein attaches to single-stranded DNA and stabilizes the molecule during replication and repair. New research shows that the protein, once thought to be stiff and immobile, actually does a lot of slipping and sliding as it stabilizes DNA by wrestling it into place.

The new observations, made possible by new ways of looking at DNA, fit into a growing body of experimental evidence that is providing researchers with a greater appreciation of the dynamic interplay between DNA and the entourage of protein courtiers that shadow and groom the molecule.

In a paper published October 11, 2009, in the journal *Nature*, Howard Hughes Medical Institute investigator Taekjip Ha at the University of Illinois, Urbana-Champaign and his former graduate student, Rahul Roy, presently at Harvard University, collaborated on the studies with Alexander Kozlov and Timothy Lohman at Washington University School of Medicine in St. Louis. The researchers have shown for the first time that single-strand binding protein (SSB) does not stand in one place like a Buckingham Palace guard, but rather scoots along single-stranded DNA (ssDNA). Visualizing this movement is important, Ha says, because it may lead to greater understanding of the machines that repair and replicate DNA, which are intimately linked to cancer and aging.

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"There is increasing evidence that the SSB protein-ssDNA complex, rather than being inert, is a dynamic, perhaps functionally important, unit," Ha says of the findings.

"This work gives us an intimate understanding of how fluid and dynamic these SSB protein-ssDNA interactions actually are," says Eric Greene, an HHMI Early Career Scientist and assistant professor in the Department of Biochemistry and Molecular Biophysics at Columbia University. "It's really rather amazing that this protein slips and slides along DNA, and makes one wonder whether this might be a much more common behavior among DNA binding proteins than previously believed."

The iconic representation of DNA depicts the molecule as a double helical structure in which two strands of genetic material are intertwined. The double helix is the more common and stable form of DNA. But the two strands must occasionally separate – creating two single strands of DNA -- so that enzymes can have access to copy or repair DNA. At these times, SSB steps in to keep the individual strands of DNA apart so these processes can proceed.

In the bacteria *Escherichia coli*, single-stranded DNA attaches to the SSB protein by winding around it like a seam on a baseball. Ha and his colleagues were curious about the relationship between SSB protein and single-stranded DNA. Their studies began by asking the simple question: How much force would it take to pull the single-stranded DNA from the SSB protein?

To find out, they planned to use a new tool that Ha's group developed to measure both tension and fluorescence from individual biological molecules.

To begin their experiments, the scientists synthesized a single strand of DNA that was just long enough to wind around the SSB protein and leave the ends protruding. This extra DNA ensured that the scientists would have something to grab and pull on. They placed a red fluorescent tag on one end of the single-stranded DNA, where it first joined the protein, and green on the other, where it left the protein.

The researchers used the fluorescent tags to measure the proximity of the DNA ends to one another. Ha explained that when the two tags are very close, they can transfer energy to one another and change color. The researchers were surprised to see significant FRET (fluorescence resonance energy transfer) fluctuations between the two tagged ends, suggesting that the single-stranded DNA ends moved in relation both to each other and to the SSB protein. That was the first clue that the SSB protein was not stuck on one spot of single-stranded DNA as had been assumed, prompting Ha's team to shelve the original plan to measure forces and instead began to look into the possibility of the proteins' migration on DNA.

"We were studying how the protein interacts with DNA but had no expectation that the protein would diffuse along the single-stranded DNA," says Ha. Diffusion describes the sliding movement of the protein along DNA. The findings were surprising, but made sense considering the job the protein has to do, he adds. If the SSB protein attaches randomly to the single-stranded DNA, with no ability to adjust its position later -- as scientists once thought -- then there would be many unprotected gaps along the single-stranded DNA. But instead if the protein can diffuse on the DNA, such gaps would be quickly filled.

In order to confirm that the single-stranded DNA was, indeed, moving in relation to the SSB protein, Ha's group next did a technically difficult experiment using three-color FRET. They used green dye to tag the SSB protein; red dye to tag one end of the single-stranded DNA; and purple dye for the other end. When the purple end approaches the green tag and the green tag is excited, the purple tag fluoresces. On the other hand, when the red tag is close to the green tag, which is then excited, the red end fluoresces. The FRET fluctuations on each single-stranded DNA end, tagged with red and purple, are inversely correlated indicating that the SSB protein indeed traveled from one end to the other.

"That's another way of showing that motion is actually going on," explains Ha.

Next the researchers wondered if the SSB protein might do more than just stabilize single strands of DNA. They made a strand of DNA in which part of the molecule was fused together in a hairpin-like structure. Hairpins occur naturally in single-stranded DNA, creating a kink that presents problems during replication and repair. Again using FRET, the researchers showed that SSB protein moves along the strand and melts hairpin structures. In effect, the protein irons out the strand – smoothing the way so that other key

proteins involved in the replication or repair process can continue their work.

Ha said that one such protein, RecA, normally gets stuck if it hits a hairpin in the DNA. "RecA does not know how to extend over this hairpin structure, but SSB protein can dissolve it," says Ha.

Ha hopes in the future to determine whether this same SSB protein diffusion mechanism occurs in human proteins. Two genes that are frequently mutated in breast and ovarian cancer -- *BRCA1* and *BRCA2* -- produce proteins that help with DNA repair requiring single-stranded DNA intermediaries. Ha says that understanding the SSB protein-single-stranded DNA interaction in human proteins might help advance cancer research.

In the longer term, Ha dreams of using multi-color fluorescence and optical tweezers to look at as many as 10 different proteins as they interact with one another in real time.

"That's a dream experiment," he says. "Of course, we start simple."