

APRIL 15, 2005

Joining Hands to Solve a DNA Replication Puzzle

Two heads and three tools are better than one. A Howard Hughes Medical Institute (HHMI) professor and a colleague who mentors HHMI-supported undergraduates in his structural biology lab are using the tools of molecular biology, biochemistry, and biophysics to solve a scientific puzzle.

What has their attention is the mysterious mechanism that enables DNA replication in simian virus 40 (SV40), a mammalian model for that vital process. “We’ve taken what began as a biochemical and molecular genetic approach, then used structural biology to learn about protein interactions, and then returned to biochemistry to validate our structural model in a functional way,” said Ellen Fanning, an HHMI professor at Vanderbilt University in Nashville, Tennessee. HHMI professors are accomplished research scientists who are working to bring the excitement of research to undergraduate teaching.

Fanning and Walter Chazin, director of Vanderbilt’s Center for Structural Biology, report their findings in the April 2005 *Nature Structural & Molecular Biology*, published online March 27, 2005.

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- Ellen Fanning

“Walter and I started working together four years ago,” Fanning explained. “He’s from a very biophysical culture, while I take a molecular biology, biochemistry approach. It required an investment of effort to learn each other’s languages and persuade our labs to communicate.” She said the payoff from those investments has begun. “This paper is the first step in what will become a series of discoveries.”

Fanning’s HHMI professorship and Chazin’s participation in her HHMI-supported undergraduate research program play important roles in their collaboration. “We have about a half dozen undergraduates and a couple

of graduate students and postdocs who are actively involved in projects between the two labs,” Fanning said. “A lot of the mutations and constructs are being made by undergraduate students.” She predicted that each undergraduate will emerge from this collaboration as an author on a published research paper.

In the research reported in *Nature Structural & Molecular Biology*, the scientists sought the mechanism by which single-stranded DNA (ssDNA) breaks free from the chains of its binding protein to allow repair or replication, a process that is not well understood. Fanning and Chazin found structural and biochemical evidence for that mechanism, providing a model of this early step in DNA processing in mammalian cells.

Every organism has an ssDNA-binding protein for DNA replication and repair pathways. In eukaryotes or organisms whose cells have a nucleus, it is called replication protein A (RPA). One of the common functions of RPA in DNA processing pathways is facilitating “hand-off,” a process that ensures that the correct proteins move into place along the ssDNA to begin DNA processing.

RPA plays an important protective role for ssDNA. “You don't want to have naked single-stranded DNA lying around in a cell,” explained Fanning. “It will get tangled, make hairpins within itself, get chewed up by nucleases. Ss binding proteins keep ssDNA straight and accessible to the right processing enzyme.”

RPA binds with at least a dozen different repair and replication proteins. The question has been how RPA gets dislodged, allowing various enzymes access to the DNA for necessary processing. Fanning and Chazin have developed a working model to answer that question.

Using SV40 as a model system, the scientists mapped atomic level interaction on the surfaces of proteins involved in DNA processing. They used biochemical and genetic tools to determine how the interactions of those proteins promote synthesis of small segments of RNA known as primers, which are required for initiation of DNA replication.

In the SV40 system, three key proteins interact. The viral protein T antigen (Tag) interacts with RPA and an enzyme known as DNA polymerase-primase (pol-prim). Tag is a helicase, or DNA unwinding enzyme. After it has unwound the DNA, it also places the pol-prim on the DNA to make primers. The researchers studied this last step: how Tag pulls RPA away sufficiently to load the pol-prim onto the DNA, allowing it to synthesize primers.

Fanning and Chazin showed that interaction between Tag and RPA requires multiple contact points. They found that, along with a domain on RPA called RPA70, a second one, RPA32C, also needs to bind to Tag before processing can begin.

The scientists suggest that Tag associates first with RPA32C and then with RPA70 as the RPA molecule sits on ssDNA. Binding at both of these points alters the conformation of RPA, scrunching it up to expose a small stretch of ssDNA. Tag brings with it pol-prim, which is deposited in the short stretch of unbound ssDNA. Once pol-prim is in place, Tag and RPA are no longer needed, so they are displaced as the third protein begins its work on the ssDNA. This is the “hand-off.”

“This provides a testable model for how the ssDNA binding protein can be displaced from single-stranded DNA to allow a DNA processing pathway,” Fanning said. “This is a general phenomenon that happens throughout all DNA processing pathways.”