

Watching DNA Repair in Real Time

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Direct observations of DNA are giving new insights into how genetic material is copied and repaired.

"We can monitor the process directly, and that gives us a different perspective," said Roberto Galletto, a postdoctoral scholar at UC Davis and first author on a paper published Sept. 20 on the Web site of the journal *Nature*.

In *E. coli* bacteria, molecules of an enzyme called RecA attach themselves along a DNA strand, stretching it out and forming a filament. A piece of complementary DNA lines up along side it, and pieces of DNA can be swapped in to repair gaps in the original strand. A similar protein, called Rad51, does the same job in humans.

"How RecA and Rad51 assemble into filaments determines the outcome of DNA repair, but very little is known about how assembly is controlled," said senior author Stephen Kowalczykowski, professor in the sections of Microbiology and of Molecular and Cellular Biology and director of the Center for Genetics and Development at UC Davis. Genes that control the human gene, Rad51, have been linked to increased risk of breast cancer.

Galletto attached a short piece of DNA to a tiny latex bead and placed it in a flow chamber, held by laser beam "tweezers." Fluid flowing past made the DNA stream out like a banner. Then he nudged it into an adjacent channel containing fluorescently-tagged RecA. After short intervals of time, he moved it back to the first chamber to observe the results.

By repeatedly dipping the same piece of DNA into the fluorescent channel, the researchers could see the RecA form clusters of four to five molecules on the DNA. Once those clusters had formed, the DNA/RecA filament rapidly grew in both directions. The measurements made in those experiments will be the baseline for future studies of both RecA and Rad51, Kowalczykowski said.

The new work adapts an approach developed by Kowalczykowski and Ronald J. Baskin, professor of molecular and cellular biology, to study single enzymes at work unwinding DNA strands. That research was first published in *Nature* in 2001.

In addition to Galletto, Kowalczykowski and Baskin, the research team included

postdoctoral scholar Ichiro Amitani. The work was funded by the National Institutes of Health and a fellowship awarded to Galletto by the Jeane B. Kempner Foundation.

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