Recombination protein dynamics observed with single monomer resolution

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Using a sensitive, single-molecule measurement technique, researchers at the University of Illinois at Urbana-Champaign have observed the life cycle of RecA, a protein that plays a major role in repairing damaged DNA.

The protein forms a filament, which grows and shrinks primarily by one monomer at a time, the researchers report in the August issue of the journal *Cell*.

RecA is a DNA recombination protein found in the gut bacterium E. coli. A human homolog, called Rad51, interacts with many proteins, including BRCA2, whose mutation increases susceptibility to breast and ovarian cancers. A better understanding of how these proteins function could help our understanding of cancer.

"Our measurement technique provides a way of counting the number of individual monomers bound to DNA in real time," said Taekjip Ha, a professor of physics at Illinois and a Howard Hughes Medical Institute investigator. "With that, we can determine the kinetic rates for reactions occurring at either end of the protein filament."

During the recombination process, RecA binds with DNA to form a filament that spirals around the DNA. The filament can grow in either direction, and can advance on the DNA by growing at the leading end and dissociating at the trailing end.

To study the dynamics of RecA, the researchers used a highly sensitive single-molecule fluorescence resonance energy transfer (FRET) technique that Ha and colleagues developed.

To use FRET, researchers first attach two dye molecules – one green and one red – to the molecule they want to study. Next, they excite the green dye with a laser. Some of the energy moves from the green dye to the red dye, depending upon the distance between them.

The researchers then measure the brightness of the two dyes simultaneously. The changing ratio of the two intensities indicates the relative movement of the two dyes, and therefore the motion of the molecule or its change in size.

The technique revealed intricate details of how RecA nucleates to form a filament, how the filament changes shape, and how the filament removes proteins from DNA.

"Contrary to our initial expectations, both ends of the RecA filament continually grow and shrink, but a higher binding rate at one end causes the filament to grow primarily in one direction," Ha said. "We also learned that as the filament grows and shrinks, it does so by one protein unit at a time."

Following recombination proteins step by step could further help researchers determine in what ways cancer-causing proteins are defective, and perhaps find ways to correct them.

Source: University of Illinois at Urbana-Champaign