Watching a single enzyme function

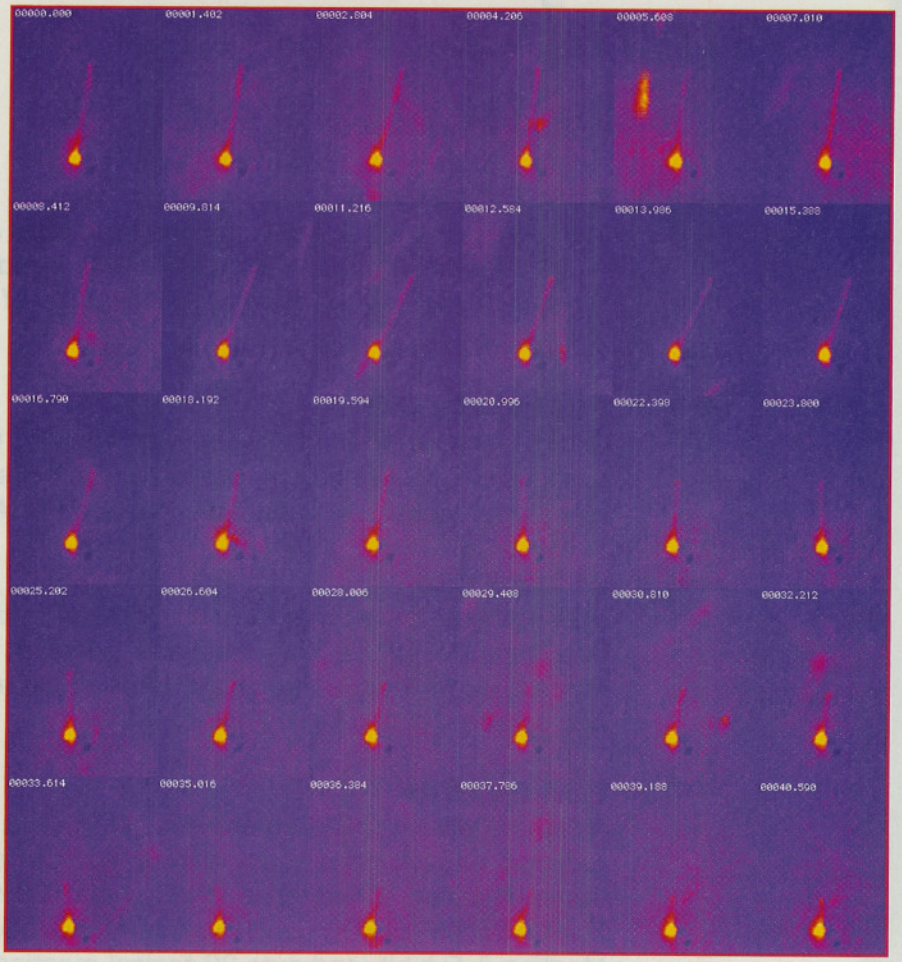
A wide variety of enzymes operate on DNA. Some assist in replication, some with transcription and others effect repairs on damaged DNA. Because our DNA contains the information about how our bodies are built, any understanding we gain about how these enzymes work adds insight into the processes that drive the healthy operation of our bodies. In particular, if we can better understand the mechanisms of DNA repair, we may be able to enhance the efficiency of the process and perhaps even extend our lives.

One enzyme involved in DNA repair is the RecBCD. Recent studies have been done on quantifying the operation of single RecBCD enzymes by measuring their effect on individual double-stranded DNA molecules.

RecBCD unwinds DNA and separates the double strands. In work reported in the Jan. 18 issue of *Nature*, professor Stephen Kowalczykowski and colleagues at the University of California in Davis and at Lawrence Livermore National Laboratory in Livermore, Calif., studied the operation of the enzyme by labeling double-stranded DNA with the YOYO-1 fluorescent marker.

YOYO-1 binds to base pairs along the strand, providing a uniform fluorescent signal along the length of the double-stranded DNA. Because it binds to the base pairs, and not to individual nucleic acids, it will separate from DNA when the two strands are dissociated. The marker's quantum efficiency drops by three orders of magnitude when separated from DNA, so it provides a unique indication of the state of a DNA molecule. YOYO-1 fluorescence signals an intact double strand; if no fluorescence is visible, no double-stranded DNA is present. But the problem remains: How is it possible to look at a single enzyme operating on a single DNA molecule?

Kowalczykowski's team solved this problem by turning to another photonic tool: optical tweezers. It introduced streptavidin-coated 1-µm polystyrene beads to a solution of biotin-labeled DNA. The biotin bound to the streptavidin, attaching the beads to the ends of the DNA. It added the RecBCD enzyme, which bound to the free end of the DNA but which remained inactive as long as no ATP was present.



The RecBCD enzyme dissociates with two strands of DNA as it begins the repair process. YOYO-1 fluorescent labels along the length of the DNA show the shortening of the double strand as the process progresses.

The DNA then flowed through a micromachined Y-shaped fluid channel placed in an epifluorescence microscope. An Nd:YLF 1.047-µm laser from Spectra-Physics of Mountain View, Calif., focused through a 1.3-NA microscope objective from Nikon Inc. in Melville, N.Y., provided the optical trap. The other channel of the flow cell contained a solution of adjustable concentrations of ATP. Where the two branches of the "Y" come together, the two solutions flowed next to each other with smooth laminar flow. One side of the 4-mm-wide channel carried the prepared DNA in an ATP-free solution, while the other carried ATP with no DNA. To initiate action of the RecBCD enzyme, the scientists trapped a bead and translated the microscope stage, moving the DNA to the ATP solution side. In the presence of ATP, the enzyme began to operate.

With ATP present, the enzyme unwound the DNA, dissociating the YOYO-1 and nullifying its ability to fluoresce. The observations show a decrease in the length of the fluorescent signal corresponding to the operation of the RecBCD. The decrease in measured length, for a given ATP concentration and a specific

RecBCD enzyme, was linear and constant. Within the 33-ms exposure window and the 3000-base-pair spatial resolution, there were no measured pauses.

For given concentrations of ATP, the average RecBCD enzyme used ATP at the same rates as previously determined with bulk ensemble measurements. But within a given population, the rate at which the enzyme used ATP differed anywhere from 1.4 to five times.

"It is simply impossible to study the instantaneous behavior of complex systems unless one can observe the behavior of an individual molecule, or assembly, in real time," Kowalczykowski said. "It is possible, with the single-molecule experiments, to determine if a property of a molecule is continuous in time, or whether it is discontinuous — jerky, pausing or episodic. The detailed dynamic behavior can be watched directly: In principle, the formation of transient intermediates and their lifetimes can be imaged and studied. This is difficult or impossible in bulk measurements where randomly behaving ensembles are being studied."

He noted the importance of understanding the enzyme's operation: "The RecBCD protein is an enzyme involved in DNA repair and its recombination. It participates in the essential task of maintaining chromosome integrity. Hence, understanding how it works in conjunction with its partner proteins will help us prevent chromosomal abnormalities, and perhaps even allow us to fix them in a nano-bioengineering sort of way."

Beyond the important information about the specific task of the RecBCD enzyme, the experiment provided more general knowledge about molecular motors. "Perhaps one day we can harness the work performed by these and other nanomotors to perform related or bioengineered 'nano-tasks,' " he said.

The group is studying other varied classes of motor proteins operating on DNA and will eventually study multiprotein interactions using multiwavelength labeling. The prospect of studying multiple protein interactions intrigues Kowalczykowski, but he also appreciates what the researchers have already accomplished. "Never did I expect, in my lifetime, to 'watch' a single enzyme — especially one that we've studied for about 15 years — function before my eyes in real time. 'Seeing' something that you've only imagined in your head for many years is most gratifying and exciting." □

Richard Gaughan

Molecular beacons detect seven targets in one shot

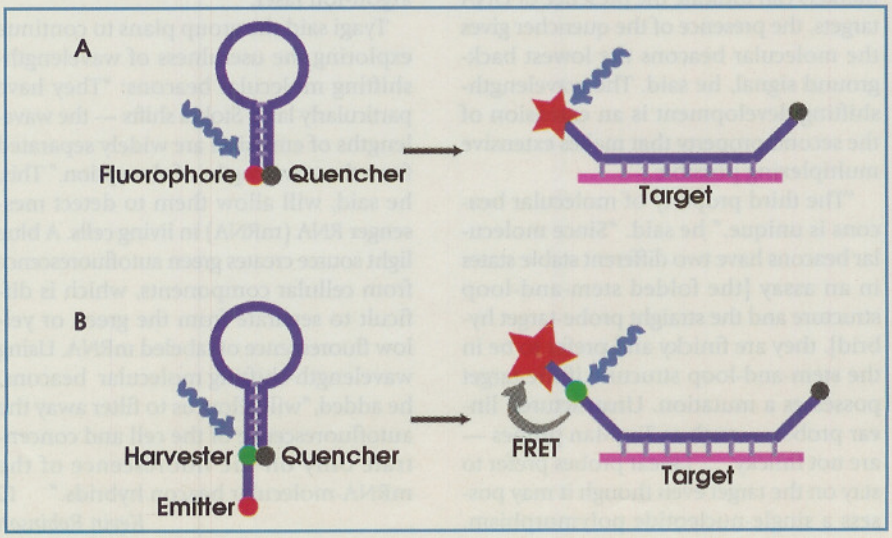
Automated DNA analysis systems typically use a blue laser or a series of blue LEDs to excite fluorescent markers that distinguish different DNA sequences. One problem with this type of system is that the number of fluorescent markers that can be used in a single test is limited by the blue light source.

Now researchers at the Public Health Research Institute in New York have developed highly selective fluorescent molecular beacons that absorb blue excitation light and fluoresce at orange or red wavelengths.

When a molecular beacon is placed in a solution, it begins to glow as soon as it finds its target, said Sanjay Tyagi, lead author of a paper discussing the development in the November issue of *Nature Biotechnology*. "Molecular beacons that are specific to different targets and can emit fluorescence of different colors can be used together to accomplish multiplex detection of many different pathogens or genetic markers in the same test tube," he said.

Using them with only blue excitation light is another task altogether. "We developed wavelength-shifting molecular beacons so that the same instruments could be used to perform multiplex DNA analyses," he said.

Fred Russell Kramer, the principal investigator on the project, explained it further: "Although blue light efficiently stimulates the fluorescence of fluorophores that emit in the green and yellow wavelengths, [it] is less efficient at stimulating fluorescence ... in the orange, red and near-infrared wavelengths. Thus, multiplexing is limited by the decreasing efficiency of stimulating fluorescent emission as redder fluorophores are used. Wavelength-shifting molecular beacons



When not bound to a target, the molecular beacon structure places the quencher next to the harvester fluorophore, suppressing fluorescence. When bound to a target, the molecular beacon spreads out, placing the quencher so far away from the harvester fluorophore that the fluorescent energy transfers to the emitter fluorophore.