

## Nano-Motion Pictures Scientists are now able to track the movements of single proteins as they shuttle along a DNA strand.

SWIFTLY, SMOOTHLY, A DOUBLE-HELIX OF DNA UNWINDS AND parts, exposing two complementary strands. Somehow each of these strands, without getting a molecule out of place, gathers the nucleotide building blocks it needs to become whole again. One double-helix thus becomes two; a cell divides; and life goes on.

HHMI investigator Taekjip Ha would love to see how this fundamental molecular act unfolds in real time. He disarmingly calls his goal a “pie in the sky dream,” since there are no microscopic techniques that can produce moving images at such fine resolution. But he has just taken a big step toward realizing his dream, with a landmark study that reveals the detailed motions of a key protein involved in DNA replication.

In the study, Ha and his colleagues at the Urbana–Champaign campus of the University of Illinois used a relatively new molecular imaging technique known as fluorescence resonance energy transfer, or FRET. This obscure quirk of quantum physics occurs when a certain kind of light-absorbing molecule invisibly donates its absorbed energy to another that lies close by. The efficiency of this energy transfer depends precisely on the distance between the donor and acceptor molecules; if both are fluorescing, then by measuring their respective luminosities one can determine the tiny distance between them with great accuracy—even as it changes rapidly.

Physicists had known about FRET for 50 years when, in 1996, a young graduate student at the University of California, Berkeley, first used it to track the distance between donor and acceptor fluorescent tags on a single biological molecule, a short stretch of DNA. That student was Ha, and since then he and his associates have developed the ability to deploy multiple fluorescent tags to track the motions of even complex molecular shapes.

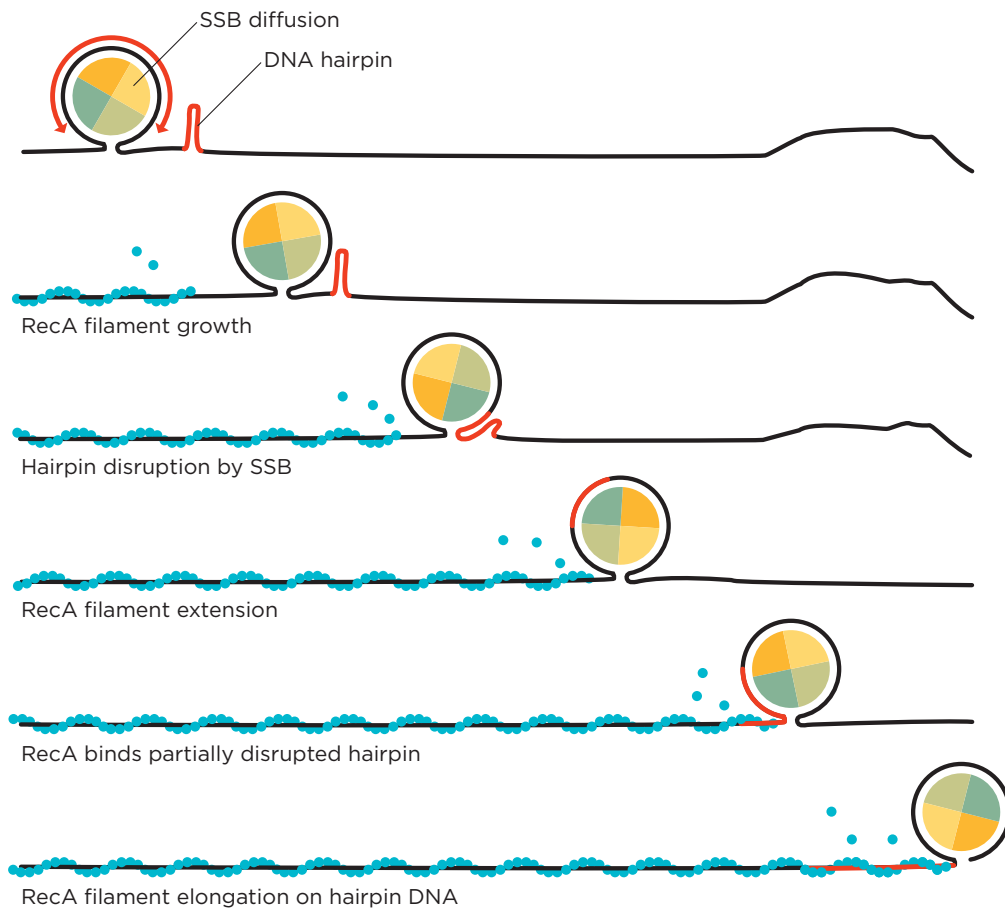
In work led by then biophysics graduate student Rahul Roy (now a postdoc at Harvard University), published in *Nature* on

October 22, 2009, Ha’s team used FRET to investigate single-stranded DNA binding (SSB) protein, a key player in DNA replication. Whenever DNA unwinds and separates itself into two single strands of nucleotides, each of these strands swiftly wraps around SSB proteins. It had been thought that SSB proteins help to protect these naked DNA single strands from the ravages of enzymes and oxidants and that they might also coordinate the work of other repair and maintenance proteins. “But the binding of SSB proteins to DNA is so tight,” says Ha. “We wondered how they are removed when they need to be.”

Using FRET with two fluorescent-dye tags, plus a more advanced tri-dye version that tagged three points, Ha’s group was able to “watch” an SSB protein as it was wrapped by a single DNA strand. To their surprise, the protein shuttled back and forth, fixing small strand defects, known as hairpins, as it went. No one had ever observed a protein moving like that on single-stranded DNA.

The team then added another DNA repair and maintenance protein, RecA, which is known to bind along the length of single-stranded DNA, somehow displacing SSB. The resulting FRET data strongly suggested that as RecA extended along the single-stranded DNA, it prodded SSB and turned its “random walk” into a one-way move, at about three nucleotide base pairs per step. SSB’s removal of the hairpins in turn allowed RecA to continue extending itself.

In follow-up work, Ha and his team brought out another tool, a nano-sized “tweezer,” based on the phenomenon of optical trapping, in which a beam of bright light effectively sucks a tiny object toward its center. Using a laser, they optically trapped a microsphere that was already linked to FRET-tagged DNA; the DNA’s other end was bound to a molecular anchor. One of Ha’s grad students, Ruobo Zhou, used the technique to gradually pull the single-stranded DNA away from SSB while recording the applied tension as well as FRET signals. The SSB



Taekjip Ha's group watched a single-stranded DNA binding (SSB) protein as it fixed small defects (red DNA hairpin) on single-stranded DNA during replication. They used FRET to measure energy transfer among multiple fluorescent tags and found that a second protein, RecA, appears to prod SSB to keep moving in one direction to do its repair work and then move off the DNA at the right time.

protein still seemed able to move back and forth, even when partly unwrapped.

To Ha, these findings indicate that repair and maintenance proteins can move along a single-stranded nucleotide structure much more easily and robustly than had been thought. RecA's apparent prodding of SSB so that it keeps moving in a single direction also suggests to him "a general mechanism for displacement of SSB."

Ha and his lab group are trying to refine their measurement techniques to be able to track dimensions smaller than a single DNA nucleotide. "That's really the ultimate resolution you can ask for," he says. But much of Ha's time these days is also taken up with the job of systematizing and disseminating these new and still somewhat arcane research tools. "Eventually I want these techniques to be used by every biologist." ■

—JIM SCHNABEL