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For RNA polymerase, it's one base at a time

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Scientists have used an optical trap to track the movement of RNA polymerase (RNAP), showing that the enzyme appears to shift along DNA one base pair at a time. Although many experts had expected this conclusion, the report, appearing in this week's *Nature*, reports the motion at a scale ten times finer than previous work, allowing the researchers to directly resolve the individual steps. This angstrom-scale resolution could shed light on less-understood aspects of gene transcription and its regulation, as well as on the tiny motions of other enzymes as they perform their chemical tasks.

The researchers, led by Steven Block of Stanford University, Ca., improved the stability of their optical trap so that it could resolve single-base-pair steps of *E. coli* RNAP in solution. In this way they were able to recognize and eliminate occasional pauses and backtracking, isolating the "bare" elongation rate. They confirmed that the progressive step-by-step movement of RNAP occurs because binding of a new ribonucleoside triphosphate (NTP) unit locks in forward movements as the enzyme jiggles back and forth along the DNA, in what is called a 'brownian ratchet.'

"This paper does the best job so far of providing evidence for the brownian ratchet mechanism," said Rui Sousa of the University of Texas Health Center in San Antonio, one of the people who proposed the mechanism in 1997. Sousa, who was not involved the study, called the new experiment a "real technical tour de force."

"This is the most precise measurement made on a single protein in an aqueous buffer," Block told *The Scientist*. To achieve this stability, the team operated a two-trap apparatus in helium gas to reduce laser deflections due to air motion. One trap held a polystyrene bead attached to the growing RNA strand. The other held a bead attached to the double-stranded DNA being transcribed. The researchers either assisted or retarded the RNAP motion using a new technique to position one bead near the edge of the trap to apply a constant force, avoiding slow and complicated external feedback.

Even so, there was significant variation in the bead position with time. Block emphasized that this noise is not added by the instrument or the bead, but reflects the normal motion of the molecules. "Any transcription that takes place in an organism…is taking place in an intrinsically noisy environment in which all the components … are jiggling around like crazy." Still, the researchers could see the RNAP move in steps that averaged 3.7± 0.6Å during elongation, consistent with the distance between single DNA base pairs. Nonetheless, Block said that other phases of the transcription process -- for example, its initiation -- may well involve a more complicated process in which the enzyme moves a larger distance.

To model the bare elongation rate as they varied the force and NTP concentration, the researchers included a second NTP binding site into the brownian ratchet model, as suggested by recent structural analyses. Future research may further investigate the pausing, backtracking, and editing that ensure accurate transcription, as well as details of how DNA sequence modifies the elongation rate. In addition, Block said that in principle the instrument could now resolve the mechanical motions that accompany the chemical activity of many other enzymes.

Thomas Steitz of Yale University in New Haven, Connecticut, said the paper "looks like it's correct" for *E. coli* RNAP, which is homologous to the primary nuclear RNAP in all branches of life. But Steitz,

who was not involved in the study, cautioned that the simpler RNAP from T7 bacteriophage, may harness chemical energy directly for motion, despite results from recent optical trap experiments that support the brownian ratchet model.

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