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Proteins Transform DNA into "Molecular Velcro"

Proteins critical for compacting DNA in preparation for cell division actually interact with the double helix to fashion it into a kind of molecular Velcro, researchers have discovered.

The proteins, called condensins, are important for a variety of housekeeping processes in chromosomes, but the mechanics behind their function have been largely unknown. When the researchers alternately stretched and compressed a single molecule of DNA with condensins attached, they found that the DNA extended in stepwise clicks, akin to Velcro unzipping.

The successful manipulation of a single DNA molecule with condensin proteins attached makes it plausible to think about using a similar strategy to explore the machinery that processes chromosomes in the cell, said one of the study's senior authors, Carlos Bustamante, a Howard Hughes Medical Institute researcher at the University of California, Berkeley.

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- Carlos J. Bustamante

Bustamante, Ryan B. Case, Nicholas R. Cozzarelli and their colleagues at Berkeley published their findings on June 3, 2004, in *Science Express*, which provides rapid electronic publication of selected articles from the journal *Science*.

Until now, little was known about the function of condensins, said Bustamante. It was known that if the gene for the protein was knocked out, chromosomes failed to segregate properly in cell division. One daughter cell might receive all the DNA and the other none.

Bustamante and his colleagues took note of earlier studies by another group of researchers that provided evidence that condensins appeared to induce

supercoiling in DNA, which occurs when two helical molecules intertwine.

We decided to try to develop a single-molecule assay, to see whether we could really understand the mechanism of this protein's effects on DNA, said Bustamante. Even though there was no bulk assay for this protein's activity, we thought that maybe we would get lucky and observe some activity at a single-molecule level.

The researchers worked with a type of condensin found in the bacterium *E. coli*. Their experimental procedure consisted of attaching one end of a DNA molecule to a tiny plastic bead held by suction onto a micropipette. They then caused the DNA molecule to extend by flowing liquid past it, and exposed it to a solution containing the bacterial condensin protein. The researchers next added the energy-containing molecule ATP to the solution. After the ATP was added, they captured the other end of the condensin-treated DNA molecule with another plastic bead and proceeded to pull on the DNA with precisely measured force.

We found that the DNA molecule had become much shorter in the presence of the condensin protein, said Bustamante. And when we began to pull it apart carefully, we saw it extend in a sawtooth pattern of force, like the click-click-click of Velcro unzipping.

When we pulled again a second time, much to our surprise, the process reproduced identically every tooth in the sawtooth pattern. We had never seen anything like that. We really thought that we were only seeing noise in the stretching of the DNA, but instead we were seeing a perfect registry in the sawtooth pattern, Bustamante said.

That perfect reproducibility strongly suggested to Bustamante and his colleagues that they were seeing a condensed structure with a well defined organization. Every time we pulled it out and relaxed it, the molecule was able to return to the same initial or condensed form, said Bustamante. In fact, the researchers pulled and relaxed the same DNA molecule dozens of times, seeing the same sawtooth pattern of extension and condensation each time.

They also found that the energy-containing ATP molecule appeared to play a regulatory role, rather than providing energy for the condensation reaction. When the researchers removed all excess ATP from the solution, they found that the condensin proteins continued to function. That finding was a big surprise, because we expected the protein to be more like a motor that had to burn ATP every time it condensed, said Bustamante. Also, when they removed the excess protein from the solution, the bound protein was able to recondense the DNA when the tension on the DNA was lowered.

The researchers' analyses led them to propose a model of how the string of condensin proteins interacts to condense the DNA molecule. They theorize that the heads of the condensin proteins attach themselves sequentially and

tightly to DNA. By attaching in this fashion, each protein cooperates with its neighbor, binding itself reversibly to the head of the next protein, thereby scrunching the DNA bit by bit into its condensed state. And when the researchers experimentally stretched the DNA molecule, the condensin heads popped apart sequentially, producing the sawtooth force extension pattern. But the heads remained bound to the DNA, so that when the force is lowered they can go back to their closed state and recondense the DNA molecule.

According to Bustamante, these studies of the bacterial condensin molecule will open the way to future studies of similar proteins that manipulate DNA and maintain chromosomal structure. The actual mechanism by which these molecules actually carry out their function is unknown, he said. And so, we are very excited that we have been able to develop an assay that, for the first time, gives us an understanding of how these molecules may be acting at the molecular level.