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Crash-Test Reveals DNA Traffic Control

Enzymes that copy DNA don't travel on a lonely highway, but instead ply their trade on crowded interstates. Now, Howard Hughes Medical Institute researchers have discovered that when those DNA-copying machines run head-on into oncoming traffic, they kick the obstacles out of their way.

A detailed view of DNA replication showing both strands of the DNA double helix acting as templates for the new DNA strands.

Video: HHMI Biointeractive

The finding, reported in the January 29, 2010, issue of *Science*, reveals new details about the "rules of the road" that help ensure that cells make accurate copies of their genetic material – essential for producing healthy new cells.

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- Michael E. O'Donnell

In preparation for cell division, cells rely on complex protein machines to pull apart and untwist opposing strands of DNA. Once the double helix is untwisted, both strands are copied to produce two complete sets of the genome. The replisome is a protein complex that moves at high speed for long distances on DNA, wrenching the helix apart as it goes. The replisome shares its tracks with other proteins that transcribe DNA into messenger RNA, which is then used to produce proteins. Sometimes these convoys move in opposite directions – and collisions are unavoidable. HHMI investigator Michael O'Donnell of Rockefeller University wondered what happens to the machines when they collide.

To find out, O'Donnell and his colleague Richard Pomerantz reconstructed a cellular traffic accident in a test tube. To do that, they first had to assemble the replisome on DNA in a test tube, an endeavor that required years of effort in O'Donnell's lab. Once his group had successfully reconstructed a replisome from the relatively simple bacteria *E. coli*, they were ready to begin their experiments.

They set an RNA polymerase—the enzyme that transcribes DNA into RNA—in motion on a piece of DNA and then stalled it. Next, they assembled the components that make up the DNA replication machine at the opposite end of the DNA and nudged this complex into action. Then they analyzed the aftermath of the resulting collision.

They found that the DNA replication machine managed to copy the full length of the DNA molecule, indicating that it had traveled the full distance and somehow got past the RNA polymerase. Further analysis suggested that the DNA replication machine stops when it encounters the RNA polymerase, shoves the RNA polymerase off the DNA, and then proceeds.

Researchers knew that a protein called Mfd kicks RNA polymerase off DNA when it has stalled out because a section of DNA is damaged. In the current experiment, RNA polymerase was stalled by another means. But O'Donnell and Pomerantz wondered whether Mfd would help the DNA replication machinery move through RNA polymerase faster even in this case.

O'Donnell and Pomerantz repeated the crash test, this time including Mfd in the mix. With Mfd present, the replisome made even more full-length copies of the DNA than it did without Mfd, suggesting that Mfd helps give RNA polymerase the boot.

Scientists have reported conflicting observations about whether replisomes fall apart when they hit a road block, and O'Donnell's results provide additional evidence that the replisome is hearty. “The replisome is very stable,” says O'Donnell. “It just sits there until it finally wins.”

It makes sense biologically to give the replisome priority, he adds. “Losing an RNA transcript is no big deal. But the consequences would be dire if the replisome fell apart every time it met an RNA polymerase. These collisions are probably common in the cell, so keeping the replisome moving ensures that DNA replication proceeds neatly and rapidly.”

O'Donnell is now searching for other factors that push the replisome through blocks. He'd also like to know whether the replisome in eukaryotic cells, such as yeast or mammalian cells, behaves similarly to the bacterial complex he and Pomerantz have studied. The replication machinery in those kinds of cells is much more complicated, and his group is still working on recreating the mammalian replisome in a test tube.