Scientists crash test DNA's replication machinery

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(PhysOrg.com) -- Important molecular machines routinely crash into one another while plying their trades on DNA. New research shows that the enzymes that copy DNA before cell division, called replisomes, are the kings of this road, kicking aside machines that are performing less critical tasks, such as transcribing instructions for proteins.

Enzymes that travel along DNA to copy or transcribe it — the crucial processes underlying cell replication and protein production — aren't coordinated by a central dispatcher. In fact, they often collide. Now, Rockefeller University researchers have discovered that when DNA-copying machines run head-on into proteins performing less critical tasks, they kick the obstacles aside and continue on their way. The finding, reported in the January 29 issue of *Science*, reveals new details about the "rules of the road" that help cells make accurate copies of their genetic material — essential for producing healthy offspring.

In preparation for cell division, cells rely on complex protein machines called replisomes to untwist and tease apart the double helix of DNA. As the two strands separate, the replisome copies the strands, producing two complete sets of the genome. The replisome moves at high speed for long distances on DNA, but it runs along the same path as the RNA polymerases that transcribe DNA into messenger RNA, the genes' instructions for manufacturing proteins. Sometimes these convoys move in opposite directions and collisions are unavoidable.

To find out what happens when they collide, Michael O'Donnell, head of

Rockefeller's Laboratory of DNA Replication and a Howard Hughes Medical Institute investigator, and his colleague Richard Pomerantz reconstructed a cellular traffic accident in a test tube. They developed a system that allowed them to assemble the replisome from the relatively simple bacteria Escherichia coli at one end of a DNA strand — a years-long endeavor in O'Donnell's lab — and then set it on a collision course with a stalled RNA polymerase from the opposite direction. The scientists found that the DNA replication machine managed to copy the full length of the DNA molecule, indicating that it had traveled the entire distance, despite the obstacle. Further analysis of the collision suggested that the replisome stops when it encounters the RNA polymerase, shoves the RNA polymerase off the DNA and then proceeds.

The scientists also reran the experiments adding a transcription repair protein called Mfd, which is known to help eliminate transcription machinery that has stalled at a damaged section of DNA. The replisome made even more full-length copies of the DNA when Mfd was present, suggesting the protein helps give RNA polymerase the boot in their experimental system as well.

A deficiency of transcription-repair coupling proteins such as Mfd causes the rare congenital recessive disorder called Cockayne syndrome, a disease that is marked by a small head and stature and accelerated aging. The experiments illustrate a new role for Mfd enabling the replisome to move past an RNA polymerase block and effectively copy DNA, which could have implications for understanding the disorder, O'Donnell says.

In addition, the research provides more evidence that the replisome is sturdy and does not fall apart when it hits a road block, as some experiments had suggested. "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.”

The recent experiments continue a line of research O’Donnell and Pomerantz first reported in Nature in 2008, which used the same experimental set-up to study what happens when the replisome rear-ends a stalled RNA polymerase rather than strikes it head on. The replisome moves along DNA at a brisk clip, about 15 to 30 times faster than RNA polymerase, and the rear-ending actually happens more frequently in nature than the head-to-head encounter, O’Donnell says. In the earlier work, the researchers found that the replisome displaced RNA polymerase but used the messenger RNA to continue leading strand synthesis.

“This discovery may explain the decades-old dilemma between work from the 1970s that observed discontinuous synthesis on the leading and lagging strands, and the current semidiscontinuous model in the textbooks based on studies of replisome mechanisms outside the context of a living cell, that is, without concurrent transcription,” O’Donnell says.

O’Donnell is now searching for factors other than Mfd 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.

More information: *Science* 327: 590-592 (January 29, 2010) Direct restart of a replication fork stalled by a head-on RNA polymerase . Richard T. Pomerantz and Mike O’Donnell

Provided by Rockefeller University