Video imaging of single molecule DNA replication

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Almost all life on Earth is based on DNA being copied, or replicated. Now for the first time scientists have been able to watch the replication of a single DNA molecule, with some surprising findings. For one thing, there's a lot more randomness at work than has been thought.

"It's a different way of thinking about replication that raises new

questions," said Stephen Kowalczykowski, distinguished professor in microbiology and molecular genetics at the University of California, Davis. The work is published June 15 in the journal *Cell* with coauthors James Graham, postdoctoral researcher at UC Davis and Kenneth Marians, Sloan Kettering Cancer Center.

Using sophisticated imaging technology and a great deal of patience, the researchers were able to watch DNA from *E. coli* bacteria as it replicated and measure how fast enzyme machinery worked on the different strands.

DNA Replication Basics

The DNA double helix is made from two strands that run in opposite directions. Each strand is made of a series of bases, A, T, C and G, that pair up between the strands: A to T and C to G.

The first step in replication is an enzyme called helicase that unwinds and "unzips" the double helix into two single strands. An enzyme called primase attaches a "primer" to each strand that allows replication to start, then another enzyme called DNA polymerase attaches at the primer and moves along the strand adding new "letters" to form a new double helix.

Because the two strands in the double helix run in opposite directions, the polymerases work differently on the two strands. On one strand - the "leading strand" - the polymerase can move continuously, leaving a trail of new double-stranded DNA behind it.

But on the other, "lagging strand," the polymerase has to move in starts, attaching, producing a short stretch of double stranded DNA then dropping off and starting again. Conventional wisdom is that the polymerases on the leading and lagging strands are somehow coordinated so that one does not get ahead of the other.

The Experiment: Rolling Circles and Fluorescent Dye

To carry out their experiment, the researchers used a circular piece of DNA, attached to a glass slide by a short tail. As the replication machinery rolls around the circle, the tail gets longer. They could switch replication on or off by adding or removing chemical fuel (adenosine triphosphate, ATP) and used a fluorescent dye that attaches to double-stranded DNA to light up the growing strands. Finally, the whole set up is in a flow chamber, so the DNA strands stretch out like banners in the breeze.

Stops, Starts and Variable Speeds

Once Graham, Kowalczykowski and Marians started watching individual DNA strands, they noticed something unexpected. Replication stops unpredictably, and when it starts up again can change speed.

"The speed can vary about ten-fold," Kowalczykowski said.

Sometimes the lagging strand synthesis stops, but the leading strand continues to grow. This shows up as a dark area in the glowing strand, because the dye doesn't stick to single-stranded DNA.

"We've shown that there is no coordination between the strands. They are completely autonomous," Kowalczykowski said.

What looks like coordination is actually the outcome of a random process of starting, stopping and variable speeds. Over time, any one strand will move at an average speed; look at a number of strands at the same time, and they will have the same average speed.

Kowalczykowski likened it to traffic on a freeway.

"Sometimes the traffic in the lane one over is moving faster and passing you, and then you pass it. But if you travel far enough you get to the same place at the same time."

The researchers also found a kind of "dead man's handle" or automatic brake on helicase, which unzips DNA ahead of the rest of the enzymes. When polymerase stops, helicase can keep moving, potentially opening up a gap of unwound DNA that could be vulnerable to damage. In fact, exposed single-strand DNA sets off an alarm signal inside the cell that activates repair enzymes.

But it turns out that when it gets uncoupled and starts to run away from the rest of the replication complex, helicase slows down about five-fold. So it can chug along until the rest of the enzymes catch up then speed up again.

This new stochastic approach is a new way of thinking about DNA replication and other biochemical processes, Kowalczykowski said. "It's a real paradigm shift, and undermines a great deal of what's in the textbooks," he said.

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