Forks colliding: How DNA breaks during re-replication

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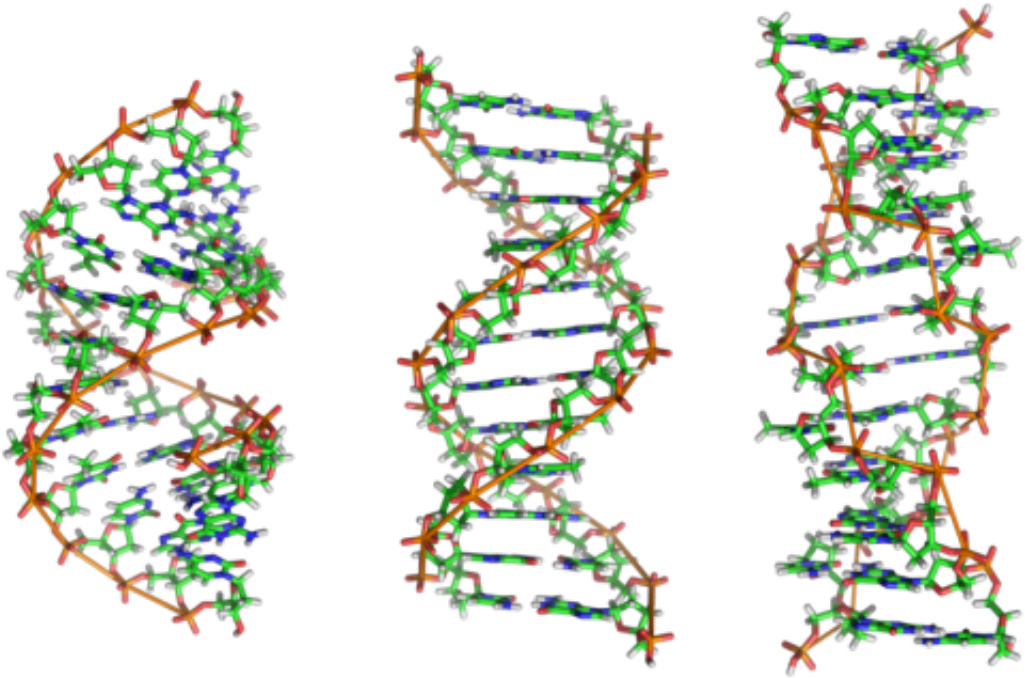

From left to right, the structures of A-, B- and Z-DNA. Credit: Wikipedia

Leveraging a novel system designed to examine the double-strand DNA breaks that occur as a consequence of gene amplification during DNA replication, Whitehead Institute scientists are bringing new clarity to the causes of such genomic damage. Moreover, because errors arising during DNA replication and gene amplification result in chromosomal abnormalities often found in malignant cells, these new findings may bolster our understandings of certain drivers of cancer progression.

At the core of system, developed in the lab of Whitehead Member Terry Orr-Weaver, are Drosophila ovarian follicle cells—a unique cell type whose DNA replication is tightly regulated during organismal development. The controlled nature of replication in these cells enabled Orr-Weaver and her lab to manipulate aspects of the process to discover the sites of double-strand breaks (DSBs), their likely cause, and the mechanisms by which the cells attempt repair. The system is described in the latest edition of *Current Biology*.

"We know that improper regulation of replication can lead to changes in copy number, and the backdrop is that there are changes in DNA copy number in cancer cells," says Orr-Weaver, who is also an American Cancer Society Research Professor of biology at the Massachusetts Institute of Technology. "We know that unregulated replication leads to genome instability, but nobody was able to really look at that in a direct way."

When successful, DNA replication transmits genetic material from mother to daughter cells (as occurs during mitotic cell division) or boosts DNA copy number in the cells of tissues that rely on multiple copies of the genome to increase in size. In the cells studied here, the process of re-replication (also referred to as amplification) originates at six specific sites known as DAFCs (for Drosophila Amplicons in Follicle Cells). Two-pronged structures known as replication forks form at the DAFCs and move along the double-stranded DNA, unwinding it to create two single strands for copying.

Although scientists had surmised that DSBs occur when replication forks—whose movement Orr-Weaver likens to a train moving along a DNA railroad track—collide with each other, none had shown it. Until know. Using a combination of imaging and sequencing technologies, Orr-Weaver and her lab found that DSBs were occurring at the sites of replication forks that had stalled, almost certainly because of collision.

"Because of the resolution of this system, we're now able to know where the forks are in relation to where the breaks are happening," says Jessica Alexander, a graduate student in the Orr-Weaver lab and first author of the *Current Biology* paper.

Alexander and Orr-Weaver also found that the DSBs must be repaired to maintain fork progression. The way in which the repair occurs, however, proved somewhat surprising. DNA breaks are mended in one of two ways: the first, known as non-homologous end-joining (NHEJ) is rapid but prone to mistakes; the second, homologous recombination (HR), takes longer but is much more accurate. Given the importance of faithful replication, one might presume that HR would be the chosen repair method. By altering various checkpoint mechanisms in DNA repair pathways, the researchers found that the cells instead rely on quick-and-dirty NHEJ to fix the DSBs and keep the re-replication forks moving. Speed, it seems is of the essence.

"Non-homologous end-joining is about 14 times faster than homologous recombination," notes Alexander. "The follicle cells don't care about using a mutagenic repair pathway, though, because they slough off the Drosophila oocyte at the end of amplification. Any mutations that arise won't affect the organism."

With this new system in hand, Orr-Weaver and her lab can begin to isolate the individual components involved in the repair pathways, resolve their precise roles, and perhaps determine whether other genomic locations are involved in the process.

"This really provides us with the fundamental knowledge to move forward, looking mechanistically at what the cell uses to repair the breaks," she says. "It's possible that where this happens in the genome could affect the way the DNA gets repaired."

More information: "Replication fork progression during re-replication requires the DNA damage checkpoint and double-strand break repair" *Current Biology*, June 4, 2015

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