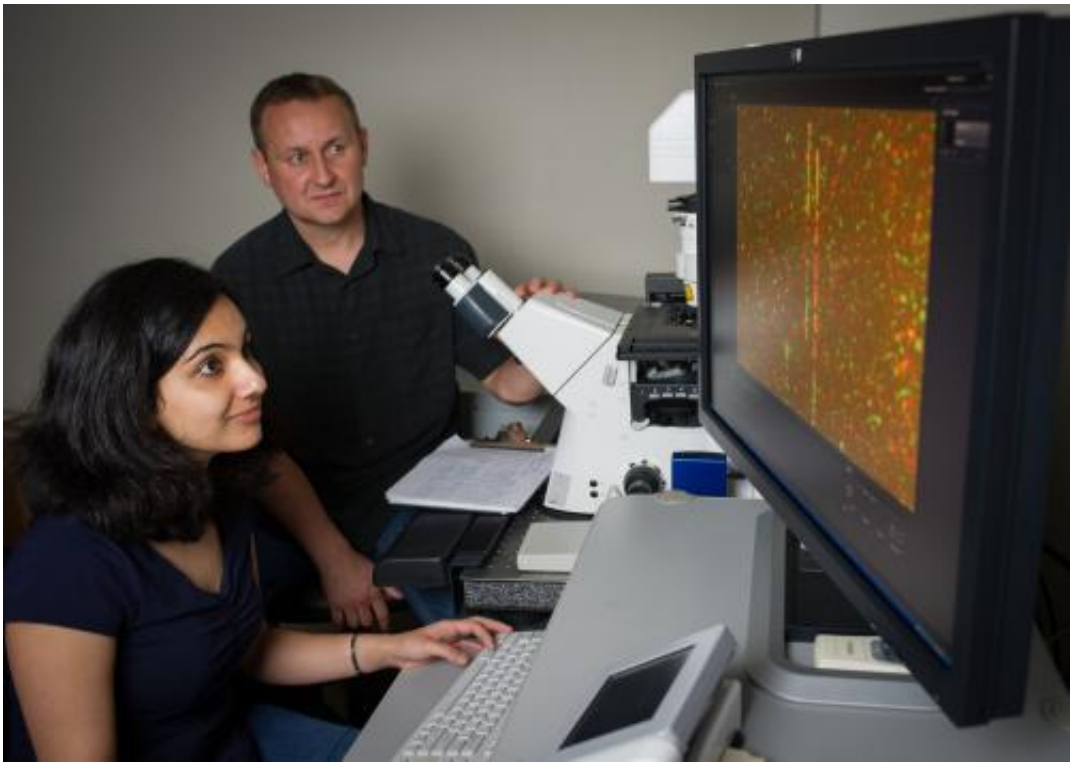
Unusual mechanism of DNA synthesis could explain genetic mutations

September 11 2013



Georgia Tech associate professor Kirill Lobachev and graduate student Natalie Saini review data from a DNA combing experiment at the Microscopy and Biophotonics Core Facility in the Parker H. Petit Institute for Bioengineering and Bioscience. Credit: Rob Felt

Researchers have discovered the details of how cells repair breaks in both strands of DNA, a potentially devastating kind of DNA damage.

When chromosomes experience double-strand breaks due to oxidation, ionizing radiation, replication errors and certain metabolic products, cells utilize their genetically similar chromosomes to patch the gaps via a mechanism that involves both ends of the broken molecules. To repair a broken chromosome that lost one end, a unique configuration of the DNA replication machinery is deployed as a desperation strategy to allow cells to survive, the researchers discovered.

The collaborative work of graduate students working under Anna Malkova, associate professor of biology at Indiana University-Purdue University Indianapolis (IUPUI) and Kirill Lobachev, associate professor of biology at the Georgia Institute of Technology, was critical in the advancement of the project. The group's research was scheduled to be published Sept. 11 in the online edition of the journal *Nature*, with two graduate students, Sreejith Ramakrishnan of IUPUI, and Natalie Saini of Georgia Tech, as first authors. Other collaborators include James Haber of Brandeis University and Grzegorz Ira of the Baylor College of Medicine.

"Previously we have shown that the rate of mutations introduced by break-induced replication is 1,000 times higher as compared to the normal way that DNA is made naturally, but we never understood why," Malkova said.

Lobachev's lab used cutting-edge analysis techniques and equipment available at only a handful of labs around the world. This allowed the researchers to see inside yeast cells and freeze the break-induced DNA repair process at different times. They found that this mode of DNA repair doesn't rely on the traditional replication fork—a Y-shaped region of a replicating DNA molecule—but instead uses a bubble-like structure to synthesize long stretches of missing DNA. This bubble structure copies DNA in a manner not seen before in eukaryotic cells.

Traditional DNA synthesis, performed during the S-phase of the cell cycle, is done in semi-conservative manner as shown by Matthew Meselson and Franklin Stahl in 1958 shortly after the discovery of the DNA structure. They found that two new double helices of DNA are produced from a single DNA double helix, with each new double helix containing one original strand of DNA and one new strand.

"We demonstrated that break-induced replication differs from S-phase DNA replication as it is carried out by a migrating bubble instead of a normal replication fork and leads to conservative DNA synthesis promoting highly increased mutagenesis," Malkova said.

This desperation replication triggers "bursts of genetic instability" and could be a contributing factor in tumor formation.

"From the point of view of the cell, the whole idea is to survive, and this is a way for them to survive a potentially lethal event, but it comes at a cost," Lobachev said. "Potentially, it's a textbook discovery."

During break-induced replication, one broken end of DNA is paired with an identical DNA sequence on its partner chromosome. Replication that proceeds in an unusual bubble-like mode then copies hundreds of kilobases of DNA from the donor DNA through the telomere at the ends of chromosomes.

"Surprisingly, this is a way of synthesizing DNA in a very robust manner," Saini said. "The synthesis can take place and cover the whole arm of the chromosome, so it's not just some short patches of synthesis."

The bubble-like mode of DNA replication can operate in non-dividing cells, which is the state of most of the body's cells, making this kind of replication a potential route for cancer formation.

"Importantly, the break-induced replication bubble has a long tail of single-stranded DNA, which promotes mutations," Ramakrishnan said.

The single-stranded tail might be responsible for the high mutation-rate because it can accumulate mutations by escaping the other repair mechanisms that quickly detect and correct errors in DNA synthesis.

"When it comes to cancer, other diseases and even evolution, what seems to be happening are bursts of instability, and the mechanisms promoting such bursts were unclear," Malkova said.

The molecular mechanism of break-induced replication unraveled by the new study provides one explanation for the generation of mutations.

More information: N. Saini, et al., "Migrating bubble during break-induced replication drives conservative DNA synthesis," *Nature*, 2013. dx.doi.org/10.1038/nature12584

Provided by Georgia Institute of Technology