Cells can read damaged DNA without missing a beat

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Scientists have shown that cells' DNA-reading machinery can skim through certain kinds of damaged DNA without skipping any letters in the genetic "text." The studies, performed in bacteria, suggest a new mechanism that can allow bacteria to develop resistance to antibiotics.

The results were published online this week in the Proceedings of the National Academy of Sciences. The senior author is Paul Doetsch, PhD, professor of biochemistry and radiation oncology at Emory University School of Medicine and associate director for basic research at Winship Cancer Institute of Emory University.

Working with Doetsch, graduate student Cheryl Clauson examined the ability of RNA polymerase (the enzyme that transcribes, or makes RNA from DNA) to handle damaged DNA templates.

RNA polymerase reads one strand of the double helix and assembles RNA that is complementary to that strand. In test tube experiments, when the enzyme comes to a gap or a blank space, it keeps reading but leaves out letters across from the damaged stretch. In contrast, in cells, RNA polymerase puts a random letter (preferring A) across from the gap.

"We were surprised to find that the transcription machinery rolls right over the damaged portion," Doetsch says. "This shows that if the cell initiates, but doesn't complete repair, it still can lead to mutagenesis."

Clauson says a challenge in planning her experiments was finding a way to sensitively detect when RNA polymerase reads through DNA damage.

She loaded damaged DNA into a gene that encodes an enzyme from fireflies, which generates light-emitting chemicals, and then introduced that gene into bacteria. A full working enzyme is produced only if RNA polymerase bypasses the DNA damage without skipping any letters.

DNA in every type of cell, whether bacterial, plant or animal, is constantly being damaged by heat, oxygen and radiation. In addition, all cells make RNA from some of their genes to produce proteins and carry out their normal functions. Cells periodically copy their DNA before dividing, but only if conditions are right for them to grow.

The experiments were performed in bacteria with mutations disabling some forms of DNA repair, Clauson says.

"This situation may resemble one where something like radiation or a mutagenic chemical has overwhelmed the normal repair mechanisms," she says.

In addition, Clauson used an antibiotic called novobiocin to shut down DNA replication in the bacteria. She says this simulates a more challenging environment when cells are not growing quickly.

"Our ability to see transcriptional mutagenesis in growth-limiting conditions is important," Doetsch says. "Out in the environment, bacteria are not constantly surrounded by the rich mix of nutrients we give them in the lab."

"Because this work hints at a simple mechanism by which bacteria could escape from growth-restricted environments, it has important implications for how pathogenic microorganisms may acquire resistance

to antibiotics," he adds. The next phase of these studies for Doetsch and colleagues will be to test whether transcriptional mutagenesis can lead directly to antibiotic resistance in bacteria and other microorganisms.

More information: C.L. Clauson, K.J. Oestreich, J.W. Austin and P.W. Doetsch. Abasic sites and strand breaks in DNA cause transcriptional mutagenesis in Escherichia coli. PNAS Early Edition (2010)

Provided by Emory University