Biologists unravel full sequence of DNA repair mechanism

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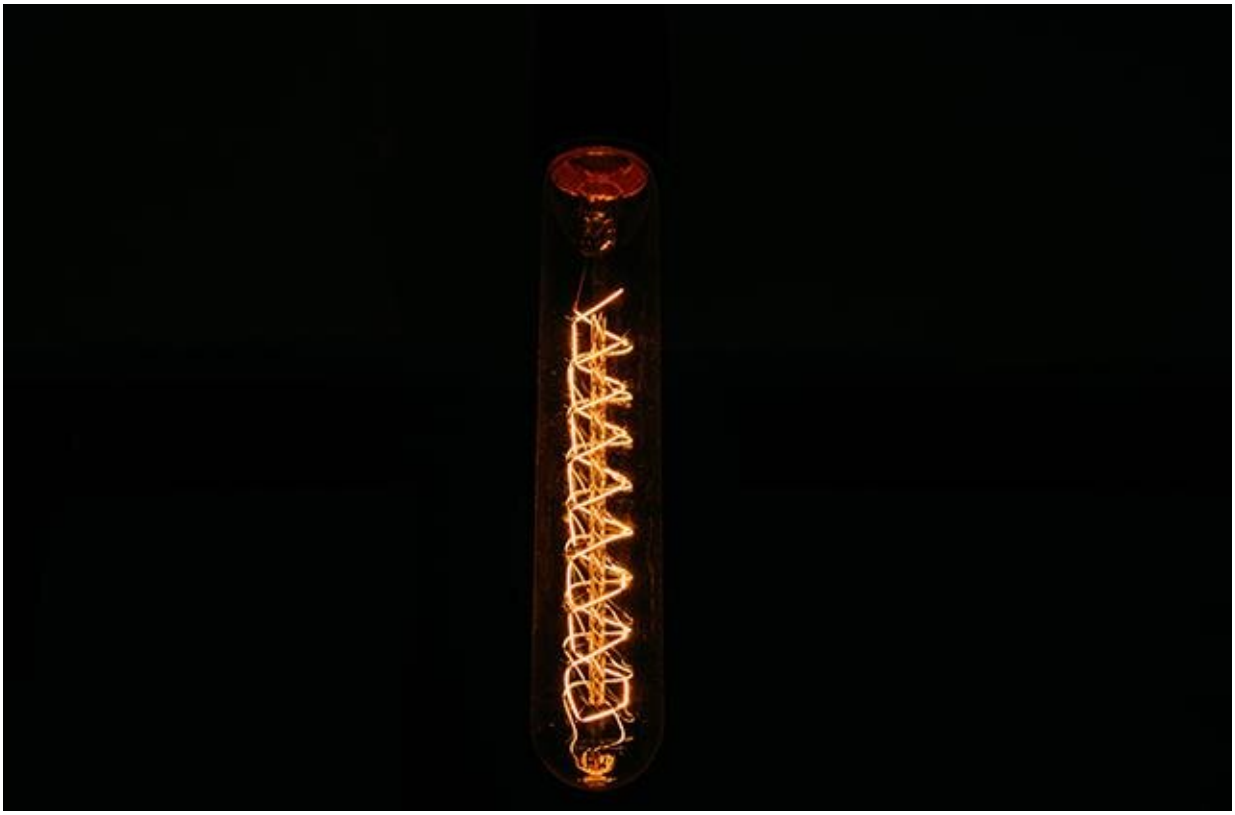

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Every living organism has DNA, and every living organism engages in DNA replication, the process by which DNA makes an exact copy of itself during cell division. While it's a tried-and-true process, problems can arise.

Break-induced replication (BIR) is a way to solve those problems. In humans, it is employed chiefly to repair breaks in DNA that cannot be fixed otherwise. Yet BIR itself, through its repairs to DNA and how it conducts those repairs, can introduce or cause genomic rearrangements and mutations contributing to cancer development.

"It's kind of a double-edged sword," says Anna Malkova, professor in the Department of Biology at the University of Iowa, who has studied BIR since 1995. "The basic ability to repair is a good thing, and some DNA breaks can't be repaired by other methods. So, the idea is very good. But the outcomes can be bad."

A new study led by Malkova, published Jan. 20 in the journal *Nature*, seeks to tease out BIR's high risk-reward arrangement by describing for the first time the beginning-to-end sequence in BIR. The biologists developed a new technique that enabled them to study in a yeast model how BIR operates throughout its repair cycle. Until now, scientists had only been able to study BIR's operations at the beginning and end stages. The researchers then introduced obstructions with DNA replication, such as transcription—the process of copying DNA to produce proteins—that are believed to be aided by BIR.

"Our study shows that when BIR comes to the rescue at these collisions, its arrival comes at a very high price," says Malkova, the study's corresponding author. "When BIR meets transcription, it can introduce even more instability, which can lead to even higher mutations. As a result, we think that instabilities that mainly were found at collisions between transcription and replication that have been suggested to lead to cancer might be caused by BIR that came to the rescue. It comes, it rescues, but it's kind of questionable how helpful it really is."

Scientists have known how BIR works at some stages. For example, they know the DNA repair apparatus forms a bubble of sorts around the

damaged DNA, then moves forward, unzipping the DNA, copying intact segments, and finally transferring those copied segments to a new DNA strand.

But what remained elusive was following BIR throughout its entire repair cycle. Using a technique involving Droplet Digital PCR and a new DNA purification method developed by biology graduate student Liping Liu, the researchers were able to observe BIR from beginning to end.

"If you imagine this as a train, Liping installed a bunch of stations, and she watched how the train proceeded at each station, tracking the increase in DNA at each station, how much increase is occurring at each station, and thus, in aggregate, how the entire process unfolds," Malkova explains.

The team then intentionally introduced obstructions at some stations—transcription and another obstruction called internal telomere sequences—to observe how BIR responded to the obstacles. One finding: when transcription is introduced near the beginning of the BIR process, the repairs fail to commence, as if they're being suppressed. Also, the researchers found the orientation of the transcription with respect to BIR can affect the repair cycle and may be an important factor affecting instability that can promote cancer in humans.

"Scientists already know there's a lot of instability in places where high transcription meets normal replication," Malkova says. "What we did not know until now is where is it coming from and why is it happening."

The first author of the study, "Tracking break-induced replication shows that it stalls at roadblocks," is Liu, who is a sixth-year graduate student in Malkova's lab.

More information: Liping Liu et al, Tracking break-induced

replication shows that it stalls at roadblocks, *Nature* (2021). DOI: 10.1038/s41586-020-03172-w

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