Scientists uncover key mechanism linking DNA replication to cancer

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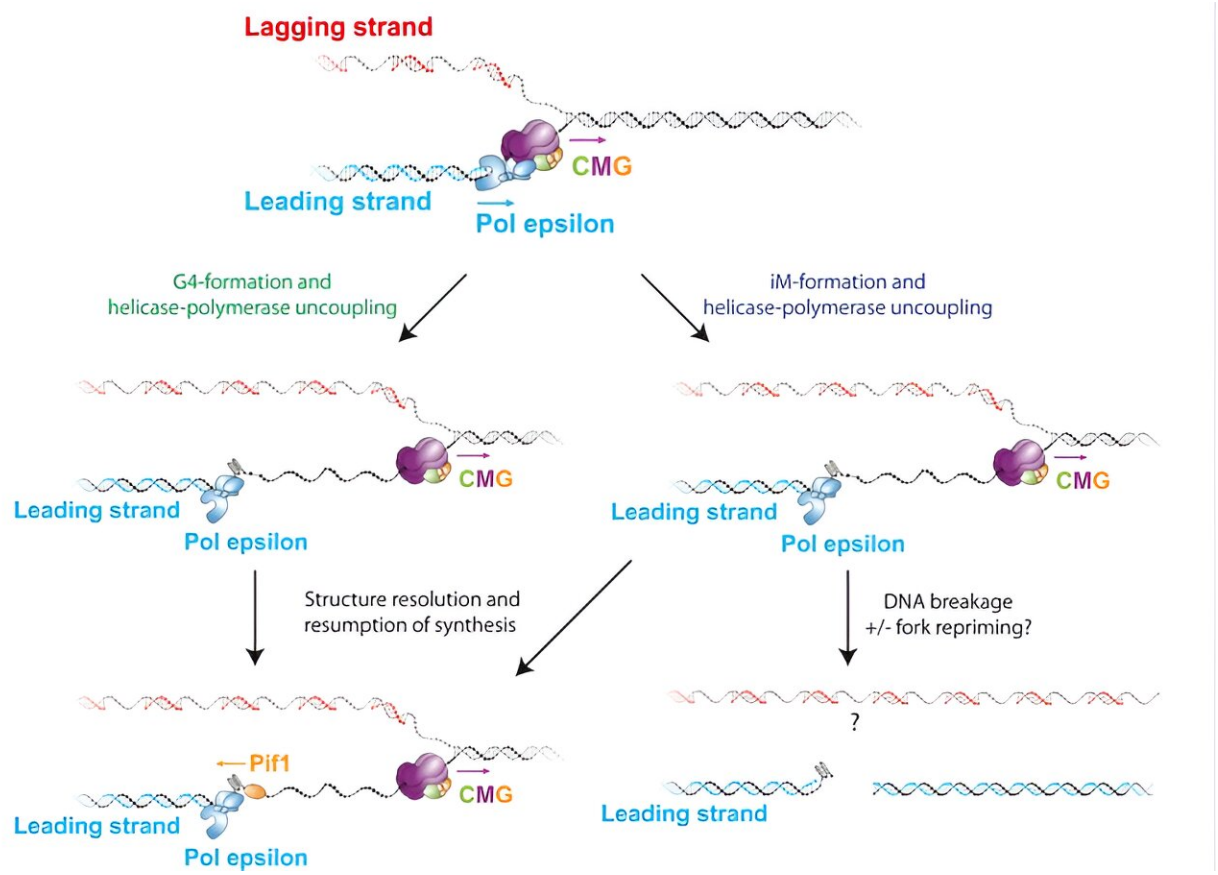

Model illustrating the effects of G4s and iMs on replication. CMG unwinds past a leading strand quadruplex-forming sequence and secondary structures form from the exposed ssDNA. These structures inhibit synthesis by polymerase ε, leading to helicase-polymerase uncoupling. Pif1 can unwind both G4s and iMs and allow synthesis to resume. iMs can be resolved in this manner or lead to nascent DNA breakage in the absence or presence of a repriming event. Lagging strand products may remain intact. Credit: *The EMBO Journal* (2023). DOI:

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Researchers have gained a clearer insight into how common alterations to the structure of DNA affect the process of DNA replication.

These structural changes can put a stop to replication, causing "replication stress," which affects the stability of the genome and is a well-established hallmark of cancer.

By uncovering how certain DNA structures drive replication stress, this work contributes to a better understanding of how cancer develops. It may also open the door to new treatment approaches that could be effective across multiple cancer types.

The study, which was led by researchers at The Institute of Cancer Research, London, has been published in the *EMBO Journal*.

It only takes one unusual structure to stop DNA replication

The researchers used purified replication factors to recreate the process of DNA replication in a test tube. In conjunction with various cutting-edge techniques, they investigated the effects of alternative DNA structures called G-quadruplexes (G4s) and i-Motifs (iMs) on DNA replication. Through a series of experiments, they demonstrated that just one G4 or iM structure is sufficient to halt the replication process. They also found that iMs can cause DNA breakage.

Obstructions to DNA replication can cause various rearrangements of the genome, in which genes can be deleted, moved elsewhere, or

mutated. These changes can also affect gene expression.

If DNA damage or altered gene expression affects cellular processes, this can lead to uncontrolled cell division and the development of cancer. Previous research suggests that about two thirds of the mutations that drive cancer result from errors made during DNA replication.

How secondary structures form during DNA replication

DNA replication is an essential part of cell division. Before a cell divides, it creates a copy of its entire genome so that each of the resulting daughter cells has all of the essential genetic information. A protein complex called a replisome assembles at each of the thousands of initiation sites across the genome, called origins of replication. An enzyme called a helicase unwinds the double helix structure, exposing the nucleotides—the building blocks of DNA—on the two separate strands.

Enzymes called DNA polymerases use each parent strand as a template, correctly pairing freely available nucleotides with the exposed ones on the strand. The parent strand and the new strand then re-coil into the usual double helix structure.

While DNA is exposed in a single-stranded form, certain sequences can fold into unusual DNA secondary structures. Sequences that contain many guanine (G) nucleotides can fold into a structure called a G-quadruplex, or G4, while sequences rich in cytosine (C) nucleotides can form a different type of quadruplex structure called an intercalated-motif, or iM. Both types of structures are associated with genetic instability and mutations.

Completing the puzzle one piece at a time

The researchers began by recreating DNA replication in a test tube, specifically looking at known G4-forming and iM-forming sequences. Both types of sequences caused DNA replication to stall, and iM-forming sequences were also able to cause breaks in DNA.

However, the team wanted to show that it was the secondary structures themselves, rather than the nucleotide sequences, that were responsible. To do this, the scientists deliberately introduced mutations to disrupt the structures. As predicted, this led to a significant reduction in stalling. Furthermore, they used state-of-the-art nanopore sensing technologies in collaboration with scientists at the University of Cambridge to show that the DNA used in their experiments had no pre-existing structures. This result confirmed that structures form as a consequence of replication.

Their next aim was to determine exactly how the structures halt DNA replication. The two major possibilities were that the helicase was arrested or that the polymerase was inhibited. The team directly tested the ability of a helicase called CMG to unwind structures and determined that it was able to bypass G4s and iMs in time. However, synthesis of the new strand continued to be inhibited, suggesting that it was the polymerase that was affected.

On this basis, the researchers propose that the DNA replication issues result from an uncoupling between the helicase and the polymerase, leading to the exposure of single-stranded DNA.

Dr. Gideon Coster, Team Leader of the Genome Replication team at the ICR, said, "While replication stress can be driven by various factors, the most common internal source is the DNA template itself. Our work shows that certain sequences in the human genome lead to secondary structures that are sufficient by themselves to halt replication. Current

estimates suggest that there are hundreds of thousands of G4-forming sequences in the human genome, so there is a lot of potential for this to occur."

Opening up new research avenues

This work has improved scientists' understanding of how genome instability is caused by repetitive and structure-forming DNA sequences. This knowledge is relevant not only to cancer but also to other diseases, including neurodegenerative conditions caused by repeat expansions, such as fragile X syndrome and Huntington's disease.

In the long term, the researchers are hopeful that health care professionals may be able to use a patient's replication stress status to help guide their treatment decisions. For example, single-stranded DNA can be a useful biomarker for the diagnosis of tumors.

They also believe that their work may lead to the identification of novel drug targets. It is known that certain small molecules that bind to G4s can affect their stability, so these may be attractive drug targets. Another option is to target the many helicases that have a role in unwinding DNA secondary structures.

First author Sophie Williams, a final year Ph.D. student in the Genome Replication team at the ICR, said, "These frequently occurring DNA secondary structures are common mutation hotspots in cancer. We have known for some time that they play a part in the development of cancer, but this work has furthered our understanding of the specific way in which they contribute to genome instability.

"Understanding the drivers of replication stress is essential for developing efficient therapies that target the cellular response to replication stress and DNA damage."

Medical Xpress

More information: Sophie L Williams et al, Replication-induced DNA secondary structures drive fork uncoupling and breakage, *The EMBO Journal* (2023). DOI: 10.15252/embj.2023114334

Provided by Institute of Cancer Research

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