How one mammalian DNA polymerase challenges the central dogma of biology

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June 14, 2021 -- New research demonstrates how an unusual mammalian DNA polymerase, called polymerase θ (Polθ), can facilitate the conversion of RNA into DNA -- the opposite of normal polymerase function. The data, published in *Science Advances* on June 11, could challenge the central dogma of molecular biology that states that DNA makes RNA, which in turn makes proteins.

In mammalian cells, DNA polymerases build RNA messages that are read by cellular machinery to be translated into proteins. According to the central dogma of molecular biology, the flow of genetic information goes from DNA to RNA, and this genetic information cannot be transferred in the opposite direction.

Of the 14 DNA polymerases in mammalian cells, only three do the bulk of the work of duplicating the entire genome to prepare for cell division. The remaining 11, including Polθ, are mostly involved in detecting and making repairs when there is a break or error in the DNA strands.

Researchers at Thomas Jefferson University noticed that Polθ shared characteristics with a reverse transcriptase enzyme that is commonly found in viruses such as HIV. A research group led by Richard Pomerantz, PhD, and his team focused on investigating this unusual DNA polymerase activity. Pomerantz is an associate professor of biochemistry and molecular biology at Thomas Jefferson University

Polθ is a DNA polymerase-helicase fusion protein that is highly error-prone and promiscuous, meaning it can catalyze reactions outside of its main function. It facilitates microhomology-mediated end-joining (MMEJ) of double-strand breaks (DSBs) by extending partially base-paired 3′ single-stranded DNA (ssDNA) overhangs at DSB repair junctions. Because Polθ has an inactive proofreading domain, it also has the ability to reverse transcribe RNA in a similar fashion to retroviral reverse transcriptases.

Therefore, the team hypothesized that Polθ has RNA-dependent DNA synthesis activity and can tolerate ribonucleotides during DNA repair activities to promote RNA-templated DNA repair.

In a series of cellular assays, the researchers tested whether Polθ reverse transcribes RNA like HIV reverse transcriptase using a DNA primer annealed to a RNA template. By quantifying complementary DNA produced by Polθ, they found that it exhibits a similar rate of reverse transcription activity as HIV reverse transcriptases, thus confirming that Polθ is capable of converting RNA to DNA. As expected, Polθ was also more efficient at converting DNA to DNA, relative to the viral reverse transcriptase, pointing to its function in DNA repair.

Notably, the team found that Polθ was more efficient and introduced fewer errors when using an RNA template to write new DNA messages than when duplicating DNA into DNA, suggesting that this function could be its primary purpose in the cell.

"Our research suggests that polymerase theta's main function is to act as a reverse transcriptase," said Pomerantz in a statement.

To test whether Polθ promoted RNA-templated DNA repair synthesis (RNA-DNA repair) in a biological setting, the researchers developed a cellular green fluorescent protein (GFP) reporter assay that simultaneously quantitated Polθ genetic repairs in different scenarios. Cotransfection of Polθ and different genetic material in mouse induced pluripotent stem cells (iPSCs) demonstrated that Polθ promotes both MMEJ (DNA to DNA repair) and RNA-DNA repair.

By teaming up with collaborators in Xiaojiang Chen's lab at the University of Southern California and using x-ray crystallography to define Polθ's structure, the scientists found that Polθ was able to change shape to accommodate more bulky RNA molecules. This high degree of structural plasticity enables Polθ to efficiently transcribe template ribonucleotides and accommodate a full RNA-DNA hybrid within its active site.

"This work opens the door to many other studies that will help us understand the significance of having a mechanism for converting RNA messages into DNA in our own cells," Pomerantz explained. "The reality that a human polymerase can do this with high efficiency raises many questions."

Pomerantz's team is exploring the role of Polθ in unhealthy cells, such as cancer cells, where it is highly expressed and promotes cancer cell growth and drug resistance. They are working to understand how Polθ contributes to cancer cell proliferation.