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# Pioneering technique reveals new layer of human gene regulation

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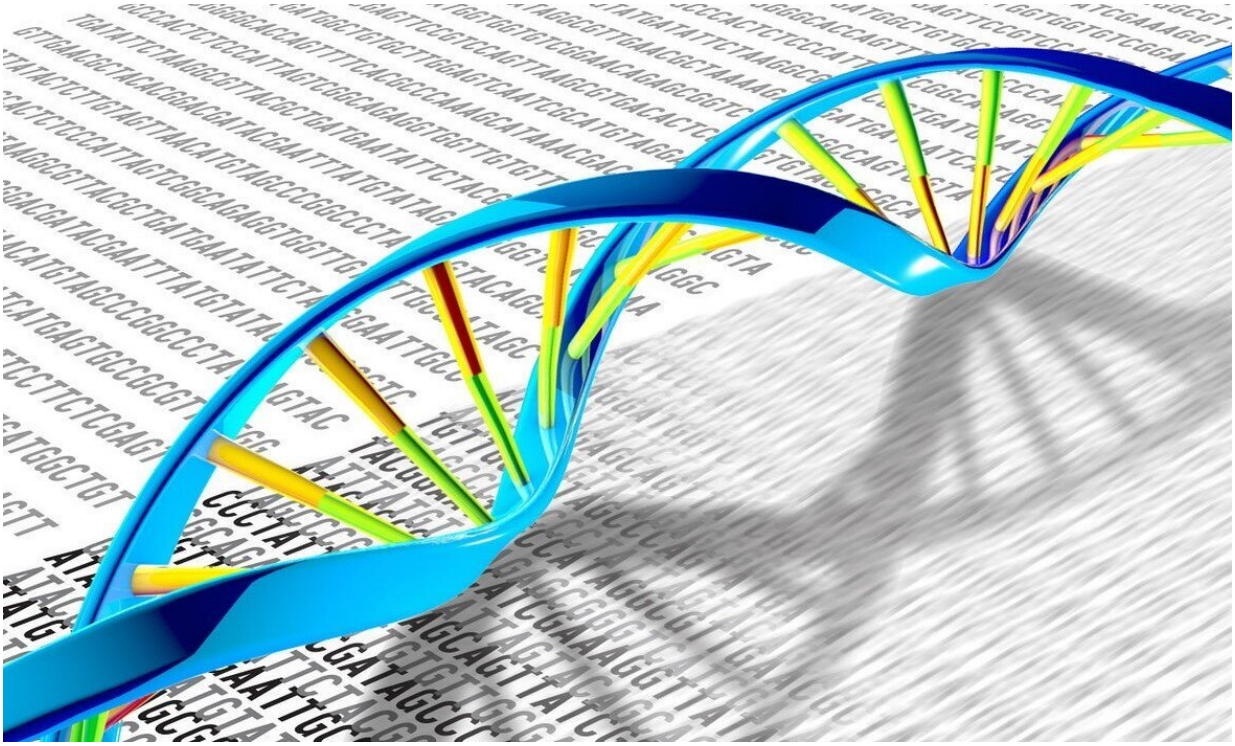

DNA, which has a double-helix structure, can have many genetic mutations and variations. Credit: NIH

A technique can determine for the first time how frequently, and exactly where, a molecular event called "backtracking" occurs throughout the genetic material (genome) of any species, a new study shows.

Published online February 9 in *Molecular Cell,* the study results support the theory that backtracking represents a widespread form of gene regulation, which influences thousands of human genes, including many involved in basic life processes like cell division and development in the womb.

Led by researchers from NYU Grossman School of Medicine, the work revolves around genes, the stretches of DNA molecular "letters" arranged in a certain order (sequence) to encode the blueprints for most organisms. In both humans and bacteria, the first step in a gene's expression, transcription, proceeds as a protein "machine" called RNA polymerase II ticks down the DNA chain, reading genetic instructions in one direction.

In 1997, Evgeny Nudler, Ph.D. and colleagues published a paper that showed RNA polymerase can sometimes slip backward along the chain it is reading, a phenomenon they named "backtracking." Studies since then have shown that backtracking occasionally takes place in living cells soon after RNA polymerase begins RNA synthesis or when it encounters damaged DNA to make room for incoming repair enzymes.

Subsequent work suggested that the backsliding and repair machinery had to work quickly and dissipate, or it might collide with DNA polymerase to cause cell-death-inducing breaks in DNA chains.

Now a new study led by Nudler's team at NYU Langone Health reveals that their new technique, Long Range Cleavage sequencing (LORAX-seq), can directly detect where backtracking events begin and end. By complementing past approaches that were indirect or limited, the new method reveals that many such events move backward further than once thought, and in doing so, last longer.

The results also suggest that persistent backtracking occurs frequently

throughout genomes, happens more often near certain gene types, and has functions well beyond DNA repair.

"The surprising stability of backtracking at longer distances makes it likely that it represents a ubiquitous form of genetic regulation in species from bacteria to humans," says Nudler, the study's senior author, and the Julie Wilson Anderson Professor in the Department of Biochemistry and Molecular Pharmacology at NYU Langone.

"If further work expands our findings to different developmental programs and pathological conditions, backtracking may be akin to epigenetics, the discovery of which revealed a surprising new layer of gene regulation without changing the DNA code."

## Central to Life?

RNA polymerase II translates DNA code into a related material called RNA, which then directs the building of the proteins. To do so, the complex moves down DNA chains in one direction, but backtracks in certain scenarios. Past studies have shown that as RNA polymerase II backtracks, it forces out (extrudes) from its interior channel the tip of the RNA chain it has been building based on the DNA code.

As prolonged backtracking is prone to causing detrimental collisions, transcription is thought to be quickly restored by the transcription factor IIS (TFIIS), which promotes the cutting off (cleavage) of the extruded, "backtracked" RNA. This clears the way for RNA polymerase II to resume its forward code reading.

Other, earlier studies, however, had shown that when polymerase backtracks beyond a certain distance (e.g. 20 nucleobase DNA building blocks), the backtracked RNA can attach to the channel through which it is extruded, holding it in place longer. Locked, backtracked complexes

are less likely to be rescued by TFIIS-driven cleavage, and more likely to delay transcription of the gene involved.

This led to the theory that backtracking, in addition to playing a key role in DNA repair pathways, may dial up or down the action of genes as a major regulatory mechanism.

According to the researchers, TFIIS likely occurs at low concentrations in living cells, and competes with hundreds of other proteins to get at and cut out backtracked RNA so transcription can continue.

In the current study, the team instead used a high concentration of purified TFIIS (no competing proteins) to precisely cut out any piece of backtracked RNA anywhere it occurs in a cell's genetic code. This made the cut-out snippets available to technologies that read code sequences and provide clues to their locations and functions.

The research team also found that the genes that control histones—protein "spools" that DNA chains wrap around within the chromatin that organizes gene expression—are highly prone to persistent backtracking.

The authors theorize that the degree to which this happens, with related changes in the transcription of certain genes, may control the timing of large-scale histone accumulation needed during cell division to rebuild chromatin. They also suggest that persistent backtracking may influence the timely transcription of genes vital to tissue development.

"Along with its potentially useful functions, persistent backtracking could also result in DNA damage and other genetic malfunctions that contribute to disease," says first study author Kevin Yang, a graduate student in Dr. Nudler's lab.

"We speculate that the measurement of backtracking in the context of aging or cancer, for instance, may help us understand why malfunctions occur in the cell stress response and cell replication, and to suggest new treatment approaches."

Along with Yang and Nudler, study authors from the Department of Biochemistry and Molecular Pharmacology at NYU Langone Health were Aviram Rasouly, Vitaly Epshtein, Criseyda Martinez, Thao Nguyen, and Ilya Shamovsky. Nudler is also an Investigator with the Howard Hughes Medical Institute.

**More information:** Persistence of backtracking by human RNA polymerase II, *Molecular Cell* (2024). DOI: 10.1016/j.molcel.2024.01.019. www.cell.com/molecular-cell/fu … 1097-2765(24)00055-8

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