Transcription elongation checkpoint discovered

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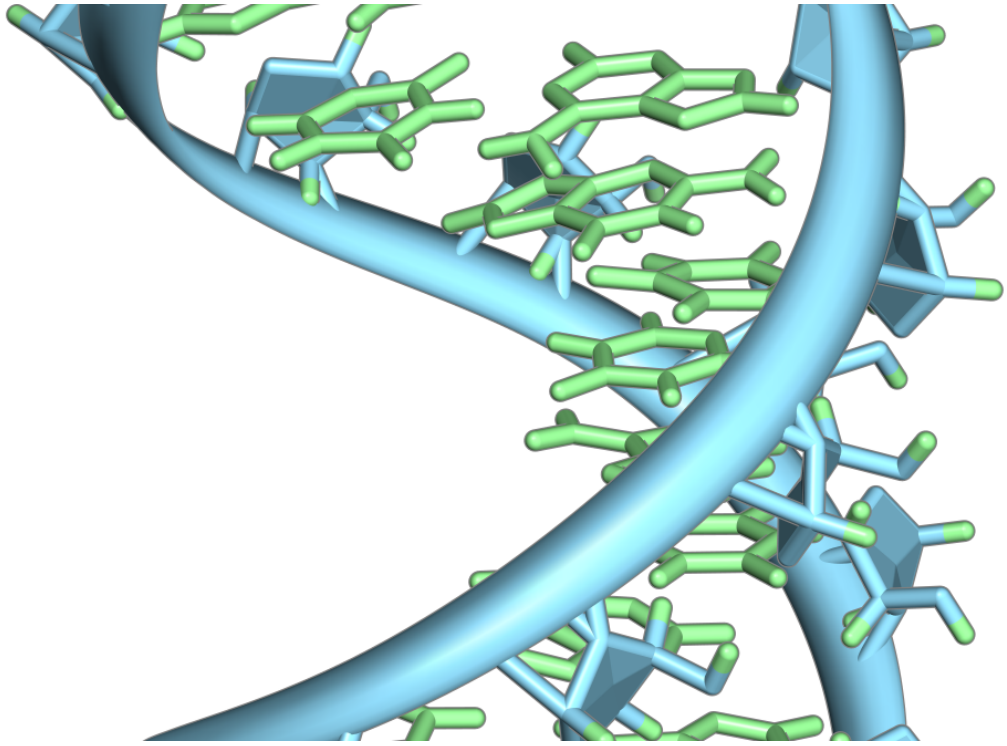

A hairpin loop from a pre-mRNA. Highlighted are the nucleobases (green) and the ribose-phosphate backbone (blue). Note that this is a single strand of RNA that folds back upon itself. Credit: Vossman/ Wikipedia

Northwestern Medicine scientists have identified a critical checkpoint in transcription elongation, the process of synthesizing RNA from a DNA template, according to findings published in *Molecular Cell*.

According to the study, the presence of a protein called SPT5 serves as a "passport," determining whether a polymerase complex is allowed to proceed down the length of DNA or is instead degraded and destroyed.

"Only RNA Polymerase IIs with SPT5 are allowed to leave the station," said Ali Shilatifard, Ph.D., the Robert Francis Furchgott Professor, chair of Biochemistry and Molecular Genetics and senior author of the study.

Many molecular biology experiments operate by deleting a gene, or the protein that gene codes for, and observing the impact, which suggests the function of that gene. However, these methods often produce other mutations or require waiting as long as 72 hours before observation, allowing for other transcription processes to occur.

"By that point, you are reporting on the quaternary effect of the knockdown," said Shilatifard, who is also a professor of Pediatrics, director of the Simpson Querrey Institute for Epigenetics and a member of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University.

The new experimental method, called auxin-inducible degradation, allows immediate observation of the effects of protein depletion. In the study, investigators used this method to interrogate the role of the protein SPT5 in transcription elongation.

During transcription elongation, the RNA polymerase II complex "walks" along one strand of DNA, copying genetic elements to a strand of RNA. Shilatifard's laboratory has previously discovered pauses in this process linked to regulatory checkpoints. Now, using this new method of gene deletion, they've discovered a crucial checkpoint before elongation even starts.

In cells with SPT5 depleted, polymerase never begin its journey down

the strand of DNA. Instead, it is recognized as faulty and degraded. How exactly it is recognized remains unknown, but it's clear that SPT5 serves as a badge of approval, according to Yuki Aoi, Ph.D., a postdoctoral fellow in the Shilatifard laboratory and lead author of the study.

"If there are issues here, and the polymerase is allowed to go, there could be more issues down the line," Aoi said. "It's only allowing polymerases that are certified to leave because they have SPT5."

The investigators also discovered that a protein called CUL3 is part of the pathway that destroys polymerases lacking SPT5. The gene that codes for this protein and its associated genes are mutated in many cancers—possibly allowing malformed polymerases to begin their transcription journey, resulting in aberrant cells that could contribute to cancer. This is a pathway of great interest, according to Aoi.

"We need to examine this link to cancer," Aoi said.

More information: Yuki Aoi et al, SPT5 stabilization of promoter-proximal RNA polymerase II, *Molecular Cell* (2021). DOI: 10.1016/j.molcel.2021.08.006

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