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Researchers identify a process that enables access to genes

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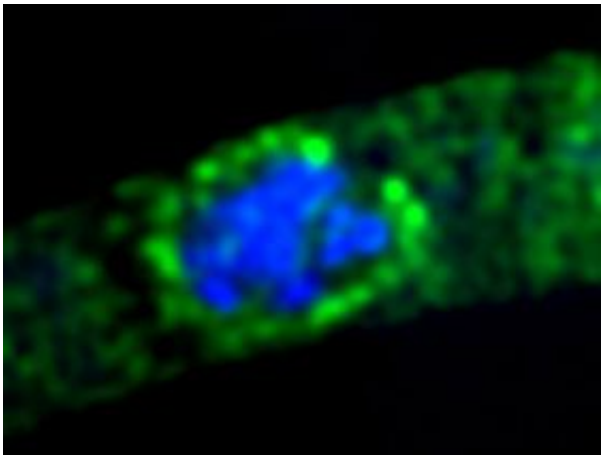

Underneath ubiquitylation. The nucleus (blue) of a mutant yeast cell appears to be deformed due to the loss of a process, ubiquitylation, that underlies the unpackaging of DNA.

It turns out there's more than one way to skin a gene. New research from Rockefeller University suggests that two closely related DNA unpackaging mechanisms may not work the way scientists thought.

Access to a gene requires a host of proteins to work in tandem to pry open DNA's protective chromatin shell, formed by complexes of DNA and special packaging proteins called histones. Research in David Allis's Laboratory of Chromatin Biology and Epigenetics focuses on understanding chemical modifications to the threadlike protein "tails"

that hang from histones. But two of these gene-activating modifications, which add chemical groups called methyl and ubiquitin to the amino acid lysine at specific locations on neighboring histones, are poorly understood. At least, they were.

Early research by Allis's lab established that these two modifications are functionally related. Experiments showed that mutations in yeast that abolished ubiquitylation also led to loss of methylation. Because ubiquitylation takes place at lysine 119 on one type of histone, called H2B, and methylation takes place at lysine 4 on another histone, H3, the finding suggested that modifications on different histones communicate with each other in a type of signaling pathway. But questions still remained about the function of each of these modifications in transcription activation.

Jason Tanny, a postdoc in Allis's laboratory, and his colleagues answer some of these questions in a cover article in the April issue of *Genes and Development*. Tanny, first author of the report, set out to determine whether ubiquitylation and methylation are functionally equivalent.

Using fission yeast as a model, Tanny created a mutation in histone H2B that knocked out its ubiquitylation site on lysine 119, and found that methylation on H3 was also impaired, confirming the relationship between the two modifications. He also found that the cells with defects in ubiquitylation became unhealthy: The mutant yeast cells grew more slowly than their normal sister cells.

"If the cells are sick, it would be because you are knocking out this pathway that leads from ubiquitylation to methylation," says Tanny. "So we thought that knocking out methylation would also make the cells sick." But when Tanny knocked out a gene called set1, which abolished methylation on H3 without affecting ubiquitylation on H2B, normal cell growth was unimpaired. So H2B ubiquitylation was functioning

“upstream” of lysine H3 methylation. Tanny reasoned that ubiquitylation was operating in a separate pathway and affecting cell growth.

Tanny used a chromosome immunoprecipitation assay to determine where RNA polymerase — the large protein machine that copies DNA into RNA — is located in these genes. He found that RNA polymerase had no problem getting to the gene promoter — the first step in transcription — but there were problems downstream, at the body of the gene.

“The real effect of the ubiquitylation mutation on transcription is on the ability of RNA polymerase to get through the gene, rather than the ability of RNA polymerase to get to the gene in the first place,” Tanny says. “This suggests that the reason we saw gene expression defects in these mutants is because there is a transcription elongation defect at specific genes.”

Nucleosomes represent a barrier to transcription — test tube studies have shown that nucleosomes need to be disrupted in some way for RNA polymerase to get through to the DNA. Scientists think that transcription progresses as the nucleosomes are taken apart and then put back together after RNA polymerase goes through. Tanny proposes that ubiquitylation directly affects nucleosome assembly as RNA polymerase goes through.

“Because these modifications are so conserved from yeast to humans, this is going to be important for understanding mechanisms for how nucleosome structures are altered during transcription,” Tanny says.

“Jason’s work demonstrates that histone ubiquitylation plays a role in RNA polymerase elongation when genes exist in a chromatin landscape, a function that can be separate from other chromatin ‘on’ marks that more closely function with transcription initiation,” says Allis, who is the Joy and Jack Fishman Professor. “Understanding how this happens

mechanistically will likely be one of the next exciting chapters in protein ubiquitylation."

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