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Unexpected findings uncover new understanding of gene expression

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Northwestern Medicine scientists have discovered that the catalytic activity of the fly enzyme Trr and mammalian MLL3/MLL4—members of the COMPASS family of proteins central to gene expression—is not required for proper development and viability in flies or gene expression in mammalian cells.

The surprising findings, published October 2 in the journal *Nature*

Genetics, suggest that the enzymatic function may not be as important as the body or the context of the protein complex itself, which has important implications in the understanding of cancer development.

Ali Shilatifard, the Robert Francis Furchgott Professor and chair of biochemistry and molecular genetics at Northwestern University Feinberg School of Medicine, was the senior author of the study. Ryan Rickels, a student in Feinberg's Driskill Graduate Program in Life Sciences, was the first author.

Trr is one of three enzymes that make up the COMPASS family of proteins in Drosophila, the genus that encompasses small fruit flies (humans, by comparison, have six COMPASS members). COMPASS, which was first characterized by Shilatifard almost 16 years ago, catalyzes methylation at a histone location called H3K4 and has since been shown to be critical to gene expression and the regulation of many other processes.

Because the human equivalents of Trr—enzymes called MLL3 and MLL4—are highly mutated in many forms of cancer, there has been significant interest in understanding this protein complex and its function during methylation, when Trr deposits a single methyl group at enhancers, influencing whether genes are turned on or off.

Trr itself is indispensable to an organism's viability; when the entire gene is deleted, flies die early in embryogenesis. MLL3 and MLL4 are also required for mammalian development.

In the current study, Shilatifard's lab investigated the outcomes for flies when just the key catalytic function of the Trr gene was knocked out. The investigators made a single inactivating point mutation to the SET domain, the part of the protein that catalyzes the process of methylation.

Previously, it had been assumed that this methylation was critical to the overall function of the enzyme. But what Shilatifard's lab discovered was entirely unexpected.

"When we made this mutation to kill its catalytic activity, we found that it was not lethal to the flies—in fact, the flies developed just fine, which was really surprising," Rickels said.

The team also investigated creating a version of the enzyme that can implement three methyl marks, instead of one, which hyper-activates the activity of the enzyme. The results were similar, with the flies living normally and experiencing only minimal changes in gene expression and phenotypes.

"We came to the conclusion that this monomethylation at enhancers is apparently not necessary for the enhancer to function under the laboratory conditions—but rather, another portion of the enzyme is doing the really important function," Rickels said.

The findings open up new avenues for research, as the scientists now investigate the importance of other portions of the Trr protein, as well as run similar experiments with other COMPASS members.

"Things have shifted now. The entire field was focused on this methyl mark; now we have to go for the whole enzyme itself and figure out what it's doing," said Shilatifard, also a professor of pediatrics and a member of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University. "What is it about the whole body of the enzyme itself that functions in development and its mutations that can result in cancer? That's what we need to figure out."

Based on this research project, Rickels received two grant awards from the National Institutes of Health (NIH) earlier this year: a F31

Predoctoral Research Fellowship, as well as a F99/K00 Predoctoral to Postdoctoral Fellow Transition Award.

More information: Histone H3K4 monomethylation catalyzed by Trr and mammalian COMPASS-like proteins at enhancers is dispensable for development and viability, *Nature Genetics* (2017). nature.com/articles/doi:10.1038/ng.3965

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