A first of its kind tool to study the histone code

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University of North Carolina scientists have created a new research tool, based on the fruit fly, to help crack the histone code. This research tool can be used to better understand the function of histone proteins, which play critical roles in the regulation of gene expression in animals and plants.

This work, published in the journal *Developmental Cell*, opens the door to experiments that are expected to uncover new biology important for a host of conditions, such as neurological diseases, diabetes, obesity, and especially cancer, which has become a hotbed of epigenetic research.

"People think cancer is a disease of uncontrolled proliferation, but that's just one aspect of it," said Robert Duronio, PhD, professor of biology and genetics and co-senior author. "Cancer is actually a disease of development in which the cells don't maintain their proper functions; they don't do what they're supposed to be doing." Somehow, the gene regulation responsible for proper cell development goes awry.

One aspect of gene regulation involves enzymes placing chemical tags or modifications on histone proteins – which control a cell's access to the DNA sequences that make up a gene. Properly regulated access allows cells to develop, function, and proliferate normally. The chemical modification of histones is thought to be a form of epigenetic information – information separate from our DNA – that controls gene regulation. This idea is based on the study of the enzymes that chemically modify histones. However, there is a flaw in this argument.

"In complex organisms, such as fruit flies, mice, and humans, scientists have only been able to infer how these enzymes mechanistically accomplish their tasks," said Daniel McKay, PhD, assistant professor of genetics and biology and first author of the paper. "It's been technically impossible to directly study the role of histone modifications. Now, through our collaboration between UNC biologists, we've been able to develop a tool in fruit flies to directly test the function of histones independently of the enzymes that modify them."

This is crucial because therapies, such as cancer drugs, can target histones. With this new research tool, scientists will be able to better study thousands of enzyme-histone interactions important for human health.

"If you think of the genome as a recipe book, then you could say we've made it possible to know that there are hidden ingredients that help explain how specific recipes turn out correctly or not," said Greg Matera, PhD, professor of biology and genetics and co-senior author of the paper. "That's the first step in scientific discovery – knowing that there are things we need to look for and then searching for them."

Beyond Yeast

Before now, a lot of this epigenetic research had been done in yeast – single cell organisms that also use enzymes to lay chemical tags on histone proteins. This work has yielded many interesting findings and has led to the development of therapeutics. But some of this work has led to an oversimplification of human biology, leaving many questions about human health unanswered.

For instance, in complex organisms, enzymes in cells typically do more than one thing. One likely reason for this is that animals undergo cellular differentiation; human life begins as a single cell that differentiates into

the various cell types needed for different organs, body parts, blood, the immune system, etc. This differentiation has to be maintained throughout life.

"Because of this, animals likely have a greater requirement for epigenetic regulation than yeast do," Matera said. "Animal cells have to 'remember' that they must express genes in specific ways." When cancer cells start dividing rapidly to form tumors, these cells are actually reverting to an earlier time in their development when they were supposed to divide rapidly. The gene regulation that was supposed to rein them in has gone haywire.

Whereas in yeast, a histone-modifying enzyme might have a single regulatory task, the human version of that same enzyme might have other regulatory tasks that involve additional proteins.

"In fact, maybe the really critical target of that one modifying enzyme is some other protein that we don't know about yet," Matera said. "And we need to know about it."

The best way to figure that out would be to make it impossible for the enzyme to modify a histone by changing – or mutating – the histone protein. If a histone protein could be disabled in this way and cells still behaved normally, then that would mean there was some other protein that the enzyme acted on. To do this, however, would require replacing a histone gene with a genetically engineered one that could not be modified by an enzyme.

The problem is that in animals, such as mice and humans, there are many histone genes and they are scattered throughout the genome. This makes replacing them with 'designer' histone genes difficult. In addition, other genes are located in between the histone genes. Therefore, deleting the portion of the chromosome with histone genes in order to replace them

with a modified one would wind up deleting other genes vital for survival. This would make such an approach in, say, a mouse, useless.

"It has been technically impossible to do this kind of research in complex organisms," Duronio said. "But fruit flies have all their histone genes in one place on the chromosome; this makes it feasible to delete the normal genes and replace them with designer genes."

Designer genes

Matera, Duronio, and McKay led an effort to delete the histone genes in fruit flies and replace them with specific designer histone genes they created. These new genes were created so they could not be the repositories of epigenetic tags or modifications. That is, the modifying enzyme would not be able to do its job on that particular protein.

As shown in the Developmental Cell paper, the researchers put their new tool to the test. They "broke" one histone protein that had been identified to interact in a specific way with a modifying enzyme, and they got the outcome in fruit flies they expected. But for another enzyme-histone interaction, the researchers got an unexpected result.

Previously, in mammalian cells, other researchers had discovered that when you mutate a specific modifying enzyme, the result is death because the cells can't replicate.

With their new fruit fly research model, the UNC researchers altered the histone gene so that this particular enzyme could not modify its histone protein target. The result was not death. In fact, the flies lived and flew as normal flies do. This meant that the enzyme, which was previously proven to be vital to life, must do something else very important.

"There must be another target for that modifying enzyme," Matera said.

"There must be another hidden carrier of epigenetic information that we don't know about."

McKay added, "This is a demonstration of the potential of our epigenetic platform. Going forward, we're going to do a lot more experiments to identify more discrepancies and hopefully other targets of these enzymes. We're on the ground floor of a long-term project."

This research shows that the epigenetic recipe book for yeast is thin. The recipe book for humans, which is genetically akin to the one for fruit flies, is much thicker, more complex, and full of hidden ingredients scientists have yet to discover.

Now, scientists have a tool to test the recipes.

More information: "Interrogating the Function of Metazoan Histones using Engineered Gene Clusters" DOI: dx.doi.org/10.1016/j.devcel.2014.12.025

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