DNA repair protein caught in act of molecular theft

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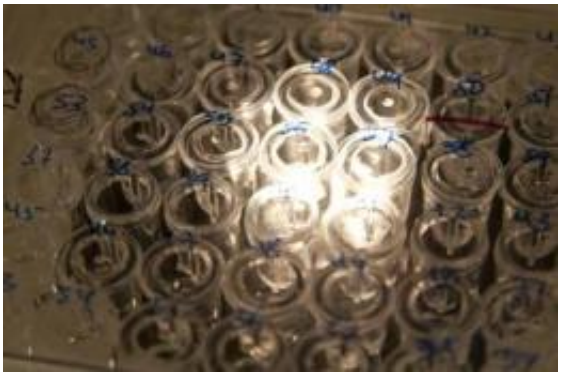


A tray of crystallized proteins awaits examination under an optical microscope in Chuan He's laboratory. Credit: Lloyd DeGrane

Scientists have observed, for the first time, an intermediate stage in the chemical process that repairs DNA methylation damage and regulates many important biological functions that impact health conditions such as obesity, cancer and diabetes.

The observations focused on the bacterial DNA repair protein AlkB, but the results also apply to several proteins in the same family that play key regulatory roles in humans. Armed with these results, researchers may one day develop methods for blocking the protein's efforts to perform the biologically important demethylation function in human cells, said Chuan He, Professor in Chemistry at the University of Chicago.

"This family of proteins is the most exciting protein family now in biology," said He, who led the study. "These proteins directly impact obesity, cancer and diabetes, and they do not go through the traditional pathways of DNA or protein modification. Most likely they go through RNA modification and demodification. It's a new area of biological research."

He and his colleagues at UChicago and the University of Wisconsin-Madison report their findings in the journal *Nature*, published online on Nov. 10.

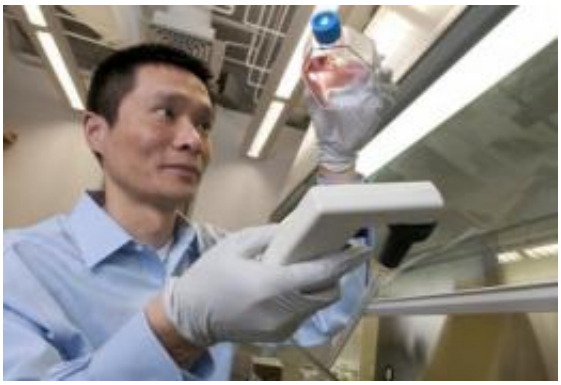
The *Nature* article presents new details about how proteins chemically alter biological molecules and their functioning via a process called oxidative demethylation. Methylation is a chemical process that helps control how DNA and other proteins carry out their functions in the body. In the case of DNA, methylation and demethylation affect how the genetic code gets made into proteins. In recent years scientists had assumed that AlkB and related proteins initiate an oxidizing reaction to remove a hydrocarbon group (the methyls) from the group's host molecule.

"Biological methylation is one of the most important processes in nature to regulate all kinds of things," He said, including how cells differentiate into their final state and how genetic information is transmitted to proteins.

The UChicago researchers recently invented a chemical technique to trap the AlkB protein when it reacts with its host molecule — a previously unobserved, ephemeral process. The technique tethers the protein to the host molecule. "It's stuck there. It can react and stop at the intermediate stage," He said.

Bizarre Observation

Two of the enzymatic intermediates that He's team trapped and observed were predicted and expected based on the chemical principles involved, but these fleeting species were directly observed for the first time. For a third intermediate, however, "we observed something bizarre," He said.



Chuan He, professor in chemistry at the University of Chicago, grows cancer cell lines in his laboratory to study DNA/RNA repair and modification and other important biological processes. Credit: Lloyd DeGrane

Researchers at UW-Madison then carried out computational calculations on the electronic and structural properties of the intermediates that He observed in his experiments. The calculations showed that the bizarrely behaving intermediate was "zwitterionic," meaning that it carried an overall neutral charge, but displayed positive or negative charges when interacting with different atoms.

"We were able to show that the intermediate captured by Chuan's beautiful experiment is zwitterionic in nature, which offers new clues regarding the chemical steps of the biological demethylation process," said Qiang Cui, professor of chemistry at UW-Madison.

The team documented the role of oxidation in demethylation using the

U.S. Department of Energy's Advanced Photon Source at Argonne National Laboratory. The APS produces the brightest X-rays in the Western Hemisphere, which permitted the team to determine the crystal structures that show the three-dimensional atomic framework of the intermediate stage in the demethylation process.

Members of He's research team visit the APS two or three times a month for a full day of experimentation. "We literally collected close to a hundred data sets there," He said. The researchers take multiple data sets at different intervals to confirm the accuracy of their results.

Provided by University of Chicago