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New Discoveries Speak Volumes about How Genes Are Silenced

In two recently published research articles, Howard Hughes Medical Institute researchers have discovered new information that shows how histones are chemically modified to control gene expression during development.

Histone proteins make up the “smart stuffing” in chromosomes—the core of proteins around which DNA winds so that it is packaged compactly. However, histones are more than inert DNA packing material - they are also involved in gene regulation. For example, chemical modifications to histones constitute an important epigenetic mechanism by which genes are activated or repressed. These epigenetic control mechanisms are distinct from the regulatory DNA element embedded in the sequences of the genes themselves.

In papers published in *Nature* and *Molecular Cell*, HHMI investigator Yi Zhang and his colleagues revealed new details about the molecular mechanisms underlying chemical modifications of two core histones, H2A and H3. Zhang is at the University of North Carolina at Chapel Hill (UNC).

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- Yi Zhang

In the *Nature* paper, which was published online December 18, 2005, Zhang, his UNC colleagues, and researchers at Memorial Sloan-Kettering Cancer Center, reported the discovery of a family of enzymes that can remove methyl groups from histones. Methyl groups are chemical “marks” that regulate expression of specific genes during development.

Although it had been known that attaching methyl groups to histones can activate or repress genes, the new discovery is important because it adds

convincing new evidence that methylation is reversible.

“Until very recently, people thought the process was not reversible,” said Zhang. “Early studies had shown that the turnover rate of methyl groups on histones was about the same as the lives of the histones themselves.” And while other researchers had identified an enzyme that can catalyze removal of a single or double methyl groups from histones, no one had identified an enzyme that use a mechanism compatible with removal of three methyl groups. Both dimethyl and trimethyl groups are widely found on histones, so their removal is likely to be important in epigenetic gene regulation.

In studies using cultured human cells, Zhang and his colleagues employed a biochemical assay they had developed to detect an enzyme that could remove a dimethyl and trimethyl group from the histone H3. They discovered that this enzyme contained a particular catalytic structure called a JmjC domain that was known to regulate chromatin function. Thus, they named the new enzyme JmjC domain-containing histone demethylase 1A (JHDM1A). Importantly, said Zhang, the catalysis mechanism of JHDM1A suggest that other members of the newly discovered enzyme family can likely remove trimethyl groups from histones as well.

The broad importance of JHDM1 was revealed by studies in which the researchers found homologous versions of JHDM1 in a wide range of other organisms—including toads, fruit flies, roundworms and two yeast species.

“Although we showed that this family of proteins acts to demethylate histones, and by a new biochemical mechanism, I have no doubt that other members of this family will also demethylate other proteins,” said Zhang. Thus, he said, the JmjC domain-containing family proteins are likely to have other important biological roles.

Further studies in his laboratory are seeking to determine how JHDM1-type enzymes are regulated; how their specificity is determined; and their biological function, he said.

In the *Molecular Cell* article, which was published online on December 15, 2005, Zhang and his colleagues at UNC investigated another histone modification, called ubiquitylation. This process, which involves adding a short protein called ubiquitin to a histone—also regulates genes by shutting them off, or “silencing” them.

In these studies, the researchers studied the enzymatic machinery underlying ubiquitylation of the histone H2A. Specifically, they explored protein subunits that make up a complex called PRC1, which the researchers had previously discovered attaches ubiquitin at a particular spot on H2A.

PRC1 belongs to a group of proteins that are of considerable biological importance because they silence the expression of *Hox* genes. These genes

play a central role in patterning the developing embryo—determining where limbs and other body segments will grow. Silencing gene expression is important for normal coordination of embryonic development

In their experiments, Zhang and his colleagues identified two subunits of PRC1—called Bmi-1 and Ring1A—that play a central role in PRC1's ability to ubiquitylate H2A. Their experiments also found that Bmi-1 and Ring1A also play an important role in silencing *Hox* genes through their participation in ubiquitylation of histone H2A.

Such insights into the roles of Bmi-1 and Ring1A could have important clinical implications, said Zhang. For example, Bmi-1 is known to be involved in silencing genes that control cell proliferation. In these silencing roles, Bmi-1 has been linked to cancer as well as the ability of stem cells to self-renew.

Further studies in Zhang's laboratory will seek to understand how H2A ubiquitylation can lead to gene silencing and how the multiple PRC1 subunits such as Ring1A contribute to ubiquitylation.

“These two papers reflect our overall strategy of using in vitro biochemical assays to identify new enzymes that play a role in histone modifications,” commented Zhang. “Then, once we identify an enzyme, we study it in vivo to put its function in a biological context. Thus, we hope to gain significant insights into the processes of epigenetic modification of gene expression and how they determine cell fate during development.”