Scientists confirm molecular clipping mechanism behind stem cell development

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(PhysOrg.com) -- Stem cells don't just become a part of the liver or the brain in a flash; it takes a complex molecular choreography and requires that specific genes be switched on and off at specific times. Some of these genes are regulated through a process by which proteins in the cell nucleus, called histones, are chemically modified by small "chemical marks" such as acetyl or methyl groups. New research from Rockefeller University scientists now shows that during specific stages of differentiation in mouse embryonic stem cells, crucial marks can be removed by cutting off the end of the histone's tail.

The research, reported in the October 17 issue of *Cell,* identifies for the first time a clipping mechanism that scientists first hypothesized nearly 30 years ago. The finding offers new clues about differentiation of embryonic stem cells and raises questions about the potential effects of a new class of cancer treatments that specifically target histones.

One key to strong activation of a gene has been methylation of a specific amino acid in histones called lysine 4, and understanding how a cell could remove this methyl mark has been one of the most important questions in gene regulation. Some researchers had suggested that an enzyme could remove the methyl group, just as there are enzymes that remove other chemical groups, or that an entirely new histone could replace the methylated histone. Evidence for the first two methods has been experimentally obtained. But scientists hypothesized a third approach: clipping of the portion of the histone tail that contains the methylated lysine.

Elizabeth Duncan, a graduate student in C. David Allis's Laboratory of Chromatin Biology and Epigenetics, found signs of clipping of a specific histone, known as H3, while studying the development of mouse embryonic stem cells. Duncan then extracted the responsible activity and collaborated with Donald Hunt's mass spectrometry group at the University of Virginia to generate a list of possible proteins, potentially including enzymes known as proteases, that could be clipping the H3 tail. Duncan eyed one, a cysteine protease called cathepsin L, that had previously been shown to operate in the cell nucleus.

This was surprising, Duncan says, because cathepsin L was originally identified in a cell organelle called the lysosome, and its role as a protease in the nucleus was less well established.

Further experiments were done with colleagues in the Peptide Synthesis Service at Rockefeller's Proteomics Resource Center, who constructed a series of peptides that mirrored the section of the H3 tail that flanked the cleavage, but with different modifications to specific amino acids. Duncan found that methylated lysine 27 in the tail of H3 enhanced the cleavage reaction. Acetylation of lysine 18 also seemed to increase the likelihood of cleavage by cathepsin L, while acetylation of lysine 23 significantly decreased this cleavage.

In addition to the finding's implications for understanding gene expression in embryonic stem cell differentiation, the role of acetylation in H3 clipping also raises questions about the effect of a new class of cancer drugs called HDAC inhibitors. HDAC inhibitors block the removal of acetyl groups, which are known gene activators, from histones. As acetyl groups begin to accumulate, genes that have been mistakenly silenced in tumors begin to be reactivated.

"It's now a formal possibility from Beth's work that when you build up acetyl marks you may perturb some of these clips," says Allis, who

provided some of the first evidence for histone clipping as a postdoc in 1980. “We are aware now that we should go back and reexamine the patient samples of people who are getting these HDAC inhibitors in clinical trials and see if this is happening.”

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