Rules governing expression of developmental genes in mouse embryonic stem cells are more nuanced than anticipated

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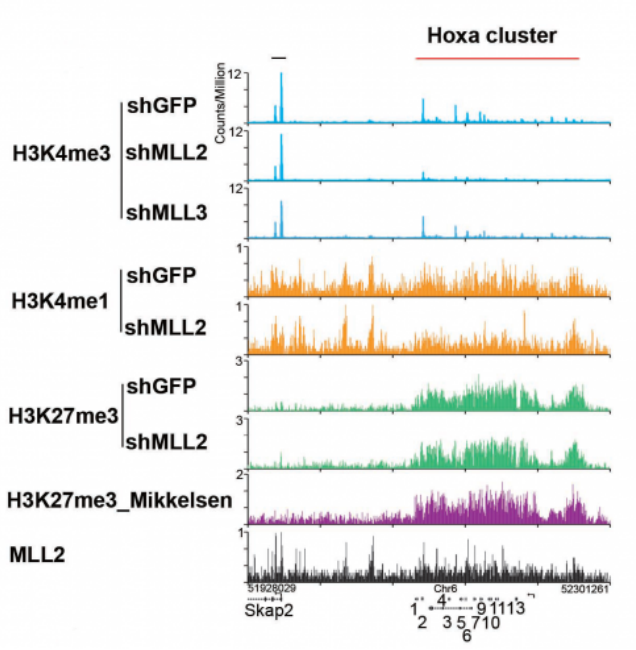

A detailed analysis of the methylation patterns of histone H3 revealed a nuanced picture of the epigenetic rules governing expression of developmental genes in mouse embryonic stem cells. The image shows a ChIP-seq track file example of H3K4me3 at mouse Homeobox (*Hox*) gene clusters. Credit: Study's authors and *Nature Structure & Molecular Biology*.

A decade ago, gene expression seemed so straightforward: genes were either switched on or off. Not both. Then in 2006, a blockbuster finding reported that developmentally regulated genes in mouse embryonic stem cells can have marks associated with both active and repressed genes, and that such genes, which were referred to as "bivalently marked genes", can be committed to one way or another during development and differentiation.

This paradoxical state—akin to figuring out how to navigate a red and green traffic signal—has since undergone scrutiny by labs worldwide. What has been postulated is that the control regions (or promoters) of some genes, particularly those critical for development during the undifferentiated state, stay "poised" for plasticity by communicating with both activating and repressive histones, a state biologists term "bivalency."

A study by researchers at the Stowers Institute for Medical Research now revisits that notion. In this week's advance online edition of the journal *Nature Structural and Molecular Biology*, a team led by Investigator Ali Shilatifard, Ph.D., identifies the protein complex that implements the activating histone mark specifically at "poised" genes in mouse embryonic stem (ES) cells, but reports that its loss has little effect on developmental gene activation during differentiation. This suggests that there is more to learn about interpreting histone modification patterns in embryonic and even cancer cells.

"There has been a lot of excitement over the idea that promoters of developmentally regulated genes exhibit both the stop and go signals," explains Shilatifard. "That work supports the idea that histone modifications could constitute a code that regulates gene expression. However, we have argued that the code is not absolute and is context dependent."

Shilatifard has a historic interest in gene regulation governing development and cancer. In 2001, his laboratory was the first to characterize a complex of yeast proteins called COMPASS, which

enzymatically methylates histones in a way that favors gene expression. Later, he discovered that mammals have six COMPASS look-alikes—two SET proteins (1A and 1B) and four MLL (Mixed-Lineage Leukemia) proteins, the latter so named because they are mutant in some leukemias. The group has since focused on understanding functional differences among the COMPASS methylases. The role of mouse Mll2 in establishing bivalency was the topic of the latest study.

Comprehending the results of the paper requires a brief primer defining three potential methylation states of histone H3. If the 4th amino acid, lysine (K), displays three methyl groups (designated H3K4me3), then this mark is a sign of active transcription from that region of the chromosome. If the 27th residue of histone H3 (also a lysine) is trimethylated (H3K27me3), this mark is associated with the silencing of that region of the chromosome. But if both histone H3 residues are marked by methylation (H3K4me3 and H3K27me3 marks), that gene is deemed poised for activation in the undifferentiated cell state.

The team already knew that an enzyme complex called PRC2 implemented the repressive H3K27me3 mark. To identify which COMPASS family member is involved in this process, the group genetically eliminated all possibilities and came up with Mll2 as the responsible factor. Mll2-deficient cells indeed show H3K4me3 loss, not at all genes, but at the promoters of developmentally regulated genes, such as the *Hox* genes.

The revelation came when the researchers evaluated behaviors of Mll2-deficient mouse embryonic stem cells. First, the cells continued to display the defining property of a stem cell, the ability to "self-renew," meaning that genes that permit stem cell versatility were undisturbed by Mll2 loss. But remarkably, when cultured with a factor that induces their maturation, Mll2-deficient mouse ES cells showed no apparent abnormalities in gene expression. In fact, expression of the very *Hox* genes that normally exhibit bivalent histone marks was as timely in Mll2-deficient cells as it was in non-mutant cells.

"This means that Mll2-deficient mouse ES cells that receive a differentiation signal can still activate genes required for maturation, even though they have lost the H3K4me3 mark on bivalent regions" says Deqing Hu, Ph.D., the postdoctoral fellow who led the study. "This work paves the way for understanding what the real function of bivalency is in pluripotent cells and development."

The study's findings also potentially impact oncogenesis, as tumor-initiating "cancer stem cells" exhibit bivalent histone marks at some genes. "Cancer stem cells are resistant to chemotherapy, making them difficult to eradicate," says Hu. "Our work could shed light on how cancer stem cells form a tumor or suggest a way to shut these genes down."

More information: The Mll2 branch of the COMPASS family regulates bivalent promoters in mouse embryonic stem cells, DOI: 10.1038/nsmb.2653

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