Research reveals how dynamic changes in methylation can determine cell fate

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Scientists at Cold Spring Harbor Laboratory (CSHL) and the University of Southern California (USC) have uncovered intriguing new evidence helping to explain one of the ways in which a stem cell's fate can be determined.

The new data show how the "marking" of DNA sequences by groups of methyl molecules – a process called methylation – can influence the type of cell a stem cell will become. The cellular maturation process, called differentiation, has long been thought to be affected by methylation. Subtle changes in methylation patterns within subsets of a particular cell type have now been observed and closely scrutinized, and they reveal some intriguing mechanisms at work in the process.

A team led by postdoc Dr. Emily Hodges, working in the laboratory of CSHL Professor and HHMI Investigator Gregory Hannon, studied how methylation changes in blood stem cells can affect whether a given stem cell will differentiate into either a myeloid cell or a lymphoid cell. These are the two major lineages of mature blood cells. Sophisticated mathematical analyses of the data were performed under the direction of USC Professor Andrew D. Smith.

The study, which will appear in print October 7 in the journal *Molecular Cell*, generated some surprising findings that challenge currently held theories about how methylation operates. First, it demonstrated that methylation patterns are more dynamic than they are often thought to be. "It's not a question of methylation being 'on' or 'off' at a given site in the

genome," explains Hodges. "We find, instead, an interesting fluctuation of the boundaries of regions that are free of methylation marks. This fact, in turn, can have a profound impact upon cell fate."

Areas lacking methylation, called hypomethylated regions, or HMRs, tend to coincide with so-called CpG islands, sites in the genome where adjacent "Cs" and "G's" – cytosine and guanine nucleotides – are seen in strings of repeats. These unmethylated regions tend to be ones associated with nearby genes that are capable of being expressed. In contrast, sites in the genome that are methylated are typically not expressed.

The new study, which looks at these areas at high resolution in cells of the different blood cell lineages and in blood stem cells, finds that in many cases, a core portion of the unmethylated region is shared in common, but that adjacent areas, sometimes called "CpG shores" – the outlying areas around CpG islands – differ markedly in breadth. The CSHL-USC team refines the notion of islands and shores, preferring to describe the narrowing and widening of the "shoreline" as a tidal phenomenon.

"We observed that the boundaries of these unmethylated regions goes in and out, like the tides," says Hodges. "The key question is what drives these changes. We found that the width of these regions depends on the gene that is associated with the region. We showed in blood cells that the variation is lineage-specific."

The team deduced this after making close study of the methylation patterns in genomic regions containing genes known from other research to be expressed specifically in lymphoid cells, but not in myeloid cells, or vice versa. In these cases, all blood cells share a narrow "core" region of hypomethylation; but only in one lineage did the unmethylated region widen – a widening that opens the promoter of the "underlying" gene to the cellular machinery initiating gene expression. In other words, the

lack of methylation over a wider area enables the underlying gene to be activated – only in the specified cell-type, but not in any of the others.

Another striking observation made from this data is the directional preference of this expansion. For example, in the widening of the unmethylated region seen in the case of the lymphoid cell, the direction of the widening was toward the area occupied by the underlying gene, which in this case was a gene encoding a B cell surface marker called CD22.

It has generally been thought that methylation is a stable epigenetic mark and that changes in methylation are unidirectional; and further, that cells become increasingly methylated as they move through the differentiation process toward their mature identity. And in fact, the only known direction of active change is from an unmethylated state to a methylated state.

The new data suggests, however, that dynamic changes in methylation status may be possible. The relevant evidence comes from blood stem cells, which were observed to have methylation patterns that the team describes as "intermediately methylated," seemingly in dynamic equilibria of the two extreme states of "methylated" and "unmethylated."

According to Hodges, this raises the possibility that methylation might in fact be bidirectional, and that there might be an as yet undiscovered, active mechanism that performs de-methylation. No known enzyme has this ability to remove methyl groups from DNA; DNA methyltransferase is the well-known enzyme that catalyzes the addition of methyl groups.

Yet another of the team's unexpected findings concerns the position of HMRs relative to know genic regions. While unmethylated regions tend to be associated with nearby genes that are capable of being expressed, the team found, according to Hodges, "a lot of HMRs located far away

from any annotated gene locus." One notable thing about these regions, she says, "is that they were highly enriched for binding sites of specific regulatory molecules that are involved in chromatin organization."

Chromatin consists of DNA and the protein complexes called histones around which genomic DNA is packed. In a given cell, chromatin organization, like methylation, helps to determine whether specific genes can be expressed or not.

More information: "Directional DNA Methylation Changes and Complex Intermediate States Accompany Lineage Specificity in the Adult Hematopoietic Compartment" appears in *Molecular Cell* October 7, 2011. The paper was published online ahead of print and can be accessed at: doi:10.1016/j.molcel.2011.08.026

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