EPIGENETICS

Dynamics of DNA demethylation

Locating the final oxidation products of methylated cytosine by enrichment and sequencing reveals that DNA demethylation is common across the genome.

Until recently, methylation was viewed as a stable DNA mark, one that disappears only during dramatic episodes of global reprogramming such as at fertilization. Work by a few labs, including those of Yi Zhang of Harvard and Chuan He of the University of Chicago, has changed that perception.

"We now come to realize that DNA methylation is not static," says He. Rather, much of the genome exists as "a dynamic equilibrium of methylation and demethylation." DNA methylation is linked to repressed gene expression. The discovery that ten-eleven translocation (TET) proteins can oxidize methylated cytosine to 5-hydroxymethylcytosine (5-hmC) prompted questions about whether this effect can be actively reversed.

Zhang's group and others showed that the TET proteins further oxidize 5-hmC to 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC). The low abundance of these marks makes profiling their location in the genome difficult. Highly specific antibodies can be used to enrich genomic fragments bearing cytosine modifications such as 5-hmC for sequencing, but its two derivatives are much rarer. Nonetheless, Zhang and colleagues surmounted the challenge using existing antibodies that recognize 5-fC and 5-caC, and they showed that the modified bases occur mainly in repeat regions in mouse embryonic stem cells (Shen *et al.*, 2013).

The researchers also used a clever form of genetic enrichment. Thymine-DNA glycosylase (TDG) replaces 5-fC and 5-caC with unmodified cytosine through base excision repair, an event suggestive of what Zhang calls a methylation-demethylation cycle run by DNA methylase, TET and TDG proteins. "We found the best way to demonstrate that this hypothesis is correct is to block one of the steps to prevent the cycling," he says. In cells depleted of TDG, the accumulation of 5-fC and 5-caC painted a dynamic picture of demethylation in nonrepetitive and regulatory elements, suggesting a role for active cycling in regulating gene expression.

Similar discoveries came from He, who worked with Peng Jin of Emory, and their colleagues (Song *et al.*, 2013). The selective labeling approach they took builds on their prior method for detecting 5-hmC by tagging it with biotin via an enzymatic conversion and 'click' chemistry step. In the new approach, dubbed fC-Seal, 5-hmC sites are enzymatically blocked, 5-fC is chemically converted to 5-hmC, and only newly converted 5-hmC sites are then tagged with biotin. Enriching DNA by biotin pulldown is efficient and is not biased by the density of tagged sites in the genome.

The researchers discovered 5-fC in regulatory regions and 'poised' enhancers bearing epigenetic marks that indicate they can be rapidly activated. They also found that 5-fC may coordinate gene activation by recruiting the transcriptional activator p300.

Challenges remain. Direct precipitation by antibodies can suffer density-dependent biases and fail when marks are sparse, whereas chemical conversions mitigate these problems but add steps that may introduce artifacts. Both methods offer limited resolution.

To achieve single-base resolution, He's team developed chemically assisted bisulfite sequencing (fCAB-seq), in which 5-fC is found by comparing sequence from a sample in which the mark is converted to uracil by bisulfite to another sample in which the mark is chemically protected from bisulfite. The rarity of 5-fC requires very deep sequencing, which makes genome-wide applications expensive, so He recommends fC-Seal for genomic profiling and fCAB-seq to follow up on interesting loci. Another option described in He's work is to first enrich for 5-fC and then profile with fCAB-seq.

These studies show that methylation is dynamic and widespread. Profiling demethylation intermediates provides rich information about gene regulation and reveals that derepression serves as an activating mechanism—as He notes, many regions annotated as 'unmethylated' are actually 'constantly demethylated'. To explore deeper, economical methods are needed to profile 5-fC and 5-caC genome wide at single-base resolution from limited starting material.

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RESEARCH PAPERS

Shen, L. *et al.* Genome-wide analysis reveals TET- and TDG-dependent 5-methylcytosine oxidation dynamics. *Cell* **153**, 692–706 (2013).

Song, C.-X. *et al.* Genome-wide profiling of 5-formylcytosine reveals its roles in epigenetic priming. *Cell* **153**, 678–691 (2013).