EPIGENETICS

Writing the histone code

Recruiting chromatin modifiers to a specific locus allows the regulation of heterochromatin formation in living cells.

Genetic memory does not live by basic DNA sequence alone. Decades of research have established the importance of histones and their various post-translational modifications in determining gene activation and passing these changes on to the next generation of cells.

Some of these modifications, such as the triple methylation on lysine 9 of histone 3, H3K9me3 for short, have well-established roles in gene silencing and heterochromatin formation. A lot is known about the enzymes that methylate the histone, but the timing and regulation of this methylation process in the context of a living cell remain elusive.

Gerald Crabtree from Stanford University was frustrated with trying to use *in vitro* chromatin remodeling assays to address these questions. "You are more or less stuck with artificial templates: short pieces of DNA that bear no relationship to the actual template, which has different histone modifications in different tissues," he says.

Instead he wanted an approach that would allow him to quickly add and remove a chromatin modification from a locus of choice in a living cell. Together with postdoctoral fellows Oliver Bell and Nathaniel Hathaway, Crabtree brought this idea to life in the form of their chromatin *in vivo* assay.

Working in mouse embryonic stem cells (ESCs), they targeted the promoter of one *Oct4* allele—one of four genes essential for stem cell renewal—with landing sites for either a zinc-finger protein or the DNA binding protein Gal4, and they replaced the first exon of *Oct4* with *GFP* to report gene expression. Next they expressed two fusion proteins: a zinc-finger protein combined with an anchor protein and the histone modifier heterochromatin protein 1 (HP1) fused to a recruiter protein. Adding a small molecule facilitates the rapid association of anchor with recruiter and brings HP1 to the targeted locus on the DNA.

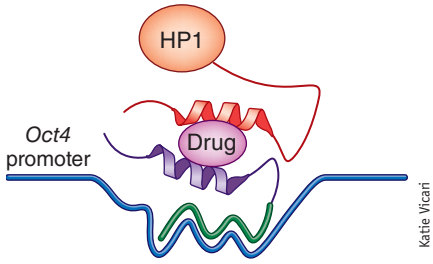
HP1 binds the lysine 9 on histone 3 and recruits histone methylases, which add methyl groups to adjacent H3 molecules, thus spreading the H3K9me3 mark on chromatin.

After the scientists had ascertained that their modified *Oct4* allele itself had no effect on the behavior of the ESCs—because *Oct4* is haplosufficient, ESCs can dispense with one allele—they induced HP1 recruitment and monitored silencing of GFP expression and the spreading of H3K9me3 via chromatin immunoprecipitation. Courtney Hodges, a postdoctoral fellow working with Crabtree, derived a mathematical model that allowed them to calculate the rate of H3K9me3 spreading.

After 3 days of permanent HP1 recruitment, they saw a population of cells with a completely silenced *Oct4* promoter. In addition to H3K9me3, the scientists also noted an increase in DNA methylation.

"I was surprised," recalls Crabtree, "that recruiting HP1 also recruits the [DNA] methylation system, but there is evidence that the cross-talk exists and reinforces the degree of repression. It makes it more stable."

Oct4 expression is silenced in differentiated cells, but Crabtree and his team wanted to know whether the heterochromatin mark could be erased by active transcription. They generated a mouse from their modified ESCs, isolated embryonic fibroblasts and targeted a transcriptional activator to the silenced *Oct4* locus. They saw reactivation of GFP expression after only 24 hours; after 5 days, 10% of the cells were GFP positive. Such reactivation of silent loci is essential during reprogramming when differentiated cells regain a pluripotent state.



The chromatin *in vivo* assay allows recruitment of chromatin modifiers to a specific locus in live cells.

Crabtree sees more potential applications of the chromatin *in vivo* assay. "The technique allows the study of the mechanistic underpinnings of reprogramming," he notes.

For Crabtree, one of the big lessons of this work lay not so much in what they discovered but in what they did not see. "It allows us to predict where the holes are," he says. When they first developed the model for H3K9me3 spreading, Crabtree recalls being pleased with the result; however, it soon became clear that during the first few hours of HP1 recruitment, something was going on that they did not understand. HP1 is recruited rapidly, but the first H3K9 methylation mark is much slower to appear. Something seems to occupy this locus that first needs to be removed. Crabtree calls this factor the 'nonvisible part' of their assay and has some ideas, such as using RNA interference or small molecule screens, for figuring out what is happening during the first few hours of chromatin silencing.

Now that the histone code can be actively (re)written, much waits to be explored.

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RESEARCH PAPERS

Hathaway, N.A. *et al.* Dynamics and memory of heterochromatin in living cells. *Cell* **149**, 1447–1460 (2012).