

## EPIGENETICS

# Detecting the dynamics and memory of heterochromatin

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Post-translational histone modifications influence gene regulation, although experimental approaches have been limited in their ability to assess the dynamics of this regulation, and their transmission across cell generations remains a matter of debate. This paper presents a novel method for measuring the kinetics of chromatin modification *in vivo* and its maintenance through cell division.

In mammalian cells, histone H3 lysine 9 trimethylation (H3K9me3) is a hallmark of heterochromatin and is involved in the transcriptional silencing of genes in this context. This transcriptional repression involves heterochromatin protein 1 (HP1), which binds to methylated H3K9 and also recruits H3K9-specific histone methyltransferases. In this way, HP1 is thought to propagate H3K9me3 modifications to mediate chromatin condensation.

In this study, Crabtree and colleagues developed a system for studying the formation of heterochromatin at a well-described genetic locus — that of *Oct4* (also known as *Pou5f1*) in mouse embryonic stem cells (ESCs). *Oct4* is highly expressed in ESCs, but following cellular differentiation it is rapidly silenced after a series of events, including histone H3K9me, HP1 binding and DNA methylation. The authors used a chemically induced proximity system, which involves a pair of chimeric proteins that associate through

a small molecule. One chimeric protein (the ‘anchor’) contains a DNA-binding domain and the other (the ‘partner’) has the functional domain of interest. In this case, binding sites for the ‘anchor’ were introduced into one allele of the *Oct4* gene, and the ‘partner’ was fused to HP1 $\alpha$ ; rapamycin was the small molecule used to induce the association of the anchor and partner and thus to trigger H3K9 methylation at *Oct4*. This system, which is called chromatin *in vivo* assay (CiA), enables induction and termination of chromatin modification in living cells.

The authors used this approach to study the stability of induced heterochromatin and its transmission through cell generations by the timed removal of rapamycin. They found that the stability of H3K9me3 domains differed among cell types and varied in the context of transcription and DNA methylation. The chemically induced H3K9me3 was stably maintained through cell divisions in fibroblasts (in which there

are no active pluripotency factors) from the mice after the removal of the HP1 $\alpha$  stimulus, and this maintenance did not rely on DNA methylation. Therefore, the authors conclude that H3K9me3 is an epigenetic mark, in that it persists after removal of the initial stimulus. However, recruitment of transcriptional activators can disrupt heritable maintenance, suggesting a reversible nature for this modification.

The kinetic data on H3K9 methylation and turnover also enabled the generation of a mathematical model that predicts the steady-state dynamics of most of all noncentromeric H3K9me3 domains in the mouse genome.

The authors propose that their novel CiA system is adaptable for high-throughput screening for small molecules and modifiers of chromatin regulation in different cell types derived from the mouse. Such studies may increase our understanding of the mechanisms that are involved in the establishment and maintenance of stable gene expression.

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**ORIGINAL RESEARCH PAPER** Hathaway, N. A. *et al.* Dynamics and memory of heterochromatin in living cells. *Cell*, 14 June 2012 (doi:10.1016/j.cell.2012.03.052)

**FURTHER READING** Beisel, C. & Paro, R. Silencing chromatin: comparing modes and mechanisms. *Nature Rev. Genet.* **12**, 123–135 (2011)

