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EPIGENETICS

Synthetic readers and writers for mammalian chromatin

> this synthetic suite of chromatin regulators could … be adapted to study and manipulate genome architecture and dynamically control gene expression

The establishment and propagation of epigenetic marks, such as DNA methylation and histone modifications, through cell division is thought to be based on a read–write system of natural chromatin modifiers. An article in *Cell* now reports simple minimal synthetic circuits that allow researchers to study the basic principles of these epigenetic regulatory systems in mammalian cells, circumventing the potential unknown confounders of natural systems, which could bias results.

Park et al. developed a system that combines modules that establish an epigenetic modification de novo, that recruit effector proteins to mediate a transcriptional output and that spread and maintain the modification — even in the absence of the inducing signal — all in agreement with the current paradigm of epigenetic regulation. The chromatin modification chosen was N6-methyladenine (m6A), a modification not or rarely present on metazoan DNA and therefore ideal to minimize interference with the endogenous systems in the target cells, human embryonic kidney cells (HEK293FT) in this work.

A fusion protein of the *Escherichia coli* DNA adenine methyltransferase (Dam), which deposits m6A at GATC motifs, and an engineered zinc finger protein that is specific for a 20-bp synthetic binding sequence (BS) was designed as the synthetic initiator module (synI). To validate the functionality of synI, two different reporter systems were used: a clustered reporter and an interspersed reporter, both of which were integrated into the HEK293FT genome. The clustered reporter, which was designed to investigate spatial dynamics, consisted of an array of 5 BS repeats followed by a 63-fold GATC array upstream of the gene for destabilized EGFP (d2EGFP), a protein with a reduced half-life relative to GFP. The interspersed design, with intermixed BS and GATC motifs upstream of the reporter gene, was particularly suited for temporal investigations.

Initially, a library of Dam variants was tested to screen for one that showed sufficient activity of m6A deposition at target sites (that is, via BS) without generating high unspecific background methylation. The Dam variant ultimately selected for the synI module (N132A) showed on-target methylation with minimal off-target activity and minimal effects on the endogenous transcriptome, cell cycle and viability of HEK293FT cells.

In the next step, a reader module, synR, was engineered by fusing the m6A reader domain of *Streptococcus pneumoniae* DpnI to various transcriptional effector domains, namely the VP64 activation domain, the KRAB repressive domain and the chromo shadow domain of HP1a. These synR modules induced or repressed transcription according to the endogenous function of the fused transcriptional effector. To achieve more versatile targeting options for the synI module (without the need for the synthetic BS), a CRISPR-guided synI module was generated that mediated methylation of the guide RNA-defined target sites.

Finally, to model the spreading of an epigenetic mark from a nucleation site, a read–write module was constructed, consisting of the m6A reader domain and the Dam writer domain. A small-molecule inducible initiator was brought into cells together with the read–write module, and spatial propagation of m6A was monitored using the clustered reporter. This propagation circuit also enabled confirmation of the principle of epigenetic memory. In the absence of the inducing small molecule, cycling cells showed partially stable expression of the reporter over approximately ten generations.

The researchers envision that in the future this synthetic suite of chromatin regulators could be expanded to include eraser modules and be adapted to study and manipulate genome architecture and dynamically control gene expression.

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ORIGINAL ARTICLE Park, M. et al. Engineering epigenetic regulation using synthetic read-write modules. *Cell* https://doi.org/10.1016/j.cell.2018.11.002 (2019)