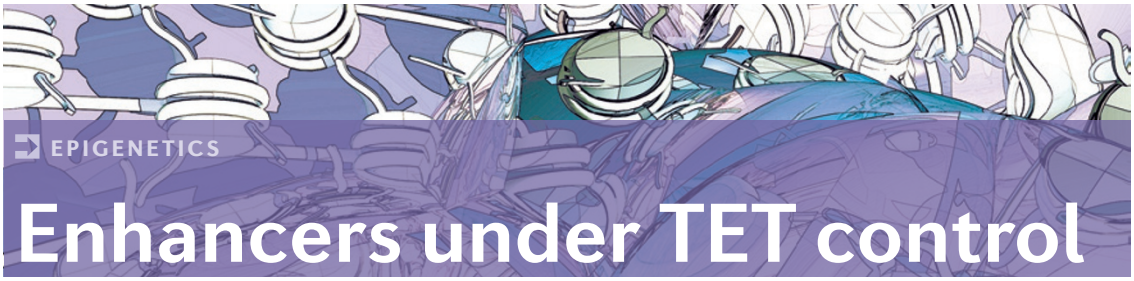

Patrick Morgan/NPG

EPIGENETICS

Enhancers under TET control

DNA methylation at the cytosine C-5 position (5mC) is an epigenetic mark of promoter gene silencing that regulates important biological processes. The establishment and maintenance of 5mC depends on several DNA methyltransferases, whereas the ten-eleven-tanslocation (TET) family of protein dioxygenases initiates active DNA demethylation. TET proteins oxidise 5mC to 5-hydroxymethylcytosine (5hmC), which is further oxidized to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) — 5hmC, 5fC and 5caC are intermediates of DNA demethylation. Although the function of DNA methylation in promoter silencing is well-established, its role in the regulation of enhancer activity is less clear. Moreover, the exact distribution of TET-mediated demethylation across the genome and its physiological function remain to be clarified. By mapping DNA methylation at base-pair resolution, two studies now report that TET-mediated demethylation occurs primarily at enhancer regions, and that it functions to modulate enhancer activity and the gene expression programmes that are important for establishing cell identity and for differentiation.

> “TET-mediated demethylation occurs primarily at enhancer regions”

Lu *et al.* generated mouse embryonic stem (ES) cells deficient for all three TET family members — TET1, TET2 and TET3 — using the CRISPR-Cas9 technology. No self-renewal defects were seen in these triple knockout (TKO) ES cells, which were used for base-resolution DNA methylome and transcriptome analyses. Whole-genome bisulphite sequencing revealed that in TKO ES cells, hypermethylation (resulting from a deficiency in TET-mediated demethylation) occurred in clusters at specific genomic loci with regulatory functions, such as promoters, enhancers and gene bodies, but that the most affected by TET-dependent demethylation were enhancer regions. Hon *et al.* found that TET2 is the major hydroxymethylase in mouse ES cells, as cells in which *Tet2* was deleted lost over 90% of their 5hmC, and report that *Tet2* deletion results in global depletion of 5hmC at promoters, gene bodies, enhancers and sequences bound by the insulator binding protein CCCTC-binding factor (CTCF), as well as in an increase in 5mC at enhancers and other distal-regulatory elements. Thus, both studies report that TET proteins are directly involved in the demethylation of regulatory elements, and in particular of enhancers.

Next, the authors of both studies went on to analyse whether the observed enhancer hypermethylation is connected to enhancer activity. In both *Tet2*-deleted cells and TET TKO cells, active or poised enhancers were extensively hypermethylated. Importantly, the expression levels of their associated genes were substantially reduced following loss of TET activity, indicating that, at least for a subset of enhancers, hypermethylation reduces their activity.

As enhancers are involved in the early steps of cell fate commitment during ES cell differentiation, Hon *et al.* analysed whether the altered epigenetic status seen in the absence of TET affects gene expression during differentiation. They observed that during their differentiation to neural progenitor cells (NPCs), *Tet2*-deleted mouse ES cells exhibited delayed induction of neuronal-specific genes during early differentiation. Moreover, the hypermethylated enhancers and the delayed genes were located in the same genomic topological domains, suggesting that TET proteins mediate enhancer and gene activity during differentiation.

In addition, Lu *et al.* found that TET TKO led to an increased number of cells in the two-cell embryo-like state, which is a transient state required for ES cell long-term maintenance. They further showed that TKO cells have longer telomeres owing to increased sister chromosome telomere exchange.

Together, these studies underscore the importance of TET in regulating enhancer methylation and activity. Further studies are required to elucidate the mechanisms that determine which specific enhancers are targeted and whether similar mechanisms regulate gene expression in somatic cells.

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ORIGINAL RESEARCH PAPERS Lu, F. *et al.* Role of Tet proteins in enhancer activity and telomere elongation. *Genes Dev.* **28**, 2103–2119 (2014) | Hon, G. C. *et al.* 5mC oxidation by Tet2 modulates enhancer activity and timing of transcriptome reprogramming during differentiation. *Mol. Cell* http://dx.doi.org/10.1016/j.molcel.2014.08.026 (2014)

FURTHER READING Pastor, W. A., Aravind, L. & Rao, A. TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. *Nature Reviews Mol. Cell Biol.* **14**, 341–356 (2013)