STEM CELLS

Signalling to DNA methylation

Four groups have investigated the role of DNA methylation in transmitting environmental signals to mouse embryonic stem (ES) cells. The findings suggest that DNA methylation levels may be modulated by the milieu of the cells, including nutritional signals.

Habibi *et al.* and Ficiz *et al.* focused on the changes to DNA methylation that occur in ES cells on culturing in a serum-free medium that contains two small molecule kinase inhibitors (2i) — this is known to create more stable ES cell lines in comparison to ES cells cultured in a serum-containing medium. Using bisulphite sequencing they showed that 2i treatment in mouse ES cells induces genome-wide demethylation. Therefore, treated cells are more hypomethylated — similar to the cells of the inner cell mass of the early embryo — than ES cells derived in serum only. DNA methylation is known to be deposited by the *de novo* methyl transferases DNMT3A and DNMT3B and may also be modified by the hydroxylases TET1 and TET2 to form 5hmC. A more detailed examination of DNA methylation

dynamics showed that, on 2i treatment, methylation decreased in a stepwise manner in demethylated regions and that this was concomitant with hydroxymethylation increase.

Both groups showed that 2i treatment resulted in the downregulation of *Dnmt3a*, *Dnmt3b* and their regulator *Dnmt3l*. Furthermore, Ficiz *et al.* showed that levels of TET2 were increased. These authors then identified a *cis*-regulatory site in the *Dnmt3b* promoter with a *Nanog* and *Prdm14* motif that if deleted reduced the response of *Dnmt3b* to 2i inhibitors. This provides a link to the fibroblast growth factor signalling pathway, as this pathway is known to sustain the expression of *Nanog*. A role for hydroxymethylation in the demethylation process was further implicated by the presence of TET1-binding sites in demethylated regions. Furthermore, Ficiz *et al.* showed that a combination of *Tet1* knockout and *Tet2* knockdown resulted in a substantial reduction in 5hmC and a delay in demethylation on 2i treatment.

The study of Blaschke *et al.* commenced with a serendipitous

observation: that vitamin C induces gene expression changes in ES cells. These authors and Yin *et al.* showed a transient increase in 5hmC levels on treatment with vitamin C in ES cell lines followed by a decrease in methylation levels. These changes were accompanied by an increase in gene expression of ~200 genes, which was consistent with the loss of a silencing mark — however, the expression of TET and DNMT were not affected. In fact, both groups showed that vitamin C is able to directly increase the catalytic activity of TET enzymes, and *Tet* double knockout ES cells show no changes in 5hmC or 5mC and had suppressed gene expression changes in response to vitamin C treatment. Thus, it is the direct modulation of this enzyme that is able to induce the change in DNA methylation.

The ability for DNA methylation to respond through signalling pathways or to nutritional factors opens the gate for other such factors to be identified and, ultimately, manipulated.

Hannah Stower

“The findings suggest that DNA methylation levels may be modulated by the milieu of the cells, including nutritional signals”

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ORIGINAL RESEARCH PAPERS Habibi, E. *et al.* Whole-genome bisulfite sequencing of two distinct interconvertible DNA methylomes of mouse embryonic stem cells. *Cell Stem Cell* <http://dx.doi.org/10.1016/j.stem.2013.06.002> (2013) | Ficiz, G. *et al.* FGF signaling inhibition in ESCs drives rapid genome-wide demethylation to the epigenetic ground state of pluripotency. *Cell Stem Cell* <http://dx.doi.org/10.1016/j.stem.2013.06.004> (2013) | Blaschke, K. *et al.* Vitamin C induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells. *Nature* <http://dx.doi.org/10.1038/nature12362> (2013) | Yin, R. *et al.* Ascorbic acid enhances Tet-mediated 5-methylcytosine oxidation and promotes DNA demethylation in mammals. *J. Am. Chem. Soc.* <http://dx.doi.org/10.1021/ja4028346> (2013)

FURTHER READING Leitch, H. G. *et al.* Naive pluripotency is associated with global DNA hypomethylation. *Nature Struct. Mol. Biol.* **20**, 311–316 (2013)