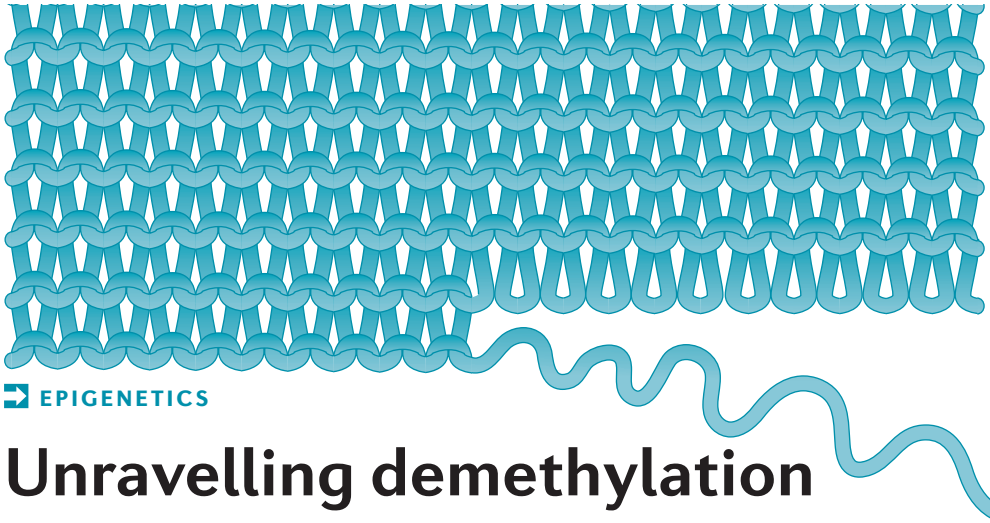

EPIGENETICS

Unravelling demethylation

DNA demethylation is essential to spatially and temporally control gene expression and to overcome gene silencing. However, despite extensive efforts, no consensus mammalian DNA demethylase has been identified. Now, in *Nature*, two studies identify two proteins — activation-induced cytidine deaminase (AID; also known as AICDA) and elongator complex protein 3 (ELP3) — required for DNA demethylation in mammals.

DNA demethylation is crucial for the reprogramming of somatic differentiated nuclei towards a pluripotent state and is known to be a limiting step in the production of induced pluripotent stem (iPS) cells. To identify factors that induce DNA demethylation, Bhutani *et al.* used non-dividing short-term heterokaryons generated by fusing mouse embryonic stem (ES) cells and human fibroblasts. Previous pioneering experiments by this group used heterokaryons to reverse the silent state of genes and reprogramme cells from one differentiated state to another. Heterokaryons complement other reprogramming strategies (nuclear transfer and iPS cells) by enabling mechanistic studies. In heterokaryons, the onset of reprogramming occurs rapidly (within 1 day) and in a large proportion of cells (70%). Using real-time PCR with species-specific primers, the authors find that the human copies of *OCT4* (also known as *POU5F1*) and *NANOG* — two key pluripotency genes — are rapidly activated following cell fusion. DNA methylation typically occurs at CpG dinucleotides in somatic tissues and, importantly, human *OCT4* and *NANOG* activation coincides with CpG demethylation in their promoters.

The mammalian deaminase AID is thought to be specific to lymphocytes and antibody diversification, but it has also been implicated in DNA demethylation in zebrafish. Bhutani *et al.* report that AID knockdown in heterokaryons blocks human *OCT4* and *NANOG* activation and inhibits promoter demethylation. Remarkably, AID binds to highly methylated silent *OCT4* and *NANOG* promoters in human fibroblasts but not in mouse ES cells, in which they are active, suggesting a direct involvement of AID in targeted DNA demethylation during somatic cell reprogramming to pluripotency.

In another study, Okada *et al.* searched for factors responsible for zygotic paternal genome demethylation in mice. Immediately after fertilization, paternal and maternal genomes are reprogrammed for the transition from germ cell to somatic cell transcription programmes, and a key event is the active demethylation of paternal DNA. The authors developed a green fluorescent protein-based probe with high affinity towards non-methyl-CpG, which allows paternal genome demethylation to be monitored. Using small interfering RNA knockdown, they tested the function of a dozen candidate genes — selected on the basis of their expression, structural features and potential demethylation activity — and found that ELP3 knockdown impairs DNA demethylation in paternal pronuclei. ELP3 is one of six subunits of the elongator complex, which is known to have diverse functions, including transcription elongation, exocytosis and cytoplasmic kinase signalling. The knockdown of two other subunits also impairs demethylation, suggesting that zygotic paternal DNA demethylation requires the elongator complex, although its direct activity on DNA remains to be proved.

These studies suggest that AID and elongator complexes are crucial for mammalian DNA demethylation, but the exact molecular mechanisms by which they function have yet to be determined.

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ORIGINAL RESEARCH PAPERS Bhutani, N. *et al.* Reprogramming towards pluripotency requires AID-dependent DNA demethylation. *Nature* 26 Dec 2009 (doi:10.1038/nature08752) | Okada, Y. *et al.* A role for the elongator complex in zygotic paternal genome demethylation. *Nature* 6 Jan 2010 (doi:10.1038/nature08732)