Technology watch

WITHOUT A TRACE

Reprogramming of somatic cells to a pluripotent state, thereby creating induced pluripotent stem (iPS) cells, requires the transient expression of one or more transcription factors. The efficiency of adenoviral and plasmid transfection to deliver reprogramming transcription factor genes is very low, and neither method has been applied successfully to human cells. Three new reports describe the non-viral transfection of mouse and human cells using a transposable element, called *piggyBac* (PB), which carries one or more transcription factor genes and inserts itself into cellular DNA.

The PB system requires inverted terminal repeats that flank the transgene cassette, and transient expression of the transposase enzyme to catalyse insertion and excision events. Efforts led by Woltjen *et al.* and by Kaji *et al.* resulted in the efficient reprogramming of mouse and human embryonic fibroblasts, which expressed characteristic pluripotency markers, indicating a reprogrammed state. This was confirmed by *in vitro* differentiation assays and chimaera development. The reprogramming cassette was efficiently excised from mouse cells by transient expression of transposase or Cre recombinase; yet, the pluripotent state was stably maintained. Guo *et al.* reprogrammed partly specialized mouse cells, called epistem cells, into iPS cells using a single reprogramming factor, KLF4. Following Cre-mediated excision of the *Klf4* transgene, the iPS cells maintained themselves using the endogenous *Klf4* gene, which had been switched on during reprogramming. So, reprogramming factors can be removed without a trace from iPS cells once exogenous expression becomes dispensable.

ORIGINAL RESEARCH PAPERS Woltjen, K. *et al.* *piggyBac* transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature* 1 Mar 2009 (doi:10.1038/nature07863) | Kaji, K. *et al.* Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature* 1 Mar 2009 (doi:10.1038/nature07864) | Guo, G. *et al.* Klf4 reverts developmentally programmed restriction of ground state pluripotency. *Development* **136**, 1063–1069 (2009)

ORIGIN OF BLOOD

The origin of blood cells has been controversial, but a new time-lapse, single-cell imaging approach sheds light on the problem. Eilken *et al.* used mesodermal cells and monitored the development of endothelial cell (EC) colonies followed by the formation of the first blood cells. Single-cell image analysis revealed that haemogenic ECs first express vascular endothelial cadherin, form tight junctions with neighbouring cells, develop endothelial morphology and take up fluorescently labelled acetylated low-density lipoprotein. The cells subsequently lose these EC markers and migrate from their EC colony, morphologically transform into blood cells, express the blood-specific marker CD45 and proliferate as suspension cells. Although the existence of other haemogenic populations is not ruled out, the simultaneous detection of morphology and multiple markers in single-cell imaging studies proved a powerful approach to demonstrate EC-derived blood cell generation.

ORIGINAL RESEARCH PAPER Eilken, H. M. *et al.* Continuous single-cell imaging of blood generation from haemogenic endothelium. *Nature* **457**, 896–900 (2009)