STEM CELLS

A recipe for reprogramming

Japanese researchers have succeeded in reverting differentiated cells to a pluripotent state through the expression of just four genes. This suggests a way of reprogramming adult cells without the need to use material from embryos.

Embryonic stem (ES) cells are able to reprogramme differentiated cells when the two cell types are cultured together. On the basis of previous studies, Takahashi and Yamanaka drew up a list of 24 genes that they suspected might be required for this reprogramming. This list included genes with known roles in ES cell pluripotency, proliferation and maintenance, and other genes that are specifically expressed in ES cells. The authors used a retroviral system to ectopically express these genes in mouse embryonic fibroblasts. To provide an assay for pluripotency of the transduced cells, they placed a gene for resistance to the drug G418 under the control of a promoter that is specifically active in pluripotent cells.

Excitingly, the application of all 24 retroviral vectors together allowed cells to grow in the presence of G418. By expressing different combinations, the authors were able to narrow this down to a set of four genes that together have the same effect. These genes were *Oct3/4*, *Klf4* and *Sox2*, which have known roles in ES cell self-renewal, and *Myc*, which is best known for its role in tumorigenesis. Surprisingly, *Nanog* was not required, although this gene is thought to be a key regulator of the pluripotent state.

How closely do cells that express these four factors resemble ES cells? Microarray expression analysis revealed a transcriptional profile that was similar, but not identical, to that of ES cells. Furthermore, like ES cells, the induced cells formed embryoid bodies in culture and produced teratomas when injected into mice. The induced cells also differentiated to produce all three embryonic germ layers, confirming their pluripotency. Importantly, similar results were achieved on expression of the same four factors in cells that were derived from adult mice.

The authors followed the fate of induced pluripotent cells derived from adult tissue, after injecting them into blastocysts. Although the injected cells contributed to the embryo up to late stages of development, they did not contribute to any of the pups that were born. This indicates that the induced cells do not have all the properties of ES cells, suggesting that reprogramming was incomplete. As well as subtle differences in the gene expression profiles of the induced cells and ES cells, the authors also saw differences in DNA methylation. They suggest that the induced cells might be caught in an intermediate epigenetic state between differentiation and pluripotency.

Understanding why the induced pluripotent cells are different from ES cells promises to provide insights into the reprogramming mechanism. If the approach described by Takahashi and Yamanaka works for human cells, and if full reprogramming can eventually be achieved, this could pave the way to an approach for reverting differentiated adult cells to a pluripotent state without the practical and ethical constraints that are imposed when ES cells are required.

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ORIGINAL RESEARCH PAPER Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–676 (2006)