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STEM CELLS

Introducing the next generation

Wouldn't it be great if differentiated cells could be switched back to their embryonic state? This would avoid the technical complications and ethical objections that are currently associated with cloning. Three studies in *Nature* and *Cell Stem Cell* show that mouse fibroblasts can be reprogrammed to an embryonic stem (ES)-cell state.

Shinya Yamanaka's group previously showed that the expression of four transcription factors in mouse fibroblasts led to pluripotent cells, which were named induced pluripotent stem cells (iPS cells). Although these cells had some characteristics of ES cells — they formed colonies, could proliferate continuously and could form teratomas — they lacked others; for example, they did not produce chimaeras.

Now, the groups of Yamanaka, Rudolf Jaenisch, Konrad Hochedlinger and Kathrin Plath present the second generation of iPS cells. The reprogramming of adult and embryonic mouse fibroblasts to a pluripotent state was induced *in vitro* through ectopic retroviral expression of four transcription factors: OCT4, SOX2, c-MYC and KLF4. But, in contrast to the previous study, the research groups all used a more stringent selection strategy for the isolation of iPS cells — activation of the endogenous genes *Oct4* or *Nanog*, which are both markers of ES-cell pluripotency.

The second generation of iPS cells shares similar patterns of gene expression, chromatin state and DNA methylation with ES cells. Jaenisch and colleagues showed that *Oct4* and *Nanog* were hypomethylated in iPS cells (as in ES cells) and that the bivalent histone modifications of developmental regulators were re-established. Similar to ES cells, iPS cells were resistant to the global demethylation that is induced by inactivation of the DNA methyltransferase DNMT1. The teams of Hochedlinger and Plath analysed female iPS cells. These cells had proper demethylation at the promoters of key pluripotency genes, they reactivated a somatically silenced X chromosome that underwent random X inactivation upon differentiation, and they had a global histone methylation pattern that was almost identical to that of ES cells. Taken together, these data show that transcription-factor-induced reprogramming leads to global reversion of the somatic epigenome into an ES-cell-like state.

iPS cells were also able to form viable chimaeras, could contribute to the germ line and led to the generation of live late-term embryos when injected into tetraploid blastocysts. Although reactivation of the *c-Myc* retroviral transgene can result in tumour formation — tumours developed in 20% of the offspring — Yamanaka and colleagues propose alternative strategies that can be used in clinical applications, such as transient expression or the use of small molecules to replace the four genes.

The improved method, which sees a handful of genes added back to fibroblasts before selection for an ES-cell marker, means that no embryos are harmed in the creation of these ES-cell-like cells. Although this method still needs some tweaking, it offers the prospect of making customized ES cells for patient-specific cell treatment from a simple skin biopsy.

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ORIGINAL RESEARCH PAPERS Okita, K. *et al.* Generation of germline-competent induced pluripotent stem cells. *Nature* 6 June 2007 (doi: 10.1038/nature05934) | Wernig, M. *et al.* *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 6 June 2007 (doi: 10.1038/nature05944) | Maherali, N. *et al.* Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* **1**, 55–70 (2007)