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STEM CELLS

Safeguarding pluripotency

The special abilities of pluripotent cells depend on the expression of a few key regulators that direct a unique transcriptional programme. New work has revised our view of one protein that was previously classed among these master regulators of pluripotency — the transcription factor Nanog. Rather than being essential for stem cell self-renewal, this protein seems instead to safeguard pluripotent cells against premature differentiation. It also has a central role in the production of germ cells.

Previous views of Nanog function focused on reports that loss or a reduced level of this protein causes differentiation. Chambers and colleagues instead asked whether pluripotency persists when Nanog is absent. They generated mouse embryonic stem cell lines that were engineered to express GFP from the endogenous *Nanog* gene, and found that in approximately 20% of these cells GFP expression was absent, although markers of the undifferentiated state persisted. Remarkably, GFP-negative cells could divide and produce colonies containing GFP-positive cells, suggesting that loss of Nanog expression is reversible. However, GFP-negative cells also produced a larger number of differentiated cells than their GFP-positive counterparts. So, cells that transcribe little or no *Nanog* do not necessarily differentiate, but are more inclined to do so.

The authors confirmed their findings in cells that completely lacked Nanog owing to deletion of the gene. The ability to form colonies containing undifferentiated cells remained in the absence of Nanog, although with a larger number of cells differentiating than when it is expressed.

How do these findings apply *in vivo*? Using a system in which deletion of *Nanog* is coupled to simultaneous GFP expression, Chambers and colleagues made chimeric aggregates between *Nanog*$^{-/-}$ cells and wild-type embryos at an early stage of development. By examining GFP expression they showed that *Nanog*$^{-/-}$ cells are present throughout the embryo at E12.5, contributing to a range of cell types. Furthermore, contributions from these cells can be detected after birth. So, Nanog is not needed for cells to contribute to embryonic development, or for pluripotency.

Another important finding from this study relates to Nanog's function in germ-cell development. Nanog is expressed in primordial germ cells (PGCs) at a stage when dramatic epigenetic changes occur. The authors found that *Nanog*$^{-/-}$ cells fail to undergo germ-cell development beyond stage E11.5, suggesting that Nanog is required for PGC progression beyond this stage. In support of this, repairing one *Nanog* allele by homologous recombination restored ongoing PGC development.

The authors compare the function of Nanog in pluripotent cells to a rheostat, rather than a switch-like mechanism: the higher the level of Nanog expression, the less likely cells are to differentiate. The timing of Nanog requirement in PGCs, and of its expression in the early embryo — both of which correlate with widespread alterations in epigenetic marks — led the authors to speculate that Nanog has a fundamental role in setting up cell states at times when the epigenetic regulation of gene expression is in a state of flux.

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ORIGINAL RESEARCH PAPER Chambers, I. *et al.* Nanog safeguards pluripotency and mediates germline development. *Nature* 20/27 December 2007 (doi:10.1038/nature06403)
FURTHER READING Spivakov, M. & Fisher, A. G. Epigenetic signatures of stem-cell identity. *Nature Rev. Genet.* **8**, 263–271 (2007) | Sasaki, H. & Matsui, Y. Epigenetic events in mammalian germ cell development: reprogramming and beyond *Nature Rev. Genet.* **9**, 128–140 (2008)

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