STEM CELLS

Troublesome memories

Thomas P. Zwaka

Methods for generating embryonic-like stem cells have been established. The focus now is on finding ways to coax these cells into matching their natural counterparts as closely as possible, should this be desired.

In F. Scott Fitzgerald's short story 'The Curious Case of Benjamin Button', the protagonist, Benjamin Button, is born with the physical appearance of a 70-year-old man but is then progressively rejuvenated until he reaches an infantile stage characterized by complete memory loss: "There were no troublesome memories in his childish sleep," Scott Fitzgerald tells us. A few years ago, Shinya Yamanaka developed a technique of reprogramming that could produce embryonic-like stem cells from differentiated cells[1]. In many ways, these cells, known as induced pluripotent stem cells (iPSCs), are the cellular equivalents of Benjamin Button. But emerging evidence indicates that they retain certain characteristics that are not typical of embryonic stem cells (ESCs), which may compromise their suitability for use in genetic engineering and regenerative medicine.

Will it be possible — or even necessary — to ensure that the ability of rejuvenated adult cells to differentiate into various cell types, called pluripotency, matches that of authentic ESCs? Three papers[2–4], including those by Kim *et al.*[2] and Ji *et al.*[3] in this issue, address this question.

Kim and colleagues[2] (page 285) compared the two most common methods of cellular reprogramming, somatic-cell nuclear transfer (SCNT) and transcription-factor-based reprogramming — the Yamanaka method — for their effect on the DNA-methylation status and other properties of reprogrammed cells. With the SCNT technique, the nucleus of a somatic (non-germ) cell is transferred into an enucleated but unfertilized, or only recently fertilized, egg[5]. Reprogramming with the Yamanaka method[1] relies on forced expression of four transcription factors in somatic cells: Myc disrupts chromatin (DNA–protein complexes), thereby allowing Oct4 and Sox2 to recognize gene-regulatory regions and restore pluripotency, and Klf4 serves as a cofactor for Oct4 and Sox2 and as an inhibitor of programmed cell death, which might otherwise be induced[6].

The authors[2] compared the ability of ESCs generated by SCNT, blood-derived iPSCs, fibroblast-derived iPSCs and fertilized-embryo-derived (authentic) ESCs to differentiate into the cells of blood (haematopoietic) or bone (osteogenic) lineages. They also assessed the methylation status of these cells' DNA, because the two reprogramming methods both reset this epigenetic modification, which can either repress or activate the expression of key, tissue-specific genes.

Remarkably, the authors found residual signatures of DNA methylation, typical of the somatic tissue of origin, in ESCs generated by SCNT and in iPSCs. This observation was especially striking in iPSCs, with the epigenetic memory of SCNT-generated ESCs being much more akin to that of authentic ESCs (Fig. 1). These differences were also seen in tests of differentiation potential, with the fibroblast- and blood-derived iPSCs showing clear propensities to differentiate into their cells of origin — osteogenic and haematopoietic cells, respectively. The authors conclude that SCNT is more effective than transcription-factor-based reprogramming at establishing 'ground-state' pluripotency in somatic cells.

In a similar study, Polo *et al.*[4] generated iPSCs from lymphocytes, granulocytes, muscle cells and fibroblasts, and — like Kim *et al.* — found that the resultant iPSCs varied in their differentiation potential, which reflected their cells of origin. The authors also noted that changes in the transcriptional activity of iPSCs were distinguishable by the cell type of origin and correlated with different methylation levels of the chromatin-associated histone proteins. The two studies[2,4] underscore the problem of residual epigenetic memory in attempts to reprogram somatic cells using transcription factors.

Ji *et al.*[3] (page 338) addressed this issue directly by determining the importance of specific DNA-methylation marks in the developmental progression of particular cell lineages. They studied overall methylation patterns of DNA repeats known as CpG islands, which are particularly rich in methyl groups, using a sophisticated analytical tool (CHARM), and conclude that very specific DNA-methylation patterns create an epigenetic map of lineage identity.

Indeed, changes in DNA methylation seemed to be one of the principal factors driving key lineage decisions, such as commitment to myeloid versus lymphoid blood-cell lineages. Although much remains to be learned about the mechanisms of DNA methylation and how to manipulate them towards specific cell fates, Ji and colleagues' work provides a useful blueprint for collecting and analysing the epigenetic information that characterizes specific cell lineages.

It is noteworthy that, although the iPSCs that Kim *et al.*[2] and Polo *et al.*[4] describe retain the epigenetic memory of their tissues of origin, they seem entirely compatible with ESCs by most other criteria (Fig. 1). This implies that, in somatic cells, manipulation with transcription factors can stably establish the ESC phenotype, even though specific subsets of genes that govern developmental decisions remain resistant to reprogramming. In other words, despite their persistence, the residual genetic and epigenetic programs reported by these authors do not seem to impinge on the network

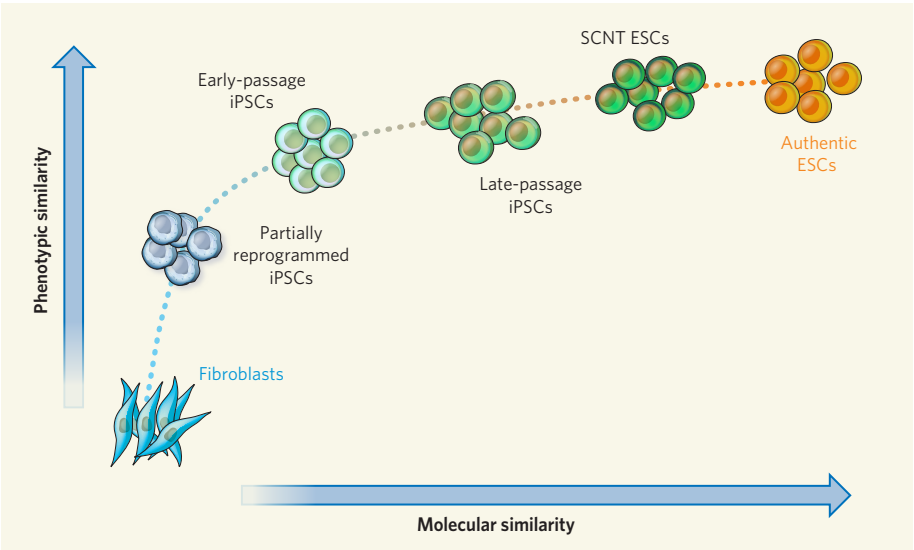

Figure 1 | An embryonic-stem-cell beauty contest. Kim *et al.*[2] used different methods and starting materials to generate different sets of stem-cell populations. They then assessed the similarities, both phenotypic (morphology, growth and ability to differentiate) and molecular (epigenetic modifications and gene-transcription patterns), of the reprogrammed stem cells to differentiated cells — fibroblasts — and to authentic embryonic stem cells (ESCs). Although the authors generated all the induced pluripotent stem cells (iPSCs) by the Yamanaka method, the cells were reprogrammed to different extents by being subcultured for different lengths of time. Of all the reprogrammed cells, ESCs generated by somatic-cell nuclear transfer (SCNT) most closely resembled authentic ESCs. In general, it seems that an ESC-like phenotype is achieved much more readily than an ESC-like molecular state.

ORIGINS OF LIFE

Shock synthesis

When asteroids or comets smash into Earth, they form craters such as the one at Gosses Bluff, Australia (pictured). But could such an impact have kick-started life on our planet? Nir Goldman and colleagues have modelled what happens inside cometary ice when it smashes into a planet (N. Goldman *et al. Nature Chem.* doi:10.1038/nchem.827; 2010). They find that, under certain circumstances, complexes form that can act as precursors to glycine, the simplest amino acid.

The idea that comets delivered organic molecules to the early Earth is disputed, because the extreme heat generated by the impact would probably have incinerated such a cargo. An alternative theory is that the heat and pressure of cometary impacts could have caused material on Earth's surface to react to form organic compounds. But the early Earth's chemical environment isn't thought to have been conducive to such reactions.

Impacts in which Earth was struck glancingly, however, would have generated much lower temperatures within cometary ice, allowing organic matter to survive. Goldman *et al.* therefore used quantum molecular dynamics to simulate events inside cometary ice during such collisions.

The authors modelled a mixture of water, methanol, ammonia, carbon monoxide and carbon dioxide under conditions of shock compression, and found that many products were formed — including oligomers that contained the carbon–nitrogen bonds required for amino acids. Subsequent quenching of the model system to lower pressures and temperatures broke the complexes into smaller fragments, including a glycine–CO_2 complex.

Of course, we may never know how life began on Earth. But Goldman *et al.* calculate that the probability that any particular cometary impact will generate shock waves of the size modelled is 17% — well within the realms of possibility.

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S. ALVAREZ/NATL GEOGR. STOCK

of genes essential to achieving the ground state of pluripotency. What, then, are the prospects for resetting the differentiation propensity of iPSCs? The results of both studies suggest that this could be achieved by either repeated reprogramming or continuous subculturing (passaging) of iPSCs — or perhaps by using demethylating drugs.

These findings[2–4] are a reminder that our understanding of the pluripotent state is still primitive, and that the mechanisms of both transcriptional and epigenetic control of reprogramming should be explored further. They also indicate that not all iPSC lines will be equal in their differentiation capacity, regardless of the care taken to ensure faithful reiteration of the ground state. These differences may simply reflect a series of glitches in the reprogramming protocol, suggesting that many more iPSCs could potentially be coaxed to follow a trajectory towards classical ESC pluripotency. If the goal of somatic-cell modification is to achieve a fully naive ground state of pluripotency, then SCNT may be preferable to transcription-factor-based reprogramming. But if differentiation to a particular cell lineage is desired, iPSC lines biased towards that lineage could be used, especially if the lineage is difficult to obtain using authentic ESCs.

The three papers[2–4] also compel us to look more carefully into reports documenting transcription-factor-driven direct reprogramming of one somatic cell into another — for instance, exocrine pancreatic cells into β-cells[7], or fibroblasts into neurons[8] or heart muscle[9]. Thus, retention of epigenetic memory may have a more significant impact on the cellular characteristics than is currently appreciated. Finally, it has been suggested that at least some somatic-cell lineages are modified at the DNA level (for example, by transposons)[10], hinting that the memory of DNA methylation may extend to DNA sequences in iPSCs obtained from such cells.

Ultimately, it seems as though we have different reprogramming tools on hand that produce slightly different pluripotent stem cells. Rather than asking which of these tools is likely to yield superior results, the focus should be on the most appropriate application for each method. It must be kept in mind, however, that authentic ESCs remain the gold standard against which all reprogramming technologies must be judged. ■

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EARTH SCIENCE

Glaciers shield mountain tops

Jean Braun

Glaciers frozen to bedrock may have protected the southernmost Andes from erosion, providing an explanation for the mountains' topography and fresh constraints on possible links between climate and tectonics.

Most geologists would agree that mountain glaciers, which appeared some 3 million to 5 million years ago in response to Earth's slowly cooling climate, are responsible for erosionally shaping most mountains into their jagged present-day morphology. However, an extensive data set collected by Thomson and co-authors (page 313 of this issue[1]) provides evidence that glaciers have protected rather than eroded the high-relief regions of southern Patagonia, which has led to a widening of the mountain belt during these geologically recent glaciations. This evidence supports the theoretical concept that climate, through erosion, or lack of it, affects the shape and dynamics of mountain belts.

It has long been recognized that Earth's upper crust behaves mechanically like a thick pile of dry sand — that is, as a frictional material in which strength, or resistance to deformation,