STEM CELLS

Transdifferentiation from the top

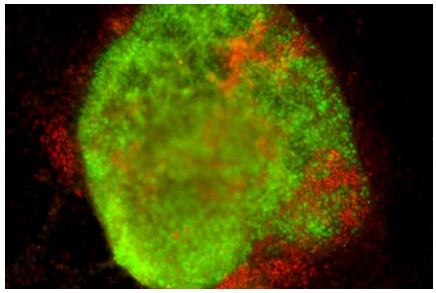
Fibroblasts pass through a pluripotent state when undergoing transdifferentiation in the presence of pluripotency factors.

Conrad Waddington famously visualized development as a landscape in which a young cell differentiates much as gravity acts on a ball, leading it down forked canals that represent alternate lineages. A young cell could as easily be a skier choosing between slopes while racing downhill. The stunning discovery of Shinya Yamanaka and others—that somatic cells can acquire a pluripotent or undifferentiated state once researchers activate four transcription factors—added a gravity-defeating ski lift to return skiers to the mountaintop. A body of work has also shown that expressing certain lineage factors can cause differentiated cells to swap fates, like snowmobiles ferrying skiers between slope termini—a process known as transdifferentiation, or direct conversion.

Some direct conversions happen more readily than others. More recently, scientists discovered that exposing skin cells to various differentiation conditions following a brief pulse of the four Yamanaka factors (abbreviated as OKSM) enabled the cells to rapidly assume a variety of fates. The prevailing theory was that these cells bypass pluripotency, instead entering a plastic intermediate state that enables facile conversions.

The idea of a nonpluripotent plastic state piqued the interest of Konrad Hochedlinger at Harvard University and Jacob Hanna, Rada Massarwa and Noa Novershtern at the Weizmann Institute. "How is it possible that the same transcription factors induce such different cell identities or cell fates, simply by changing the extracellular milieu or environment?" Hochedlinger recalls asking. The word 'plasticity' itself has a malleable definition. "What is that state, and how can we track it?" asked Hanna. "Is there a molecular consequence of bypassing pluripotency?"

Both groups reproduced OKSM transdifferentiation experiments to generate induced neural stem cells (iNSCs) and



O. Bar-Nur

Neural stem cells (red) derived from female skin cells using a transdifferentiation protocol with Yamanaka factors first pass through a pluripotent stem cell state (green, indicating X-chromosome reactivation).

cardiomyocytes (iCMs) from mouse fibroblasts but soon began questioning the mechanism of conversion. Hochedlinger's group detected cells that express the Oct4-GFP pluripotency marker at low frequency in cultures fated to become iNSCs or iCMs (Bar-Nur *et al.*, 2015). Replating these cells in embryonic stem cell medium confirmed that they were true induced pluripotent stem cells that could be expanded in culture and form germline-transmitting chimeric mice.

For Hanna, an important part of the discussion is the quality of a reprogrammed cell type, or how closely it resembles its native counterpart in the organism. "We suspected that there might be some pluripotency because the quality of the [transdifferentiated] cells was so high," he says. Researchers in his lab also noticed the expression of pluripotency markers Nanog-GFP and Oct4-GFP in some cells during OKSM transdifferentiation, which they subsequently showed behave like pluripotent stem cells (Maza *et al.*, 2015).

These initial experiments did not address what fraction of transdifferentiated cells had passed through a pluripotent stage. The teams turned to classical lineage-tracing strategies in which the expression of Nanog (Hanna) or Oct4 (Hochedlinger) would result in permanent activation of a fluorescent reporter based on inducing Cre recombinase. The results were striking. "Essentially all of the induced neural stem cells were fluorescently labeled," says Hochedlinger, and the other team found that 80–100% of clones passed through this state.

Epigenetic lineage tracing showed similar hallmarks of pluripotency: Hochedlinger's group observed robust silencing of an integrated retroviral reporter, Hanna's group detected global demethylation, and both teams found that female somatic cells stably reactivated silent X chromosomes.

The results support the ski-lift function of Yamanaka factors in the alpine model, even under suboptimal reprogramming conditions. Hanna and Hochedlinger stress that their observations are restricted to the conversions that they tested in mouse cells, though they expect the findings to generalize to any OKSM transdifferentiation scenario. Neither group detected pluripotent states in non-OKSM conversion, but they leave the possibility open, especially for slower conversions, and encourage the use of lineage tracing to study fate transitions.

There may be ramifications for therapy; "one has to exclude the possibility that you still may have…rare pluripotent cells that could give you a teratoma," says Hochedlinger. But both researchers are quick to point out that the OKSM protocols are very valuable. Hanna goes a step further to suggest that reprogrammed cells lacking any epigenetic 'baggage' from a prior cell fate will make the highest-quality cells for therapy; there may be molecular consequences for bypassing pluripotency during direct transdifferentiation, bearing a closer look.

Tal Nawy

RESEARCH ARTICLES

Bar-Nur, O. *et al.* Lineage conversion induced by pluripotency factors involves transient passage through an iPSC stage. *Nat. Biotechnol.* **33**,761–768 (2015).

Maza, I. *et al.* Transient acquisition of pluripotency during somatic cell transdifferentiation with iPSC reprogramming factors. *Nat. Biotechnol.* **33**,769–774 (2015).