Cell

Speeding to Pluripotency

Josh G. Chenoweth[1] and Ronald D. McKay[1,*]
[1]Lieber Institute for Brain Development, 855 N. Wolfe Street, Baltimore, MD 21205 USA
*Correspondence: ronald.mckay@libd.org
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Finding a cell that reprograms in a nonstochastic manner without genetic manipulation has proven elusive. In this issue, Guo et al. report the identification of a cell defined by an ultrafast cycle whose progeny reprogram in a synchronous and rapid manner.

The discovery of induced pluripotency has revealed the remarkable plasticity of differentiated cells. However, the inefficiency of this process has presented considerable barriers to defining the rules of reprogramming. Competing hypotheses have been developed to account for bottlenecks. An elite model proposes that only a subset of determined founder cells exist within a population and have the potential to yield reprogrammed progeny. A stochastic model allows for all cells within a population to initiate reprogramming, albeit with few successful events. Genetic strategies have been used to identify roadblocks that inhibit deterministic reprogramming. Recently, it has been shown that depletion of the nucleosome and remodeling deacetylase (NuRD) complex member Mbd3 allows for near 100% reprogramming efficiency (Rais et al., 2013). In this issue, Guo and colleagues apply a different approach to this problem. Using in vitro live-cell imaging of clonal granulocyte monocyte progenitor (GMP) lineages, they identify a "privileged" cell that is not bound by intrinsic reprogramming bottlenecks (Guo et al., 2014).

Previous studies by this group established the technical capacity to image reprogramming at minute intervals using GMPs from mice that carry an *Oct4:GFP* allele (Megyola et al., 2013). The authors now use this technology to search for a cell that can be reprogrammed by a deterministic mechanism. The ideal properties of this cell are progeny that synchronously transition to the pluripotent state with a short latent period following Yamanaka factor induction. Amazingly, the reconstruction of complete GMP lineages from successful reprogramming events reveals a subset of cells with these properties. The privileged cells and all of their progeny adopt the pluripotent state within 48 hr of transgene expression. The high-content data yielded by cell imaging of the somatic GMPs revealed a striking phenotype. The cycle time of the first division in the privileged founders was an ultrafast 8 hr, significantly shorter than the derivative pluripotent cells themselves (Figure 1).

To extend their finding, the authors show that the ultrafast cycle can be induced by cytokine stimulation of the relatively slow cycling $Lin^{-}Kit^{+}Sca^{+}$ hematopoietic stem cell, resulting in privileged reprogramming. Surprisingly, Guo et al. also show that the ultrafast phenotype is a function of Yamanaka factor expression in mouse embryonic fibroblasts (MEFs). This is consistent with a previous study using live-cell imaging of reprogramming MEFs that reveals a rapid increase in proliferation rates of founders (Smith et al., 2010). Inhibition of the p53 tumor suppressor in clonal B cells also promotes proliferation and enhances reprogramming (Hanna et al., 2009). Guo et al. argue that depletion of p53 in MEFs promotes the emergence of the ultrafast cycling population and that this specifically accounts for the increased efficiency of reprogramming. Rapid rates of proliferation have been positively correlated with reprogramming before. However, the key finding reported here is that you can reprogram somatic cells by a deterministic mechanism independent of any other genetic manipulation.

The hematopoietic lineage has been exploited previously to show that primitive cells reprogram more efficiently than their differentiated progeny (Eminli et al., 2009). This is consistent with epigenetic models of development in which cell identity is increasingly restricted by changes in chromatin status. These new results show that the privileged cell is not the hematopoietic stem cell (HSC); rather, it is a restricted progenitor. Molecular characterization reveals elevated levels of the cell-cycle inhibitor Cdkn1c (p57) in the slow cycling HSCs versus the GMP cells. Reduction of p57 levels specifically leads to an increase in HSC reprogramming efficiencies. These results are consistent with other recent results linking the action of cell-fate regulators to cell-cycle

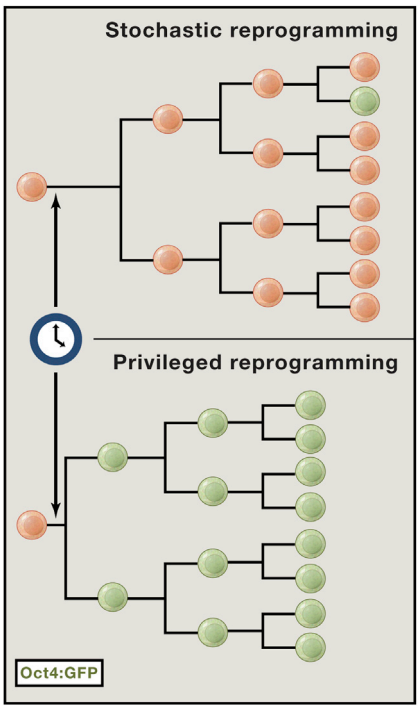

Figure 1. Privileged Reprogramming in Granulocyte Monocyte Progenitor Lineages
Induced pluripotency is nonstochastic in a subset of isolated granulocyte monocyte progenitors (GMPs) that are recognized by an ultrafast cell cycle in the founder cell. The ultrafast cycle is independent of Yamanaka factor expression or genetic manipulation, and nearly all of the privileged cell's progeny will express Oct4:GFP.

inhibitors. A deeper understanding of the way that cell-cycle inhibitors are controlled in somatic lineages may have important implications in regenerative medicine.

The restoration of function in diseased tissue is the major goal of regenerative medicine. There is significant interest in leveraging the rules of cellular reprogramming to inform clinical strategies. To regulate homeostasis, resident somatic stem and progenitor cells must maintain tissue for the life of the organism while allowing response to acute injury. In the gut epithelium, differentiated cells are constantly replaced by progenitors in the intestinal crypts. Distinct lineages (secretory and enterocyte) are specified by Δ-like ligand mediated lateral inhibition. Strikingly, the chromatin of all progenitor cells in the crypt, including the $Lgr5^+$ stem cell, is very similar and broadly permissive based on enhancer mapping (Kim et al., 2014). This design allows for Notch-based cell interactions and specific transcription factors to efficiently specify fates without the requirement for dramatic epigenetic changes. In another experiment, individual Yamanaka factors have different effects in the intestinal crypt. Whole-animal overexpression of Sox2 targets the $Lgr5^+$ cell in the gut, inducing expansion of the entire crypt, whereas Oct4 overexpression only expands the transit-amplifying cell (Kuzmichev et al., 2012). Consistent with a profound role for Sox2 in specifying cell identity, activation of the endogenous gene is associated with a late deterministic phase of reprogramming (Buganim et al., 2012). It will be interesting to see how the different Yamanaka factors act in the privileged cell reported by Guo et al.

The results presented by Guo et al. force us to consider the behaviors of different cells in a lineage. Recent work shows that oncogenic Nras increases competiveness through a bimodal effect on early hematopoietic precursor cells, increasing the self-renewal potential of one subset while increasing the proliferation of another (Li et al., 2013). This occurs through a Stat5-dependent signaling mechanism, but we don't know exactly how differences in this early cellular compartment generate these two outcomes. These results remind us that watching the behavior of cells in the hematopoietic lineage can reveal powerful new biological insights.

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