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Epigenetic reprogramming: Prdm14 hits the accelerator

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The release of epigenetic boundaries during epigenetic reprogramming is poorly understood. In the recent issue of *Cell Stem Cell Journal*, Gillich and colleagues identify a unique role for Prdm14 in the acceleration of this process (Gillich *et al*, 2012).

Pluripotent stem cells can be established from pre-implantation blastocyst embryos (embryonic stem cells, ESCs) as well as from the post-implantation epiblast stem cells (EpiSCs; Chenoweth *et al*, 2010). Murine ESCs and EpiSCs both express central pluripotency factors such as Oct4, Nanog and Sox2, yet the different developmental origins of these two cell types is clearly reflected in their molecular, epigenetic and functional properties. Murine ESCs appear to exist in a unique 'naive' state reminiscent of the pre-implantation epiblast. They are characterized by the expression of germ cell–related genes, a remarkably open chromatin structure with two active X chromosomes, and the functional ability to contribute to chimera formation upon blastocyst complementation (Nichols and Smith, 2011). In contrast, EpiSCs reflect the properties of the post-implantation epiblast, characterized by low-level expression of early determinants of somatic differentiation, a near-absence of germ cell gene expression, inactivation of one of the X chromosomes and negligible ability to support the development of chimeric mice. The conversion of primed to naive pluripotent state requires the release of epigenetic restrictions that are established in the post-implantation epiblast. It is thus a reprogramming process akin to the derivation of induced pluripotent stem cells (iPSCs) from somatic cells. The results on Prdm14 from Gillich and colleagues offer new insights into the underlying molecular mechanisms governing epigenetic reprogramming.

Interconvertible pluripotent states

Murine ESCs convert into EpiSCs upon substitution of the growth factor leukaemia inhibitory factor (LIF) for EPiSC growth factors, bFGF and ActivinA (Figure 1). Conversely, EpiSCs can revert to naive state and generate so-called reverted ESCs (rESCs) through prolonged culture in the presence of LIF (Bao *et al*, 2009). Yet this spontaneous, growth factor–induced reprogramming of EpiSCs is inefficient and occurs at a frequency of approximately 1 in 10^6 over a period of 2–4 weeks. LIF signalling was shown by Yang *et al* (2010) to play an active role in the conversion of EpiSCs to rESCs; they demonstrated that LIF-induced activation of Stat3 signalling is a prerequisite for

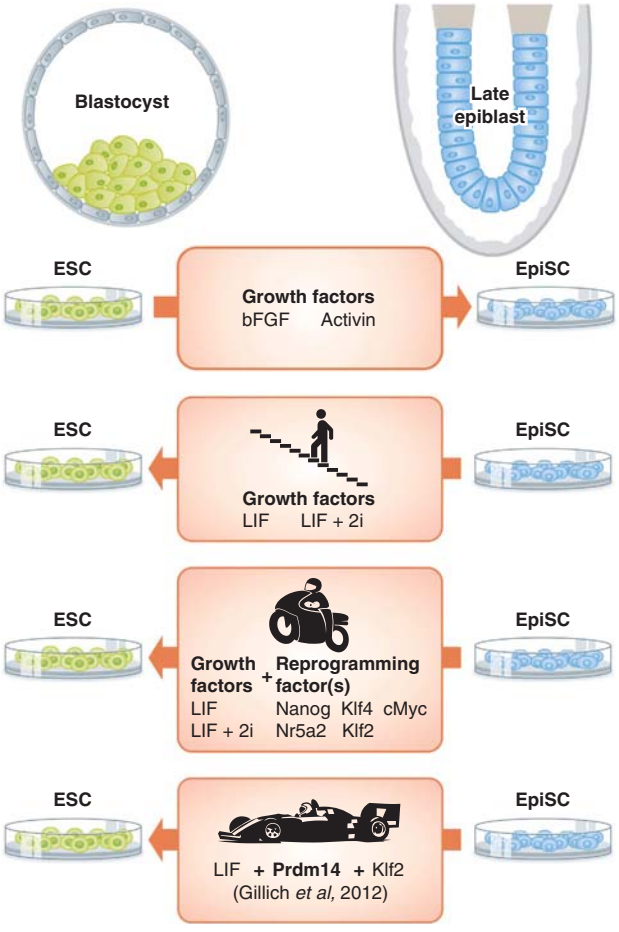

Figure 1 Interconvertible pluripotent states. Schematic representation of the molecular events that govern the conversions of 'naive' ESCs to 'primed' EpiSCs and vice versa. The conversion of ESCs to EpiSCs is a relatively efficient process that is induced when the ESC self-renewal factor LIF is substituted for the EpiSC maintenance factors bFGF and ActivinA. Conversely, EpiSCs can spontaneously revert to an ESC-like cell upon prolonged culture in LIF or LIF + 2i conditions, but this process is highly inefficient and takes several weeks to complete. Addition of reprogramming factors (single factors or combinations of two) enhances both the efficiency and the rate of EpiSC to ESC conversion. In the recent issue of *Cell Stem Cell Journal*, Gillich and colleagues demonstrate that the combination of Klf2 and Prdm14 uniquely accelerates the EpiSc to ESC conversion, shortening the reprogramming process to 3–4 days (for references see the text).

successful EpiSC to rESC conversion. LIF–Stat3 signalling acts upstream of several key pluripotency genes, including Klf4 and Nanog, which are known to enhance epigenetic reprogramming and iPSC formation. Similarly, the EpiSC to rESC conversion is greatly enhanced by the ectopic expression of pluripotency factors Klf4, cMyc, Nanog or Nr5A2 (Silva *et al*, 2008; Guo *et al*, 2009; Hanna *et al*, 2009; Guo and Smith 2011), yielding ESC-like colonies at a frequency of 0.1–1% in about 8–10 days.

In the recent study, Gillich and co-workers demonstrate that Prdm14 can greatly accelerate the EpiSC reprogramming process, but only when expressed in combination with Klf2. Individually these factors only modestly affect EpiSC conversion: Klf2 seems to display similar effects as ectopic expression of Klf4 or Nanog, and primarily enhances the frequency of the reprogramming events; Prdm14 alone does not affect EpiSC to rESC conversion. Together, however, these factors demonstrate a strong synergetic effect, reducing the reprogramming window to 3–4 days. The Prdm14–Klf2 combination uniquely induces a subset of ESC-related genes that are not, or only weakly upregulated by the individual factors, suggesting that the combination of these two factors makes the pluripotency network more robust. Indeed, the authors demonstrate that Prdm14 enhances the recruitment of Klf2 to essential pluripotency loci, including the distal Oct4 enhancer, which specifically drives Oct4 expression in ESCs.

Germ cell factors in epigenetic reprogramming

Prdm14 is a key regulator of mammalian germ cell development (Yamaji *et al*, 2008). In fact, the expression of many germ cell–related genes is a hallmark property of naive pluripotent stem cells (Tesar *et al*, 2007), and the striking similarities between ESCs and PGCs have prompted the idea that these two cell types may be ontologically linked. Recent genetic marker experiments by Chu *et al* (2011) demonstrated that during the derivation of ESCs, cells in the inner cell mass transiently express Blimp1 (Prdm1), the earliest known marker of PGC specification (Ohinata *et al*, 2005). These data suggest that ESCs may be derived from an early PGC precursor that arises in the early epiblast cells *in vitro*, upon plating of the blastocyst. However, transition through a Blimp1-positive state was not an absolute requirement for ESC derivation, since many ESCs derived in the presence of so-called '2i + LIF' conditions were not labelled by the Blimp1 genetic marker. This indicates that the 2i inhibitor cocktail activates a genetic programme allowing direct ESC derivation directly from the pre-implantation epiblast. Interestingly, in this study, Gillich and colleagues demonstrate that the transcriptional changes induced by ectopic expression of Prdm14–Klf2 are similar to those induced by 2i culture conditions. Prdm14–Klf2 efficiently allows the conversion of Blimp1-knockout EpiSCs to rESCs demonstrating that, similar to ESC derivation in the presence of 2i inhibitors, the Prdm14–Klf2-induced EpiSC to ESC conversion does not involve an obligatory transition through a Blimp1-positive state. Blimp1 interacts with Prdm14 during early PGC specification (Ancelin *et al*, 2006), and it is possible that these two factors have redundant activities during the establishment of the naive pluripotent state, while Blimp1 has an additional specific function during early PGC specification. Indeed, PGC formation is not entirely abrogated in Blimp1-knockout mice, but the specified PGCs demonstrate severe proliferation defects. The advances reported by Gillich and colleagues have shed new light on the complex molecular pathways that govern epigenetic reprogramming and establishment of naive pluripotency. Further exploration of the mechanism by which these factors synergize to enhance reprogramming and pluripotency may help us understand how this unique stem cell state is maintained in pluripotent stem cells of other species, including man.

Conflict of interest

The author declares that he has no conflict of interest.

References

Ancelin K, Lange UC, Hajkova P, Schneider R, Bannister AJ, Kouzarides T, Surani MA (2006) Blimp1 associates with Prmt5 and directs histone arginine methylation in mouse germ cells. *Nat Cell Biol* **8:** 623–630

Bao S, Tang F, Li X, Hayashi K, Gillich A, Lao K, Surani MA (2009) Epigenetic reversion of post-implantation epiblast to pluripotent embryonic stem cells. *Nature* **461:** 1292–1295

Chenoweth JG, McKay RD, Tesar PJ (2010) Epiblast stem cells contribute new insight into pluripotency and gastrulation. *Dev Growth Differ* **52:** 293–301

Chu LF, Surani MA, Jaenisch R, Zwaka TP (2011) Blimp1 expression predicts embryonic stem cell development in vitro. *Curr Biol* **21:** 1759–1765

Gillich A, Bao S, Grabole N, Hayashi K, Trotter MWB, Pasque V, Magnúsdóttir E, Surani M (2012) A epiblast stem cell-based system reveals reprogramming synergy of germline factors. *Cell Stem Cell* **10:** 425–439

Guo G, Smith A (2011) A genome-wide screen in EpiSCs identifies Nr5a nuclear receptors as potent inducers of ground state pluripotency. *Development* **137:** 3185–3192

Guo G, Yang J, Nichols J, Hall JS, Eyres I, Mansfield W, Smith A (2009) Klf4 reverts developmentally programmed restriction of ground state pluripotency. *Development* **136:** 1063–1069

Hanna J, Markoulaki S, Mitalipova M, Cheng AW, Cassady JP, Staerk J, Carey BW, Lengner CJ, Foreman R, Love J, Gao Q, Kim J, Jaenisch R (2009) Metastable pluripotent states in NOD-mouse-derived ESCs. *Cell Stem Cell* **4:** 513–524

Nichols J, Smith A (2011) The origin and identity of embryonic stem cells. *Development* **138:** 3–8

Ohinata Y, Payer B, O'Carroll D, Ancelin K, Ono Y, Sano M, Barton SC, Obukhanych T, Nussenzweig M, Tarakhovsky A, Saitou M, Surani MA (2005) Blimp1 is a critical determinant of the germ cell lineage in mice. *Nature* **436:** 207–213

Silva J, Barrandon O, Nichols J, Kawaguchi J, Theunissen TW, Smith A (2008) Promotion of reprogramming to ground state pluripotency by signal inhibition. *PLoS Biol* **6:** e253

Tesar PJ, Chenoweth JG, Brook FA, Davies TJ, Evans EP, Mack DL, Gardner RL, McKay RD (2007) New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature* **448:** 196–199

Yamaji M, Seki Y, Kurimoto K, Yabuta Y, Yuasa M, Shigeta M, Yamanaka K, Ohinata Y, Saitou M (2008) Critical function of Prdm14 for the establishment of the germ cell lineage in mice. *Nat Genet* **40:** 1016–1022

Yang J, van Oosten AL, Theunissen TW, Guo G, Silva JC, Smith A (2010) Stat3 activation is limiting for reprogramming to ground state pluripotency. *Cell Stem Cell* **7:** 319–328

