A liaison between intrinsic and extrinsic regulators of pluripotency

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A pluripotent cell state is regulated by complex transcription factor network under the control of extrinsic signals, so that undifferentiated cells can readily commit to any cell lineages in response to appropriate stimuli. A number of recent studies using pluripotent stem cells *in vitro* have shown that maintenance of pluripotency can be achieved with different combinations of extrinsic factors, yet by activating common intrinsic regulators. Two papers in this issue of *The EMBO Journal* identified Tfc2l1 (also known as Crtr-1 or Tfc2l1) as a factor on which activating and repressing stimuli for pluripotency converge. The results provide insight into how intrinsic pluripotency regulatory network is stably organized downstream of diverse inputs.

Pluripotent stem cells in early embryos are transient cell populations that exist in the epiblast of the peri-implantation embryos, but can be captured *in vitro* as embryonic stem cells (ESCs). ESCs self-renew indefinitely while keeping the ability to differentiate into any cell lineages. Maintenance of self-renewal in ESCs depends on self-organizing transcriptional regulatory network, in which continuous inputs from extrinsic cues are required to sustain an intrinsic transcription factor (TF) circuitry. The most critical extrinsic regulators are leukaemia inhibitory factor (LIF) and its downstream effector Stat3. Although loss of zygotic Stat3 does not affect the embryonic development before gastrulation, a recent analysis of embryos lacking both maternal and zygotic Stat3 clearly revealed its indispensable role in the specification of the epiblast lineage around implantation (Do *et al*, 2013). However, consistent with other studies showing that lineage identities in early embryos are progressively consolidated by the interplay between intrinsic and extrinsic regulators, *Stat3*-null embryos have a defect in maintaining the epiblast identity after initial apparent segregation of this lineage. Numerous TFs are implicated in pluripotency in ESCs, with only a few absolutely required for pluripotency *in vivo*. Such core pluripotency determinants include Oct3/4, Sox2 and Nanog, while the function of Nanog in the maintenance of self-renewal seems auxiliary. Considering the strong dependence of pluripotent network on these core TFs, their expression should be robustly maintained. Indeed, they form a mutually regulated TF network together with numerous ancillary transcriptional

regulators, in which positive and negative feedback loops are supposed to be dedicated to buffering fluctuations while keeping sensitivity to differentiation cues. Nonetheless, the expression level of pluripotency genes are somehow variable in individual ESCs (Faddah *et al*, 2013), which would be due to both intrinsic fluctuations arising from network regulation and negative inputs from counteracting signals. MEK–ERK signalling and a transcriptional repressor Tcf7l1 (also known as Tcf3) are the most detrimental components for self-renewal of ESCs. Simultaneous blockage of these factors using inhibitors of MEK and GSK3 (2i) can sustain self-renewal in serum-free medium even in the absence of Stat3 (Ying *et al*, 2008). Importantly, Stat3, MEKi and GSK3i have additive

Figure 1 Maintenance of self-renewal in pluripotent ESCs by the interplay between intrinsic and extrinsic regulators. Self-renewal depends on the sustainability of self-organizing transcriptional regulatory network. Ground state TF circuitry embraces the inherent vulnerability. Induction of Fgf4 by the pluripotency core activates ERK signalling, which in turn restricts the expression of pluripotency genes. A transcriptional repressor Tcf7l1, which is robustly expressed in ESCs, frequently occupies the genomic regions together with Oct3/4/Sox2/Nanog, allowing for Tcf7l1 to serve as a specific limiter of pluripotency. In the presence of these detrimental components, continuous inputs from LIF signalling are required to sustain the TF circuitry. LIF/Stat3, MEKi and GSK3i additively stabilize the pluripotency circuitry via activation of partially overlapping sets of TFs (only critical targets are shown).

effects on facilitating self-renewal, in which any combinations of two are minimally sufficient (Wray *et al.*, 2010), suggesting partially redundant roles of these independent signalling in stabilizing pluripotency (Figure 1). The details of the interface between intrinsic and extrinsic regulators, however, are poorly understood, especially how the different signalling cues drive the common pluripotency core. Two new papers in *The EMBO Journal* (Martello *et al.*, 2013; Ye *et al.*, 2013) attempt to answer this question by characterizing a pleiotropic TF Tfcp2l1 mediating pluripotency-promoting effects of Stat3 and 2i.

To understand how Stat3 contributes to transcriptional control of pluripotency, Martello *et al.* (2013) utilized *Stat3*-null ESCs, which exhibit a gene expression profile and differentiation capacity comparable to wild-type ESCs when maintained in 2i. Using an RNA-seq approach, the authors identified 183 genes that were induced as early as 1 h after LIF stimulation only in the presence of Stat3. After narrowing down the list of candidate genes, the authors focused on Tfcp2l1, which has not been implicated in LIF/Stat3 signalling. In the second paper, Ye *et al.* (2013) analysed C57BL/6 ESCs cultured with or without 2i to investigate the effect of 2i. Consistent with the fact that 2i is required for efficient self-renewal of ESCs derived from the recalcitrant mouse strain C57BL/6, a substantial number of genes were upregulated or downregulated 12 h after treatment of 2i. The authors focused on TFs upregulated by 2i and found Tfcp2l1 in this list. The induction of *Tfcp2l1* by 2i was confirmed in 129 ESCs, in which MEKi and GSK3i had redundant effects.

Both authors then performed gain- and loss-of-function experiments and found that sustained expression of *Tfcp2l1* robustly promoted self-renewal by bypassing the requirement of LIF/Stat3 or MEKi, but only partially of GSK3i (Martello *et al.*, 2012, 2013; Ye *et al.*, 2013). Self-renewal was impaired when *Tfcp2l1* was knocked down in the presence of minimal inputs like serum + LIF and MEKi + LIF. However, depletion of *Tfcp2l1* was largely tolerated in 2i, 2i + LIF, or by constitutive expression of *Nanog*. To gain more insight, both authors tested its role in reprogramming of post-

implantation epiblast stem cells (EpiSCs) to a more primitive ESC state, in which LIF/Stat3 and 2i play instructive roles. As expected, Tfcp2l1 was found to be required and sufficient to reprogram EpiSCs in combination with 2i.

Taken together, the two papers identify Tfcp2l1 as an intrinsic pluripotency regulator interfacing with multiple environmental inputs. How Tfcp2l1 contributes to pluripotency remains elusive because of its promiscuous binding to a large number of genomic regions by itself or, albeit less commonly, together with Oct3/4/Sox2/Nanog (Chen *et al.*, 2008). Nevertheless, Ye *et al.* (2013) suggest that activation of Nanog by Tfcp2l1 is essential for its function. Tfcp2l1 physically interacts with other TFs, including Oct3/4 and Esrrb, and distinct chromatin-modifying complexes (van den Berg *et al.*, 2010), suggesting a context-dependent role of Tfcp2l1. The authors' studies also emphasize functional redundancy between extrinsic and intrinsic regulators of pluripotency (Figure 1). Indeed, ablation of one of these modules seems to be well tolerated both *in vitro* and *in vivo*, as is the case in *Tfcp2l1* knockout (Yamaguchi *et al.*, 2006). Thus, these functionally redundant modules may additively contribute to the robustness under suboptimal environments during the development. Considering that most TFs relaying extrinsic stimuli in ESCs, including Tfcp2l1, are also expressed in the pre-implantation epiblast (Guo *et al.*, 2010), the robustness of pluripotency network in ESCs may also account for that seen in embryos. It would be interesting to see the effect of simultaneous ablation of two redundant modules on pluripotency *in vitro* and *in vivo*. One can expect that such individual characterization of each functional module helps to understand how dynamically and how flexibly pluripotency network behaves and how much such network regulation also accounts for the establishment of and exit from pluripotency.

Conflict of interest

The authors declare that they have no conflict of interest.

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