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New Balance in Pluripotency: Reprogramming with Lineage Specifiers

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Induction of pluripotency in somatic cells has been achieved by myriad combinations of transcription factors that belong to the core pluripotency circuitry. In this issue, Shu et al. report reprogramming with lineage specifiers, lending support to the view of the pluripotent state as a fine balance between competing differentiation forces.

The induction of pluripotency in somatic cells by transcription factors that govern the pluripotent circuitry (Takahashi and Yamanaka, 2006) has changed the way that we understand pluripotency and cellular states in general. The idea that forced activation of master regulators can induce lineage conversion into the specific cell type that is controlled by these transcription factors has become canonical by now (reviewed in Vierbuchen and Wernig, 2011). Since Yamanaka's seminal experiment, various cocktails of transcription factors have been used for pluripotency induction; more recently, general epigenetic regulators that change chromatin state through histone modifications or DNA methylation have been shown to facilitate reprogramming by directly or indirectly activating pluripotency genes (reviewed in Papp and Plath, 2013). In this issue of *Cell*, Shu et al. (2013) reveal that mouse fibroblasts can be reprogrammed into induced pluripotent stem cells (iPSCs) using nuclear factors that control lineage specification and are not considered to be core factors of pluripotent stem cells.

According to the prevailing model, pluripotency factors prohibit differentiation and thereof enable the maintenance of the undifferentiated state (Hanna et al., 2010; Young, 2011). However, an alternative view of pluripotency has been suggested, according to which pluripotency factors act as rival specifiers that compete to specify differentiation along mutually exclusive lineages (Loh and Lim, 2011). Shu et al. now provide experimental and computational support for this idea.

In their study, Shu et al. performed a large-scale search for genes that can replace Oct4 in the reprogramming of somatic cells. Their analysis demonstrates that Gata3, as well as other mesendodermal (ME) specifiers, can replace Oct4, presumably by counteracting the upregulation of ectodermal (ECT) genes induced by Sox2. They further show that RNAi against Dlx3, an important ECT gene, can recapitulate this effect. Reciprocally, they demonstrate that Gmnn, an ectodermal specifier, can replace Sox2 in reprogramming, as it attenuates the elevation of ME genes induced by Oct4. Most interestingly, reprogramming can be achieved with Gata3 and Gmnn in the absence of both Oct4 and Sox2. What makes the current report so surprising is that the combination of nuclear factors used did not include any of the core pluripotency factors; rather, the authors describe the induction of pluripotency in somatic cells by the introduction of counteracting lineage specifiers.

Based on these results, the authors propose a "seesaw" model that places in the center of the reprogramming process the balance between counteracting differentiation cues. According to this model, a pluripotent state can be reached only if all specification forces are well balanced, and such a "balanced state" is sufficient for reprogramming induction in the presence of Klf4 and c-Myc. It has been shown before that Oct4 and Sox2, the core pluripotency activators, can also induce lineage specification (reviewed in Loh and Lim, 2011). Shu et al. take a critical step in advancing this observation to suggest that the counter-effects of pluripotency genes on differentiation play a major role in the induction of pluripotency. Furthermore, they suggest that these effects are so critical that introducing lineage-specific nuclear factors is sufficient to induce pluripotency. Their model thus implies a mirror image in the function of the pluripotency genes Oct4 and Sox2 and the lineage specifiers Gata3 and Gmnn (Figure 1A). Therefore, the interaction between the close cellular states of pluripotency and early specification may be more complex than has been previously perceived.

Several interpretations can explain these surprising results. First, as is advocated by the authors, preventing lineage specification into the main lineages may by itself be sufficient for pluripotency induction (Figure 1B, I). The fact that RNAi against Dlx3 can replace Oct4 in reprogramming lends support to this idea. The balance between counteracting differentiation cues can intuitively explain how cells are maintained in the undifferentiated state, but it is much more difficult to speculate why such balance would also be sufficient for pluripotency induction. Moreover, this interpretation does not account for the authors' finding that only some, but not all, master regulators of specification can successfully replace Yamanaka's reprogramming factors. The mechanism that links specification cues and pluripotency activation is still a "black box" in the model.

An alternative explanation is that lineage specifiers execute their role in pluripotency induction regardless of their main activity in lineage specification yet not through activation of pluripotency genes

(Figure 1B, II). This hypothesis is somewhat vague, as it does not explain how these factors trigger pluripotency without being wired into the pluripotency network. An appealing solution would therefore be that some lineage specifiers do regulate core pluripotency factors (Figure 1B, III). The authors provide evidence that Gata3 is not a direct activator of Oct4; however, it may still directly activate other pluripotency factors not tested in the current study. Furthermore, these lineage specifiers may indirectly activate pluripotency regulators through the activation of epigenetic regulators (Figure 1B, IV). Such indirect activation would be consistent with the reprogramming kinetics presented in the study. Importantly, Gmnn, which was used in this study as an ectodermal specifier, is also expressed in undifferentiated pluripotent stem cells, where it mediates the expression of Oct4, Sox2, and Nanog through the chromatin-remodeling factor Brg1 (Yang et al., 2011). This observation supports the notion that some lineage specifiers may function through the core pluripotency network. ChIP-seq analyses of pluripotency-inducing lineage specifiers and their comparison to pluripotency-refractory lineage specifiers may help to further examine these possibilities.

Though the exact mechanism of reprogramming by lineage specifiers remains to be elucidated, the suggested model generates a novel conceptual framework and raises testable predictions. Future studies will examine the robustness of the new reprogramming cocktail(s) and whether they can apply more broadly to other settings of reprogramming and to other cellular states. For example, previous work has shown that exogenous Oct4 together with either Klf4 or c-Myc is sufficient to generate iPSCs from neural stem cells (Kim et al., 2008); will Gata3 and Klf4 or c-Myc be able to reprogram neural stem cells? Will systematic changes in the relative levels of Oct4 and Sox2 in pluripotent stem cells correlate well with their differentiation tendencies toward different lineages? Can direct reprogramming of one somatic cell type into another be achieved by balancing differentiation cues instead of expressing master regulators that govern the desired cellular state? The current work raises fascinating questions and may shift the balance of our view of pluripotency, from focusing mostly on the pluripotency state to the analysis of its early differentiating progenies.

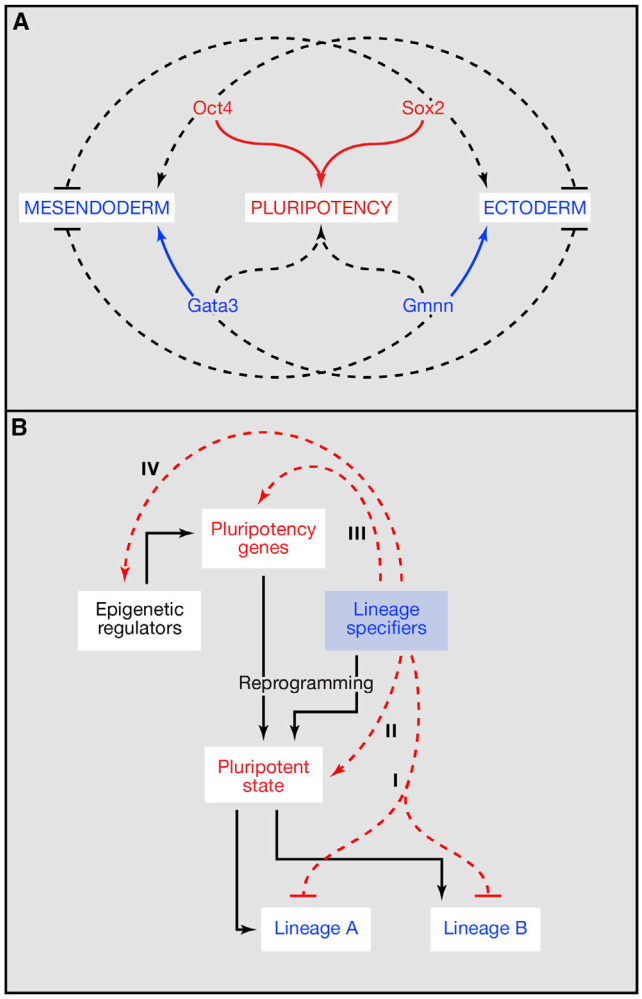

Figure 1. Mechanisms by which Lineage Specifiers May Induce Pluripotency

(A) Cooperative activation of Oct4 and Sox2 induces pluripotency, but their discordant activation may lead to mesendodermal or ectodermal differentiation, respectively. Shu et al. (2013) demonstrate that coordinate activation of mesendodermal specifiers such as Gata3 and the ectodermal specifier Gmnn is sufficient to induce pluripotency. Bold arrows indicate canonical activity, whereas dashed arrows indicate noncanonical activity. Red denotes the pluripotency state and its core transcription factors, and blue denotes the specified states and their nuclear factors.

(B) Reprogramming somatic cells into pluripotent cells can be achieved by induction of core pluripotency genes and can be facilitated by epigenetic regulators, such as chromatin modifiers. The current study suggests that pluripotency can also be induced by coordinated effects of lineage specifiers. This induction of pluripotency can be due to: (I) coordinated inhibition of specification; (II) a yet-undetermined mechanism that is related neither to specification inhibition nor to activation of pluripotency genes; (III) direct activation of core pluripotency factors by lineage specifiers; or (IV) indirect activation of core pluripotency factors through the activation of epigenetic regulators.

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