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Order from Chaos: Single Cell Reprogramming in Two Phases

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The generation of induced pluripotent stem cells (iPSCs) is considered to be stochastic with a minute fraction of cells becoming pluripotent. Recently in *Cell*, Buganim et al. (2012) changed this view using single cell analyses to reveal a stochastic early and hierarchical late phase, with implications for productive alternative reprogramming strategies.

The dramatic reprogramming of a somatic cell into a pluripotent one equivalent in terms of developmental potentials to those only found in early embryo such as a blastocyst is a tremendous achievement. Although three approaches are known to accomplish this feat, somatic cell nuclear transfer (SCNT) (Gurdon et al., 1958), cell fusion, and transcription factor (TF)-based reprogramming, the generation of induced pluripotent stem cells (iPSCs) by four TFs represents a breakthrough that has enriched our understanding of cell fate decisions in a fundamental way (Takahashi and Yamanaka, 2006). Despite intense interest in this topic, how TF-based reprogramming actually occurs remains unclear at this time, due in part to the low efficiency of iPSCs generation. Experimental evidence and mathematic modeling suggest that reprogramming to iPSCs is a stochastic process (Hanna et al., 2009; Yamanaka, 2009), in contrast to reprogramming by SCNT, which is mostly considered to be deterministic (Figure 1A). In a recent issue of *Cell*, Buganim et al. (2012) addressed this issue using single cell analysis of reprogramming by Yamanaka factors, which indicates a two-stage process that is stochastic at the early phase followed by a more deterministic or hierarchical late phase governed by specific regulatory factors (Buganim et al., 2012). Remarkably, these analyses allowed them to replace the original Yamanaka factors with downstream factors identified in the late phase. The new findings, along with the single cell approach, may change the way we view reprogramming.

The Yamanaka factors, Oct4, Sox2, Klf4, and Myc (OSKM), previously known for their role in development and cancer, were discovered in 2006 for their combined ability to reprogram mouse embryonic fibroblast (MEF) cells to iPSCs (Takahashi and Yamanaka, 2006). Earlier attempts to understand how the Yamanaka factors work focused on population-based global analyses such as microarrays, proteomics and functional genomics/epigenetics that have generated large data sets documenting the molecular changes triggered by the Yamanaka factors. One such data set suggested that reprogramming goes through three distinct phases: initiation, maturation, and stabilization (Samavarchi-Tehrani et al., 2010), consistent with the sequential activation of various known stem cell markers and epigenetic changes (Mikkelsen et al., 2008). At the cellular level, the first morphological change upon the transduction of the Yamanaka factor is the acquisition of epithelial properties by MEFs, suggesting that a mesenchymal-to-epithelial transition (MET) initiates the reprogramming process (Li et al., 2010), a conclusion corroborated by functional genomics (Samavarchi-Tehrani et al., 2010). Detailed analysis of the MET process revealed a clear division of labor among the Yamanaka factors at the transcriptional level, i.e., the suppression of mesenchymal genes such as Snail, TGF-b and TGF-b receptor 2 by Sox2, Oct4, and c-Myc, followed by the induction of epithelial genes including E-cadherin by Klf4 (Li et al., 2010). Despite these early insights on the role of the Yamanaka factors in reprogramming, population-based investigations might have missed minor yet critical regulators due to the inherent low signal-to-noise ratio because only a tiny fraction of cells eventually become iPSCs.

In order for a somatic cell to eventually give rise to iPSCs, one can imagine that the forced expression of the Yamanaka factors would need to bind to accessible genomic sites and start to activate the expression of those targets; then this first wave of genes, termed first responders, in turn would not only activate further downstream regulators but also open up the chromatin domains previously not accessible in MEFs for transcription activations (Pei, 2009); finally, the first responders along with their targets would then be able to activate the core pluripotency circuitry, turn off somatic genes, and even silence the exogenous reprogramming factors, effectively rewriting the cell fate code from a somatic into a pluripotent one (Pei, 2009). Because reprogramming is a lengthy process, the original cell keeps on dividing and only a small fraction of its progeny contributes to an iPSC colony. The rest of the cells would have aborted the reprogramming process and assumed cell fates that are short of full pluripotency, e.g., partially reprogrammed cells. Thus, the ideal approach to track the molecular events essential to successful reprogramming is to analyze individual cells during reprogramming.

The Jaenisch group deployed the latest technology for single cell analysis

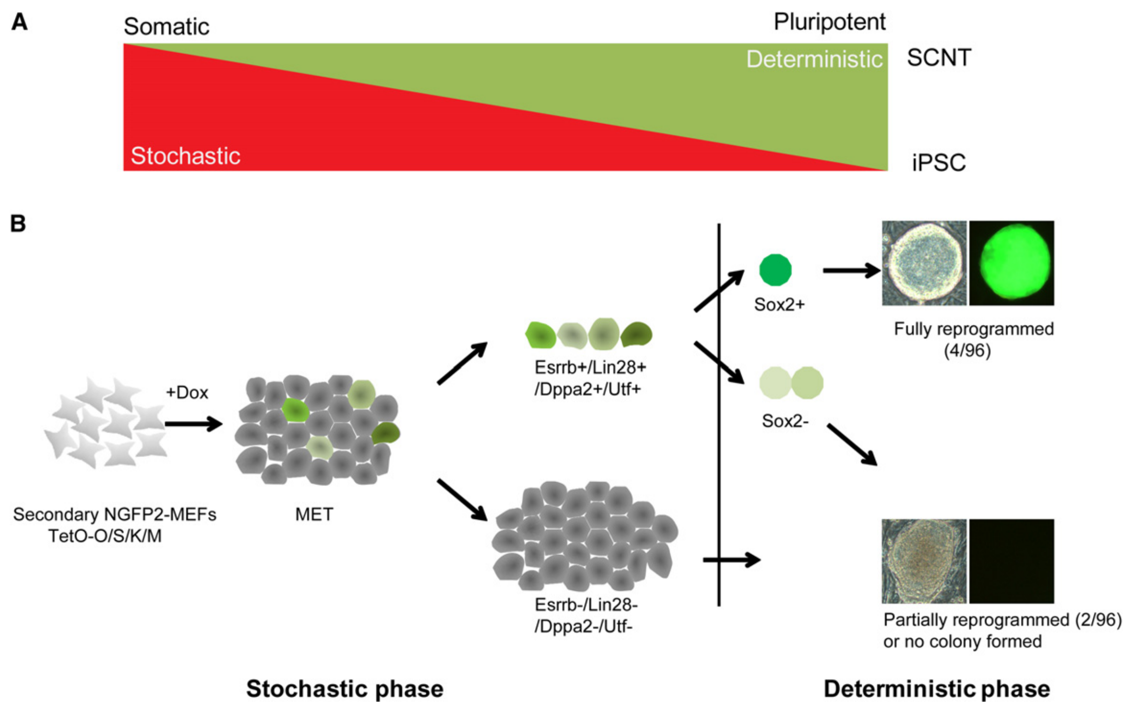

Figure 1. Schematic View of Reprogramming Models and Phases
(A) Two models exist to explain the reprogramming of somatic cells to pluripotent ones. SCNT is thought to reprogram by a deterministic mechanism (top). SCNT is known to be fast. The generation of iPSCs is thought to be stochastic (bottom). The process is lengthy and inefficient. A combined model may have both stochastic and deterministic phases (the sliding slope).
(B) The likely cellular events of reprogramming as revealed by single cell analysis.

to reexamine the molecular events associated with the mechanism of action for the Yamanaka factors. Using arrays, they analyzed the expression of 48 genes including those for ESC chromatin remodeling, cell cycle regulation, signal transduction, and pluripotent markers in single cells sorted from different stages of OSKM-induced reprogramming representing early, intermediate, and fully reprogrammed iPSCs (Buganim et al., 2012). They showed that at the very early stage of reprogramming, the expression of these genes exhibited large variations between different individual cells as predicted by the stochastic model. An individual cell with each activation profile was then clonally expanded, and the resulting sister cells were dynamically traced individually for the expression of the same set of 48 genes at different stages during further reprogramming. This single-cell tracing system provided a comprehensive view of the molecular events over time and in terms of differentiation potential from MEFs to iPSCs. As a result, they made several key observations on the reprogramming process.

They observed that the cell cycle regulators and MET markers are detected at the early stage in both iPSC-producing and non-iPSC-producing cells. This is consistent with earlier work on cell cycle and MET (Mikkelsen et al., 2008) (Li et al., 2010). Then, they found a surprise: the early activation of Oct4, the most critical gene for pluripotency as its expression is essential to maintain pluripotency for the inner cell mass of mouse blastocysts in vivo and mouse ESCs in vitro, is not predictive for the generation of full iPSCs. In contrast, cells expressing a particular set of genes heterogeneously at the early phase progressed into a full pluripotent state later. This set of ‘‘iPSC predictive’’ genes, *Esrrb*, *Utf1*, *Lin28*, *and Dppa2*, appear to be more informative in forecasting the reprogramming outcome. Remarkably, single cell profiling at the later stage of reprogramming identified Sox2 as a reliable mark for those cells that eventually progress into iPSCs. Sox2 is a well-known partner for Oct4 and has been in its shadow in previous literature on stem cell pluripotency because it can be substituted functionally by a small molecule during reprogramming. Yet, single cell analysis revealed that the activation of endogenous Sox2 sets a hierarchical course of action leading to the eventual acquisition of pluripotency, suggesting that the post-Sox2 events are no longer stochastic. One might argue that reprogramming becomes deterministic after the activation of Sox2 at the single cell level (Figure 1B).

The story did not end at Sox2. Buganim et al. (2012) took advantage of the predictive power of their model and proposed a bold move: to replace the Yamanaka factors with their downstream factors. This is brilliant because the outcome not only validates their model but also opens up a new direction. To this end, they found that as a group, Lin28, Sall4, Esrrb, and Dppa2 were able to reprogram MEFs to iPSCs. Although these four factors have been shown previously to be able to enhance reprogramming, it is remarkable that they are sufficient by themselves without the original Yamanaka factors and Nanog (Figure 1). Although the efficiency remains very low for this new

combination, it can be further improved by optimizing the reprogramming environment as has been achieved with the Yamanaka factors in the near future (Chen et al., 2011).

The realization that reprogramming by defined factors is a stochastic early and deterministic late process may encourage further efforts to manipulate the ratio between stochastic and deterministic phases (Figure 1A). Because reprogramming is a collaboration between the defined factors and the culture environment (Li et al., 2010), one may speculate that ultimately, a "perfect" reprogramming environment may allow reprogramming to proceed with no or very short stochastic phase (Figure 1A). If so, it may be feasible to achieve all deterministic reprogramming with defined factors, thus, narrowing or reconciling the difference between SCNT and iPSC (Figure 1A). As pointed out by the authors, single cell analysis is at its infancy. Yet, it has already helped the reprogramming field so nicely. Much should be anticipated from this line of inquiry.

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Hematopoietic Stem Cells Burn Fat to Prevent Exhaustion

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Ito et al. (2012) recently report in *Nature Medicine* that fatty acid oxidation (FAO) regulated by PPARδ controls asymmetric division in hematopoietic stem cells (HSCs). This metabolic mechanism prevents HSC exhaustion and is downstream of the promyelocytic leukemia protein PML, suggesting therapeutic implications for HSC function and disease.

Hematopoietic stem cells (HSCs) are some of the most mysterious entities of an organism, subdivided into an incredible variety of subsets. HSCs are exquisitely sensitive to changes in transcriptional networks and external informatory molecules, such as those provided by the niche microenvironment. The metabolism of these essentially quiescent cells has been the focus of many recent studies (reviewed in Suda et al., 2011) but the contribution of lipid metabolism remains unexplored (Suda et al., 2011). Ito et al. now report findings that not only bridge nuclear organization, transcriptional control, and lipid metabolism in decisions underlying asymmetric cell division, but that also have major implications for therapeutic manipulation of HSCs.

In a previous study, the Pandolfi group reported that deletion of *pml* leads to loss of HSC quiescence, resulting in their transient amplification and subsequent exhaustion (Ito et al., 2008). Here, the authors demonstrate that PML activates PPARδ, a nuclear receptor that has a key role in stem cell maintenance. Indeed, in multiple in vivo or ex vivo assays, conditional loss of *ppar*δ was found to decrease HSC abundance and repopulating ability while treatment with specific agonists improved HSC function. Loss of self-renewal likely results from an increase in HSC cycling, so that loss of either *pml* or *ppar*δ results in the accumulation of committed progenitors. Conversely, defects in $pml^{-/-}$ HSCs were partly rescued by PPARδ agonists. PPARs are central regulators of metabolism and control mitochondrial function, in particular fatty acid oxidation (FAO).